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HALOPHYTES FOR THE TREATMENT OF SALINE AQUACULTURE EFFLUENT

by

Jonathan Jed Brown

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A Dissertation Submitted to the Faculty of the
SCHOOL OF RENEWABLE NATURAL RESOURCES

In Partial Fulfillment of the Requirements
For the Degree of

DOCTOR OF PHILOSOPHY
WITH A MAJOR IN WILDLIFE AND FISHERIES SCIENCE

In the Graduate College
THE UNIVERSITY OF ARIZONA

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and recommend that it be accepted as fulfilling the dissertation
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ABSTRACT

The discharge of untreated aquaculture effluent can pollute receiving water bodies. I tested the feasibility of using salt-tolerant plants (halophytes) with potential as forage and oilseed crops, as biofilters to treat saline aquaculture effluent. Plants were grown in draining lysimeters in greenhouses and irrigated with effluent salinized with NaCl. Irrigation water came from a recirculating tilapia culture system. I measured yield potential, water use and capacity for nitrogen and phosphorus uptake.

In Experiment 1, *Suaeda esteroa*, *Salicornia bigelovii* and *Atriplex barclayana* (Chenopodiaceae) were grown in sand in 0.02 m³ lysimeters. Plants were irrigated with effluent of 0.5 ppt, 10 ppt and 35 ppt salinity, to meet evapotranspiration demand and to allow 30% of the applied water to leach past the plant root zone. Despite the high leaching fraction and short residence time of water in the pots, the plant-soil system removed 98% and 94% of the applied total and inorganic nitrogen, respectively, and 99% and 97% of the applied total and soluble reactive phosphorus respectively. For all species, salt inhibited ($P \leq 0.05$) the growth rate, nutrient removal, and volume of water the plants could process. The salt marsh species *S. esteroa* and *S. bigelovii* performed better than the desert saltbush, *A.*

barclayana, at 35 ppt.

In Experiment 2, *Suaeda esteroa*, was grown in lysimeters containing approximately 0.8 m³ sandy loam soil and irrigated three times per week with 31 ppt NaCl effluent. I used five irrigation treatments, ranging in volume from 50 to 250% of the potential evaporation rate. Plant biomass and water consumption increased significantly ($P \leq 0.05$) with increasing irrigation volume. Nitrate concentrations in water draining from the lysimeters decreased during the experiment, and were significantly lower in the high-volume treatments than in the low-volume treatments. Phosphorus concentrations in the leachate water increased during the experiment as a function of increasing irrigation volume. Irrigating halophyte crops with aquaculture wastewater of seawater-salinity may be a viable strategy for disposal of effluent.

INTRODUCTION TO THE DISSERTATION

1. Aquaculture and the environment

Aquaculture has expanded rapidly over the past decade. The global aquaculture production of fish, crustaceans and molluscs was 21.3 million mt in 1995, an increase of 177% over production in 1985 (New, 1997). Aquaculture contributes about 25% of the global supply of food fish, and production has approximately doubled in the decade from 1985-1995 (New, 1997). The increase in the production of several marine species has been particularly spectacular. Salmon production increased over ten fold and marine shrimp over four fold from 1985 to 1994. Aquaculture production accounts for about a third of the total production of both of these species (New, 1997). Aquaculture production is projected to continue to increase in the near future to meet the world's growing protein demand (New, 1997).

The expansion of aquaculture has caused some environmental degradation. The major problem is the discharge of untreated effluent into receiving water bodies. The discharge of this effluent can lead to hypereutrophication (increased dissolved nutrient levels) and eutrophication in the receiving water body.

Effluent is generated through the exchange of water or draining ponds for harvest. Water exchange is used to flush nutrients and phytoplankton from aquaculture ponds to limit

phytoplankton blooms, to remove toxic metabolites such as ammonia, to temporarily reduce salinity and to substitute for aeration (Boyd, 1990). A typical brackish water pond aquaculture operation may exchange from 2-40% of water per day, with an average rate of about 8-10% per day (Boyd, 1990).

The composition of effluent is determined by feed and fertilizer inputs and by water exchange rates (Hopkins et al., 1995). Generally, effluent is high in suspended solids, organic matter, inorganic nutrients, biological oxygen demand, and low in dissolved oxygen (Hopkins et al., 1995). The dissolved nutrients are derived from fish or shrimp excretion, dissolved food particles, and recycling from sediments from the pond bottom.

Effluent can increase phytoplankton and bacteria levels or reduce the dissolved oxygen content in the receiving water body due to the high biological oxygen demand of the effluent (Hopkins et al., 1995). Generally nitrogen and phosphorus are implicated in eutrophication because they are considered to be the nutrients limiting phytoplankton growth--nitrogen in marine systems, phosphorus in freshwater systems (Rhyther and Dunstan, 1971; Goldman et al., 1973).

Environmental impacts resulting from the discharge of untreated effluent into adjacent receiving water bodies have been the subject of several reviews (Gowen and Bradbury, 1987; Iwama, 1991; Handy and Poxton, 1993; Hopkins et al.,

1995; Wu, 1995; Beveridge 1996; Dierberg and Kaattisimkul, 1996; Costa-Pierce, 1996). Whereas aquaculture effluent has not been implicated in large scale eutrophication in marine systems, localized hypernutrification and or lowered dissolved oxygen levels in the water column have been reported in both freshwater and marine environments (Bergheim et al., 1982; Penczak et al., 1982; Bergheim et al., 1984; Wisemann et al., 1988; Iwama, 1991; Wildish et al., 1992; Cornell and Whoriskey, 1993; Hargrave et al., 1993; Briggs and Funge-Smith, 1994; Wu et al., 1994). In general, negative environmental effects of the discharge of aquaculture waste are more pronounced in enclosed or semi-enclosed areas with poor or limited water circulation. Other negative environmental effects associated with aquaculture wastes include toxic algal blooms (Kaartvedt et al., 1991), reduced and or anoxic benthic sediments (Holmer and Kristenen, 1992; Hargrave et al., 1993; Wu et al., 1994), decreased benthic faunal biodiversity (Brown et al., 1987; Weston, 1990; Tsutsumi et al., 1991, Ye et al., 1991; Hargrave et al., 1993), and accumulation of pathogenic and antibiotic-resistant bacteria (where antibiotics are added to feed) (Husevåg et al., 1991; Samuelson et al., 1992; Kerry et al., 1994).

The discharge of nutrients from a 1 ha intensive shrimp pond is estimated to be about 1570 kg nitrogen and 430 kg phosphorus per year (Roberston and Phillips 1996). Briggs

and Funge-Smith (1994) estimate that only 24% of the total nitrogen and 13% of the total phosphorus added via feed is incorporated into shrimp biomass, and the remainder is ultimately exported to the surrounding environment.

Similarly, it has been estimated that of the total nutrient inputs into a salmon cages, 21-22% of the carbon, 27-28% of the nitrogen, and 17-19% of the phosphorus was recovered in the fish at harvest, and 75-78% of the carbon, 67-71% of the nitrogen, and 78-82% of the phosphorus was lost to the environment (Hall et al., 1990; Holby and Hall, 1991; Hall et al., 1992).

Aquaculture effluent is generally less concentrated than domestic or industrial wastewater, but environmental problems arise from the large volumes of water discharged. The nutrient waste load from intensive shrimp ponds in Thailand are estimated to be equivalent to the load of 3.1-3.6 million people or 5-6% of the population for nitrogen, and from 4.6-7.3 million people or 7-11% of the population for phosphorus (Briggs and Funge-Smith, 1994).

Self-pollution results when many ponds are concentrated in estuaries that have low flushing rates (Phillips, 1995). Self-pollution occurs when ponds are taking in water of poor quality, water that may have been recently discharged from other farms and that may contain pathogens. Self-pollution has been blamed for the collapse of the shrimp industry in Taiwan and the Upper Gulf of Thailand (Phillips, 1995).

There are several options currently available to reduce the quantity, and to improve the quality of the effluent discharged by aquaculture operations. The quantity of exchanged water can be reduced, but there is still debate about how reduced water exchange affects the yield and the quality of the final product (Hopkins et al., 1993; Martinez-Cordova et al., 1995). In addition, greater farm management skills are required to operate low discharge systems (Phillips, 1995). Alternatively, the quality of the effluent can be improved by optimizing feeding rates, increasing food conversion ratios and using environmentally friendly feeds that have lower nitrogen and phosphorus content (Phillips, 1995).

There is particular interest in the treatment of mariculture effluent because of the rapid increase in the number of shrimp farms and the associated environmental problems these farms create (Dierberg and Kaattisimkul, 1996). There are currently fewer treatment options available in marine or estuarine systems than with freshwater systems. The need for more environmentally benign fish-farming practices, combined with increased government regulation of effluent discharge has led to a demand for treatment technologies for saline aquaculture effluent.

Technologies currently available for the treatment of effluent include both physical and biological methods. The physical methods include settlement ponds and mechanical

filtration. Settlement ponds require large areas and provide no economic return. Additionally, there is a large expense associated with pond construction, and buoyant phytoplankton do not settle out easily (Phillips, 1995). Mechanical filtration effectively removes solids, but is prohibitively expensive, with a capital cost of \$137,500/hectare of pond and an annual operating cost of \$75,000 (Phillips, 1995).

Biological methods include using filter-feeding fish or bivalves to filter and cleanse effluent. However, many of these organisms are sensitive to salinity fluctuations and further degrade water-quality through the production of feces and pseudofeces (Phillips, 1995). Seaweeds have been used effectively as biofilters in intensive, closed systems (Neori et al., 1996). However, on-site in effluent canals, seaweeds tend to become light-limited due to the high turbidity of the water. They also commonly become covered by solid particles from the high concentration of solids in effluent (Phillips, 1995). Constructed wetlands can be used to remove nutrients and settle solids from effluent (Schwartz and Boyd, 1995). However, they provide no economic return to the grower, require a large area, and the nutrients are not ultimately removed from the ecosystem unless the plant biomass is harvested.

Freshwater aquaculture effluents have been used successfully to irrigate traditional crops (Olsen et al.,

1993; D'Silva and Maughan, 1996). Plants can remove a significant fraction of the nutrients supplied in the irrigation (effluent) water and incorporate them into their phytomass. Additionally, the crop provides an economic return to the grower, the nutrients are removed from the area when the phytomass is harvested, and much of the effluent volume is actually disposed of through evapotranspiration of the plants. It is potentially feasible to use halophytes, or salt-tolerant crop plants similarly to treat saline aquaculture effluent.

2.0. Review of halophytes as crops

Halophytes are plants that are capable of growing and reproducing in areas subject to constant or periodic high salinities. They dominate the world's salt marsh and salt desert floras (O'Leary and Glenn, 1994). The family with the greatest number of halophyte species is the Chenopodiaceae; over half of its 550 species are halophytic (Aronson, 1989). Halophytes can be grown to produce forage and fiber, seed (oilseed and grain) and vegetable crops, landscape plants, or ornamentals.

2.1. Irrigation of halophytes

Miyamoto (1995) estimated the optimum soil salinity for several euhalophyte species at approximately $20 \text{ mg l}^{-1} \text{ NaCl}$ or less. Growth was observed to decline rapidly beyond soil

salinities of 20-30 mg l⁻¹. Salinity of the soil solution can be 2-3 times higher than the salinity of the irrigation water, thus indicating an optimum irrigation water salinity of about 10 ppt. Therefore true halophytes (euhalophytes) possess an optimum salinity range for maximum growth, above which growth will be reduced. High salinity water such as seawater (32 ppt), is above the soil salinity optimum for halophytes. Therefore, if irrigation with high salinity water is attempted, special measures must be taken to ensure the salinity of the soil solution is maintained at levels that do not greatly inhibit plant growth. Lowering soil salinity is generally accomplished by leaching--applying sufficient water so that a portion of the applied water percolates past the plant root zone and carries away excess salts. The leaching fraction (LF) is the proportion of the applied water that percolates below the plant root zone (Ayers and Wescot, 1985) and is given by the equation:

$$LF = \frac{\text{Depth of water leached below root zone}}{\text{Depth of water applied at the surface}}$$

With everything else constant, the higher the leaching fraction, the lower the salinity of the soil water. However, higher leaching fractions and greater irrigation depths are associated with higher pumping costs and larger discharges of water to the aquifer or drainage system. Large discharges may be a problem where best management

practices must be followed (Glenn et al., 1998). In general there is no straightforward rule to determine LF. Ayers and Wescott (1985) estimated the minimum Leaching Requirement (LR) as:

$$LR = \frac{EC_w}{5EC_e - EC_w}$$

where:

EC_w = salinity of the applied irrigation water in $dS\ m^{-1}$

EC_e = average soil salinity tolerated by the crop as measured on a soil saturation extract.

Miyamoto (1995) gave the following equation to calculate the first approximation of the leaching required to maintain soil salinity levels:

$$LF = \frac{C_I}{2C_S - C_I}$$

where: C_I = salinity of the irrigation water

C_S = salinity of the soil solution in the root zone.

However, Miyamoto (1995) cautions that leaching requirements estimated by simple steady state formulas may be excessive for seawater or high salinity water, especially with respect to handling drainage water.

Glenn et al. (1997) irrigated the euhalophyte *Salicornia bigelovii* in sand in lysimeters with seawater at irrigation depths ranging from 0.7-3.8 m, and found that all irrigation treatments produced a leaching fraction of about 0.35. Increasing the irrigation depth lowered the soil salinity, and resulted in higher plant growth and water use, and hence leaching fractions were approximately equal over all treatments. In general, Glenn et al. (1995) recommended that a leaching fraction of at least 25% be used when irrigating with seawater to maintain soil solution salinity below levels detrimental to plant growth. Much lower leaching fractions can be used for less saline water. Glenn et al. (1998) successfully grew *Atriplex nummularia* on water of 1149 and 4100 mg l⁻¹ TDS with leaching fractions of only 0.074.

Once a leaching fraction for the particular halophyte crop is established, the irrigation depth (D_I) can be calculated by:

$$D_I = \frac{ET}{1 - LF}$$

where: ET is the evapotranspiration during the irrigation interval.

In early experiments, Glenn and O'Leary (1985) used irrigation depths of 18 m yr⁻¹ to grow halophyte crops using

seawater irrigation. This amount of water is greatly in excess of the amount of water applied to grow conventional crops with freshwater. More recent experiments have shown that it is possible to grow the halophyte crop *Salicornia bigelovii* on seawater using irrigation depths of 2.3-3.8 m yr^{-1} . This rate of water application is about 30-40% higher per unit of dry matter production than the amount of fresh water required to grow a conventional crop such as alfalfa or Sudangrass under similar environmental conditions. This 30-40% excess is attributable to the higher leaching requirement when irrigating with seawater.

2.2. Frequency of irrigation

Another strategy to maintain acceptable soil solution salinities is to increase the irrigation frequency. For conventional crops grown with freshwater irrigation, irrigations are typically scheduled when the soil moisture is depleted by 25-50%. If this protocol were to be followed for seawater irrigation, root zone soil water salinities could reach detrimental levels. Therefore when irrigating with seawater it is necessary to avoid depleting the soil moisture content by more than 25%. (Glenn et al., 1995). Glenn and O'Leary (1985) and Miyamoto et al. (1995) demonstrated that to achieve high yields of halophytes under seawater irrigation in sandy soil, the soil must be kept continually moist through daily irrigation, and that

dropping to an irrigation frequency of 3 days or longer will markedly reduce yield even when large volumes of irrigation water are applied.

2.3. Quality of irrigation water

Another concern when irrigating with water with high sodium concentrations is that infiltration problems will result from soil dispersion and structural breakdown. The Sodium Adsorption Ratio (SAR) is the most commonly used method to assess potential infiltration problems. The SAR equation is:

$$SAR = \frac{[Na^+]}{[Ca^{+2} + Mg^{+2}]}$$

where: concentrations are expressed in mmoles l⁻¹.

Generally soils are classified as sodic when the SAR > 15. However, because the electrolyte concentration in seawater is high relative to the sodium concentration, slow infiltration rates are generally not a hazard for soil under seawater irrigation (Quirk, 1971).

2.4. Useful products from halophytes

Animal feeds that can be produced from halophytes include forage from dried plant shoots, oil, seed meal and grains. In general, halophytes have mixed characteristics

as forages. On the positive side they generally have high protein content, ranging from 10-20% of dry matter (Le Houérou, 1995). On the negative side, they generally have high salt (ash) content (15-50% salts on the leaf dry matter) and are fairly poor energy sources (Le Houérou, 1995). Additionally, halophytes can occasionally contain oxalate levels in the toxic range (Le Houérou, 1995).

Whereas many halophyte species have been tested for use as forages, the salt bush genus *Atriplex* has received the most attention. *Atriplex* species tend to have high protein content, generally ranging from 12-22% (Glenn et al., 1994; Gihad and el-Shaer, 1994). Crude protein of other halophyte species tends to be lower (Gihad and el-Shaer, 1994); for example the protein content of *Salicornia bigelovii* ranges only from 4-6% (Glenn et al., 1995). However, estimates of crude protein in halophytes overestimate the digestible protein content of the plant (O'Leary et al., 1985) because crude protein estimates may include non-protein nitrogen sources such as quaternary ammonia compounds and proline. These substances are used as osmocompatible solutes by halophytes. Normally, these compounds, which can account for about 50% of the plant nitrogen content, are excreted (urine and feces) and not digested. However, Le Houérou (1995) believes that non-protein nitrogen can be metabolized where animals have evolved rumen bacteria that are capable of using the non-protein nitrogen, so long as there is

sufficient energy in the diet to allow the bacteria to break down these compounds.

Despite all the drawbacks associated with utilization of halophytes as forages, Swingle et al. (1996) demonstrated that halophytes could be incorporated in a lamb diet with no affect on growth performance. In that study *Suaeda esteroa*, *Atriplex barclayana*, and *Salicornia bigelovii* straw were compared with conventional *Cynodon dactylon* hay (control) at 30% of the diet. The halophyte forages contained a much higher ash content than the *Cynodon* hay (24-34% vs. 5%). Lambs fed diets containing halophytes consumed more dry matter to compensate for the lower organic matter content of this diet compared to the control diet. Halophyte-fed lambs were able to gain weight at the same rate as the control lambs, but the feed efficiency was lower and water intake was higher.

O'Leary (1985) stated that the most promising use of halophytes may be as seed crops. Unlike the shoot tissues which accumulate large amounts of salts, the seeds of halophytes have a very low salt content, even under saline irrigation. Halophyte seeds have been used as grains. *Distichlis palmeri* seeds were harvested and used as a grain crop by the Cocopa Indians of the Colorado River Delta region (Felger, 1979). Halophytes can also be grown for oilseed production. At least two products can be derived from an oilseed. The oil can be extracted from the seed,

and used either as a vegetable oil for human consumption, or as an industrial oil. Seed meal can be used as a protein-rich food supplement similar to soybean meal. O'Leary (1988) notes that because two products are produced, there is an increased chance for an oilseed crop becoming a commercial success. Glenn et al. (1991) examined the oil of *Salicornia bigelovii* seeds and found that it was similar in caloric value to soybean oil. No difference in growth rate was found in chickens fed a diet containing 2% soybean oil, versus those fed with 2% *S. bigelovii* oil. However, there was growth inhibition of chickens fed the *S. bigelovii* seed meal. Growth depression was probably caused by saponins. Growth inhibition was reversed when the *S. bigelovii* seed meal was supplemented with 1% cholesterol at 1%; cholesterol counteracts the antigrowth properties of saponins (Glenn et al., 1991). Similar results were found by Attia et al. (1997). Ruminants are less susceptible to saponins than poultry, and seed meal from *S. bigelovii* was able to replace cottonseed meal as a protein supplement (10% inclusion rate) with no effect on lamb growth performance (Swingle et al., 1996).

2.5. Field trials of halophytes

Hugo and Elizabeth Boyko (1959) pioneered the utilization of saline water to grow salt tolerant glycophytes and halophytes on diluted and full strength

seawater. More recently three research groups have conducted additional field trials: The Environmental Research Laboratory of the University of Arizona, the Halophyte Biotechnology Center, University of Delaware, and the Institutes for Applied Research, Ben Gurion University of the Negev, Israel.

Glenn and O'Leary (1985) reported yields ranging from 13.6-17.9 t ha⁻¹ dry weight for the most productive halophyte species grown on 40 ppt seawater in field trails in a coastal desert environment (Puerto Peñasco, Sonora, Mexico). These yields are comparable to those for conventional forage crops such as alfalfa grown on freshwater (7-12 t ha⁻¹ dry matter). Native species outperformed exotics and the minimum effective irrigation rate was 18m year⁻¹.

Glenn et al. (1991) reported biomass yields of 12.7-24.6 t ha⁻¹ dry weight for the oilseed halophyte *Salicornia bigelovii*, an annual with a 200 day growing season, under 40 ppt seawater irrigation. Seed yields ranged from 1.39-2.46 t ha⁻¹, with a 5-year mean of 1.99 t ha⁻¹. These yields are comparable to or greater than yields of traditional oilseed crops such as soybeans and sunflower. The seed contained 28% oil, was high in polyunsaturated fatty acids, particularly linoleic acid, and had 31% protein, 5% fiber, and 5% ash.

In 1994 and 1995 Glenn et al. (1997) grew *Salicornia*

bigelovii irrigated with 40 ppt seawater in lysimeters. Irrigation treatments ranged from 46-225% of potential evaporation. Biomass yields ranged from 13.6-23.1 t ha⁻¹, and both biomass and seed yields increased with increasing irrigation depth. Increased irrigation depth lowered the salinity of moisture of the top 15 cm of the soil profile below the 70-75 ppt threshold required to achieve acceptable yields, and thus increased plant yields. Seed yield ranged from 0.002-0.037 t ha⁻¹, only 20% of the yields reported by Glenn et al. (1991). The lower seed yields were attributed to the use of non-native germplasm (Glenn et al., 1997).

Yensen et al. (1988) reviewed the potential of using the salt grass *Distichlis palmeri* as a grain crop that could tolerate seawater irrigation. The grain produced by *D. palmeri* has a nutritional composition similar to wheat, but had a lower protein and higher fiber content. In experimental plantings, yields were sharply reduced at 25 ppt salinity compared to those at 5 ppt. The authors estimated potential grain production at approximately 1 t ha⁻¹ (salinity of irrigation water not indicated).

Watson et al. (1987) achieved estimated dry weight biomass yields of 14.7 and 12.3 t ha⁻¹ for *Atriplex lentiformis* and *A. nummularia*, respectively under irrigation with water with an electrical conductivity (EC) of 10 ds m⁻¹. Watson (1990) used saline (EC ranged between 10-13 ds m⁻¹) drainage water to grow several accessions of *Atriplex*.

Dry matter yield of *A. barclayana*, the most productive of the species investigated, was estimated at 11.1 t ha^{-1} . Mean crude protein for all species ranged from 14.4 to 19%, somewhat lower than the crude protein content of alfalfa hay (22-29%). Average ash content ranged from 16.6-37.0% with an overall mean of 29.7%. Watson and O'Leary (1993) grew several *Atriplex* species with saline drainage water ($\text{EC}, 18 \text{ ds m}^{-1}$). Plants were mechanically harvested and baled four times over 27 months. Mean annual yields ranged from $2.2\text{--}5.3 \text{ t ha}^{-1}$, with a mean of 3.8. Mean crude protein levels ranged from 6-13%. The authors concluded that *Atriplex deserticola* and *A. undulata* had the best set of agronomic characteristics for hay production, including tolerance to low cuts by machine and multiple harvests, resistance to damage from harvest equipment wheel traffic and adaptability to harsh field conditions. Glenn et al. (1998) grew *Atriplex nummularia* in outdoor lysimeters. Plants were irrigated with two sources of saline wastewater, one with 1149 mg l^{-1} TDS and the other 4100 mg l^{-1} TDS. Biomass production did not differ between plants irrigated with waters of different salinity. Mean dry weight biomass production was 44 t ha^{-1} , nearly twice as high as the yield of conventional forage crops, such as alfalfa grown in the same county. Mean protein content (10%) was lower than that of alfalfa. Leaf ash content was 21.2% for plants grown on the less saline water, and 25.5% for plants grown

on the more saline water.

Researchers at Ben Gurion University of the Negev (Aronson et al., 1988) tested 120 halophyte species under seawater irrigation (56 dS m^{-1}) and 15% seawater (5.5 dS m^{-1}). They had greatest success with *Atriplex* species. Dry weight yields and percent crude protein of seven *Atriplex* species ranged from $12.6\text{--}20.9 \text{ t ha}^{-1}$ and $9.9\text{--}19.5\%$, respectively, on full strength seawater and from $10.4\text{--}28.9 \text{ t ha}^{-1}$ and $9.0\text{--}18.0\%$, respectively, on 15% seawater. *Atriplex barclayana* was the outstanding performer. Other halophyte species with potential for use as landscape, ornamental, and forestry crops were identified (Pasternak and Nerd, 1995).

Gallagher (1985) examined several species of salt marsh grasses with potential as forage crops under seawater irrigation in Delaware. Yields of *Distichlis spicata* ranged from $5.2\text{--}9.5 \text{ t ha}^{-1}$, depending on harvest date. Several other forage grasses were tested in Egypt (salinity of irrigation water not indicated). Yields for *D. spicata* there ranged from $1.8\text{--}5.6 \text{ t ha}^{-1}$, depending on accession. Two accessions of *Sporobolus virginicus* produced 15.5 and 7.8 tons of dry weight biomass per hectare. *Spartina patens* grown in Delaware and Egypt yielded between $7.2\text{--}14.4 \text{ t ha}^{-1}$.

Gallagher (1985) also investigated the use of *Atriplex triangularis* as a fresh vegetable crop. The plant has a high vitamin C content, and estimated yield was 21.3 t ha^{-1} fresh weight when irrigated with 30 ppt seawater. The

perennial seashore mallow *Kosteletzkya virginica* produced a grain that is 32% protein and 22% oil. Grain yield ranged from 0.8-1.4 t ha⁻¹ when irrigated with 25 ppt salinity water but survival and growth was poor when plants were irrigated with 30 ppt water.

Species of *Juncus* have been investigated for use as a fiber plant for paper production (Zahran, 1994). Wild stands of *Juncus rigidus* were estimated to produce 38-43 dry tons of culms per hectare. However, under experimental conditions of irrigation once every 5 days in the summer and once every 7 days in the winter, *J. rigidus* produced 2 kg dry weight of culms per 7 X 6 m plot (0.47 t ha⁻¹) and *J. acutus* produced 1.1 kg culms per plot (0.26 t ha⁻¹). Salinity of the irrigation water was not reported. Decreased irrigation frequency led to decreased yields. One ton of *J. rigidus* culms is estimated to produce 375-400 kg pulp.

Kochia is an annual chenopod halophyte that has received attention as a forage plant (Durham and Durham, 1979). Sherrod (1971, 1973) showed that kochia had protein levels and digestibility similar to alfalfa hay. Irrigated fields of *K. scoparia* in saline soils yielded from 8-13 t dry matter ha⁻¹. Seed production ranged from 1.1-1.7 t ha⁻¹ (Garduño, 1993).

Coxworth et al. (1969) conducted field trials of three chenopod halophytes: *Kochia scoparia*, Russian thistle,

Salsola kali, and *Atriplex hortensis*. Plants received 193 mm rainfall over the growing season. Mean seed yields and percent protein in the seed were 2.2, 1.6 and 0.9 t ha⁻¹ and 29.0, 42.6, and 30.5%, respectively, for *K. scoparia*, *S. kali*, and *A. hortensis*. *Kochia* and *A. hortensis* seeds contained significant amounts of saponins. Comparable yields in order to obtain protein yields similar to those obtained with *Kochia* and Russian thistle would be: flaxseed (27% protein) 2.3-2.7 t ha⁻¹ and rapeseed (29% protein) 2.2-2.4 t ha⁻¹.

Zahran (1994) carried out experiments on *Kochia indica*. Shoots were harvested three times over a 7 month period. Total shoot dry weight production ranged from 6-22.5 tons ha⁻¹ depending on fertilizer treatment in plants irrigated with water ranging from 3.7-5.3 mmhos cm⁻¹.

Zaruyk and Baalbaki (1996) proposed *Inula crithmoides*, a perennial halophyte shrub, as a forage plant for saline agriculture. The authors estimated a dry weight yield of 4 t ha⁻¹ using 40 ds m⁻¹ water as the irrigation source. Crude protein content averaged 12.7% and was not affected by variation in salinity.

In summary, halophyte crops can produce satisfactory yields under irrigation with water of varying salinities. Irrigation with full strength seawater can produce plant yields comparable to glycophyte crops under freshwater irrigation. Yields of halophytes when irrigated with lower

salinity water can actually exceed yields of conventional crops under certain circumstances.

3. Halophytes and nitrogen

Desert and salt-marsh plants tend to assimilate nitrogen efficiently because nitrogen appears to be the major nutrient limiting growth. In deserts, plants need to take up nitrate after rains in order to maintain growth as the soil dries out (Smirnoff and Stewart, 1985). In salt marshes, plants receive nutrients in pulses via tidal flooding, and must take up nitrogen as efficiently as possible to prevent growth inhibition (Stewart et al., 1979). The basic characteristics of nitrogen assimilation in halophytes appear qualitatively similar to those in glycophytes; however, there are differences with respect to intermediary nitrogen metabolism. Many halophytes contain high levels of soluble nitrogen compounds, most notably proline and glycine betaine. These compounds are probably used for osmoregulation by halophytes in saline environments. In some halophytes, glycine betaine and/or proline account for more than 20% of total plant nitrogen (Stewart et al. 1979). Most species are able to maintain a consistent rate of nitrogen assimilation under saline conditions, but exhibit changes in the pattern of nitrogen metabolism with respect to the level and nature of soluble nitrogen compounds (i.e., higher concentrations of soluble

nitrogen compounds at higher salinities).

Chenopodiaceae are widely distributed in salt-affected, estuarine, or arid habitats, have high leaf nitrogen content, and accumulate glycine betaine (Storey et al., 1977). Forty-four percent of the genera in this family contain halophytic members, many of which are true halophytes or euhalophytes (O'Leary and Glenn, 1994). Many of these Chenopod halophytes are classified as nitrophilous (nitrogen-accumulating plants).

Stewart et al. (1979) noted that halophyte species exhibit three different patterns of nitrogen metabolism under saline conditions: (1) species that have a constitutive capacity for the accumulation of organic solutes and inorganic ions; (2) species that exhibit an adaptive accumulation of osmotic solutes and inorganic ions; (3) species which are able to regulate the accumulation of inorganic ions.

4. Summary

The objective of this dissertation is to evaluate the feasibility of using halophytes as biofilters to treat saline aquaculture effluent. Halophytes require a relatively large leaching fraction to ensure adequate growth. Therefore, it is important to understand whether this large volume of water leaching past the root zone will also carry with it a large load of nutrients. If excessive

amounts of nutrients are lost, then there is little point in using halophytes to treat effluent. Two experiments tested the ability of several species of halophytes to grow, consume water and remove nitrogen and phosphorus from saline aquaculture wastewater under different salinities and different irrigation rates.

This dissertation contains two appendices in journal manuscript format that represent the bulk of my experimental work. I designed experiments, collected and analyzed data, and prepared manuscripts. "Halophytes for the treatment of saline aquaculture" (Appendix A), is in review in the journal *Aquaculture* (Elsevier). There are three co-authors on this paper. Ed Glenn provided general experimental advice and minor manuscript editing. Kevin Fitzsimmons provided the majority of the funding for this study. Steve Smith provided statistical advice. "Reuse of highly saline aquaculture effluent to irrigate a potential forage halophyte, *Suaeda esteroa*" (Appendix B) will be submitted to the journal *Aquacultural Engineering* (Elsevier). Ed Glenn provided experimental advice and minor manuscript editing.

PRESENT STUDY

The methods, results, and conclusions of this study are presented in the papers appended to this dissertation. The following is a summary of the most important findings in these papers.

Halophytes for the treatment of saline aquaculture effluent (Appendix A)

In order to use halophytes as biofilters it is necessary to know which species are the most effective at sequestering nutrients from the wastewater stream. In addition, because the salinity of aquaculture effluent may range from fresh water to hypersaline, it is necessary to know at what salinities the halophyte species perform best.

The objectives of this study were to determine which of several species of chenopod halophytes would be the most effective biofilters, and to determine how the different species grew, used water, and removed nitrogen and phosphorus under a range of salinities.

I examined the ability of *Suaeda esteroa*, *Salicornia bigelovii* and *Atriplex barclayana* (Chenopodiaceae) to grow and sequester nitrogen and phosphorus when irrigated with saline aquaculture effluent. *Salicornia bigelovii* is a leafless salt-marsh succulent that produces an edible oil-seed and a forage straw (Glenn et al., 1991). *Atriplex*

barclayana is a xerohalophyte endemic to the northern Gulf of California that has been used as a forage plant for ruminants (Swingle et al., 1996). *Suaeda esteroa* is a succulent salt-marsh plant that also has been used as a forage plant (Swingle et al., 1996). Plants were grown in sand in 0.02 m³ drainage lysimeters in a greenhouse. They were irrigated with aquaculture effluent generated from an intensive tilapia culture system. Enough effluent was provided to meet evapotranspiration demand and to produce a 0.3 leaching fraction. The effluent was salinized with NaCl to make salinity treatments of 0.5, 10, and 35 ppt. The plant-soil system removed 98% and 94% of the applied total and inorganic nitrogen, respectively, and 99% and 97% of the applied total and soluble reactive phosphorus, respectively (mean over all treatments), despite the high leaching fraction. For all three species, salt inhibited ($P \leq 0.05$) plant growth rate, nutrient removal, and the volume of water consumed by plants. *Suaeda esteroa* and *Salicornia bigelovii*, which are succulent salt marsh species, grew and removed more nitrogen and phosphorus than the desert saltbush, *Atriplex barclayana*, at 35 ppt. The main limitation of these biofilter systems was the low volume of water they could process at the highest salinity due to the low growth rate and evapotranspiration of the plants.

Reuse of highly saline aquaculture effluent to irrigate a potential forage halophyte, *Suaeda esteroa* (Appendix B)

Satisfactory plant growth and filtering capacity was not achieved at the highest salinity treatment in the first experiment. Therefore, I decided to focus my attention on strategies to increase plant growth under high salinity irrigation. Increasing growth rates of plants irrigated with highly saline water is important, because many aquaculture species are grown using full-strength seawater. I increased the irrigation frequency to three times per week (Monday, Wednesday, Friday) to avoid the high soil salinities that would have occurred with weekly irrigation.

Secondly, larger lysimeters were used (0.78 m^3 soil volume vs. 0.02 m^3 in the first experiment) to reduce edge effects and better simulate field conditions. Additionally, a sandy loam soil was used, which had a higher water holding capacity than the sand used in the first experiment. By increasing the irrigation frequency, increasing the soil volume, and using a finer-textured soil, I sought to maintain soil water salinity at acceptable levels and therefore achieve better plant growth than in the first experiment under irrigation with highly saline aquaculture effluent.

Suaeda esteroa, the species that performed best in the first experiment, was grown to determine yield potential, water use and capacity for nitrogen and phosphorus uptake

when irrigated with highly saline (31 ppt NaCl) effluent from a tilapia culture system. Water was applied to soil in five treatments, ranging in volume from 50 to 250% of the potential evaporation rate. The higher irrigation volumes produced significantly ($P \leq 0.05$) greater plant biomass than lower-volume treatments. Higher plant growth caused high-volume treatments to have higher water consumption than in the low-volume treatments ($P < 0.05$). Nitrate concentrations in water draining from the lysimeters decreased over the course of the experiment, and were significantly lower in the high-volume treatments than in the low volume treatments. Toward the end of the experiment, nitrate leachate concentrations in the high volume treatments were below the mean limits set by the United States Environmental Protection Agency for effluent discharge. Phosphorus concentrations in the leachate water increased over the course of the experiment and also increased with increasing irrigation volume. The use of seawater-salinity aquaculture wastewater to irrigate halophyte crops can be a viable strategy for treatment of effluent, especially where phosphorus is not a nutrient limiting phytoplankton growth.

REFERENCES

- Aronson, J.A., 1989. Haloph: A Data Base of Salt Tolerant Plants of the World. Arid Land Studies, University of Arizona, Tucson, AZ.
- Aronson, J.A., Pasternak, D., Danon, A., 1988. Introduction and first evaluation of 120 halophytes under seawater irrigation. In: Whitehead, E.E., Hutchinson, C.F., Timmerman, B.N, Varady, R.G. (Eds.), Arid Lands Today and Tomorrow: Proceedings of an International Research and Development Conference. Westview Press, Boulder Colorado, pp. 737-746.
- Attia, F.M., Alsobayel, A.A., Kriadees, M.S., Al-Saiady, M.Y., Bayoumi, M.S., 1997. Nutrient composition and feeding value of *Salicornia bigelovii* Torr. meal in broiler diets. Animal Feed Science Technology 65, 257-263.
- Ayers, R.S., Wescott, D.W., 1989. Water quality for agriculture. FAO irrigation and drainage paper 29 Rev. 1. FAO, Rome.
- Bell, P.R.F., Greenfield, P.F., Hawker, D., Connell, D., 1989. The impact of waste discharges on coral reef regions. Water Sci. Tech. 21 (1), 121-130.
- Bergheim, A., Sivertsen, A., Selmer-Olsen, A.R., 1982. Estimated pollution loadings from Norweigan fish farms. I. Investigations 1978-1979. Aquaculture 28, 347-361.

- Bergheim, A., Hustveit, H., Kittelsen, A., Selmer-Olsen, A.R., 1984. Estimated pollution loadings from Norweigan fish farms. II. Investigations 1980-1981. *Aquaculture* 36, 157-168.
- Beveridge, M.C.M., 1996. *Cage Aquaculture* 2nd edition. Fishing News Books, Oxford.
- Boyd, C.E., 1990. *Water Quality in Ponds for Aquaculture*. Birmingham Publishing Co., Birmingham, Alabama.
- Boyko, H, Boyko, E., 1959. Seawater irrigation--a new line of research on a bioclimatic plant-soil complex. *Int. J. Bioclim. Biomet.* III, II., Sec. B.1, 1-24.
- Briggs, M.R.P., Funge-Smith, S.J., 1994. A nutrient budget of some intensive marine shrimp ponds in Thailand. *Aquaculture and Fisheries Management* 25, 789-811.
- Brown, J.R., Gowen, R.J., McLusky, D.S., 1987. The effect of salmon farming on the benthos of a Scottish sea loch. *J. Exp. Mar. Biol. Ecol.* 109, 39-51.
- Cornell, G.E., Whoriskey, F.G., 1993. The effects of rainbow trout (*Onchorynchus mykiss*) cage culture on the water quality, zooplankton, benthos and sediments of Lac du Passage, Quebec. *Aquaculture* 109, 101-117.

- Costa-Pierce, B.A., 1996. Environmental impacts of nutrients from aquaculture: towards the evolution of sustainable aquaculture systems. In: Baird, D.J., Beveridge, M.C.M, Kelly, L.A. and Muir, J.F. (Eds.), *Aquaculture and Water Resource Management* Blackwell Science, Oxford, pp.81-113.
- Coxworth, E.C.M., Bell, J.M., Ashford, R., 1969. Preliminary evaluation of Russian thistle, kochia and garden atriplex as potential high protein content seed crops for semi-arid areas. *Can. J. Plant Sci.* 49, 427-434.
- Dierberg, F.E., Kaittisimkul, W., 1996. Issues, impacts and implications of shrimp aquaculture in Thailand. *Environmental Management* 20 (5), 649-666.
- D'Silva, A.M., Maughan, O.E., 1996. Optimum density of red tilapia *Oreochromis mossambicus* × *O. hornorum* in a pulsed-flow culture system. *Journal of the World Aquaculture Society* 27, 126-129.
- Durham, R.M., Durham, J.W., 1979. *Kochia*: Its potential for forage production. *Proc. Int. Arid Land Plant Resources Conf.* Texas Technical University, Lubbock, pp. 444-450.
- Felger, R.S., 1979. Ancient crops for the twenty-first century. In: Ritchie, G.A. (Ed.), *New Agricultural Crops*. Westview Press, Boulder, pp. 5-20.

- Gallagher, J.L., 1985. Halophytic crops for cultivation at seawater salinity. *Plant and Soil* 89, 323-336.
- Garduño, M.A., 1993. *Kochia*: A new alternative for forage under high salinity conditions of Mexcio. In: Lieth, H., Al Massoon, A. (Eds.), *Towards the rational use of high salinity tolerant plants*, Vol. 1. Kluwer Academic Publishers, Netherlands, pp. 459-464.
- Gihad, E.A., El Shaer, H.M., 1994. Utilization of halophytes by livestock on rangelands--problems and prospects. In: Squires, V.R., Ayoub, A.T., (Eds.), *Halophytes as a Resource for Livestock and for Rehabilitation of Degraded Lands*. Kluwer Academic Publishers, Netherlands, pp. 77-96.
- Glenn, E.P., O'Leary, J.W., 1985. Productivity and irrigation requirements of halophytes grown with seawater in the Sonoran Desert. *Journal of Arid Environments* 9, 81-91.
- Glenn, E.P., O'Leary, J.W., Watson, M.C., Thomas, T.L., Kuehl, R.O., 1991. *Salicornia bigelovii* Torr: an oilseed halophyte for seawater irrigation. *Science* 251, 1065-1067.

- Glenn, E.P., Swingle, R.S., Riley, J.J., Squires, V.R.,
1994. North American halophytes: Potential use in
animal husbandry. In: Squires, V.R., Ayoub, A.T.
(Eds.), *Halophytes as a Resource for Livestock and for
Rehabilitation of Degraded Lands*. Kluwer Academic
Publishers, Netherlands, pp. 165-174.
- Glenn, E.P., Hicks, N., Riley, J., Swingle, S., 1995.
Seawater irrigation of halophytes for animal feed. In:
Chakour-Allah, R., Malcolm, C.V., Hamdy, A. (Eds.),
Halophytes and Biosaline Agriculture. Marcel Dekker,
New York pp. 221-236.
- Glenn, E.P., Miyamoto, S., Moore, D., Brown, J.J., Thompson,
T.L., Brown, P., 1997. Water requirements for
cultivating *Salicornia bigelovii* Torr. with seawater on
sand in a coastal desert environment. *Journal of Arid
Environments* 36, 711-730.
- Glenn, E.P., Tanner, R., Miyamoto, M., Fitzsimmons, K.,
Boyer, J., 1998. Water use productivity and forage
quality of the halophyte *Atriplex nummularia* grown on
saline waste water in a desert environment. *Journal of
Arid Environments* 38, 45-62.
- Goldman, J., Tenore, K., and Stanley, H., 1973. Inorganic
nitrogen removal from wastewater: effect on
phytoplankton growth in coastal marine waters. *Science*,
180, 955-956.

- Gowen, R.J., and Bradbury, N.B., 1987. The ecological impact of salmonid farming in the coastal waters: A review. In: Barnes, M. (Ed.), *Oceanogr. Mar. Biol. Ann. Rev.* Aberdeen University Press, Aberdeen, pp. 563-575.
- Hall, P.O.J., Anderson, L.G., Holby, O., Kollberg, S., Samuelson, M.O., 1990. Chemical fluxes and mass balances in a marine fish cage farm. I. Carbon. *Marine Ecology Progress Series* 61, 61-73.
- Hall, P.O.J., Holby, O., Kollberg, S., Samuelson, M.O., 1992. Chemical fluxes and mass balances in a marine fish cage farm. IV. Nitrogen. *Marine Ecology Progress Series* 89, 81-91.
- Handy, R.D., Poxton, M.G., 1993. Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. *Reviews in Fish Biology and Fisheries* 3, 205-241.
- Hargrave, B.T., Duplisea, D.E., Pfeiffer, E., Wildish, D.J., 1993. Seasonal changes in benthic fluxes of dissolved oxygen and ammonium associated with marine cultured Atlantic salmon. *Marine Ecological Progress Series* 96, 249-257.
- Holby, O., Hall, P.O.J., 1991. Chemical fluxes and mass balances in a marine fish cage farm. II. Phosphorus. *Marine Ecology Progress Series* 70, 263-272.

- Holmer, M., Kristensen, E., 1992. Impact of marine fish culture on metabolism and sulfate reduction of underlying sediments. Marine Ecological Progress Series 80, 191-201.
- Hopkins, J.S., Hamilton II, R.D., Sandifer, P.A., Browdy, C.L., Stokes, A.D., 1993. Effect of water exchange on production, water quality, effluent characteristics and nitrogen budgets of intensive shrimp ponds. Journal of the World Aquaculture Society 24, 304-320.
- Hopkins, J.S., Sandifer, P.A., Devoe, M.R., Holland, A.F., Browdy, C.L., Stokes, A.D., 1995. Environmental impact of shrimp farming with special reference to the situation in the Continental United States. Estuaries 18 (1A), 25-42.
- Husevåg, B., Lunstead, B.T, Johannessen, P.J., Enger, Ø., Samuelson, O.B., 1991. Simultaneous occurrence of *Vibrio salmonicida* and antibiotic-resistant bacteria in sediments at abandoned aquaculture sites. Journal of Fish Diseases 14, 631-640.
- Iwama, G.K., 1991. Interactions between aquaculture and the environment. Critical Reviews in Environmental Control 21 (2), 177-216.

- Kaartvedt, S., Johnsen, T.M., Aksnes, D.L., Lie, U., Svendsen, H., 1991. Occurrence of the toxic phytoflagellate *Prymnesium parvum* and associated fish mortality in a Norweigan fjord system. Can. J. Fish. Aquat. Sci. 48, 2316-2323.
- Kerry, J., Hiney, M., Coyne, R., Cazabon, D., NicGabhainn, S., Smith, P., 1994. Frequency and distribution of resistance to oxytetracycline in micro-organisms isolated from marine fish farm sediments following therapeutic use of oxytetracycline. Aquaculture 123, 43-54.
- Le Hourou, H. N., 1995. Forage halophytes in the Mediterranean basin. In: Chakour-Allah, R., Malcolm, C.V., Hamdy, A. (Eds.), Halophytes and Biosaline Agriculture. Marcel Dekker, New York, pp. 115-136.
- Martinez-Cordova, L.R., Villarreal, H., Porchas, M.A., 1995. Culture of white shrimp *Penaeus vannamei* in reduced water exchange ponds in Sonora, Mexico. World Aquaculture 26, 47-48.
- Miyamoto, S., 1995. Salt tolerance, water use, and potential irrigation scheduling of halophytes. In: Chakour-Allah, R., Malcolm, C.V., Hamdy, A. (Eds.), Halophytes and Biosaline Agriculture. Marcel Dekker, New York pp. 181-220.

- Miyamoto, S., Glenn, E.P., Olsen, M.W., 1996. Growth, water use and salt uptake of four halophytes irrigated with highly saline water. *Journal of Arid Environments* 32, 141-159.
- Neori, A., Krom, M.D., Ellner, S.P., Boyd, C.E., Popper, D., Rabinovitch, R., Davidson, P.J., Divr, O., Zuber, D., Ucko, M., Angel, D., Gordin, H., 1996. Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. *Aquaculture* 141, 183-199.
- New, M.B., 1997. Aquaculture and capture fisheries--balancing the scales. *World Aquaculture* 28 (2), 11-30.
- O'Leary, J.W., 1988. Saline environments and halophytic crops. In: Whitehead, E.E., Hutchinson, C.F., Timmerman, B.N, Varady, R.G. (Eds.), *Arid Lands Today and Tomorrow: Proceedings of an International Research and Development Conference*. Westview Press, Boulder Colorado, pp. 773-790.
- O'Leary, J.W., Glenn, E.P., Watson, M.C., 1985. Agricultural production of halophytes irrigated with seawater. *Plant and Soil* 89, 311-321.
- O'Leary, J.W. & Glenn, E.P., 1994. Global distribution and potential for halophytes. In: Squires, V.R., Ayoub, A.T. (Eds.), *Halophytes as a resource for livestock and for rehabilitation of degraded lands* Kluwer Academic Publishers, Netherlands, pp. 7-17.

- Olsen, M.W., Fitzsimmons, K.M., Moore, D.W., 1993. Surface irrigation of cotton using aquaculture effluent. In: Wang, J. K. (Ed.), Techniques for Modern Aquaculture. ASAE, St. Joseph, MI, pp. 159-165.
- Pasternak, E., Nerd, A., 1995. Research and utilization of halophytes in Israel. In: Chakour-Allah, R., Malcolm, C.V., Hamdy, A. (Eds.), Halophytes and Biosaline Agriculture. Marcel Dekker, New York pp. 325-348.
- Penczak, T., Galicka, W., Molinski, M., Kusto, E., Zalewski, M., 1982. The enrichment of a mesotrophic lake by carbon, phosphorus and nitrogen from the cage aquaculture of rainbow trout, *Salmo gairderi*. Journal of Applied Ecology 19, 371-393.
- Phillips, M., 1995. Aquaculture and the environment--striking a balance. In: Nambiar, K.P.P., Singh, T. (Eds.), International Conference on Aquaculture: Aquaculture towards the 21st Century. Infofish, Kuala Lumpur, pp. 26-55.
- Quirk, J.P., 1971. Chemistry of saline soils and their physical properties. In: Salinity and Waster Use. Talsma, T., Philip, J.R. (Eds.), Macmillian, London, pp. 79-91.
- Rakocy, J.E., Hargreaves, J.A., 1993. Integration of vegetable hydroponics with fish culture: A review. In: Wang, J.K. (Ed.), Techniques for Modern Aquaculture. ASAE, St. Joseph, MI, pp. 159-165.

- Robertson, A.I., Phillips, M.J., 1995. Mangroves as filters of shrimp pond effluent: predictions and biogeochemical research needs. *Hydrobiologia* 295, 311-321.
- Ryther, J., Dunstan, W., 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science*, 171, 1008-1013.
- Samuelson, O.B., Torsvik, V., Ervik, A., 1992. Long-range changes in oxytetracycline and bacterial resistance towards oxytetracycline in a fish farm sediment after medication. *The Science of the Total Environment* 114, 25-36.
- Schwartz, M.F., Boyd, C.E., 1994. Channel catfish pond effluents. *Progressive Fish-Culturist* 56, 273-281.
- Schwartz, M.F., Boyd, C.E., 1995. Constructed wetlands for treatment of channel catfish pond effluents. *Progressive Fish-Culturist* 57 (4), 255-266.
- Sherrod, L.B., 1971. Nutritive value of *Kochia scoparia*. I. Yield and chemical composition at three stages of maturity. *Agron. J.* 63, 343-344.
- Sherrod, L.B., 1973. Nutritive value of *Kochia scoparia*. III. Digestibility of kochia hay compared with alfalfa hay. *J. Dairy Sci.* 56, 923-926.
- Shpigel, M., Neori, A., 1996. The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: I. Proportions of size and projected revenues. *Aquacultural Engineering* 15 (5), 313-326.

- Skeen, B., Brown, J.J., Fitzsimmons, K.M., Dickenson, G., 1997. Performance characteristics of open and closed bead filters in a recirculating tilapia production system. In: Timmons, M., Losordo, T. (Eds.), *Advances in Aquacultural Engineering: Proceedings from the Aquacultural Engineering Society Technical Sessions at the Fourth International Symposium on Tilapia in Aquaculture*. Northeast Regional Agricultural Engineering Service, Ithaca, New York, pp. 276-286.
- Smirnoff, N., Stewart, G.R., 1985. Nitrate assimilation and translocation by higher plants: comparative physiology and ecological consequences. *Physiol. Plant.* 64, 133-140.
- Stewart, G.R., Larher, F., Ahmad I., Lee, J.A., 1979. Nitrogen metabolism and salt tolerance in higher plant halophytes. In: Jeffries, R.L., Davy, A.J. (Eds.), *Ecological processes in coastal environments*. Blackwell, Oxford.
- Storey, R., Ahmad, N. & Wyn Jones, R.G., 1977. Taxonomic and ecological aspects of the distribution of glycine betaine and related compounds in plants. *Oecologia* 27, 319-327.
- Swingle, R.S., Glenn, E.P., Squires, V.R., 1996. Growth performance of lambs fed mixed diets containing halophyte ingredients. *Animal Feed Science Technology* 63, 137-148.

- Syers, J.K., Iskander, I.K., 1981. Soil-phosphorus chemistry. In: Iskander, I.K. (Ed.), Modeling Wastewater Renovation, Land Treatment. John Wiley & Sons, New York, pp. 569-599.
- Tsutsumi, T., Kikuchi, T., Tanaka, M., Higashi, T., Imasaka, K., Miyazaki, M., 1991. Benthic faunal succession in a cove organically polluted by fish farming. Marine Pollution Bulletin 23, 233-238.
- Watson, M.C., O'Leary, J.W., Glenn, E.P., 1987. Evaluation of *Atriplex lentiformis* (Torr.) S. Wats. and *Atriplex nummularia* Lindl. as irrigated forage crops. Journal of Arid Environments 13, 293-303.
- Watson, M.C., 1990. *Atriplex* species as irrigated forage crops. Agriculture, Ecosystems and Environment 32, 107-118.
- Watson, M.C., O'Leary, J.W., 1993. Performance of *Atriplex* species in the San Joaquin Valley, California, under irrigation and with mechanical harvests. Agriculture, Ecosystems and Environment 43, 255-266.
- Weston, D.P., 1990. Quantitative examination of macrobenthic community changes along an organic enrichment gradient. Marine Ecology Progress Series 61, 233-244.

- Wiesmann, D., Scheid, H., Pfeffer, E., 1988. Water pollution with phosphorus of dietary origin by intensively fed rainbow trout (*Salmo gairdneri* Rich.). Aquaculture 69, 263-270.
- Wildish, D.J., Keizer, P.D., Wilson, A.J., Martin, J.L., 1993. Seasonal changes and dissolved oxygen and plant nutrients near salmonid net pens in the macrotidal bay of fundy. Canadian Journal of Fisheries and Aquatic Science 50, 303-311.
- Wu, R.S.S., 1995. The environmental impact of marine fish culture: towards a sustainable future. Marine Pollution Bulletin 31, 159-166.
- Wu, R.S.S., Lam, K.S., MacKay, D.W., Lau, T.C., Yam, V., 1994. Impact of marine fish farming on water quality and bottom sediment: A case study in the sub-tropical environment. Marine Environmental Research 38, 115-145.
- Ye, L.X., Ritz, D.A., Fenton, G.E., Lewis, M.E., 1991. Tracing the influence on sediments of organic waste from a salmonid farm using stable isotope analysis. J. Exp. Mar. Bio. Ecol. 145, 161-174.

- Yensen, N.P., Yensen, S.B., Weber, C.W., 1988. A review of *Distichlis* spp. for production and nutritional values. In: Arid Lands Today and Tomorrow: Proceedings of an International Research and Development Conference. Whitehead, E.E., Hutchinson, C.F., Timmerman, B.N, Varady, R.G. (Eds.), Westview Press, Boulder Colorado. pp. 809-822.
- Zahran, M.A., 1994. *Juncus* and *Kochia*: Fiber and fodder-producing halophytes under salinity and aridity stress. In Handbook of Plant and Crop Stress, Pessarkli, M. (Eds.), Marcel Dekker, New York, pp. 505-528.
- Zurayk, R.A., Baalbaki, R., 1996. *Inula crithmoides*: A candidate plant for saline agriculture. Arid Soil Research and Rehabilitation 10, 213-223.

**APPENDIX A. HALOPHYTES FOR THE TREATMENT OF SALINE
AQUACULTURE EFFLUENT**

Title: Halophytes for the Treatment of Saline
Aquaculture Effluent

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Abstract

We determined the feasibility of using salt-tolerant plants (halophytes) as biofilters to remove nutrients from saline aquaculture wastewater. *Suaeda esteroa*, *Salicornia bigelovii* and *Atriplex barclayana* (Chenopodiaceae), species with potential as forage and oil seed crops, were grown in sand in drainage lysimeters in a greenhouse experiment. They were irrigated to meet evapotranspiration demand and to produce a 0.3 leaching fraction, using aquaculture effluent generated from an intensive tilapia culture system. The effluent was salinized with NaCl to make salinity treatments of 0.5 ppt, 10 ppt and 35 ppt. The plant-soil system removed 98% and 94% of the applied total and inorganic nitrogen respectively, and 99% and 97% of the applied total and soluble reactive phosphorus respectively, despite the high leaching fraction. For all three species, salt inhibited ($P < 0.05$) the growth rate, nutrient removal, and volume of water the plants could handle. *Suaeda* and *Salicornia*, which are succulent salt marsh species, performed better than the desert saltbush, *Atriplex*, at the higher salinities.

Keywords: Halophytes, Effluent, Seawater Agriculture, *Suaeda esteroa*, *Salicornia bigelovii*, *Atriplex barclayana*

1. Introduction

Untreated aquaculture effluent is usually less concentrated than domestic or industrial wastewater, but the large volumes of water discharged from coastal aquaculture projects can become a serious source of pollution, and may damage coastal ecosystems and fisheries (Bell et al, 1989; Hopkins et al., 1995; Dierberg and Kaittisimkul, 1996; Costa-Pierce, 1996). Several methods of biological treatment have been tested, however, no single treatment method always works. Seaweeds can be effective biofilters in intensive, closed systems (Neori et al., 1996; Shpigel and Neori, 1996), but in open systems where effluent is discharged into ditches or canals, seaweeds can become light-limited and fouled with settled solids (Phillips, 1995). Constructed wetlands, utilizing higher plants, have also been used to treat aquaculture effluent (Schwartz and Boyd, 1995). These wetlands filter solids out of the wastewater stream, but they are less effective at removing inorganic N and P than they are at removing solids, and they do not provide an economic return to the grower. Integrating vegetable hydroponics with fish culture is also an effective but expensive technique to remove nutrients from the fish culture system (Rakocy and Hargreaves, 1993).

Another option that has been used successfully for the disposal of freshwater aquaculture effluent is application

to field crops (Olsen et al., 1993; D'Silva and Maughan, 1996). Crop plants can remove a significant fraction of the nutrients in the irrigation stream and provide an economic return to the grower. Unlike seaweed scrubbing systems, this method actually reduces effluent water volume through evapotranspiration and could be extended to saline effluent streams if suitable, salt-tolerant crops were available. Such crops might be developed from wild, salt-tolerant plants (halophytes), with the potential to produce biomass, forage and oilseed crops using saltwater irrigation (Glenn et al., 1991; Glenn et al., 1994; Miyamoto et al., 1996; Swingle et al., 1996; Glenn et al., 1997a).

Under high-salinity irrigation, halophytes require a leaching percentage of 30-50% above consumptive use by the plants (Miyamoto et al., 1996). The leaching fraction is required to flush excess salts below the root zone and could result in significant discharge of N and P to the aquifer if these nutrients are not efficiently absorbed by the soil-plant system. We tested the capacity of three halophyte species grown at three salinities to remove N and P from a highly concentrated aquaculture effluent source when irrigated at a rate of 30% above evapotranspiration demand.

2. Methods and Materials

Experimental design and procedures

The study was conducted in July-October, 1995 in a controlled environment greenhouse in Tucson, Arizona. Plants were started from seed (*Salicornia bigelovii* and *Suaeda esteroa*) or cuttings (*Atriplex barclayana*) from wild plants collected at Estero Morua, near Puerto Peñasco, Sonora, Mexico (Glenn et al., 1991; Glenn and O'Leary, 1985). Seedlings or rooted cuttings were transplanted into 20 l, white plastic pots (drainage lysimeters), containing 25 kg (dry weight) of washed river sand and equipped with bottom tubes leading to collection bottles. Water, salt and nutrients leaching past the root zone were quantified (Miyamoto et al., 1996).

The experiment was designed as a split plot with plant species as the subplot, and salinity as the main plot. Three salinity treatments (0.5 ppt, 10 ppt, 35 ppt) were used with three replicates per species per salinity treatment level. A set of control lysimeters (soil without plants) was included at each salinity to determine the effect of the soil alone on nutrient removal (total lysimeters = 36). The lysimeters were placed in 6 rows (2 rows per each of the three blocks) near the middle of the greenhouse in a randomized complete block design.

Wastewater for irrigation was generated in two 1 m³ tanks that each held 10 kg hybrid tilapia in freshwater. Fish were fed pelleted food at 2% of body weight per day. Each tank contained a 100 l bead filter with 20 l of beads. Water was recycled within the tanks. Once a week the filters were backflushed, and solids and wastewater were withdrawn to irrigate the lysimeters. The irrigation water was thoroughly mixed then split into 3 batches to keep the nutrient content equal among treatments. Each batch was salinized by addition of NaCl to produce the treatment salinities. NaCl (the main salt in seawater) was used instead of synthetic sea salt in order to avoid using an undefined mixture of salts, some of which can precipitate at high salinities. The three salinities, 0.5, 10 and 35 ppt, simulated the salinity of non-saline irrigation water used in the western United States, brackish estuarine water, and seawater, respectively.

The lysimeters were weighed weekly on a balance to estimate water use over the past week. Lysimeters were irrigated weekly with salinized effluent to replace evapotranspiration (ET) losses and to provide for $LF = 0.3$, a typical value for irrigated crops in arid regions (Miyamoto et al., 1996). The irrigation volume (W_i) was calculated as: $W_i = (ET/1-LF)$. This design simulated best management practices for irrigating with saline water and kept the fraction of water discharged past the root zone as

a constant among treatments. A sample of weekly irrigation water was retained for water quality analysis. Lysimeters were allowed to drain overnight and the leachate from all treatments was collected on the morning following irrigation. At the end of the 12-week experiment, above-ground biomass was harvested and dried at 60 C to determine dry matter yield per lysimeter.

Water, plant and soil analyses

Electrical conductivity, pH, total ammonia nitrogen, nitrate, total nitrogen, soluble reactive phosphorus, and total phosphorus were measured in irrigation and leachate water using methods of the American Public Health Association (1995). Total nitrogen was measured by first reducing nitrate to ammonia followed by the Kjeldhal nitrogen procedure (Bremner and Mulvaney, 1982). Soil samples were analyzed for electrical conductivity, pH, total ammonia nitrogen, nitrate, total nitrogen, plant-available phosphate (sodium bicarbonate extraction), and total phosphorus following Page et al. (1982). Total nitrogen in samples of plant shoot tissue was measured with a CNS analyzer and total phosphorus was analyzed by acid digestion followed by colorimetry (Jones et al., 1991). Relative growth rate (RGR) was calculated as $RGR = \ln(\text{final weight (g)}) - \ln(\text{initial weight (g)}) / 86 \text{ days (length of experiment)}$.

Plant water use efficiency (WUE) was calculated as $WUE = \text{plant dry weight accumulated (g)}/\text{water consumed (l)}$.

The amount of nutrients removed by the plant-soil system was calculated by adding the total amount added from the irrigation water (the concentration multiplied by the irrigation volume) and subtracting from this sum the total amount of nutrients that exited the lysimeter in the leachate water. The percentage of the total amount of nutrients applied that were removed was calculated by $(1 - (\text{total out}/\text{total in})) \times 100$.

Statistical analyses

Data were first tested for homogeneity of variances using Bartlett's test. Heterogeneous data were transformed using log, reciprocal or square root transformations for continuous data, and arcsine and logit transformations for percentages (Sokal and Rohlf, 1981). Data were analyzed by parametric methods using a split plot analysis of variance with salinity as the main plot (F value: MS salinity/MS replications $\times$ salinity) and plant species as the subplot (F value: MS salinity/MS replications). The analyses were carried out using statistical software packages (JMP IN and Costat). A Student-Neuman-Kuels *a posteriori* test tested for significant differences among means. Differences were

considered statistically significant when $P < 0.05$.

3. Results

Greenhouse environmental conditions

Mean weekly maximum and minimum air temperatures in the greenhouse over the course of the experiment were (mean $\pm$ SEM) 36.4 ± 1.02 C and 22.6 ± 0.75 C respectively. Light transmission through the greenhouse was about 59% of ambient, and the mean daily quantum flux over the course of the experiment in the greenhouse was $28.6 \text{ E m}^{-2} \text{ d}^{-1}$ (Brown and Russell, 1995). Relative humidity ranged from 60-80% (the greenhouse was evaporatively cooled throughout the experiment).

Plant growth and water use

The actual mean experiment-wide leaching fraction (0.308 ± 0.021) varied little from the desired 0.3 target and did not differ significantly ($P > 0.05$) among species or salinity treatments. Mean salinity values for the leachate water for the 0.5, 10 and 35 ppt treatments were 1.6 ± 0.039 , 28.6 ± 0.81 and 84.7 ± 0.52 ppt respectively. The ratio of irrigation salinity to leachate salinity can be used as a check of the volumetric measurement of LF. LF's calculated from leachate salinity were 0.31, 0.35 and 0.41, indicating that some salt storage occurred at the middle and

high salinity treatments.

Water consumption was greatest in the low salt treatment, and lowest in the high salt treatment ($P < 0.0001$). *Suaeda* consumed more water than the other species (Table 1). On the 35 ppt salinity treatment, *Atriplex* did not use significantly more water than the bare-soil, control lysimeters. Plants produced more dry matter at the lowest and middle salinity than plants at the highest salinity ($P = 0.0016$). *Atriplex* plants were significantly ($P = 0.0017$) smaller than those of the other 2 species at 35 ppt. RGR followed the same trend as plant dry weight. WUE was reduced by salinity because soil evaporation dominated water loss at high salinity due to low plant water use.

Irrigation water quality

Nitrogen and phosphorous parameters in the irrigation source throughout the experiment were: total nitrogen 77.22 ± 0.47 mg/l; total ammonia nitrogen-N 9.69 ± 0.91 mg/l; nitrate-N 3.33 ± 0.28 mg/l; total phosphorus 25.27 ± 0.27 mg/l; soluble reactive phosphorus-P 6.40 ± 0.94 mg/l. The high nutrient levels reflected the concentrated nature of the effluent source (filter washings). The pH was 7.31 ± 0.0009 .

Nitrogen removal

Removal of both total and inorganic (total ammonia nitrogen + nitrate) by the plant-soil system was significantly ($P < 0.0001$, and $P = 0.0012$, respectively) inhibited by increasing salinity (Table 2). The lysimeters at the lowest salinity treatment processed the most water and therefore removed the most total nitrogen. *Suaeda* removed more total and inorganic nitrogen than *Atriplex* or *Salicornia* at the lowest salinity, while *Atriplex* did not remove significantly more total nitrogen than the soil control at the highest salinity. All planted lysimeters removed 96% or more of the applied total nitrogen. Removal efficiency declined slightly at the lower salinities (i.e. although lysimeters in the lower salinity treatments received and removed a greater amount of nitrogen, the percentage of nitrogen removed relative to the amount applied declined). Lysimeters with plants removed a significantly greater percentage of inorganic nitrogen than the control lysimeters; the overall mean percent inorganic nitrogen removed by lysimeters with plants was 94%, compared to 58% for soil control lysimeters.

Salinity had a significant, inhibitory effect on total nitrogen content in the plant shoot ($P < 0.0001$), and at the highest salinity, *Atriplex* contained significantly less nitrogen than the other two species (Table 3). The

percentage of the applied total nitrogen incorporated into the plant shoot was significantly greater at the 0.5 and 10ppt salinity treatment (21% -- mean of 0.5 and 10 ppt treatment), than at the 35 ppt salinity treatment.

Higher concentrations of ammonia were found in leachates from the 35 ppt lysimeters than the other salinities (Table 4). However, 96% of leachate inorganic nitrogen was in the form of nitrate. The mean concentrations of both the inorganic nitrogen and the nitrate in the leach water were significantly greater (six-fold higher) in the control lysimeters without plants, than in the lysimeters with plants. Soil inorganic nitrogen content was significantly greater ($P = 0.0006$) in the 0.5 ppt treatment than in 10 and 35 ppt treatments. In the 10 and 35 ppt treatments, soil inorganic nitrogen content was significantly higher ($P = 0.008$, $P = 0.0005$, respectively) in the control lysimeters containing no plants than in planted lysimeters (Table 4).

Phosphorus removal

The lysimeters in the lowest salinity treatment removed significantly more total and soluble reactive phosphorus than the lysimeters in the 10 ppt treatment, which in turn removed significantly more phosphorus than the lysimeters in the 35 ppt treatment ($P < 0.0001$) (Table 5). *Suaeda* removed

a significantly greater amount of total and soluble reactive phosphorus at the lowest salinity treatment ($P = 0.0022$, $P = 0.0018$, respectively). *Atriplex* did not perform significantly differently from the soil control lysimeter at the 35 ppt treatment. Overall, the lysimeters removed 99.4% and 97.4% (mean of all non-control lysimeters over all treatments) of the applied total and soluble reactive phosphorus respectively. Unlike nitrogen removal, the control lysimeters without plants had similar removal efficiency as lysimeters with plants. Removal efficiency for soluble reactive phosphorus was significantly greater at the 35 ppt treatment compared to the two lower salinity treatments ($P = 0.0074$).

Salinity had a significant inhibitory effect on total phosphorus in the plant shoot ($P < 0.0001$). At the highest salinity *Atriplex* had significantly less shoot phosphorus than the other two species ($P = 0.006$) (Table 6). The percentage of the applied total phosphorus removed by the plant shoot was significantly greater ($P < 0.0001$) at the lowest and the middle salinity (14.9% -- mean of 0.5 and 10 ppt treatment) than at the highest salinity treatment.

Mean concentration of soluble reactive phosphorus was significantly lower ($P = 0.0018$) in the highest salinity treatment than the concentration in the two lower salinity treatments (Table 7). Concentrations of both soluble reactive and total phosphorus in the control lysimeters were

as low or lower than the concentrations in the leachates from planted lysimeters. However, the total phosphorus measurements may be in error, because the measured soluble reactive phosphorus was greater than the mean concentration of total phosphorus in 6 of the 12 treatments. The samples were stored for several months before analyses for total phosphorus were completed and absorption of phosphorus onto the sides of the storage containers may have occurred. Salinity did not affect the amount of plant-available phosphate in the soil, and there were no significant differences among the species within each salinity treatment with respect to plant-available phosphate in the soil (Table 7).

4. Discussion

Overall, the plant-soil system was effective in sequestering nitrogen and phosphorus from the applied effluent across treatments and species. The lysimeters with plants removed over 98% and 94% and 99% and 97% of the applied total and inorganic nitrogen and total and soluble reactive phosphorus respectively. The high removal occurred in spite of the fact that 31% of the applied water leached from the lysimeters. In addition, the aquaculture effluent used for irrigation in this experiment was substantially higher in nitrogen and phosphorus concentration than typical mariculture effluent. In fact, the nutrient concentration of the irrigation water in this experiment was close to an order of magnitude greater than the nutrient concentrations found in typical shrimp pond effluent (Phillips, 1995). Removal efficiencies tended to increase with the higher salinity treatments probably because at lower salinities a much greater volume of water was processed than at higher salinities.

Removal efficiencies exceeded those reported by Schwartz and Boyd (1995) for a constructed wetland which received catfish pond-effluent. In addition to greater removal efficiency, the halophytes grown here might provide an economic return for the grower if they are utilized in animal feeds. Biomass of the three species used in my study

has been used to replace grass hay in sheep fattening rations (Swingle et al., 1996).

In the 0.5 and 10 ppt treatment the plant shoot sequestered approximately 21% and 15% of the applied total nitrogen respectively. Three percent of the applied total nitrogen and 1% of applied total phosphorus was lost from the system via leaching in these two treatments. The remainder of the nitrogen (76-82%) was either incorporated into plant root tissues, retained in the soil as organic matter or inorganic nutrients, or lost to the system via ammonia volatilization or denitrification. Similarly the remainder of the phosphorus was either in the plant root, or in the soil as organic matter or as inorganic phosphate. Thus, most of the nutrient removal was not due to uptake into harvestable plant tissues. The ultimate fate of the nutrients remaining in the soil can only be determined through multi-year experiments.

In the sand soil in this experiment, nitrate was the predominant form of inorganic nitrogen. Nitrate is typically very mobile in the soil water, and therefore a very high proportion of soil nitrate is available to the plant (Tisdale and Nelson, 1985). Therefore the lysimeters with plants removed a much greater percentage of the applied inorganic nitrogen than did the control lysimeters without plants. By contrast, the soil itself was responsible for the majority of phosphorus removal from the wastewater.

Phosphate is readily absorbed to soil particles, and therefore, typically only a very small percentage of soil phosphorus is available to the plant (Tisdale and Nelson, 1985). The control lysimeters (without plants) removed as much or more of the applied phosphorus on a percentage basis as the lysimeters with plants. However, the lysimeters with plants removed a greater amount of both nitrogen and phosphorus because they received more water to meet the evapotranspiration demands of the plant.

One of the main drawbacks of land disposal systems is the potential for nutrients to leach past the plant root zone and contaminate the aquifer. However, despite the uncharacteristically concentrated irrigation water used, the mean concentrations of ammonia and nitrate in the leach water were always lower than the mean concentration limits recommended by the U.S. Environmental Protection Agency for effluents (Schwartz and Boyd, 1994). The mean Environmental Protection Agency standards are 1.77 mg/l for TAN and 16.9 mg/l for nitrate-nitrogen. The mean leach water concentration for all plant-containing lysimeters was 0.28 mg/l for TAN and 2.6 mg/l for nitrate-N. On the other hand, the mean EPA standard for total phosphorus is 0.17 mg/l, a level that was exceeded in all the treatments (mean for plant-containing lysimeters = 0.53 mg/l). However, this concentration would have certainly been lower if the lysimeters had been irrigated with a more typical effluent

or if the soil had been other than sand. A deeper soil profile or higher clay content would provide more binding sites for phosphorus than were present in the sand lysimeters.

Suaeda and *Atriplex* generally performed better than *Salicornia* at low salinity whereas *Atriplex* performed very poorly relative to the other two species on high salinity water. *Atriplex* clearly is not as salt tolerant as the other two species. Plant dry matter production, RGR, ET and the amount of nitrogen and phosphorus removed decreased with increasing salinity. At seawater salinity, the plant-soil system was not capable of treating a large volume of water because the plants grew very little and therefore had low evapotranspiration rates. Slow growth occurred because root zone salinity reached levels high enough to cause serious inhibition. Had we designed the experiment to provide the same volume of wastewater to all treatments, the plants receiving 35 ppt water would have received more water and nutrients. These plants would have probably grown better because root zone salinity would decrease as the additional volume of water would flush salts from the soil. However, the volume of water leached from the high salinity treatments would have certainly been much greater than the volume leached from the low salinity treatments, and therefore would not represent a best management irrigation practice.

The weekly irrigation schedule was probably insufficient to keep soil salinity at levels that would support adequate plant growth. On the other hand, daily irrigation with seawater under a 0.35 LF produced high yields and ET by *Salicornia* grown on sand in a coastal desert environment (Glenn et al., 1997a). If halophytes are to be used to treat aquaculture effluent approaching seawater salinity, different irrigation strategies, employing greater irrigation frequency will be necessary to achieve efficient biofiltration of nutrients. The findings from the present experiments were used to conduct further experiments utilizing larger soil volumes and more frequent irrigations. At seawater salinity, much greater yields were produced than in this study (Brown et al., 1998).

Extensive field testing and site considerations would be needed before saline aquaculture effluent could be used to grow crops at a given location, due to the potential hazards presented by the salt as well as the nutrient load. However, where coastal soils overlay saline aquifers with hydraulic connection to the sea, this strategy offers a potential low-cost and effective method of removing nutrients as well as pathogens by using a soil-plant-water system for biotreatment of effluent.

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References

- American Public Health Association, American Water Works Association, Water Environment Federation, 1995. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC.
- Bell, P.R.F., Greenfield, P.F., Hawker, D., Connell, D., 1989. The impact of waste discharges on coral reef regions. Water Sci. Tech. 21 (1), 121-130.
- Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen-total. In: Page, A.L., Miller, R.H., Keeney, D.R., (Eds.), Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. American Society of Agronomy, Soil Science Society of America, Madison, WI, pp. 595-624.
- Brown, J.J., Glenn, E.P., Fitzsimmons, K.M., 1998. Forage crop production on highly saline aquaculture effluent. In: World Aquaculture '98. Book of Abstracts. World Aquaculture Society, Baton Rouge, LA., pp. 72.
- Brown, P.W., Russell, B., 1995. AZMET, The Arizona Meteorological Network. Arizona Cooperative Extension, Tucson, Arizona, <http://ag.arizona.edu/azmet/>.

- Costa-Pierce, B.A., 1996. Environmental impacts of nutrients from aquaculture: towards the evolution of sustainable aquaculture systems. In: Baird, D.J., Beveridge, M.C.M., Kelly, L.A., Muir, J.F. (Eds.), *Aquaculture and Water Resource Management* Blackwell Science, Oxford, pp. 81-113.
- Dierberg, F.E., Kaattisimkul, W., 1996. Issues, impacts and implications of shrimp aquaculture in Thailand. *Environmental Management* 20 (5), 649-666.
- D'Silva, A.M., Maughan, O.E., 1996. Optimum density of red tilapia *Oreochromis mossambicus* × *O. hornorum* in a pulsed-flow culture system. *Journal of the World Aquaculture Society* 27, 126-129.
- Glenn, E.P., O'Leary, J.W., 1985. Productivity and irrigation requirements of halophytes grown with seawater in the Sonoran Desert. *Journal of Arid Environments* 9, 81-91.
- Glenn, E.P., O'Leary, J.W., Watson, M.C., Thomas, T.L., Kuehl, R.O., 1991. *Salicornia bigelovii* Torr: an oilseed halophyte for seawater irrigation. *Science* 251, 1065-1067.
- Glenn, E.P., Swingle, R.S., Riley, J.J., Squires, V.R., 1994. North American halophytes: Potential use in animal husbandry. In: Squires, V.R., Ayoub, A.T. (Eds.), *Halophytes as a Resource for Livestock and for Rehabilitation of Degraded Lands*. Kluwer Academic Publishers, Netherlands, pp. 165-174.

- Glenn, E.P., Miyamoto, S., Moore, D., Brown, J.J., Thompson, T.L., Brown, P., 1997a. Water requirements for cultivating *Salicornia bigelovii* Torr. with seawater on sand in a coastal desert environment. *Journal of Arid Environments* 36, 711-730.
- Glenn, E.P., Brown, J.J., Khan, J., 1997b. Mechanisms of salt tolerance in plants. In: A. Basra (Ed.), *Mechanisms of Environmental Stress Resistance in Plants*. Harwood Academic Publishers, Amsterdam, pp. 83-110.
- Hopkins, J.S., Sandifer, P.A., Devoe, M.R., Holland, A.F., Browdy, C.L., Stokes, A.D., 1995. Environmental impact of shrimp farming with special reference to the situation in the Continental United States. *Estuaries* 18 (1A), 25-42.
- Jones, J.B., Wolf, B., Mills, H.A., 1991. *Plant Analysis Handbook: A Practical Sampling, Preparation, Analysis and Interpretation Guide*. Micro-Macro Publishing, Inc., Athens, Georgia.
- Miyamoto, S., Glenn, E.P., Olsen, M.W., 1996. Growth, water use and salt uptake of four halophytes irrigated with highly saline water. *Journal of Arid Environments* 32, 141-159.

- Neori, A., Krom, M.D., Ellner, S.P., Boyd, C.E., Popper, D., Rabinovitch, R., Davidson, P.J., Divr, O., Zuber, D., Ucko, M., Angel, D., Gordin, H., 1996. Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. *Aquaculture* 141, 183-199.
- Olsen, M.W., Fitzsimmons, K.M., Moore, D.W., 1993. Surface irrigation of cotton using aquaculture effluent. In: Wang, J. K. (Ed.), *Techniques for Modern Aquaculture*. ASAE, St. Joseph, MI, pp. 159-165.
- Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), 1982. *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*. American Society of Agronomy, Soil Science Society of America, Madison, WI, 1159 pp.
- Phillips, M., 1995. Aquaculture and the environment--striking a balance. In: Nambiar, K.P.P., Singh, T. (Eds.), *International Conference on Aquaculture: Aquaculture towards the 21st Century*. Infofish, Kuala Lumpur, pp. 26-55.
- Rakocy, J.E., Hargreaves, J.A., 1993. Integration of vegetable hydroponics with fish culture: A review. In: Wang, J.K. (Ed.), *Techniques for Modern Aquaculture*. ASAE, St. Joseph, MI, pp. 159-165.
- Schwartz, M.F., Boyd, C.E., 1994. Channel catfish pond effluents. *Progressive Fish-Culturist* 56, 273-281.

- Schwartz, M.F., Boyd, C.E., 1995. Constructed wetlands for treatment of channel catfish pond effluents. *Progressive Fish-Culturist* 57 (4), 255-266.
- Shpigel, M., Neori, A., 1996. The integrated culture of seaweed, abalone, fish and clams in modular intensive land-based systems: I. Proportions of size and projected revenues. *Aquacultural Engineering* 15 (5), 313-326.
- Sokal, R.R., Rohlf, F.J., 1981. *Biometry: The Principles and Practice of Statistics in Biological Research*. 2nd ed. Freeman, New York.
- Swingle, R.S., Glenn, E.P., Squires, V.R., 1996. Growth performance of lambs fed mixed diets containing halophyte ingredients. *Animal Feed Science Technology* 63, 137-148.
- Tisdale, S.L., and Nelson, W.L., 1985. *Soil Fertility and Fertilizers*. The Macmillan Company, New York.

Table 1. Mean values ($\pm$ SE, N=3) for plant shoot final dry weight (DW), relative growth rate (RGR), water use over the experiment and plant water use efficiency (WUE). *P* values are given for salinity (the main plot factor: MS salinity/MS replications $\times$ salinity) and for species (or control) within each salinity treatment, (the subplot: MS species/MS error). Means followed by the same letter are not significantly different.

	Final DW (g)	RGR (/day)	Water Use (l)	WUE (g/l)
Salinity				
<i>P</i> -value	0.0016	<0.0001	<0.0001	0.0067
0.5 ppt	a	a	a	a
<i>Suaeda</i>	66.77(10.23)	0.049(0.002)	35.8(1.6)a	1.7(0.2)
<i>Salicornia</i>	34.83(5.51)	0.041(0.002)	24.7(1.9)b	1.3(0.1)
<i>Atriplex</i>	58.17(14.18)	0.047(0.003)	26.4(2.8)b	2.1(0.3)
Soil control	-	-	17.2(1.1)c	-
Species				
<i>P</i> value	ns	ns	0.002	ns
10 ppt	a	a	b	a
<i>Suaeda</i>	43.4(3.11)	0.043(0.001)	26.1(1.0)a	1.5(0.08)
<i>Salicornia</i>	40.97(2.42)	0.043(0.0007)	23.4(1.2)ab	1.6(0.02)
<i>Atriplex</i>	45.07(5.77)	0.044(0.002)	20.3(0.8)b	2.0(0.2)
Soil control	-	-	14.9(0.5)c	-
Species				
<i>P</i> value	ns	ns	0.0013	ns
35 ppt	b	b	c	b
<i>Suaeda</i>	5.56(0.25)a	0.028(0.002)a	12.3(0.3)a	0.9(0.09)a
<i>Salicornia</i>	2.55(0.14)a	0.030(0.0008)a	11.4(0.3)b	1.1(0.09)a
<i>Atriplex</i>	0.81(0.05)b	0.008(0.0006)b	10.2(0.2)c	0.2(0.008)b
Soil control	-	-	10.8(0.3)c	-
Species				
<i>P</i> value	0.0017	0.0005	0.0002	0.001

Table 2. Mean values ($\pm$ SE, N=3) for applied total nitrogen, the percent of the applied total nitrogen, the applied inorganic nitrogen (IN--ammonia + nitrate), and the percent of the applied inorganic nitrogen removed by the lysimeters. *P* values are given for salinity (the main plot factor: MS salinity/MS replications $\times$ salinity) and for species (or control) within each salinity treatment, (the subplot: MS species/MS error). Means followed by the same letter are not significantly different.

	TN removed (mg)	% TN removed	IN removed (mg)	%IN removed
Salinity				
<i>P</i> -value	<0.0001	0.0046	0.0012	0.0167
0.5 ppt	a	c	a	b
<i>Suaeda</i>	3992.9(189.6)a	96.2(1.2)	640.7(18.3)a	93.4(2.3)a
<i>Salicornia</i>	2691.1(263.9)b	96.3(1.1)	392.6(50.3)b	83.5(4.2)a
<i>Atriplex</i>	2972.7(333.1)b	97.5(0.2)	481.4(42.9)b	95.7(1.6)a
Soil Control	1763.3(108.0)c	95.3(1.4)	138.5(18.1)c	45.4(8.8)b
Species				
<i>P</i> value	0.0028	ns	0.0002	0.0029
10 ppt	b	b	a	a
<i>Suaeda</i>	2878.2(122.8)a	98.0(0.2)a	476.6(19.4)a	97.0(0.6)a
<i>Salicornia</i>	2632.0(158.3)ab	98.4(0.03)a	433.0(23.6)ab	97.1(0.5)a
<i>Atriplex</i>	2251.2(92.9)b	98.6(0.2)a	374.6(13.4)b	97.9(0.5)a
Soil control	1544.1(28.7)c	94.4(1.1)b	151.0(5.4)c	55.1(2.1)b
Species				
<i>P</i> value	0.0009	0.004	<0.0001	<0.0001
35 ppt	c	a	b	ab
<i>Suaeda</i>	1345.6(41.0)a	99.1(0.1)	214.5(4.8)a	94.3(0.6)a
<i>Salicornia</i>	1249.8(35.4)b	99.1(0.2)	202.8(5.8)a	95.8(1.0)a
<i>Atriplex</i>	1092.5(33.1)c	98.7(0.1)	169.5(6.0)b	90.7(0.1)a
Soil control	1154.7(41.9)c	98.2(0.4)	142.2(11.1)c	72.6(3.2)b
Species				
<i>P</i> value	0.012	ns	0.0005	0.0003

Table 3. Mean values ($\pm$ SE, N=3) for plant shoot nitrogen, percent of the applied total nitrogen sequestered by the plant shoot. *P* values are given for salinity (the main plot factor: MS salinity/MS replications $\times$ salinity) and for species within each salinity treatment, (the subplot: MS species/MS error). Means followed by the same letter are not significantly different.

	Plant N (mg)	% TN Removed by plant
Salinity		
<i>P</i> -value	<0.0001	0.002
0.5 ppt	a	a
<i>Suaeda</i>	877.94(89.85)	21.49(3.44)
<i>Salicornia</i>	659.20(37.59)	23.83(1.46)
<i>Atriplex</i>	683.67(123.14)	22.11(1.69)
Species		
<i>P</i> value	ns	ns
10 ppt	b	a
<i>Suaeda</i>	533.99(26.52)	18.21(0.82)
<i>Salicornia</i>	582.05(18.11)	21.88(1.04)
<i>Atriplex</i>	427.26(49.49)	18.72(2.18)
Species		
<i>P</i> value	ns	ns
35 ppt	c	b
<i>Suaeda</i>	182.36(7.36)a	13.43(0.40)a
<i>Salicornia</i>	192.55(1.75)a	15.28(0.31)a
<i>Atriplex</i>	35.61(3.83)b	3.20(0.24)b
Species		
<i>P</i> value	<0.0001	<0.0001

Table 4. Mean values ($\pm$ SE, N=3) for concentrations of total ammonia nitrogen (TAN), nitrate, and inorganic nitrogen (IN-ammonia + nitrate), in the leach water averaged over the course of the experiment, and soil inorganic nitrogen concentration. P values are given for salinity (the main plot factor: MS salinity/MS replications $\times$ salinity) and for species (or control) within each salinity treatment, (the subplot: MS species/MS error). Means followed by the same letter are not significantly different.

	TAN-N (mg/l)	NO ₃ -N (mg/l)	IN (mg/l)	Soil IN (mg/kg)
Salinity				
<i>P</i> -value	0.0103	0.0191	0.0197	0.0006
0.5 ppt	b	a	a	a
<i>Suaeda</i>	0.32(0.055)a	3.0(1.0)c	3.29(1.09)bc	5.34 (0.89)
<i>Salicornia</i>	0.19(0.003)b	7.3(1.9)b	7.49(1.93)b	7.68 (0.77)
<i>Atriplex</i>	0.26(0.015)ab	1.9(0.8)c	2.20(0.78)c	7.50 (1.61)
Soil control	0.19(0.006)b	22.4(3.5)a	22.55(3.55)a	8.44 (1.79)
Species				
<i>P</i> value	0.0145	0.0021	0.0022	ns
10 ppt	b	b	b	b
<i>Suaeda</i>	0.21(0.023)	1.3(0.2)b	1.55(0.22)b	0.90(0.056)b
<i>Salicornia</i>	0.22(0.024)	1.2(0.2)b	1.48(0.17)b	1.57(0.78)b
<i>Atriplex</i>	0.20(0.009)	0.9(0.2)b	1.09(0.24)b	1.26(0.35)b
Soil control	0.21(0.018)	17.3(1.4)a	17.53(1.41)a	4.83(0.56)a
Species				
<i>P</i> value	ns	<0.0001	<0.0001	0.008
35 ppt	a	ab	ab	b
<i>Suaeda</i>	0.32(0.100)	2.3(0.2)bc	2.63(0.295)b	1.54(0.23)c
<i>Salicornia</i>	0.48(0.216)	1.7(0.4)c	2.14(0.58)b	1.51(0.081)c
<i>Atriplex</i>	0.31(0.049)	3.8(0.2)b	4.06(0.19)b	2.48(0.24)b
Soil control	0.47(0.105)	11.2(1.3)a	11.67(1.33)a	3.39(0.21)a
Species				
<i>P</i> value	ns	0.0003	0.0006	0.0005

Table 5. Mean values ($\pm$ SE, N=3) for applied total phosphorus (TP), the percent of the applied total phosphorus, the applied soluble reactive phosphorus (SRP), and the percent of the applied orthophosphate removed by the lysimeter. *P* values are given for salinity (the main plot factor: MS salinity/MS replications $\times$ salinity) and for species (or control) within each salinity treatment, (the subplot: MS species/MS error). Means followed by the same letter are not significantly different.

	TP removed (mg)	% TP removed	SRP removed (mg)	%SRP removed
Salinity				
<i>P</i> -value	<0.0001	ns	<0.0001	0.0074
0.5 ppt	a		a	b
<i>Suaeda</i>	1396.9(70.6)a	98.7(0.4)bc	355.6(17.1)a	94.0(1.6)b
<i>Salicornia</i>	927.3(93.5)b	99.6(0.1)ab	236.3(24.6)b	97.9(0.4)ab
<i>Atriplex</i>	1020.2(119.4)b	99.2(0.2)bc	260.8(30.7)b	96.1(1.1)ab
Soil control	589.9(44.3)c	99.7(0.003)a	144.0(11.1)c	98.4(0.2)a
Species				
<i>P</i> value	0.0022	0.0105	0.0018	0.0303
10 ppt	b		b	b
<i>Suaeda</i>	977.8(42.7)a	98.9(0.1)b	250.4(11.1)a	96.0(0.3)b
<i>Salicornia</i>	892.1(55.2)ab	99.6(0.1)a	229.1(14.4)a	98.1(0.4)a
<i>Atriplex</i>	751.3(30.7)b	99.5(0.1)a	190.1(7.8)b	98.2(0.4)a
Soil control	510.6(15.0)c	99.7(0.1)a	124.2(4.2)c	98.5(0.2)a
Species				
<i>P</i> value	0.0010	0.0099	0.0007	0.0276
35 ppt	c		c	a
<i>Suaeda</i>	435.4(15.7)a	99.7(0.02)	107.6(4.5)a	99.0(0.1)
<i>Salicornia</i>	401.7(13.7)b	99.5(0.2)	98.2(3.6)ab	98.3(0.7)
<i>Atriplex</i>	350.1(11.5)c	99.7(0.02)	86.2(3.1)c	99.1(0.1)
Soil control	374.8(16.3)bc	99.7(0.02)	93.4(4.2)bc	99.0(0.1)
Species				
<i>P</i> value	0.0034	ns	0.0082	ns

Table 6. Mean values ($\pm$ SE, N=3) for plant shoot phosphorus, and the percent of the applied total phosphorus sequestered by the plant shoot. *P* values are given for salinity (the main plot factor: MS salinity/MS replications $\times$ salinity) and for species within each salinity treatment, (the subplot: MS species/MS error). Means followed by the same letter are not significantly different.

	Plant P (mg)	% TP removed by plant
Salinity		
<i>P</i> -value	<0.0001	<0.0001
0.5 ppt	a	a
<i>Suaeda</i>	208.91(21.41)	14.93(2.03)
<i>Salicornia</i>	136.52(26.84)	14.44(1.43)
<i>Atriplex</i>	170.93(14.09)	16.79(0.93)
Species		
<i>P</i> value	ns	ns
10 ppt	b	a
<i>Suaeda</i>	105.95(17.11)a	10.68(1.57)b
<i>Salicornia</i>	168.59(6.76)a	18.90(0.92)a
<i>Atriplex</i>	105.34(11.11)a	13.94(1.32)ab
Species		
<i>P</i> value	0.0468	0.0490
35 ppt	c	b
<i>Suaeda</i>	23.07(2.38)a	5.27(0.42)a
<i>Salicornia</i>	21.33(2.25)a	5.31(0.62)a
<i>Atriplex</i>	3.49(0.33)b	0.99(0.10)b
Species		
<i>P</i> value	0.006	0.0056

Table 7. Mean values ($\pm$ SE, N=3) for concentrations of soluble reactive phosphorus (SRP) and total phosphorus in the leach water averaged over the course of the experiment, and soil plant-available phosphorus/kg soil. P values are given for salinity (the main plot factor: MS salinity/MS replications $\times$ salinity) and for species (or control) within each salinity treatment, (the subplot: MS species/MS error). Means followed by the same letter are not significantly different.

	SRP (mg/l-P)	Total phosphorus (mg/l-P)	Soil phosphate (mg-P/kg)
Salinity			
P-value	0.0018	ns	ns
0.5 ppt	a		
<i>Suaeda</i>	1.46(0.399)a	1.16(0.370)a	6.32 (0.014)
<i>Salicornia</i>	0.48(0.105)ab	0.33(0.069)b	6.21 (1.50)
<i>Atriplex</i>	0.93(0.311)a	0.70(0.240)a	5.04 (0.84)
Soil control	0.29(0.045)b	0.20(0.0033)bc	3.52 (0.52)
Species			
P value	0.0281	0.0026	ns
10 ppt	a		
<i>Suaeda</i>	0.95(0.12)a	0.95(0.088)a	6.73 (5.34)
<i>Salicornia</i>	0.44(0.107)b	0.39(0.098)b	4.79 (0.54)
<i>Atriplex</i>	0.39(0.082)b	0.39(0.070)b	4.54 (0.88)
Soil control	0.26(0.02)b	0.23(0.039)b	4.17 (0.44)
Species			
P value	0.0143	0.0038	ns
35 ppt	b		
<i>Suaeda</i>	0.19(0.007)	0.23(0.012)	7.38 (0.74)
<i>Salicornia</i>	0.31(0.123)	0.37(0.112)	5.72 (0.64)
<i>Atriplex</i>	0.16(0.003)	0.21(0.003)	5.57 (0.63)
Soil control	0.19(0.018)	0.24(0.012)	5.38 (0.71)
Species			
P value	ns	ns	ns

**APPENDIX B. REUSE OF HIGHLY SALINE AQUACULTURE EFFLUENT TO
IRRIGATE A POTENTIAL FORAGE HALOPHYTE, *SUAEDA ESTEROA***

Title: Reuse of highly saline aquaculture effluent
to irrigate a potential forage halophyte,
Suaeda esteroa

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Abstract

We tested the feasibility of reusing saline aquaculture effluent to produce a salt-tolerant shrub with potential as a forage crop, *Suaeda esteroa*. Plants were grown in sandy loam soil in drainage lysimeters to determine forage yield potential, water use, and capacity for nitrogen and phosphorus uptake when irrigated with highly saline (31 ppt NaCl) effluent from a tilapia culture system. Water was applied to soil three times per week in five treatments, ranging in volume from 50 to 250% of the potential evaporation rate. The higher irrigation volumes produced significantly ($P \leq 0.05$) greater plant biomass than lower-volume treatments. Due to higher plant growth, the high-volume treatments also used more water than the low-volume treatments ($P \leq 0.05$). Nitrate concentrations in water draining from the lysimeters decreased during the experiment and were significantly lower in the high-volume treatments than in the low-volume treatments. Toward the end of the experiment, concentrations of nitrate in the leachate in the high volume treatments were below the mean limits set by the United States Environmental Protection Agency for effluent discharge. Phosphorus concentrations in the leachate water increased during the experiment and increased with increasing irrigation volume. We conclude that using seawater-salinity aquaculture wastewater to irrigate halophyte crops can be a viable strategy for disposal of

effluent, especially where phosphorus is not a limiting nutrient.

Keywords: Halophytes, Effluent, Seawater Agriculture, *Suaeda* *esteroa*, Biofilter

1. Introduction

The growth of the aquaculture industry has been associated with negative environmental impacts from the discharge of untreated effluent into adjacent receiving water bodies (Gowen and Bradbury, 1987; Iwama, 1991; Handy and Poxton, 1993; Hopkins et al., 1995; Wu, 1995; Beveridge 1996; Costa-Pierce, 1996; Dierberg and Kaattisimkul, 1996). Detrimental environmental effects associated with aquaculture wastes include localized hypernutrification and/or lowered dissolved oxygen levels in the water column (Bergheim et al., 1982; Penczak et al., 1982; Bergheim et al., 1984; Wisemann et al., 1988; Wildish et al., 1992; Hargrave et al., 1993; Cornell and Whoriskey, 1993; Briggs and Funge-Smith, 1994; Wu et al., 1994) toxic algal blooms (Kaartvedt et al., 1991), reduced and/or anoxic benthic sediments (Holmer and Kristenen, 1992; Hargrave et al., 1993; Wu et al., 1994), changes in benthic fauna (Brown et al., 1987; Weston, 1990; Tsutsumi et al., 1991, Ye et al., 1991; Hargrave et al., 1993), and accumulation of pathogenic and where antibiotics are added to feed, antibiotic-resistant bacteria (Husevåg et al., 1991; Samuelson et al., 1992; Kerry et al., 1994).

We have attempted to develop a consumptive approach to the disposal of saline aquaculture effluent, by utilizing it to irrigate salt-tolerant crop plants (halophytes) on land (Brown et al., in review). Recycling strategies that use

effluent to produce other aquaculture crops may clean the water but they do not appreciably reduce the volume of discharge. By contrast, agricultural crops can be expected to eliminate a large part of the effluent through evapotranspiration. Wild halophytes are being developed as biomass, forage and oilseed crops using saltwater irrigation (Glenn et al., 1991; Glenn et al., 1994; Miyamoto et al., 1996; Swingle et al., 1996; Glenn et al., 1997). Irrigating halophyte crops with saline effluent from aquaculture facilities might be a useful strategy to prevent eutrophication of coastal waters by the direct discharge of aquaculture effluent.

Halophytes were effective biofilters when plants were grown in a small volume (0.02 m^3) of sand with low water-holding capacity and irrigated once per week with aquaculture effluent containing up to 10 ppt salinity (Brown et al, in review). However, the growth and filtering capacity of plants at seawater salinity was poor because the weekly irrigation and low water holding capacity of the sand led to unrealistically high soil salinities between irrigations. The present study was undertaken to determine if satisfactory plant growth and removal of nitrogen and phosphorus could be achieved using seawater-salinity aquaculture effluent under soil conditions more typical of field conditions, and at increased irrigation frequency to better control soil salt levels. We grew *Suaeda esteroa*, a

succulent salt marsh shrub, that was the most salt tolerant species tested in the earlier trial. Biomass from this species was able to replace conventional grass hay in fattening diets for sheep in previous studies (Swingle et al., 1996). Plants were grown in large-volume lysimeters in natural soil, to determine yield and nutrient removal capacity of this species as a function of irrigation volume.

2. Methods and Materials

Experimental design and procedures

The experiment was conducted in at the Environmental Research Laboratory, Tucson, Arizona. *Suaeda esteroa* plants were started from seed collected from wild plants from Estero Morua, near Puerto Peñasco, Sonora, Mexico (Glenn and O'Leary, 1985; Glenn et al., 1991). Plants were grown in drainage lysimeters made from cylindrical fiberglass tanks holding approximately 0.78 m³ soil (0.76 m deep, 1.02 m² surface area). The soil was excavated from a site near Oracle, Arizona and was a sandy loam containing 72.3% sand, 19% silt and 8.7% clay. The clay mineral fraction contained moderate amounts of montmorillonite and mica and a small quantity of kaolinite. The soil was mixed and screened (1 cm mesh) and was placed in the lysimeter tanks in 1988. Within each lysimeter, soil was placed on a sheet of subsurface drainage matting (Geotextile, AKZO, Inc., Asheville, NC, USA) that overlaid a layer of hollow concrete blocks. The matting served as a barrier for soil but was permeable to water, which exited the lysimeters through bottom tubes leading to collection containers outside the tanks. This design allowed us to quantify the amount of water, salt, and nutrients leaching past the root zone (Miyamoto et al., 1996).

Wastewater for irrigation was generated from a freshwater recirculating system containing hybrid tilapia (Skeen et al., 1997). Once a week, two upflow granular biofilters (Armant Aquaculture Inc. Model CBF-4, Vacherie, Louisiana, USA) were backflushed; solids and wastewater were withdrawn to irrigate the lysimeters. The irrigation water was salinized by addition of NaCl to produce water approximately 31 ppt NaCl, the salinity of water commonly used to produce mariculture crops. NaCl was used rather than an artificial sea-salt mixture because seawater is greater than 90% NaCl on a molar basis, and previous experiments showed that NaCl (Glenn et al., 1996) artificial seawater (Glenn and O'Leary, 1984; Glenn, 1987) and natural seawater (Miyamoto et al., 1996) have similar effects on halophyte growth in lysimeter experiments.

A Class A evaporation pan was placed in the middle of the experimental area to estimate weekly pan evaporation (E_{pan}). This value was used to adjust irrigation volumes over the experiment. The five irrigation treatments were 50, 100, 150, 200, and 250% E_{pan} , adjusted weekly. A set of control lysimeters (soil without plants) was included at the 150% treatment to determine the effect of the soil alone on nutrient removal. There were three replicate lysimeters per treatment. However, two lysimeters were removed from analysis because extraneous water accidentally entered from an overhead sprinkler system (total experimental units =

16). Lysimeters were randomly assigned to treatments.

Lysimeters were pre-irrigated with sufficient amounts of 30 ppt water to produce drainage, in order to salinize the soil medium. The study was conducted in July-October, 1996 in a controlled environment greenhouse. Seedlings were transplanted into the lysimeters (12 per lysimeter) two days before irrigation with effluent began. A subsample of plants was taken at the time of transplanting to obtain initial weights. Lysimeters were irrigated three times per week (Monday, Wednesday, Friday with one-third of the predetermined weekly volume per irrigation) with salinized effluent from the tilapia tanks. A sample of irrigation water was retained for water quality analysis after each irrigation. Lysimeters were allowed to drain overnight, and the leachate water from all treatments was collected on the morning following the irrigation. The experiment was terminated at the end of 14 weeks, by which time plants on the fastest-growing treatment had produced a dense canopy of fully-developed plants. Most of the plants flowered and produced seed during the experiment. Surviving plants were counted, and above-ground biomass was harvested and air dried to constant weight in a greenhouse to determine dry matter yield per lysimeter.

Water, plant and soil analyses

Electrical conductivity, pH, total ammonia nitrogen (TAN), nitrate, total nitrogen (TN), soluble reactive phosphorus (SRP), and total phosphorus (TP) were measured in irrigation and leachate water (American Public Health Association, 1995). Measurements of TAN, electrical conductivity and pH of the irrigation water were taken immediately after completing each irrigation. TAN in the leachate was measured for the first several weeks of the experiment, but measurement was discontinued because values were consistently below detectable limits, and thus did not contribute significantly to the nitrogen mass balance.

It was not feasible to measure every parameter at every irrigation and leaching event. Therefore, for determination of nitrate and SRP of the leachate and irrigation water, each of the thrice-weekly irrigation and leachate samples were first frozen. At the end of the week, the three subsamples from the three weekly irrigations and leachates were pooled by volume to create one composite sample that represented a weekly value for the irrigation water and for each leachate replicate. TN and TP in the irrigation water were measured for each of the pooled weekly irrigation samples. TN and TP in the leachate were measured for each replicate on samples that were pooled by volume over the

entire experiment (i.e. leachate was pooled over all weeks to create one composite sample for each replicate). TN was measured by first reducing nitrate to ammonia followed by the Kjeldhal nitrogen procedure (Bremner and Mulvaney, 1982).

Initial soil samples were taken from the top 30 cm of soil profile. Final soil samples were taken with a soil augur through the full 60 cm soil profile and separated into three samples based on depth: the top 20 cm, 20-40 cm below the surface and 40-60 cm below the surface. Soil samples were analyzed for TAN, nitrate, plant-available phosphate (sodium bicarbonate extraction) (Page et al., 1982). Soil moisture was determined gravimetrically on soil samples taken on the day the experiment was terminated, a day following an irrigation. Soil was dried in an oven to constant weight at 100 °C. Dry weight was subtracted from wet weight to ascertain the moisture content. The salt content of the dried soil was determined by measuring the electrical conductivity of the water extract made from 10 g of soil in 50 ml distilled water. The salinity of the soil water was then calculated by dividing the salt content by the soil moisture.

Net biomass of plant shoots was obtained by subtracting the final dry weight from the mean initial seedling dry weight. TN in samples of plant shoots was measured with a CNS analyzer, and TP was analyzed by acid digestion followed

by colorimetry (Jones et al., 1991). Plant water-use efficiency (WUE) was calculated as $WUE = \text{plant dry weight accumulated (g)}/\text{water consumed (l)}$.

Statistical analyses

Data were first tested for homogeneity of variances using Bartlett's test. Data with heterogeneous variance were $\log_{10}$ transformed. Percentages and proportions were arcsine transformed to ensure normality (Sokal and Rohlf, 1981). Fertilizer had been applied to the soil in previous experiments. Therefore, a series of one-way analyses of covariance was performed with irrigation volume treatment as the independent variable to test whether the initial nutrient concentrations in the soil (the covariate) affected the results. The initial concentration of inorganic nitrogen was used as a covariate for the following dependent variables: final concentration of IN in the soil, nitrate leachate, percent and grams of nitrogen in the plant shoot, carbon:nitrogen ratio in the plant shoot, and net dry weight of plants. Similarly, the initial concentration of phosphate in the soil was used as a covariate for the following dependent variables: the final concentration of phosphate in the soil, SRP leachate, the percent and grams of phosphorus in the plant shoot, and net dry weight of plants. If the covariate was significant ($P < 0.05$),

adjusted least square means and standard errors are reported. If the covariate was not significant ($P \geq 0.05$), it was removed from the analysis, and analysis was repeated as a one-way ANOVA. For the other independent variables, data were analyzed as one-way ANOVAs with irrigation volume as the independent variable. When ANOVA indicated that there were significant differences among treatments, a Tukey-Kramer *a posteriori* test was carried out to determine which means differed. Differences were considered statistically significant when $P < 0.05$. Regression analyses were performed on data sets that did not contain the control treatment. Irrigation volume, a continuous value, was used as the independent variable for all regressions. Correlation analyses were conducted to test for the relationship between production of plant shoot biomass and soil water salinity, which was expected to be the limiting factor for plant growth.

3. Results

Irrigation water quality

Mean weekly values of water quality parameters of irrigation water ($\pm$ standard error) were: TN 36.92 ± 4.75 mg/l; TAN-N 4.50 ± 0.31 mg/l; nitrate-N 8.77 ± 2.31 mg/l; TP 16.55 ± 0.95 mg/l; SRP-P 14.57 ± 0.81 mg/l; salinity 31.0 ± 0.2 ppt; pH 7.72 ± 0.06 . The high nutrient levels reflected the concentrated nature of the effluent source (filter washings).

Greenhouse environmental conditions

Mean weekly air temperatures in the greenhouse for the first 11 weeks of the experiment (no data available for last 3 weeks of the experiment due to equipment failure) were 28.0 ± 0.83 °C. Light transmission through the greenhouse was about 59% of ambient, and mean daily quantum flux in the greenhouse over the course of the experiment was $26.3 \text{ E m}^{-2} \text{ d}^{-1}$ (Brown and Russell, 1996). Relative humidity ranged from 60-80% (the greenhouse was evaporatively cooled throughout the experiment).

Water use and plant growth

Consumptive water use (total irrigation minus leachate volume) was significantly affected by irrigation volume (ANOVA, $F=74.50$, $df=5/10$, $P<0.0001$); as more water was added, plants grew more rapidly and therefore consumed more water (Table 1). The ratio of E_t/E_{pan} increased up to the 11th week and then proceeded to decrease, reflecting the development of the crop (Figure 1). The leaching fraction (leachate divided by irrigation volume) was also significantly affected by irrigation treatment (ANOVA, $F=173.86$, $df=5/10$, $P<0.0001$). The rank order was similar to consumptive water use except that the 150% control treatment was grouped with the 200 and 250% treatment. The mean leaching fraction was relatively low for the 50% treatment, and these lysimeters did not produce any leachate at all after the eighth week of the experiment.

Net production of plant shoot biomass was also significantly affected by irrigation volume (ANOVA, $F=5.54$, $df=4/8$, $P=0.019$), with the 200 and 250% treatment producing significantly more biomass than the other treatments. A regression of plant shoot dry weight on irrigation volume for the planted (non-control) lysimeters was significant ($Y=0.788X + 258.71$, $R^2=0.621$, $P=0.0014$), indicating that production of plant shoot biomass increased with increasing irrigation volume (Figure 2). Mean plant survival over all

treatments was 90%. The number of plants surviving (out of 12 planted per treatment) through the end of the experiment was (mean $\pm$ SE); 50%, 9.0 ± 1.4 ; 100%, 10.7 ± 0.3 ; 150% 10.0 ± 1.4 ; 200%, 12 ± 0 ; 250%, 11.3 ± 0.5 . Water use efficiency was not significantly affected by irrigation treatment (ANOVA, $F = 2.34$, $df = 4/8$, $P = 0.14$).

Leachate salinities were compared during the final week of the experiment, by which time the initial (non-saline) soil solution had been replaced by saline solution, and the lysimeters were assumed to be in a steady state (Miyamoto et al., 1996). Irrigation volume exerted a significant effect on the salinity of the leachate water (ANOVA, $F = 55.66$, $df = 4/9$, $P < 0.0001$) (Table 2). A regression of leachate salinity on irrigation volume for the planted (non-control) lysimeters was significant ($Y = 77.34 - 0.929X$, $R^2 = 0.864$, $P = 0.0035$), indicating that leachate salinity decreased with increasing irrigation volume. Salinity of the soil water measured at the end of the experiment was also significantly higher in the 50% treatment in the top 20 cm (ANOVA, $F = 91.20$, $df = 5/10$, $P < 0.0001$) and middle 20 cm (ANOVA, $F = 14.36$, $df = 5/10$, $P = 0.0002$) of the soil profile than in the higher-volume treatments. Regressions of salinity of the soil water at top 20 and middle 20 cm of the soil profile on irrigation volume for the planted (non-control) lysimeters were significant ($Y = -0.707X + 184.07$, $R^2 = 0.500$, $P = 0.0069$, $Y = -0.785X + 102.02$, $R^2 = 0.616$, $P = 0.0015$, respectively),

indicating that soil salinity decreased with increasing irrigation volume. Irrigation treatment also affected soil salinity in the bottom 20 cm of the soil profile (ANOVA, $F=11.61$, $df=5/10$, $P=0.0007$); the lower irrigation volume treatments tended to have higher soil water salinities. A regression of soil salinity in the bottom 20 cm of the soil profile on irrigation volume for the planted (non-control) lysimeters was significant ($Y=-0.819X + 81.55$, $R^2=0.671$, $P=0.00061$). In general the soil water salinity was inversely correlated with soil depth across all treatments.

Correlation between mean salinity of the soil column and net production of plant shoot biomass was marginally non-significant ($r=-0.586$, $P=0.058$, $N=11$). This result suggests that there was a trend toward decreased production of above-ground biomass with higher soil water salinities.

Nitrogen removal

The concentration of inorganic (TAN + nitrate) nitrogen (IN) in the soil prior to commencing the irrigation did not vary significantly (ANOVA, $F=0.64$, $df=5/10$, $P=0.67$) among the treatments (Table 3). The concentration of soil IN at the end of the experiment was significantly affected by the irrigation treatment (ANCOVA, $F=5.91$, $df=5/9$, $P=0.011$), with the lower irrigation treatments having higher

IN concentrations. A regression of final soil IN on irrigation volume for the planted (non-control) lysimeters was significant ($Y = -0.585X + 1.08$, $R^2 = 0.343$, $P = 0.036$), indicating that the concentration of soil IN decreased with increasing irrigation volume. Over all treatments the concentration of soil IN was lower at the end of the experiment than at the beginning (paired t-test, $t = 7.01$, $df = 15$, $P < 0.0001$) indicating a net removal of nitrogen from the soil by irrigation, despite the presence of high levels of nitrogen in the water supply.

There was no significant difference (ANOVA, $F = 1.57$, $df = 5/10$, $P = 0.25$) among treatments in the concentration of nitrate in the leachate water in week 2 (water quality not analyzed on week 1) (Table 4). Nitrate concentration in the leach water decreased over the experiment for all treatments (Figure 3). By the last two weeks of the experiment (weeks 13 and 14), the irrigation treatment had a significant effect on the concentration of leachate nitrate (ANOVA, $F = 93.91$, $df = 4/9$, $P < 0.0001$, ANOVA, $F = 26.08$, $df = 4/9$, $P < 0.0001$, respectively). The 200 and 250% treatments had significantly lower nitrate concentrations (0.59 mg/l, mean of week 13 and 14), followed by the 150% treatment (10.43 mg/l, mean of week 13 and 14), the 100% treatment (25.65 mg/l, mean of week 13 and 14). The 150% control tended to have the highest concentrations (28.86 mg/l mean of week 13 and 14). Regression of log week 13 and week 14 leachate

nitrate concentration on irrigation volume for the planted (non-control) lysimeters was significant ($Y = -0.932X + 2.34$, $R^2 = 0.868$, $P = 0.00003$; $Y = -0.899X + 2.18$, $R^2 = 0.809$, $P = 0.00016$, respectively), indicating that leachate nitrate concentration decreased with increasing irrigation volume. In order to determine whether most of the TN in the leachate was nitrate I compared the nitrogen concentration of the pooled TN sample to that of the mean nitrate concentration of the leachate. There was no significant difference (paired t-test, $t = -1.62$, $df = 15$, $P = 0.127$) between TN concentration of leachate samples pooled over the course of the experiment and mean leachate nitrate concentration over the course of the experiment. This result indicates that nitrogen leaching from the lysimeter system was almost exclusively in the form of nitrate.

The percentage of nitrogen in the plant shoot was also affected by the irrigation treatment (ANOVA, $F = 4.65$, $df = 4/8$, $P = 0.031$), however the *a posteriori* test was unable to separate the means (Table 5). A regression of arcsine percentage of nitrogen in the plant shoot on irrigation volume for the planted (non-control) lysimeters was significant ($Y = -0.774X + 9.87$, $R^2 = 0.599$, $P = 0.0019$), indicating that the percent nitrogen decreased with increasing irrigation volume. There was no significant difference among the treatments with respect to the total g of nitrogen in the plant shoot (ANOVA, $F = 0.33$, $df = 4/8$, $P =$

0.85), and the regression of total g of nitrogen on irrigation volume was not significant ($Y=0.308X + 9.24$, $R^2=0.095$, $P = 0.3044$). The ratio of plant N to inorganic N applied from the irrigation water was 1.15:1 (mean over all treatments). The plant shoot carbon: nitrogen ratio was affected by irrigation treatment (ANOVA, $F= 4.35$, $df=4/8$, $P =0.037$), but because of the marginal P value, the *a posteriori* test was unable to separate the means. However, a regression of plant carbon: nitrogen ratio on irrigation volume for the planted lysimeters was significant ($Y=0.800X + 8.22$, $R^2=0.640$, $P = 0.001$), indicating that the C:N ratio increased with increasing irrigation volume.

Phosphorus removal

The concentration of the soil phosphate prior to commencing the irrigation did not vary significantly among the treatments (ANOVA, $F= 1.33$, $df=5/10$, $P =0.33$) (Table 6). The concentration of soil phosphate at the end of the experiment was not significantly affected by the irrigation treatment (ANOVA, $F= 0.39$, $df=5/10$, $P =0.84$). Soil phosphate concentration was significantly lower at the end of the experiment than at the beginning (paired t-test, $t= 2.29$, $df=15$, $P =0.037$).

There was no significant difference among treatments with respect to the leachate concentration of SRP at the beginning of the experiment (mean over all treatments on Week 2 = 0.64 mg/l P) (Table 7), but the concentration of SRP in the leachate increased over the course of the experiment (Figure 4). By week 13 there was still no significant difference in the concentration of SRP among the treatments (ANOVA, $F= 1.72$, $df=4/9$, $P =0.23$), however the mean over all treatments had increased to 2.37 mg/l P. On week 14, irrigation treatment had a significant affect on the concentration of leachate SRP (ANCOVA, $F= 4.10$, $df=4/8$, $P = 0.043$), and the 200 and 250% treatments had higher concentrations than the lower volume treatments. Regression of week 13 and week 14 leachate SRP concentration on irrigation volume for the planted (non-control) lysimeters was significant ($Y=-0.616X - 1.46$, $R^2=0.380$, $P = 0.043$; $Y=-0.772X - 1.87$, $R^2=0.596$, $P = 0.0054$, respectively), indicating that the concentration of leachate SRP increased with increasing irrigation volume. In order to determine whether most of the phosphate in the leachate was in the dissolved form, the SRP concentration of the leachate was compared to the TP concentration of the leachate. The mean concentration of SRP in the leachate (mean of all treatments averaged over the course of the experiment) was 7.5% higher (paired t-test, $t= 3.88$, $df=15$, $P =0.015$), than the leachate TP concentration of a pooled sample (mean of all

treatments). This result indicates that the majority of the phosphorus leaching from the lysimeters was in the dissolved form, and the 7.5% difference was attributed to the different analytical techniques.

Neither the percentage nor grams of phosphorus in the plant shoot were significantly affected (ANOVA, $F=0.87$, $df=4/8$, $P=0.52$, ANOVA, $F=2.47$, $df=4/8$, $P=0.13$, respectively), by the irrigation treatment (Table 8). However, a regression of g of phosphorus in the plant shoot on irrigation volume for the planted (non-control) lysimeters was significant ($Y=0.712X + 0.719$, $R^2=0.506$, $P=0.0064$), indicating that g of phosphorus in the plant shoot increased with increasing irrigation volume. The treatment-wide mean ratio of plant shoot phosphorus to SRP applied in the irrigation water was 0.20:1.

4. Discussion

We achieved high plant yields irrigating with highly saline aquaculture effluent. The 250‰ irrigation treatment (1.04 m of water) produced the equivalent of 6.8 t per ha over 98 days or $6.9 \text{ g m}^{-2} \text{ d}^{-1}$. This production is greater than that of Sudan grass ($6.1 \text{ g m}^{-2} \text{ d}^{-1}$), which is grown as a conventional forage crop in Arizona using 1.2 m of water (Daugherty et al., 1996). However, plant production in greenhouses tends to overestimate yields compared to yields obtained in the field. Kahn and Glenn (1996) achieved biomass yields of $12.9 \text{ g m}^{-2} \text{ d}^{-1}$ on fresh water and $5.4 \text{ g m}^{-2} \text{ d}^{-1}$ on 250 mM NaCl (15 ppt) for barley grown in the same lysimeters used in this experiment. The lysimeters were of sufficient diameter and contained sufficient numbers of plants to simulate a field planting of this low-growing plant. Field trials with this and other halophytes have also shown that high biomass yields can be achieved under high-frequency irrigation with seawater (Glenn and O'Leary, 1985; Glenn et al., 1991). The ability to use full-strength seawater is important, because much of the shrimp, salmon, and other mariculture crops are raised at seawater salinities.

Of the total water applied to the two highest-volume irrigation treatments, 41% was consumed in evapotranspiration while 59% was discharged past the root

zone. Thus, unlike other disposal options for saline aquaculture effluent, this method actually consumes much of the water in the biofiltration process. Plants had a significant effect on both consumptive water use and the leaching fraction. The control lysimeters that were irrigated at 150% E_{pan} used about as much water as the 100% E_{pan} treatment but significantly less than the 150% E_{pan} planted treatment. Similarly, the leaching fraction for the control treatment was about the same as the leaching fraction for the two highest irrigation volume treatments, and was significantly greater than the leaching fraction of the 150% E_{pan} planted treatment.

The area of halophytes needed to treat the effluent from one ha of shrimp pond would depend on the pond water exchange rate (% volume per day, if water exchange is used at all), the depth of the pond and potential evapotranspiration. The highest irrigation volume treatment received a weekly average of 8.1 cm of effluent water. For recirculating systems that only discharge water at harvest, the harvest water from a 1 ha pond 1.5 m deep could be used to irrigate 1 ha halophytes for about 18 weeks. For a low-end estimate for systems that exchange water, a pond 1 m deep discharging an average of 20% of its water per week would require about 2.5 ha of halophytes to continuously treat water from each ha of shrimp pond. At the high end, a pond 1.5 m deep discharging an average of 70% of its water

per week would require about 13 ha of halophytes to treat water from each ha of shrimp pond. These estimates compare favorably to those made by Robertson and Phillips (1995) who estimated that it would take between 2 and 22 ha of mangrove forest to treat the effluent from a 1 ha shrimp pond. Neori et al. (1996) designed a sustainable fish recirculating system that utilized a seaweed biofilter to maintain water quality. The ratio of the area of seaweed biofilter to fish production was 3:1. However, construction of halophyte fields will generally be far less expensive than construction of seaweed biofilter ponds.

The soil in our lysimeters had been used to grow a series of experimental crops, and it contained residual nitrogen from those crops. For this reason, the leachate nitrate concentration was high at the beginning of the experiment. As the experiment progressed, concentrations of nitrate in the leachate decreased due to plant uptake and leaching from the lysimeters. At the beginning of the experiment, the leachates from all treatments exceeded the mean effluent concentration limit recommended by the U.S. Environmental Protection Agency for nitrate (16.9 mg/l N) (Schwartz and Boyd, 1994). However, by the end of the experiment, the 150, 200 and 250% treatments were lower than the mean EPA standard for nitrate concentration in effluents. The 50% treatment did not produce any leachate after the 8th week, so this treatment would have also met

the mean EPA standard after that week. The nitrate leachate concentration in the 150% soil control treatment on week 14 was about 3 times greater than the concentration in the 150% planted treatment, indicating that plants had a marked effect on nitrate removal from the system. Similarly, the soil inorganic nitrogen concentration in the 150% control treatments was significantly higher than the concentration in the 150% planted treatment.

The results for phosphorus were opposite to those for nitrate. The concentration of SRP in the leachate water tended to increase over the course of the experiment, and higher irrigation volumes tended to leach higher concentrations of SRP. The concentration of phosphorus in the soil solution is controlled by 1) precipitation-dissolution reactions, 2) sorption-desorption reactions, 3) immobilization-decomposition and 4) plant uptake (Syers and Iskandur, 1981). Of these, sorption-desorption reactions are considered to be the most important in controlling the concentration of phosphorus in the soil solution when wastewater is used for irrigation purposes. The soil used in this experiment had a low clay content, therefore there were relatively few sites available for phosphorus sorption and low contact time between solution and solid phases (Syers and Iskandur, 1981). At higher irrigation volumes binding sites for phosphorus probably became saturated, and therefore leachate SRP concentrations were higher for the

high volume treatments than for the low volume treatments. Plants did not appear to exert a significant effect on phosphorus removal. Plant uptake of phosphorus was less than one fifth than that of nitrogen. Therefore, it appeared phosphorus removal was largely attributable to the physical soil processes described above. Leachate values for SRP exceeded the mean EPA standard for TP (0.17 mg/l P). However, the EPA standards were developed primarily for freshwater systems where phosphorus is considered to be the nutrient limiting phytoplankton growth. If phosphorus reached the ocean or estuary, it would probably not be problematic, because in the coastal and estuarine areas where mariculture is practiced, nitrogen, not phosphorus, is considered to be the nutrient limiting phytoplankton growth (Ryther and Dunstan, 1971; Goldman et al., 1973).

The effluent used for irrigation in this experiment came from filter backwashings from a recirculating system. Therefore, the effluent had a heavy nutrient load, close to an order of magnitude higher than typical shrimp pond effluent (Briggs and Funge-Smith, 1994; Phillips, 1995; Dierberg and Kaattisimkul, 1996). Had we irrigated with a more typical, less concentrated effluent, the leachate concentrations for both nitrate and phosphate would have certainly been lower.

It is feasible to use high salinity aquaculture effluent to irrigate *Suaeda esteroa*. Aquaculture farm

managers who can not discharge effluent but have adequate coastal land where soils overlay saline aquifers, and where irrigated crops produced can sold or used as forage, can utilize aquaculture effluent for irrigation of halophyte crops.

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References

- American Public Health Association, American Water Works Association, Water Environment Federation, 1995. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC.
- Bergheim, A., Sivertsen, A., Selmer-Olsen, A.R., 1982. Estimated pollution loadings from Norweigan fish farms. I. Investigations 1978-1979. *Aquaculture* 28, 347-361.
- Bergheim, A., Hustveit, H., Kittelsen, A., Selmer-Olsen, A.R., 1984. Estimated pollution loadings from Norweigan fish farms. II. Investigations 1980-1981. *Aquaculture* 36, 157-168.
- Beveridge, M.C.M., 1996. Cage aquaculture 2nd edition. Fishing News Books, Oxford.
- Bremner, J.M., Mulvaney, C.S., 1982. Nitrogen-total. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*. American Society of Agronomy, Soil Science Society of America, Madison, WI, pp. 595-624.
- Briggs, M.R.P., Funge-Smith, S.J., 1994. A nutrient budget of some intensive marine shrimp ponds in Thailand. *Aquaculture and Fisheries Management* 25, 789-811.

- Brown, J.J., Glenn, E.P., Fitzsimmons, K.M., Smith, S., In Review. 1998. Halophytes for the treatment of saline aquaculture effluent. *Aquaculture*.
- Brown, J.R., Gowen, R.J., McLusky, D.S., 1987. The effect of salmon farming on the benthos of a Scottish sea loch. *J. Exp. Mar. Biol. Ecol.* 109, 39-51.
- Brown, P.W., Russell, B., 1996. AZMET, The Arizona Meteorological Network. Arizona Cooperative Extension, Tucson, Arizona, <http://ag.arizona.edu/azmet/>.
- Cornell, G.E., Whoriskey, F.G. 1993. The effects of rainbow trout (*Onchorynchus mykiss*) cage culture on the water quality, zooplankton, benthos and sediments of Lac du Passage, Quebec. *Aquaculture* 109, 101-117.
- Costa-Pierce, B.A., 1996. Environmental impacts of nutrients from aquaculture: towards the evolution of sustainable aquaculture systems. In: Baird, D.J., Beveridge, M.C.M, Kelly, L.A. and Muir, J.F. (Eds.), *Aquaculture and Water Resource Management* Blackwell Science, Oxford, pp. 81-113.
- Daugherty, L., Howell, D., Tickles, B., 1996. 1996-1997 Arizona Field Crop Budgets, Yuma County. Arizona Cooperative Extension, Tucson, Arizona.
- Dierberg, F.E., Kaittisimkul, W., 1996. Issues, impacts and implications of shrimp aquaculture in Thailand. *Environmental Management* 20 (5), 649-666.

- Glenn, E.P., 1987. Relationship between cation accumulation and water content of salt-tolerant grasses and a sedge. *Plant, Cell, and Environment* 10, 205-212.
- Glenn, E.P., O'Leary, J.W., 1985. Productivity and irrigation requirements of halophytes grown with seawater in the Sonoran Desert. *Journal of Arid Environments* 9, 81-91.
- Glenn, E.P., O'Leary, J.W., Watson, M.C., Thomas, T.L., Kuehl, R.O., 1991. *Salicornia bigelovii* Torr: an oilseed halophyte for seawater irrigation. *Science* 251, 1065-1067.
- Glenn, E.P., Swingle, R.S., Riley, J.J., Squires, V.R., 1994. North American halophytes: Potential use in animal husbandry. In: Squires, V.R., Ayoub, A.T. (Eds.), *Halophytes as a Resource for Livestock and for Rehabilitation of Degraded Lands*. Kluwer Academic Publishers, Netherlands, pp. 165-174.
- Glenn, E.P., Pfister, R., Brown, J.J., Thompson, T.L., O'Leary, J. 1996. Na and K accumulation and salt tolerance of *Atriplex canescens* (Chenopodiaceae) genotypes. *American Journal of Botany*, 83(8), 997-1005.

- Glenn, E.P., Miyamoto, S., Moore, D., Brown, J.J., Thompson, T.L., Brown, P., 1997. Water requirements for cultivating *Salicornia bigelovii* Torr. with seawater on sand in a coastal desert environment. *Journal of Arid Environments* 36, 711-730.
- Goldman, J., Tenore, K., and Stanley, H., 1973. Inorganic nitrogen removal from wastewater: effect on phytoplankton growth in coastal marine waters. *Science* 180, 955-956.
- Gowen, R.J., Bradbury, N.B., 1987. The ecological impact of salmonid farming in the coastal waters: A review. In: Barnes, M. (Ed.), *Oceanogr. Mar. Biol. Ann. Rev.* Aberdeen University Press, Aberdeen, pp.563-575.
- Handy, R.D., Poxton, M.G., 1993. Nitrogen pollution in mariculture: toxicity and excretion of nitrogenous compounds by marine fish. *Reviews in Fish Biology and Fisheries* 3, 205-241.
- Hargrave, B.T., Duplisea, D.E., Pfeiffer, E., Wildish, D.J., 1993. Seasonal changes in benthic fluxes of dissolved oxygen and ammonium associated with marine cultured Atlantic salmon. *Marine Ecological Progress Series* 96, 249-257.
- Holmer, M., Kristensen, E., 1992. Impact of marine fish culture on metabolism and sulfate reduction of underlying sediments. *Marine Ecological Progress Series* 80, 191-201.

- Hopkins, J.S., Sandifer, P.A., Devoe, M.R., Holland, A.F., Browdy, C.L., Stokes, A.D., 1995. Environmental impact of shrimp farming with special reference to the situation in the Continental United States. *Estuaries* 18 (1A), 25-42.
- Husevåg, B., Lunstead, B.T, Johannessen, P.J., Enger, Ø., Samuelson, O.B., 1991. Simultaneous occurrence of *Vibrio salmonicida* and antibiotic-resistant bacteria in sediments at abandoned aquaculture sites. *Journal of Fish Diseases* 14, 631-640.
- Iwama, G.K., 1991. Interactions between aquaculture and the environment. *Critical Reviews in Environmental Control* 21 (2), 177-216.
- Jones, J.B., Wolf, B., Mills, H.A., 1991. *Plant Analysis Handbook: A Practical Sampling, Preparation, Analysis and Interpretation Guide*. Micro-Macro Publishing, Inc., Athens, Georgia
- Kaartvedt, S., Johnsen, T.M., Aksnes, D.L., Lie, U., Svendsen, H., 1991. Occurrence of the toxic phytoflagellate *Prymnesium parvum* and associated fish mortality in a Norweigan fjord system. *Can. J. Fish. Aquat. Sci.* 48, 2316-2323.
- Kahn, M.J., Glenn, E.P., 1996. Yield and evapotranspiration of two barley varieties as affected by sodium chloride salinity and leaching fraction in lysimeter tanks. *Commun. Soil Sci. Plant Anal.* 27(1&2), 157-177.

- Kerry, J., Hiney, M., Coyne, R., Cazabon, D., NicGabhainn, S., Smith, P., 1994. Frequency and distribution of resistance to oxytetracycline in micro-organisms isolated from marine fish farm sediments following therapeutic use of oxytetracycline. *Aquaculture* 123, 43-54.
- Miyamoto, S., Glenn, E.P., Olsen, M.W., 1996. Growth, water use and salt uptake of four halophytes irrigated with highly saline water. *Journal of Arid Environments* 32, 141-159.
- Neori, A., Krom, M.D., Ellner, S.P., Boyd, C.E., Popper, D., Rabinovitch, R., Davidson, P.J., Divr, O., Zuber, D., Ucko, M., Angel, D., Gordin, H., 1996. Seaweed biofilters as regulators of water quality in integrated fish-seaweed culture units. *Aquaculture* 141, 183-199.
- Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), 1982. *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*. American Society of Agronomy, Soil Science Society of America, Madison, WI.
- Penczak, T., Galicka, W., Molinski, M., Kusto, E., Zalewski, M., 1982. The enrichment of a mesotrophic lake by carbon, phosphorus and nitrogen from the cage aquaculture of rainbow trout, *Salmo gairderi*. *Journal of Applied Ecology* 19, 371-393.

- Phillips, M., 1995. Aquaculture and the environment--striking a balance. In: Nambiar, K.P.P., Singh, T. (Eds.), International Conference on Aquaculture: Aquaculture towards the 21st Century. Infofish, Kuala Lumpur, pp. 26-55.
- Robertson, A.I., Phillips, M.J., 1995. Mangroves as filters of shrimp pond effluent: predictions and biogeochemical research needs. *Hydrobiologia* 295, 311-321.
- Ryther, J., Dunstan, W. 1971. Nitrogen, phosphorus, and eutrophication in the coastal marine environment. *Science*, 171, 1008-1013.
- Samuelson, O.B., Torsvik, V., Ervik, A., 1992. Long-range changes in oxytetracycline and bacterial resistance towards oxytetracycline in a fish farm sediment after medication. *The Science of the Total Environment* 114, 25-36.
- Schwartz, M.F., Boyd, C.E., 1994. Channel catfish pond effluents. *Progressive Fish-Culturist* 56, 273-281.

- Skeen, B., Brown, J.J., Fitzsimmons, K., Dickenson, G.,
1997. Performance characteristics of open and closed
bead filters in a recirculating tilapia production
system. In: Timmons, M. and Losordo, T. (Eds.),
Advances in Aquacultural Engineering: Proceedings from
the Aquacultural Engineering Society Technical Sessions
at the Fourth International Symposium on Tilapia in
Aquaculture. Northeast Regional Agricultural
Engineering Service, Ithaca, New York, pp. 276-286.
- Sokal, R.R., Rohlf, F.J., 1981. Biometry: The Principles
and Practice of Statistics in Biological Research. 2nd
ed. Freeman, New York.
- Swingle, R.S., Glenn, E.P., Squires, V.R., 1996. Growth
performance of lambs fed mixed diets containing
halophyte ingredients. Animal Feed Science Technology
63, 137-148.
- Syers, J.K., Iskander, I.K., 1981. Soil-phosphorus
chemistry. In: Iskander, I.K. (Ed.), Modeling
Wastewater Renovation, Land Treatment. John Wiley &
Sons, New York, pp. 569-599.
- Tsutsumi, T., Kikuchi, T., Tanaka, M., Higashi, T., Imasaka,
K., Miyazaki, M., 1991. Benthic faunal succession in a
cove organically polluted by fish farming. Marine
Pollution Bulletin 23, 233-238.

- Weston, D.P., 1990. Quantitative examination of macrobenthic community changes along an organic enrichment gradient. *Marine Ecology Progress Series* 61, 233-244.
- Wiesmann, D., Scheid, H., Pfeffer, E., 1988. Water pollution with phosphorus of dietary origin by intensively fed rainbow trout (*Salmo gairdneri* Rich.). *Aquaculture* 69, 263-270.
- Wildish, D.J., Keizer, P.D., Wilson, A.J., Martin, J.L., 1993. Seasonal changes and dissolved oxygen and plant nutrients near salmonid net pens in the macrotidal Bay of Fundy. *Canadian Journal of Fisheries and Aquatic Science* 50, 303-311.
- Wu, R.S.S., 1995. The environmental impact of marine fish culture: towards a sustainable future. *Marine Pollution Bulletin* 31, 159-166.
- Wu, R.S.S., Lam, K.S., MacKay, D.W., Lau, T.C., Yam, V., 1994. Impact of marine fish farming on water quality and bottom sediment: A case study in the sub-tropical environment. *Marine Environmental Research* 38, 115-145.
- Ye, L.X., Ritz, D.A., Fenton, G.E., Lewis, M.E., 1991. Tracing the influence on sediments of organic waste from a salmonid farm using stable isotope analysis. *J. Exp. Mar. Bio. Ecol.* 145, 161-174.

Table 1. Mean values ($\pm$ SEM) for irrigation volume, consumptive water use, leaching fraction, net plant shoot dry weight, and water use efficiency. Means within a column followed by the same or no letter are not significantly different ($P > 0.05$) by Tukey-Kramer test.

Irrigation treatment (% of pan)	Irrigation volume (l)	Consumptive water use (l)	Leaching Fraction	Net plant dry weight(g)	Water use efficiency (g/l)
50 ¹	212.2	190.5 $\pm$ 1.7 ^d	0.10 $\pm$ 0.008 ^d	384.9 $\pm$ 21.8 ^b	2.0 $\pm$ 0.1
100 ²	424.7	273.3 $\pm$ 8.5 ^c	0.36 $\pm$ 0.02 ^c	425.7 $\pm$ 60.7 ^b	1.6 $\pm$ 0.2
150 ¹	636.9	320.3 $\pm$ 15.9 ^b	0.50 $\pm$ 0.03 ^b	393.7 $\pm$ 92.4 ^b	1.2 $\pm$ 0.2
150(control) ^{2,3}	636.9	262.0 $\pm$ 9.6 ^c	0.59 $\pm$ 0.02 ^a	-----	-----
200 ²	849.2	375.4 $\pm$ 6.4 ^a	0.56 $\pm$ 0.008 ^{ab}	581.1 $\pm$ 8.0 ^{ab}	1.5 $\pm$ 0.02
250 ²	1059.9	401.1 $\pm$ 7.5 ^a	0.62 $\pm$ 0.007 ^a	693.7 $\pm$ 70.9 ^a	1.7 $\pm$ 0.2

¹n=2

²n=3

³Soil control that contained no plants.

Table 2. Mean values ($\pm$ SEM) for week 14 leachate salinity, and the salinity of the soil water profile at 0-20, 20-40, and 40-60 cm below the surface. Means followed by the same or no letter are not significantly different ($P > 0.05$) by Tukey-Kramer test.

Irrigation treatment (% of pan)	Week 14 leachate salinity (ppt)	Soil water salinity (0- 20 cm) (ppt)	Soil water salinity (20- 40 cm) (ppt)	Soil water salinity (40- 60 cm) (ppt)
50 ¹	-----	227.9 $\pm$ 8.5 ^a	107.6 $\pm$ 13.7 ^a	77.1 $\pm$ 2.7 ^a
100 ²	66.7 $\pm$ 1.1 ^a	68.0 $\pm$ 5.2 ^b	65.8 $\pm$ 2.1 ^b	67.8 $\pm$ 3.2 ^{abc}
150 ¹	56.1 $\pm$ 2.4 ^b	74.9 $\pm$ 1.9 ^b	72.0 $\pm$ 2.8 ^b	71.0 $\pm$ 2.1 ^{ab}
150 (control) ^{2,3}	42.0 $\pm$ 0.8 ^d	83.1 $\pm$ 9.1 ^b	53.7 $\pm$ 4.4 ^b	47.0 $\pm$ 4.3 ^d
200 ²	50.0 $\pm$ 1.1 ^{bc}	57.6 $\pm$ 4.7 ^b	54.8 $\pm$ 3.4 ^b	52.9 $\pm$ 2.1 ^{cd}
250 ²	48.3 $\pm$ 1.4 ^c	57.8 $\pm$ 3.3 ^b	53.5 $\pm$ 3.4 ^b	55.4 $\pm$ 3.6 ^{bcd}

¹n=2

²n=3

³Soil control that contained no plants.

Table 3. Mean values ($\pm$ SEM) for soil inorganic (nitrate + ammonia) nitrogen (IN) concentration at the beginning and termination of the experiment. Means followed by the same or no letter are not significantly different ($P > 0.05$) by Tukey-Kramer test.

Irrigation treatment (% of pan)	Initial soil IN concentration (mg/kg)	Final soil IN concentration (mg/kg) ⁴
50 ¹	14.33 $\pm$ 2.54	12.76 $\pm$ 1.30 ^a
100 ²	18.73 $\pm$ 4.76	7.50 $\pm$ 1.24 ^a
150 ¹	19.57 $\pm$ 5.16	2.02 $\pm$ 1.30 ^b
150 (control) ^{2,3}	21.29 $\pm$ 5.00	6.49 $\pm$ 1.25 ^a
200 ²	14.76 $\pm$ 1.79	4.83 $\pm$ 1.24 ^{ab}
250 ²	13.12 $\pm$ 4.06	4.43 $\pm$ 1.25 ^{ab}

¹ $n=2$

² $n=3$

³Soil control that contained no plants.

⁴Adjusted least square means and standard errors, initial soil IN used as covariate.

Table 4. Mean values ($\pm$ SEM) for leachate nitrate concentration on week 2 (first week measured), and week 13 and 14 (final 2 weeks of the experiment). Means followed by the same or no letter are not significantly different ($P > 0.05$) by Tukey-Kramer test.

Irrigation Treatment (% of pan)	Week 2 leachate nitrate concentration (mg/l)	Week 13 leachate nitrate concentration (mg/l)	Week 14 leachate nitrate Concentration (mg/l)
50 ¹	74.52 $\pm$ 37.73	-----	-----
100 ²	99.79 $\pm$ 15.51	24.25 $\pm$ 6.74 ^{ab}	27.05 $\pm$ 9.03 ^a
150 ¹	58.95 $\pm$ 11.08	11.44 $\pm$ 2.31 ^b	9.42 $\pm$ 0.19 ^a
150 (control) ^{2,3}	82.95 $\pm$ 2.10	29.58 $\pm$ 0.98 ^a	28.19 $\pm$ 2.35 ^a
200 ²	66.33 $\pm$ 4.00	0.34 $\pm$ 0.25 ^c	1.07 $\pm$ 0.58 ^b
250 ²	56.29 $\pm$ 5.49	0.17 $\pm$ 0.14 ^c	0.78 $\pm$ 0.27 ^b

¹n=2

²n=3

³Soil control that contained no plants.

Table 5. Mean values ($\pm$ SEM) for percent nitrogen (N), total mass of nitrogen, and the carbon: nitrogen (C:N) ratio in the plant shoot. Means followed by the same or no letter are not significantly different ($P > 0.05$) by Tukey-Kramer test.

Irrigation			
treatment	% N in plant		
(% of pan)	shoot	Plant shoot N (g)	Plant C:N ratio
50 ¹	2.57 $\pm$ 0.17 ^a	10.06 $\pm$ 1.24	10.02 $\pm$ 0.09 ^a
100 ²	2.45 $\pm$ 0.13 ^a	10.57 $\pm$ 1.82	11.17 $\pm$ 0.83 ^a
150 ¹	2.49 $\pm$ 0.03 ^a	9.88 $\pm$ 2.18	11.49 $\pm$ 0.07 ^a
200 ²	1.87 $\pm$ 0.25 ^a	10.99 $\pm$ 1.65	14.68 $\pm$ 1.49 ^a
250 ²	1.77 $\pm$ 0.12 ^a	12.27 $\pm$ 1.24	15.49 $\pm$ 1.27 ^a

¹_{n=2}
²_{n=3}

Table 6. Mean values ($\pm$ SEM) for soil plant-available phosphorus (P) concentration at the beginning and termination of the experiment.

Irrigation treatment (% of pan)	Initial soil P concentration (mg/kg)	Final soil P concentration (mg/kg)
50 ¹	22.5 $\pm$ 5.5	25.5 $\pm$ 8.5
100 ²	27.7 $\pm$ 5.2	24.7 $\pm$ 5.6
150 ¹	41.0 $\pm$ 14.0	20.5 $\pm$ 3.5
150 (control) ^{2,3}	24.7 $\pm$ 6.6	24.7 $\pm$ 5.2
200 ²	35.3 $\pm$ 4.8	29.7 $\pm$ 1.2
250 ²	44.3 $\pm$ 9.6	24.7 $\pm$ 1.3

¹n=2

²n=3

³Soil control that contained no plants.

Table 7. Mean values ($\pm$ SEM) for leachate soluble reactive phosphorus (SRP) concentration on week 2 (first week measured), and week 13 and 14 (final 2 weeks of the experiment). Means followed by the same or no letter are not significantly different ($P > 0.05$) by Tukey-Kramer test.

Irrigation treatment (% of pan)	Week 2 leachate SRP concentration (mg/l)	Week 13 leachate SRP concentration (mg/l)	Week 14 leachate SRP concentration (mg/l) ⁴
50 ¹	0.57 $\pm$ 0.14	-----	-----
100 ²	0.66 $\pm$ 0.22	1.09 $\pm$ 0.53	1.17 $\pm$ 0.55 ^b
150 ¹	0.50 $\pm$ 0.05	1.01 $\pm$ 0.44	0.59 $\pm$ 0.66 ^b
150 (control) ^{2,3}	0.59 $\pm$ 0.10	1.75 $\pm$ 0.39	2.09 $\pm$ 0.57 ^b
200 ²	0.64 $\pm$ 0.12	3.37 $\pm$ 1.56	2.26 $\pm$ 0.53 ^{ab}
250 ²	0.81 $\pm$ 0.19	4.17 $\pm$ 1.40	3.65 $\pm$ 0.58 ^{ab}

¹n=2

²n=3

³Soil control that contained no plants.

⁴Adjusted least square means and standard errors, initial soil P used as covaritate.

Table 8. Mean values ($\pm$ SEM) for percent phosphorus (P) and the total mass of phosphorus in the plant shoot.

Irrigation treatment		
(% of pan)	% P in plant shoot	Plant shoot P (g)
50 ¹	0.31 $\pm$ 0.07	1.20 $\pm$ 0.34
100 ²	0.38 $\pm$ 0.02	1.62 $\pm$ 0.31
150 ¹	0.38 $\pm$ 0.05	1.54 $\pm$ 0.55
200 ²	0.40 $\pm$ 0.01	2.32 $\pm$ 0.05
250 ²	0.40 $\pm$ 0.05	2.83 $\pm$ 0.61

¹_{n=2}

²_{n=3}

Figure Captions

Figure 1. Ratio of evapotranspiration to pan evaporation by week over the course of the experiment for the 250% irrigation volume treatment. Error bars represent standard errors of the mean (n=3).

Figure 2. Regression of net shoot dry weight on total irrigation volume.

Figure 3. Leachate nitrate concentration (mg/l) by week over the experiment, shown for all treatments that produced leachate over the course of the whole experiment.

Figure 4. Leachate soluble reactive phosphorus concentration (mg/l) by week over the experiment, shown for all treatments that produced leachate over the course of the whole experiment.

IMAGE EVALUATION TEST TARGET (QA-3)

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