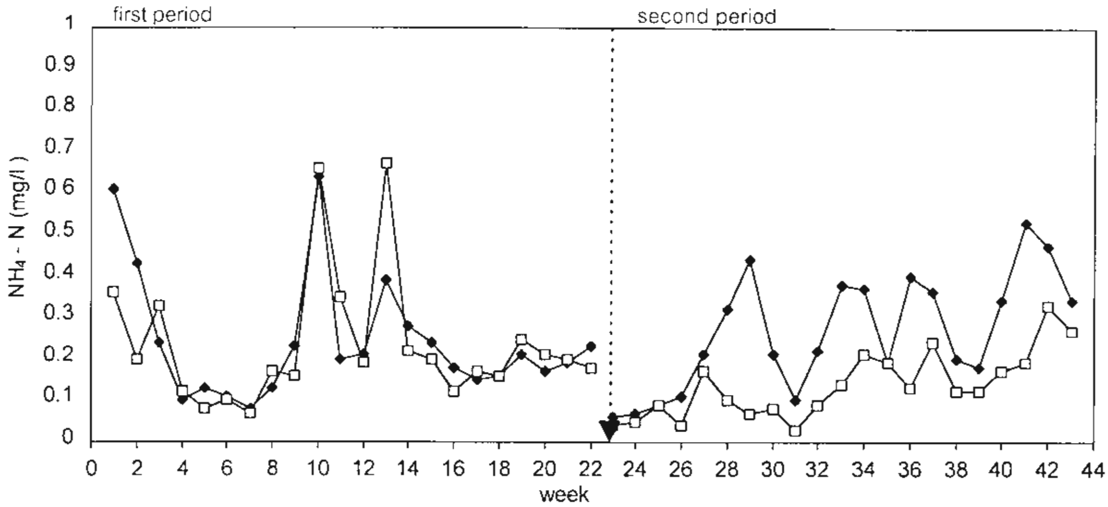


Figure 2

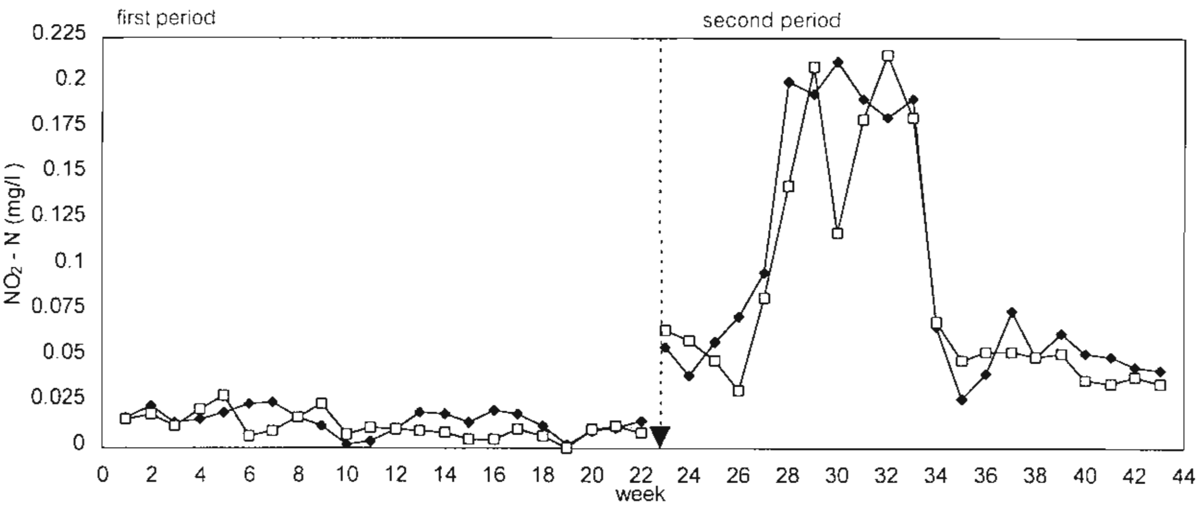


Figure 3

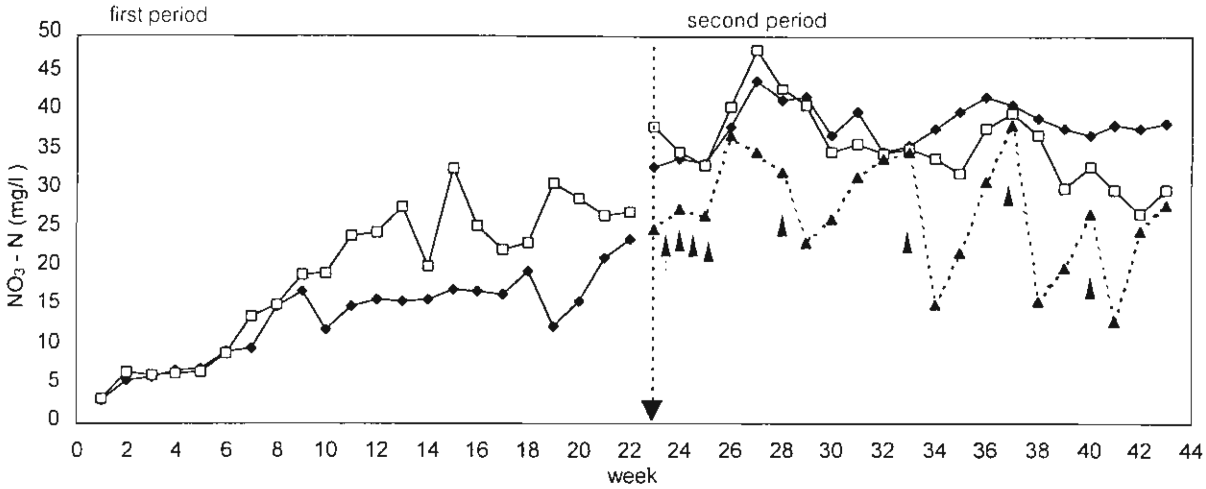
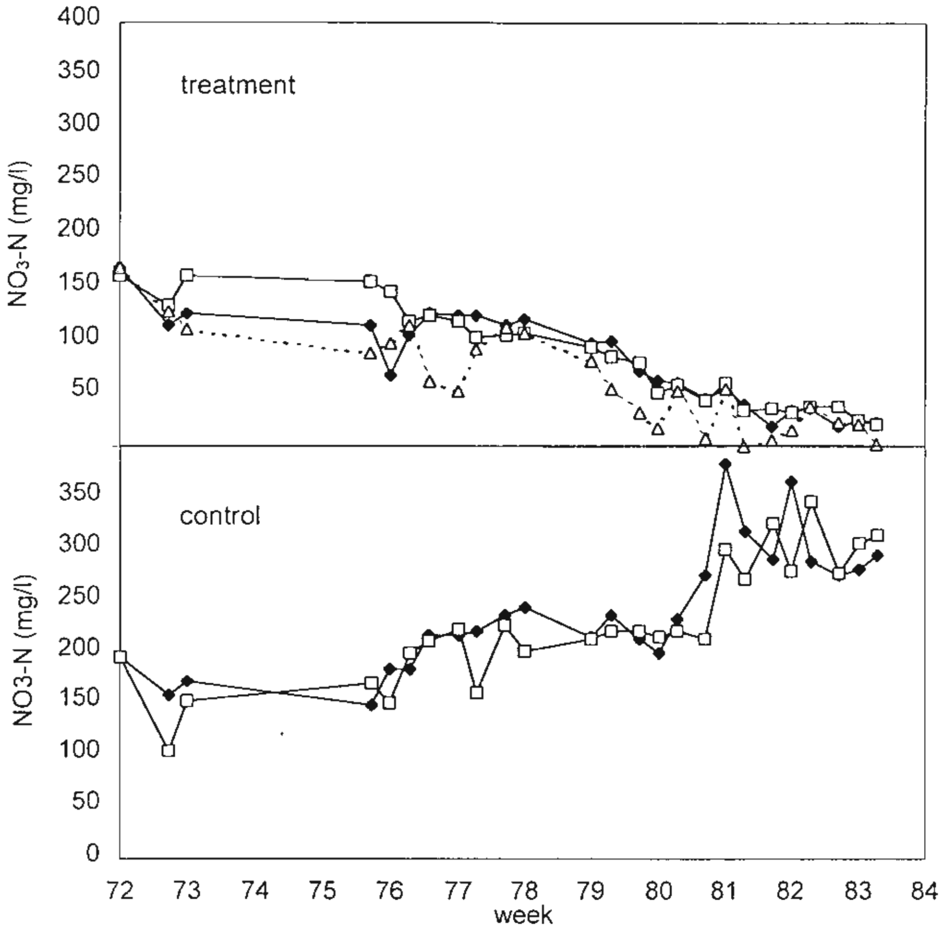


Figure 4

Figure 5



treatment
control
NO3-N (mg/l)
NO3-N (mg/l)
week
400
350
300
250
200
150
100
50
0
72
73
74
75
76
77
78
79
80
81
82
83
84

Figure 6

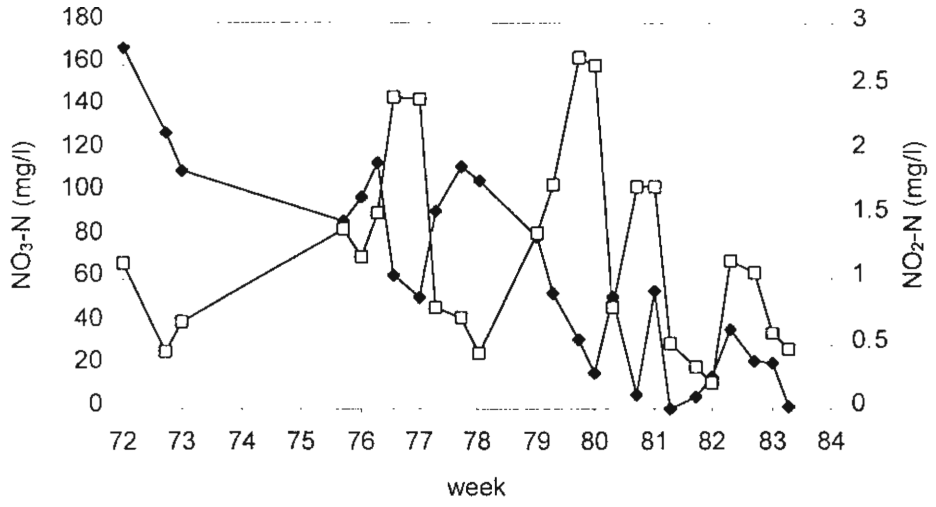

180
160
140
120
100
80
60
40
20
0
NO3-N (mg/l)
3
2.5
2
1.5
1
0.5
0
NO2-N (mg/l)
72 73 74 75 76 77 78 79 80 81 82 83 84
week

Figure 7

Optimal Photosynthetic Activities of two Macrophytes used in Recirculating Seawater Systems in Thailand

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ABSTRACT

Caulerpa lentillifera and *Acanthophora* sp. are two aquatic macrophytes often used in shrimp wastewater treatment ponds in Thailand. These algae rapidly assimilate ammonia and nitrate during growth. As part of our research, we evaluated these algae for use with small, recirculating seawater systems. A primary consideration for this application is light intensity at which photosynthetic activity is maximum (P_{max}). We measured photosynthesis by oxygen evolution method and found P_{max} at light intensities of 15,000-20,000 lux (200-270 μ mol m^{-2} s^{-1} photon flux density) with both algae. We did not observe photoinhabition at light intensities up to 60,000 lux. With small, indoor recirculating seawater systems, 4 to 5 fluorescent lights provide sufficient illumination for P_{max}.

INTRODUCTION

In Thailand, two macrophytes, a green alga (*Caulerpa*) and a red alga (*Acanthophora*) have been used recently for nitrogen waste removal with commercial shrimp culture in ponds, using recirculating seawater systems. These algae were grown in outdoor ponds of about 0.5-ha with 50-cm water depth. Algal ponds were part of the water treatment systems, following sedimentation ponds. These algae removed nutrients and iron from seawater. *Caulerpa* has also been used for water treatment with indoor aquariums, such as the invertebrate aquarium at Bangsaen Institute of Marine Science, Chonburi, Thailand. Algae reduced nitrate, which can harm invertebrates such as corals and sea anemones, even at low concentration (V. Muthuwan, personal communication, 1999). Algae growing in indoor aquariums received much lower light intensity than in outdoor ponds, but still showed high nutrient uptake.

There are many published research articles describing wastewater treatment with algae in shrimp culture systems. For example, Chaiyakam and Tunvilai (1992) used the red macrophyte *Gracilaria* sp. in combination with green mussel for biological wastewater treatment in shrimp culture ponds, and Tunsutapanich *et al.* (1998) integrated macrophytes species with fishes as the biological filter in reservoir and treatment ponds. However, physiological optimization studies with *Caulerpa* and *Acanthophora* have not been conducted.

Algae take up nitrogen and phosphorus for cellular metabolism and during growth. Maximum nutrient uptake rate is achieved by optimizing growth, and therefore by optimizing photosynthetic rate.

Photosynthesis is usually measured by two techniques, oxygen evolution or chlorophyll fluorescence. Although a modulated chlorophyll fluorescence technique provides quick response and accurate evaluation of algal photosynthesis, it also requires sophisticate and expensive instrument (Rodrigues *et al.*, 1993). Oxygen evolution, on the other hand, is a classical but less sophisticated technique. Since oxygen is produced during algal photosynthesis, changes in dissolved oxygen are used to measure photosynthetic activity of algae. During our study, we evaluated optimum light intensities for two algae, *Acanthophora* sp. and *Caulerpa lentillifera* using light saturation curves determined by photosynthetic oxygen evolution.

MATERIALS AND METHODS

Algae and stock culture condition

Caulerpa lentillifera and *Acanthophora* sp. were collected from seawater treatment ponds of Bunchong Farm, Chachoengsao Province, Thailand (Figure 1). Algae were kept in 50-l fiberglass tanks with 25-l of 30-ppt seawater supplemented with nutrients (F/2 medium) under ambient light and temperatures (27-34°C).

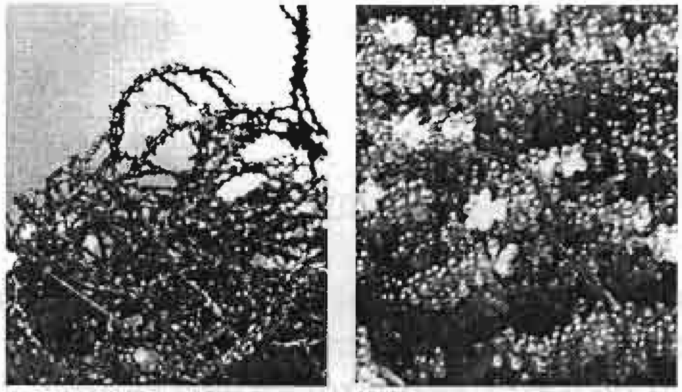

Figure 1. Photographs of the red alga *Acanthophora* sp. (left) and the green alga *Caulerpa lentillifera* (right). Both algae are used for wastewater treatment of shrimp farm effluents in Thailand.

Photosynthesis oxygen measurements in laboratory

Oxygen evolution was measured in a custom built Plexiglas chamber. The chamber was 10-cm diameter by 14 cm high, and contained 20-g of algal sample in 700-ml of sterile seawater. Water movement in the chamber was achieved using a magnetic stirrer to prevent oxygen depletion on the oxygen probe's membrane surface. Illumination was provided by 500 W or 1000 W halogen lamps in conjunction with heat reduction using a water jacket. Sodium bicarbonate (0.5 g/l) was added to the chamber to prevent carbon deficiency. Before each measurement, algae were kept in the chamber, in darkness for 10 min to equilibrate. Oxygen concentrations were measured using a Hanna 964400 dissolved oxygen (DO) meter with automatic temperature compensation, which was connected to a computer for data acquisition. Light intensity was measured inside the chamber using a Digicon LX-50 lux meter. Temperature was controlled at 29±1°C. Light intensity was adjusted by changing light sources (500 W or 1000 W halogen lamps), and using neutral density filters.

Oxygen evolution in ambient, outdoor light condition

Algae (100-g) were placed in a 10-l transparent jar with 4-l of seawater under ambient, outdoor light. Nitrogen gas was bubbling into the water to reduce DO to 1 mg/l. Nitrogen gas injection was terminated and measurements began. Oxygen evolution was monitored continuously for 1-hr, together with light intensity. Oxygen evolution from algae was always measured in parallel with controls (seawater in jars without algae). Net oxygen evolution rate was calculated by subtracting oxygen increase in the control from evolution rate with algae. Finally, net oxygen evolution rate was plotted against average light intensity for each data set.

RESULTS

Figure 2 illustrates the effect of light intensity on oxygen evolution rate with *Acanthophora* sp. during five independent trials. Oxygen evolution rate increased with light intensity until approximately 15,000 lux (photo flux density (PFD) = 200 μ mol photon m^2 s^{-1}). No further increase in oxygen evolution rate occurred after 15,000 lux.

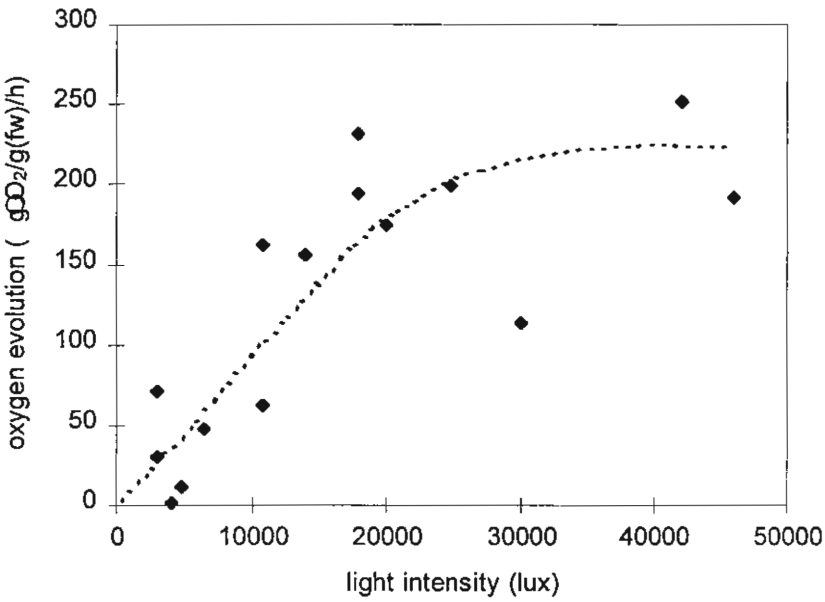

Figure 2. Photosynthetic oxygen evolution by *Acanthophora* sp. in laboratory conditions. Data were combined from five independent trials.

Caulerpa lentillifera had the same oxygen evolution pattern as *Acanthophora*, but was lower magnitude. Light saturation also occurred at about 15,000 lux (Fig. 3). However, at the highest light intensity of 45,000 lux (600 μ mol photon m^2 s^{-1} PFD), oxygen evolution appeared to decline. Since we could not provide light intensities greater than 45,000 lux with our indoor equipment, we measure oxygen evolution outdoors with natural light at higher intensities. The results are shown in Figure 4.

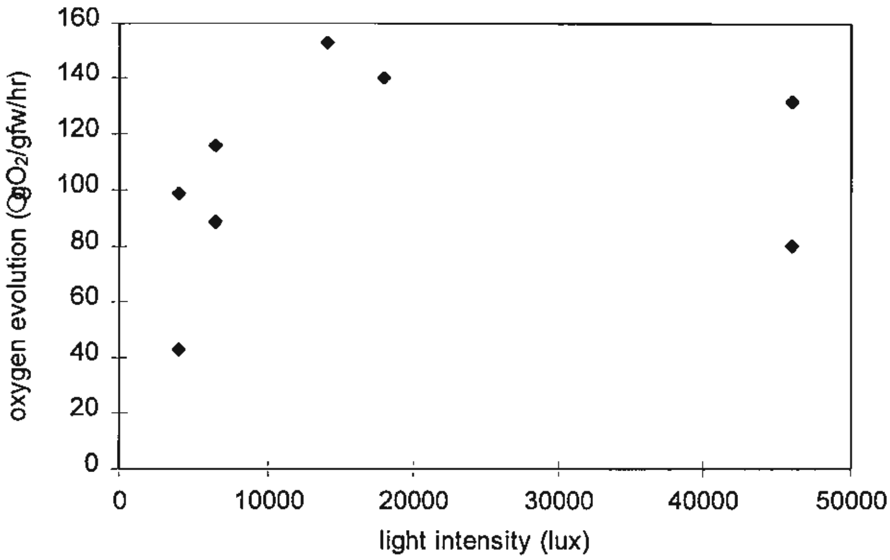

Figure 3. Photosynthetic oxygen evolution by *Caulerpa lentillifera* in laboratory conditions. Data were from three independent trials.

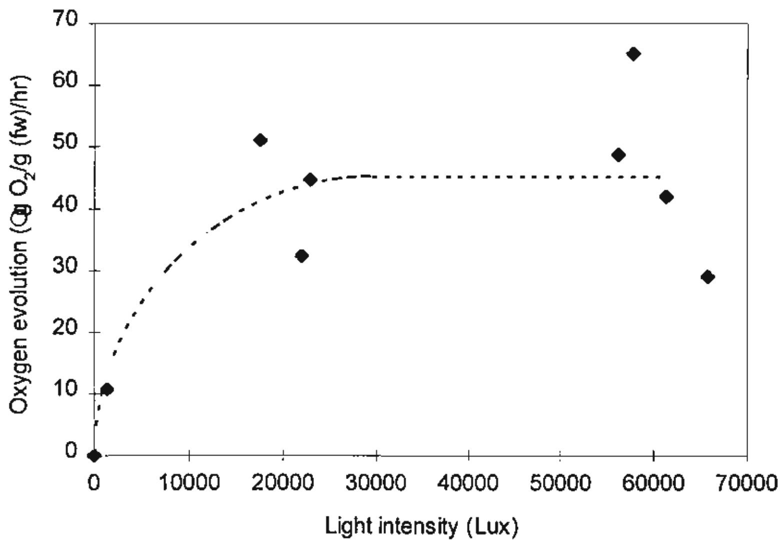

Figure 4. Photosynthetic oxygen evolution from *Caulerpa lentillifera* in outdoor conditions. Data were from nine independent trials.

Caulerpa photosynthetic DO production patterns were the same under both outdoors and laboratory conditions. There was no difference between oxygen evolution rates at 20,000 lux (270 μ mol m^{-2} s^{-1} PFD) and 65,000 lux (870 μ mol m^{-2} s^{-1} PFD). However, oxygen evolution was lower with the outdoor trials. This was probably due to atmospheric oxygen losses since the outdoor containers were open to the atmosphere.

DISCUSSION

With both laboratory and outdoor trials, *Acanthophora* sp. and *Caularpa lentillifera* had the same photosynthetic (P_{max}) light saturation intensities of 15,000-20,000 lux (200-270 μ mol photon m^2 s^{-1} PFD). This was similar to reported P_{max} values for other algae, such as brown algae *Feldmannia* spp. (Robledo *et al.*, 1994) and *Laminaria abyssalis* (Rodrigues *et al.*, 1993). Normally, P_{max} values correlate with an algae's preferred habitat. For example, P_{max} for intertidal species are in the range of 400-600 μ mol photon m^{-2} s^{-1}, while upper and mid-sublittoral species saturate at 150-250 μ mol photon m^{-2} s^{-1} (Lobban & Harrison, 1994). In Thailand, *Caulerpa* spp. is usually found attached to rocks or sand in shallow water, below low-tide level, close to coral reef (Lewmanomont and Ogawa, 1995). Light saturation of 200 μ mol photon m^2 s^{-1} PFD is expected. However, *Acanthophora* spp., especially on the Eastern Coast of the Gulf of Thailand, is normally found in the intertidal zone, 30-50 m from shore (Supowkit *el al.*, 1991). In our trials, *Acanthophora* sp. exhibited lower light saturation than other common, intertidal algae. One explanation for this is that *Acanthophora* used in our experiment were obtained from shrimp wastewater treatment ponds. Algae in these ponds were submerged at all times. Einav *et al.* (1995) found that *Acanthophora najadiformis* had much higher photosynthetic rates in air (during desiccation) than in water. This is an advantage for species growing in the mid-intertidal zone, since they are often exposed to air during low tides. In our case, *Caulerpa* may have adapted to lower light intensities while submerged in the ponds, and therefore had higher P_{max} values.

Very high light intensity can cause non-permanent damage to a plant's photosynthesis mechanism. This is called photoinhibition. Photoinhibition is the main factor affecting algal growth and metabolism when algae are exposed to strong light. With a blue-green alga (*Spirulina*), photoinhibition after midday caused a 30% decrease in growth (Richmond *et al.*, 1990). Normally, photoinhibition occurs with intertidal seaweed during desiccation stress during low tide (Herbert, 1990). In our trials, photoinhibition in *Caulerpa* was not clearly observed since our maximum light intensity was only 65,000 lux. However, photoinhibition can occur in seaweeds. For example, photoinhibition in Mediterranean species of *Caulerpa* (*C. prolifera*) was observed in the field (Häder *et al.*, 1997), and with a brown alga (*Fucus serratus*) when submerge (Huppertz *et al.*, 1990).

Algal photosynthesis and growth are highly correlated since energy and organic compounds used for cellular metabolism are all derived from photosynthesis. Light intensities that maximize photosynthesis therefore maximize growth rate. This was shown with *Laminaria* gametophyte cell culture in a photobioreactor, where specific growth rate remained unchanged at light intensities higher than 100 μ mol photon m^{-2} s^{-1} (Qi & Rorrer, 1995). Normally, with optimum photosynthesis and maximum growth rate, maximum nutrient uptake occurs. Nutrient uptake in algae is an active process involving transport proteins and specific enzymes, such as nitrate reductase and nitrite reductase. Nitrate transport and assimilation processes require energy. Oxygen evolution by blue-green alga (*Phormidium laminosum*) declined when nitrate starvation occurred. When nitrate was later be added, algal photosynthesis increased in response to nitrate uptake (Ochoa de Alda *et al.*, 1996). Energy requirements for nutrient uptake also depend on nutrient types. Ammonium-nitrogen uptake occurs at lower light intensity (requires less energy) than nitrate-nitrogen uptake, because nitrate needs eight additional reductant molecules to reduce nitrate (NO_3^-) to ammonium (NH_4^+) during assimilation. This was confirmed by Lomas *et al.* (1996).

In summary, optimum light intensity for growing *Acanthophora* and *Caulerpa* is 15,000 to 20,000 lux. Direct outdoor light is not necessary for photosynthesis and can cause lethal water temperatures. Temperatures greater than 40°C sometimes occur in the treatment ponds and cause massive algal deaths. Plastic shade netting (30-40%) over algal wastewater treatment ponds could solve this problem. With indoor aquariums, 4 to 5 white daylight fluorescence lamps are enough to provide maximum photosynthesis efficiency for both *Caulerpa lentillifera* and *Acanthophora* sp.

ACKNOWEDGEMENTS

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OZONE WATER TREATMENT IN MARINE SHRIMP CULTURE

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Abstract

Impacts of ozone on bacterial growth, shrimp (*Penaeus monodon*) postlarvae (PL) survival, and water quality were investigated in laboratory systems. Maximum safe ozone concentration (when continuously administered) for shrimp PL was 97.49 mg O_3/l soluble ozone (0.42 mg O_3/l residual ozone) produced from a 100 mg O_3/l/hr Ozoniser, and 154.27 mg O_3/l soluble ozone (0.28 mg O_3/l residual ozone) produced from a 2 g O_3/l/hr Ozone Generator. There were no negative effects on PL respiratory rate, but histological gill damage occurred after 8-hrs exposure (2 g O_3/l/hr, Ozone Generator). At 25.6 mg O_3/l soluble ozone (0.34 residual ozone), 3 log units of *Vibrio harveyi* D331 were inactivated, however, most of the bacteria recovered within 9 hrs. Greater bacterial reductions (24-hr bacteria inactivation) were achieved at 128 mg O_3/l soluble ozone (2.5 mg O_3/l residual ozone). Ozone inhibition of *Bacillus* growth was slightly less. Higher ozone concentration (420-mg O_3/l soluble ozone or 0.09 mg O_3/l residual ozone) with shrimp pond wastewater improved water quality better than aeration, although the differences were not statistically different. Ozone can be used at high concentrations to successfully eliminate bacteria and improve water quality prior to stocking shrimp.

Introduction

Disease is the major cause of cultured shrimp losses in Thailand. Efforts have been made to control or lessen this problem, including use of recirculation closed-water systems to prevent disease spread, and use of disinfectants to eliminate pathogens. Well-known disinfectants like formalin or chlorine can suppress disease outbreak (Majumdar and Sproul, 1974; Rosenthal, 1980), but their residuals create potential environmental problem (Rosenthal, 1980; Matsumura et al., 1998; Strong et al, nd). Ozone has been used routinely for water treatment for human consumption (Tate, 1991). Comparing the effects of chlorination and ozonation on *E. coli*, ozone destroyed this bacteria 600-3000 more quickly (Majumdar and Sproul, 1974). Ozone possess strong oxidising properties, but it is unstable (Rosenthal, 1980). Ozone's strong oxidising capacity destroys organic and inorganic compounds in water (Rosenthal, 1980). Ozone is therefore considered a good candidate for shrimp culture to solve disease problems, and at the same time improve water quality (Rosenthal, 1980; Menasveta, 1980; Honn and Chavin, 1976; Colberg and Lingg, 1978; Matsumura et al, 1998). Despite ozone's potentials, few shrimp culturists embrace its use (Matsumura et al, 1998), perhaps because practical details are often lacking, and because of inconsistent results from prior investigations.

The aims of our study included measurements of; total ozone production, ozone toxicity to shrimp, effects of ozone on water quality, and ozone's bactericidal properties. The results of our experiments will be used to design effective dosages and appropriate applications for killing pathogens, reducing harmful water quality conditions; while at the same time not injuring shrimp. The possibility of using ozone in growout ponds is also discussed.

Material and Methods

Experiment 1. Ozone measurement

We tested two ozone generator models: Ozoniser (100 mg O_3/l/hr), and Ozone Generator (2 g O_3/l/hr). Their respective ozone concentrations when ozone outputs were injected into seawater was measured. This information was used for subsequent experiments.

Ozone gas produced from either the Ozoniser (100 mg O_3/l/hr) or the Ozone Generator (model OZ 3050, EBASE CORP, Ltd.; 2 g O_3/l/hr) was trapped in 200 ml of 20% potassium iodide solution for 1, 5, 10, 20, 30, 40, 50 and 60 min. The potassium bi-iodate compound was titrated against 0.005 N sodium thiosulfate until end point. The data were analyzed using regression analysis.

Experiment 2. Ozone toxicity to shrimp postlarvae

2.1 Effect of residual ozone on shrimp postlarvae

Two thousand *Penaeus monodon* postlarvae (PL_{15}) from Chachoengsao Province were stocked and acclimatised in 6 ppt seawater for one week prior to the trials. PL were fed twice daily with postlarval feed pellets. Water was changed as needed.

Five litres of seawater (6 ppt) were ozonated respectively with the Ozoniser (100-mg O_3/l/hr) and the Ozone Generator (2-g O_3/l/hr) for 1, 5, 10, 30 min and 1 hr. Fifty PL were placed in each container. During the experiments, PL were fed live Artemia nauplii. Container water was static with no air added. Water temperature was 25°C, and pH was 7.4-7.6. Observations were made every 2 hrs for 24 hrs. Death was assumed when PL were immobile and showed no response when touched with a glass rod. Dead PL were removed immediately to prevent water pollution.

2.2 Effects of continuous ozone exposure on shrimp postlarvae

A 22-l glass container was divided into the sections (represented three replications), with each receiving 100 shrimp PL. Each ozonator was operated separately at maximum capacity (100 mg O_3/hr or 2 g O_3/l/hr) for 24 hr. Shrimp survival and ozone concentrations were checked every 2 hrs. Residual ozone concentrations were measured using a Spectroquant test kit (Merck).

2.3 Physiological aspects of ozone toxicity to shrimp postlarvae

Shrimp PL (PL_{15}-PL_{20}) were exposed to direct ozonation for 4, 6, 8, 10, 12, 14 and 16 hrs. At designated times, each PL was transferred into a 5 ml respiratory chamber with 25 ppt sterilised seawater, which was already acclimatised at 25°C for 1 hr. Shrimp were allowed to acclimate in these chambers for one hr, after which oxygen consumption rates were recorded every 10-min for 1 hr. Upon termination of the trials, PL were fixed in Davidson's fixative, transferred into wax, and 5-µm sections were cut and stained with haematoxylin and eosin. Histology examination of shrimp gill tissue, at different ozone exposure times was conducted using light microscopy at Srinakarintaravirot University.

<u>Experiment 3</u>. Bacteria inhibition by ozone

3.1 Inactivation of *Vibrio harveyi* D331

Seawater with 25-ppt salinity was autoclaved, and each separate 500-ml container of seawater was exposed to ozone for 0, 1, 5, 10, and 20 min at standard output (2 g/h/l Ozone Generator). Ten ml of *Vibrio harveyi* D331 culture at approximately 10^6 CFU/ml were immediately placed in the ozonated seawater to achieve a final concentration of approximately 10^5 CFU/ml, then exposed for 0, 30 sec, 15 min, 30 min and 1, 2, 4, 6,9 12, 24, 36 and 48 hr. Samples were withdrawn and placed into sterile 10 ml test tubes containing 0.1 ml 10% $Na_2S_2O_3$ to neutralize any remaining ozone and to stop bactericidal action of residual oxidant during sample transit (APHA, 1985)

Seawater samples were serially diluted with 0.85% NaCl and inoculated to obtain CFU for *V. harveyi*. The spread plate method on TCBS was used for viable bacteria count prior to, and during exposure to the disinfectants. Numbers of *V. harveyi* were enumerated using two tubes.

3.2 The inactivation of *Bacillus* S11

Bacillus S11 were cultured in BHI media at 37°C for 18 hrs, after which about 10^5 CFU/ml were inoculated into 500 ml of 25 ppt sterilized seawater and exposed to ozone for 0, 1, 5, 10, and 20 min at a standard output (2 g/h/l Ozone Generator). *Bacillus* was sampled at 0, 0.5, 15, and 30 min, and at 1, 2, 4, 6, 9, 12, 24, 36 and 48 hrs in the same manner as with *Vibrio*.

<u>Experiment 4</u>. Water quality treatment by ozone

Shrimp pond waste waters were collected and analysed for total ammonia-nitrogen, nitrate-nitrogen, nitrite-nitrogen, BOC, total suspended solids, total-phosphate and alkalinity (APHA, 1985). A 1-l water sample was ozonated for 1-hr while the control was aerated with compressed air. All parameters were measured after the designated time, except BOD, which was analysed after five days incubation.

Results

<u>Experiment 1</u>. Ozone production

Ozone concentrations in water increased linearly with increased ozonation time for each ozonator; with [y = 2.2341x] for the Ozoniser (100 mg O_3/l/hr; Fig. 1), and [y = 7.0707x] for the Ozone Generator (2g O_3/l/hr; Fig. 2). With pure oxygen gas, ozone production from the Ozone Generator increased greatly [y = 25.616x] (Fig. 3). Not all of the ozone gas produced by the ozonation machines dissolved in water. Ozone which did dissolve in water dissipated quickly, and after 24 hrs residual ozone was almost non-detectable.

<u>Experiment 2</u>. Toxicity of ozone to shrimp

<u>2.1 Effect of residual ozone on shrimp postlarvae</u>

No shrimp mortality occurred with residual ozone concentrations of 26.8 mg total soluble O_3/l (0.45mg/l residual ozone, from 100 mg O_3/l/hr running for one hr); nor with 84.8 mg total soluble O_3/l (0.06 mg/l residual ozone, from 2 g O3/hr/l running for one hr), compared with controls. Shrimp losses were mostly from cannibalism.

<u>2.2 Effect of continuous ozone exposure on shrimp postlarvae</u>

With the Ozoniser (100 mg/h/l) operating up to 12 hrs, all shrimp showed normal behaviour until 14 hrs (Table 1; total soluble ozone concentration = 97.49 mg O_3/l, 0.42 mg O_3/l residual ozone). Initial signs of shrimp weakness appeared from 14 hrs onward. Most shrimp PL were badly damaged after 24hrs exposure to direct ozonation (total soluble ozone concentration = 146.2 mg O_3/l, 0.16 mg O_3/l residual ozone).

With the Ozone Generator (2 g/h/l), effect of continuous ozonation on shrimp PL were more prominent (Table 2). At 8 hrs, signs of shrimp weakness were evident (total soluble ozone concentration = 154.27 mg O_3/l, 0.28 mg O_3/l residual ozone). After that, 50% of the shrimp lost balance, became immobile, and were carried around by water currents. They exhibited occasional, feeble and spasmodic movements of pleopods and swimmerets. Eventually, some shrimp recovered their equilibrium, but remained lethargic.

We concluded that if direct ozone is used in the water system with shrimp, total ozone concentrations should not exceed 97.49 mg total soluble O_3/l.

2.3 Physiological aspects of ozone toxicity to shrimp postlarvae

There were no significantly differences in oxygen consumption rates ($p<0.05$) of shrimp exposed to ozonation (100 mg O_3/l/hr) from 4 to16 hrs (residual ozone between 0.12 to 2.00 mg O_3/l), compared with control. However, histology studies revealed increased gill degeneration of ozone treated shrimp with time, although swelling of gill lamellae were also observed in all treatments including controls (Fig. 4). Gills had increased hypertrophy at 10-hrs exposure. At advance stages, pycnotic nuclei of gill nucleus occurred. Finally, after 16 hrs of ozonation, the gills were severely deteriorated (Fig. 5).

Experiment 3. Bacteria inactivation

Figure 6 shows the logarithmic number of colony forming units (CFU) versus contact time for *Vibrio harveyi* D331 when microbial suspensions were treated with residual ozone produced from pure dry oxygen with the same flow rates. A 3-fold, log reduction within 60 sec occurred with all four treatments. The rate of CFU reduction was fastest during the first 60 sec, but then leveled off. This suggests reduced bactericidal activity after initial ozone exposure. Residual ozone might have been too low to damage bacterial cells. This was confirmed by a decline in residual ozone concentrations in the waters, which dropped from 0.34 to 0.1 mg O_3/l during 1 hr.

Comparing all treatments, 1 min ozonation (25.6 mg total soluble O_3/l; 0.34 mg residual O_3/l) was the least effective. Within 9 hrs, *Vibrio* recovered to its original values. Other treatments (5, 10, and 20 min ozonation) also strongly affected *Vibrio* concentrations. However, there were no significant difference between 5 min treatments (128 mg total soluble O_3/l; 2.5015 mg O_3/l residual ozone) and 10 or 20 min ozonation. Therefore, we included that 5 min ozonation was sufficient to inactivate 4 log units of the *Vibrio* compared to control after 24 hrs. It is worth noting that although ozone exposed *Vibrio* recovered after being initially inhibited, their colony sizes were reduced.

In a separate study, where the source of oxygen changed from pure oxygen gas to compressed air, ozone production capacity from the same machine was reduced greatly. The Ozone Generator coupled with pure oxygen gas gave 25.6 mg total soluble O_3/l/min, compared with only 7.0 mg total soluble O_3/l/min with compressed air. It was inconsistent that 2 min ozonation (4.47 mg O_3/l total soluble ozone; 0.14 mg O_3/l residual ozone) was more effective in *Vibrio* inactivation than 5 (11.17 ppm) and 10 min ozonation (22.3 ppm; Fig. 7). A 1.75 log units reduction was achieved from 2 min ozonation. *Vibrio* recovered faster than in the first experiment. CFU values returned to the initial values within 6 hrs. Oxygen source was the main factor effecting ozone concentrations.

Bacillus tolerated ozone slightly better than *Vibrio*. A maximum of 2.2 log units reduction was achieved at 30 sec with 25.6 mg O_3/l (Fig. 8), while a 3-log unit reduction occurred with *Vibrio* at the same ozone concentration. *Bacillus* also recovered after first inactivation, but at a slower rate than *Vibrio*. This may be due to a slower growth rate of *Bacillus* compared to *Vibrio*. However, the effects of each treatment did not differ greatly. A reduction in measured residual ozone concentration was observed in all four treatments during bacterial exposure. The reduction in ozone ranged from 0.26 to 0.04 mg/l in 24hr.

Experiment 4. Water quality

At an ozone concentration of 424.2 mg O_3/l (2 g O_3/hr/l running for 1 hr; residual ozone 0.09 ppm) NH_4-N was reduced 12%, NO_2-N increased 933%, and BOD was reduced 18% (Table 4). Aeration reduced NH_4-N by 6%, increased NO_2-N by 422%, and reduced BOD by 11%. Ozonation improved water quality more than aeration, although the data were not statistically different.

Discussion

Ozone (O_3) is produced from oxygen (O_2) with energy input either from an electric discharge or by ultraviolet radiation (Yanco Industries, 1999). Different commercial, ozone generation machines are available in the market place. Their ozone production capacities, as stated by the manufacturer is based on measurements of ozone gas generated by each machine, which is much less than ozone concentrations dissolved in water. Seawater contains various minerals and impurities, which can reduce ozone potency (Liltved et al., 1995). Effective ozone concentrations measured in one situation may not apply to another situation because of differences in respective water qualities. Accordingly, actual ozone concentrations produced from an ozone generator must be measured during each trial.

From our results based on seawater of 25-ppt salinity, which is common for shrimp culture, resultant ozone in water was much less than might be expected. Ozone solubility depends on various factors such as the salinity, hardness, pH, temperature, source of oxygen, and the ozone application technique (Colberg and Lingg, 1978; Liltved et al., 1995; Wongchrinda, 1994). Ozone is lost from water by three main processes (Liltved et al., 1995); reaction with water impurities, decomposition to O_2, and ozone loss to the atmosphere. These losses are rapid and make ozone determinations in water problematic (Rosenthal, 1980). Residual ozone concentrations are not always related to ozoniser operating time, and therefore the term "residual ozone" is inappropriate. We therefore considered ozone dosage as the amount of O_3 added to water per unit time, which agrees with conventions used by other researchers (Liltved et al., 1995).

Although ozone has widely proven benefits for potable and waste water treatments (Majumdar and Spoul, 1974; Colberg and Lingg, 1978; Rosenthal, 1980), caution is necessary with aquaculture applications due to its potential harmful effects on cultured animals. Ozone toxicity in seawater is due in part to hypobromous acid formed when ozone gas combines with bromine, similar to its reaction with chlorine in seawater (Blogoslawski et al., 1976). The oxidation residual of ozone gradually decreases with time (Majumdar and Sproul, 1974). Ozone concentrations for aquaculture applications should be sufficient to inhibit pathogens of concern, and/or otherwise improving water quality with minimum risk to animal safety. Optimum ozone dosages vary according to species and age of animals. For example, concentrations of more than 0.5 mg/l residual ozone can damage oyster larvae (Maclean et al., 1973; Blogoslawski et al., 1978). With fish, lethal thresholds are lower than those for crustaceans. The 96-hr LC50, using 10-13 cm rainbow trout (*Salmo gairdneri*) was 9.3 μg/l residual ozone. Ozone causes massive destruction of the gill lamellae epithelium together with severe hydro-mineral imbalance in juvenile rainbow trout. At lower ozone concentrations, but longer exposure times (2 μg O_3/l, 96hr), hyperplasia of the lamella epithelium was noticed (Wedemeyer et al., 1979).

Our findings suggest that shrimp PL had higher tolerance to residual ozone exposure than oyster larvae or rainbow trout. Shrimp PL were able to live normally in direct ozonation with ozone concentrations of up to 97.49 mg total soluble O_3/l (0.42 mg/l residual ozone).

Physiological disturbance of animals can be evaluated by calculating their oxygen consumption rate. Often, detrimental treatments cause increased oxygen consumption, as determined by a Gilson differential respirometer (Cebrian et al., 1990). This method is also capable of verifying metabolic rate changes of eggs, such as with egg pore respiration of *Callosobruchus maculatus* (Daniel and Smith, 1994). With our experiments, ozone had no effect on oxygen consumption of *P. monodon* PL at the maximum total soluble ozone concentration of 154.27 mg/l.

We observed that at 25.2 mg/l total soluble ozone (0.34 mg/l residual ozone), 3 log units of *Vibrio harveyi* D331 were suppressed for 9 hrs, and 128 mg/l of total soluble ozone (2.5 mg/l residual ozone) inhibited the same amount of *Vibrio* for longer time (24 hrs). Therefore, for disinfecting purposes, ozone at 128 ppm should be used in order to achieve greater disinfecting power. With shrimp present, however, lower ozone concentrations (i.e. 97.49 mg total soluble O_3/l) should be used. Bacteria inactivation can be achieved with both proper time of contact and ozone concentration (Majumdar and Sproul, 1974). Ozone can inactivate both *Vibrio* and *Bacillus,* although at slightly different rates. Therefore, it is important to apply ozone well before stocking shrimp, or adding probiotics.

Biocide action of ozone is the result of membrane component disruption leading to loss of their barrier function (Trukhacheva et al., 1993). Bactericidal effects of ozone after an appropriate dosage is reached are sudden and total, and no further inactivation is achieved (Yang and Chen, 1979). This might be because of a reduction of residual ozone (Yang and Chen, 1979). which occurs from oxidation of the most reactive organic groups on the cells' surfaces, and on decreased O_3 absorbed after ozone interacts with these organic compounds (Trukhacheva et al., 1993).

Sodium chloride concentrations affect microorganism destruction by ozone. At lower salinity (NaCl below 2.5%), the bactericidal effect of ozone was enhanced, while 5% NaCl showed a slight protective effect (Yang and Chen, 1978). These results were similar to Wongchrinda's (1994) studies, which showed higher solubility of ozone in lower salinity. However, Liltved et al. (1995) stated that salinity differences did not cause any substantial differences in bactericidal activity of ozone.

A number of researchers have reported on the efficacy of ozone for water treatment. For example, ozone can reduce total suspended solids (Rueter and Johnson, 1995), reduce colour (Otte et al., 1977), reduce odor ((Millamena, 1992), improve nitrification in hatcheries (Colberg and Lingg, 1978; Menasveta, 1980), and improve water quality in growout ponds (Mutsumura et al., 1998; Honn and Chavin, 1976). However, ozone's effectiveness depends on various factors, including raw water hardness, initial suspended solids concentration (Rueter and Johnson, 1995), temperature, and pH (Colberg and Lingg, 1978)

Millamena (1992) reported that low ozone concentrations (0.11 g/hr) did not effectively remove most organic matter in slaughterhouse wastewater. Higher ozone concentrations were recommended. This agrees with our findings where ozone concentration of 424.2 mg total soluble O_3/l resulted in only a 12% reduction in total ammonia-nitrogen. Nevertheless, when compared to controls (air only), water quality was improved more with ozone. In particular, ozonation enhanced conversion of ammonia-nitrogen to nitrite-nitrogen, although the final step of nitrification to NO_3 was not observed. Millamena (1992) explained that the highly polluted slaughterhouse waste prevented complete oxidation of organic matter by ozone. However, with wastewater pretreatment, overall ozonation performances was improved with 42% reduction in BOD, total suspended solids reduction of 34%, and reduced COD by 57.5%. Majumdar and Sproul (1974) noted a similar range of water quality improvements when a high residual ozone concentration (2.17 mg/l) was used with secondary wastewater (34% reduction of total suspended solids and 54% reduction of COD).

In conclusion, we found that with shrimp PL culture, ozone is most applicable for disinfecting purposes, especially in hatchery, recirculating water systems (Menasveta, 1980; Colberg and Lingg, 1978; Rueter and Johnson, 1995). When PL are present, ozonation should be combined with carbon filtration to remove offending substances, or to ensure their dissipation (Colberg and Lingg, 1978). Ozone can be routinely used to disinfect water supplies and hatchery effluents to prevent spread of diseases, and to reduce virus or bacteria infection of fertilized eggs (Arimoto et al., 1996). Moreover, ozone application in shrimp growout ponds has not been ruled out (Matsumura et al., 1998; Strong et al., nd). Ozone generator capability of 15 kg/hr is recommended with a water recirculation system on a 550 ha farm to prevent outbreaks of infectious disease, blooms of blue-green algae, to reduce inorganic nutrient concentrations, and to improve effluent water quality (Strong et al., nd.).

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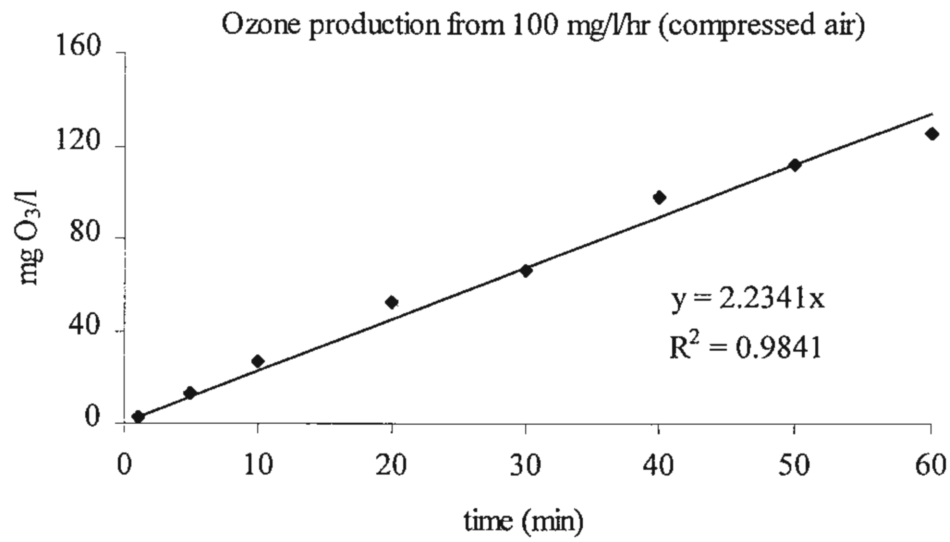

Figure 1 Regression of ozone concentrations in water produced from operating a 100 mg O_3/l/hr ozone generator up to one hour using compressed air.

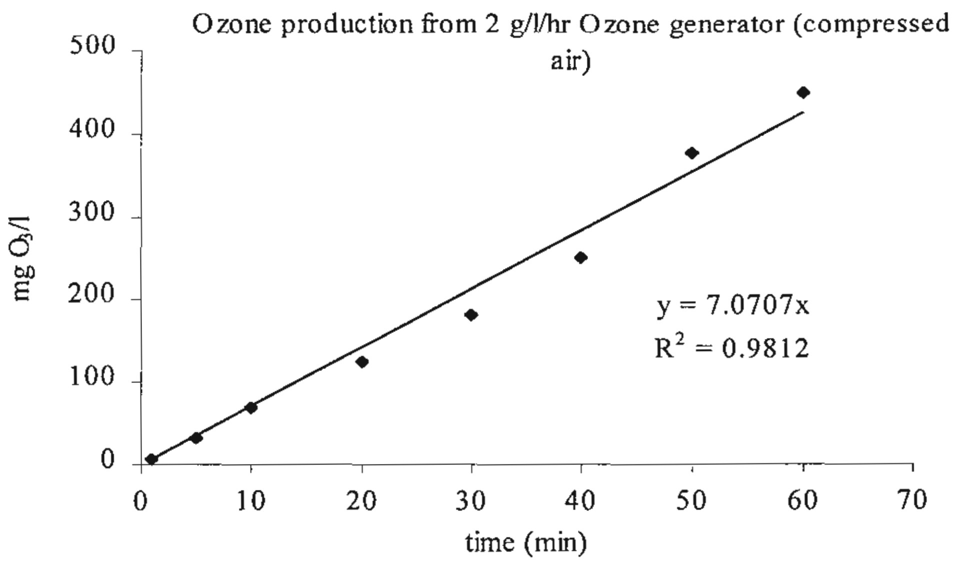

Figure 2.Regression of ozone concentrations in water produced from operating a 2 g O_3/l/hr ozone generator up to one hour using compressed air.

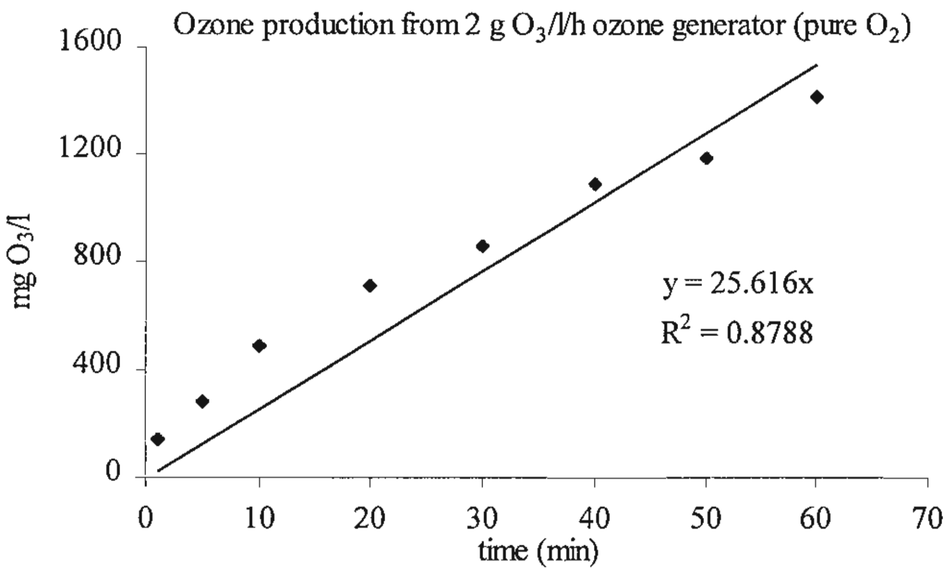

Figure 3. Regression of ozone concentrations in water produced from operating a 2 g O_3/l/hr ozone generator up to one hour using pure oxygen gas.

Figure 4. Gill histology of unexposed, control shrimp in ozone. Haematoxylin and eosin stain. Magnification at x1700.

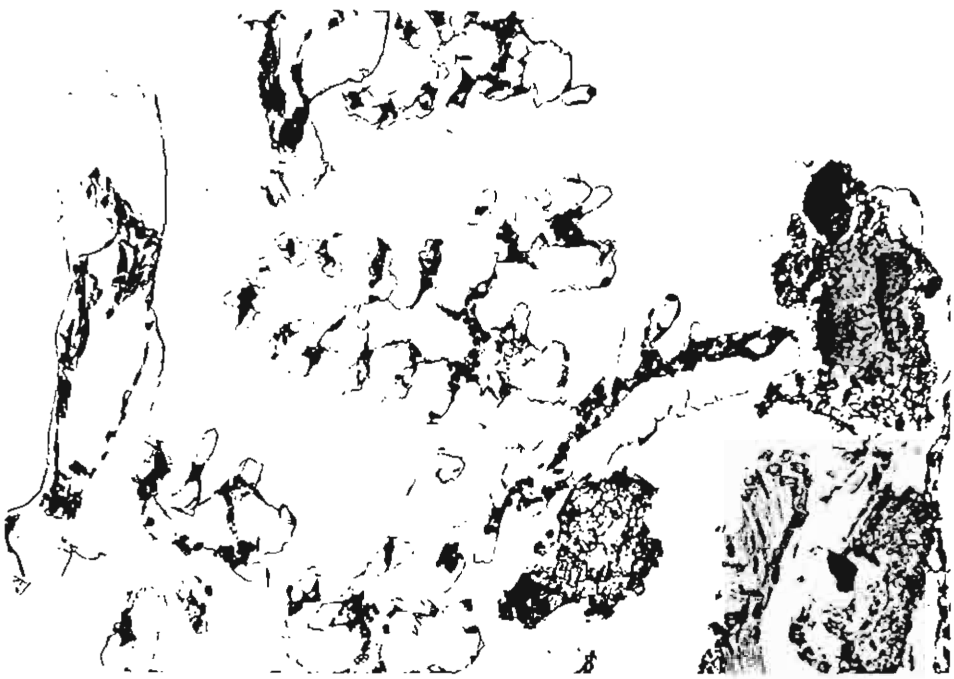

Figure 5. Histopathological changes of shrimp gills after 16 hrs exposure to ozone. Notice the pycnotic nucleoi and gill deterioration. Magnification at x1700.

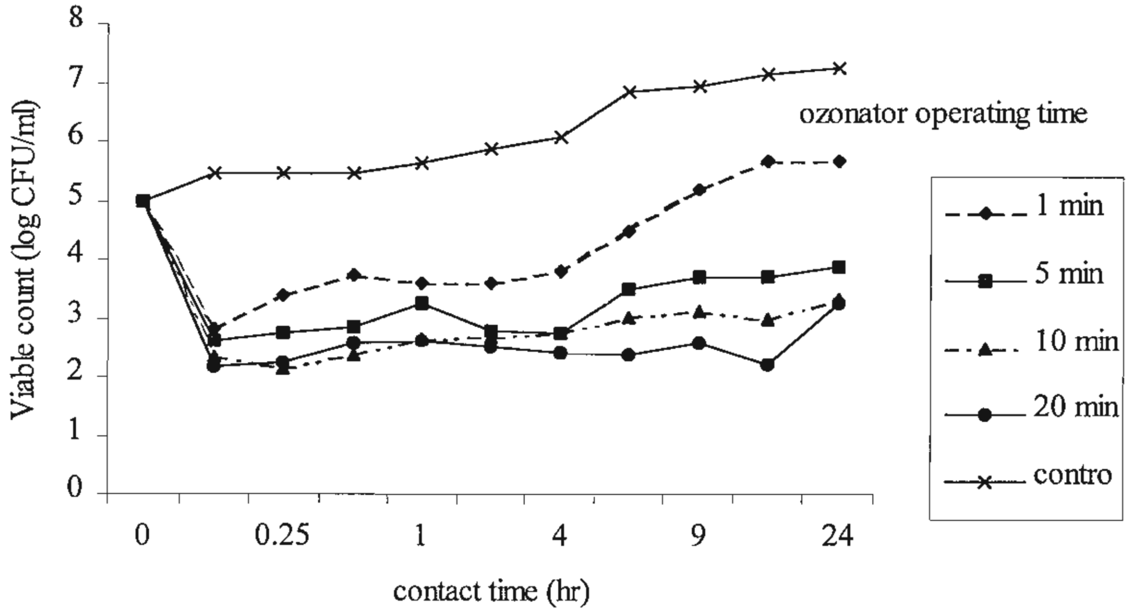

Figure 6. *Vibrio* activity after ozone exposure of up to 24 hours. Ozone was generated by using a 2 g O_3/l/hr Ozone Generator with pure oxygen gas. The Ozone Generator was operated from 0 to 20 min before contact time = 0.

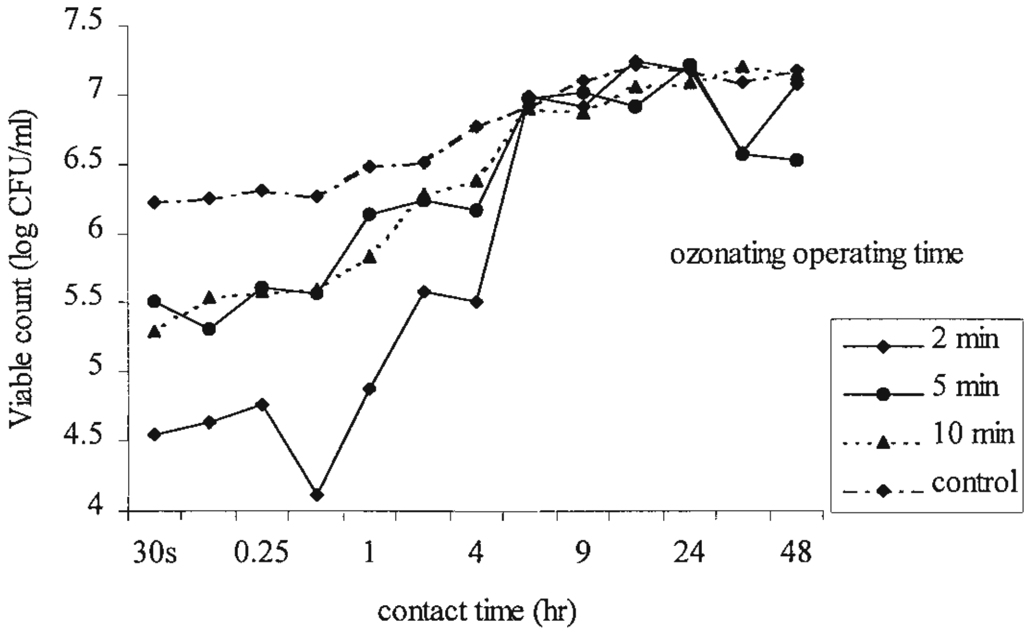

Figure 7 *Vibrio* activity after ozone exposure of up to 24 hours. Ozone was generated by using a 2 g O_3/l/hr Ozone Generator with compressed air. The Ozone Generator was operated from 0 to 20 min before contact time = 0.

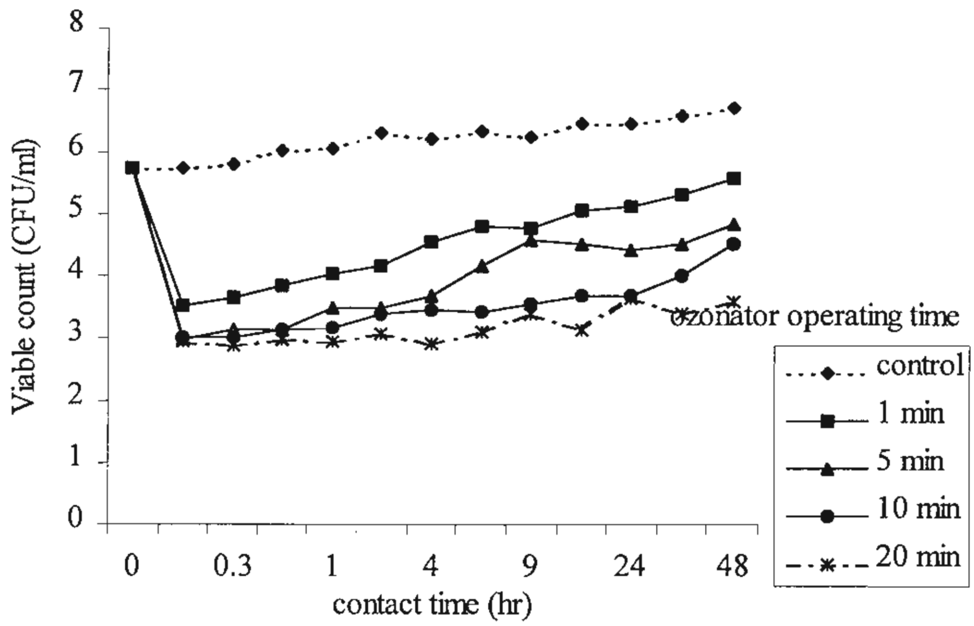

Figure 8 *Bacillus* S11 activity after ozone exposure of up to 24 hours. Ozone was generated by using a 2 g O_3/l/hr Ozone Generator with pure oxygen gas. The Ozone Generator was operated from 0 to 20 min before contact time = 0.

Table 1. Number of shrimp PL affected by ozonation from 100 mg O_3/l/hr Ozone Generator using compressed air. Number of dead PL observed, and total residual ozone (TRO) for different exposure times are shown.

TRO (ppm)	0.154	0.408	0.454	0.408	0.388	0.392	0.413	0.417	0.354	0.4	0.167	0.158
Rep	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	14 hr	16 hr	18 hr	20 hr	22 hr	24 hr
1	0	0	0	0	0	0	0	0	0	2	4	24
2	0	0	0	0	0	0	0	0	0	3	4	72
3	0	0	0	0	0	0	0	1	0	2	4	73

Table 2. Number of shrimp PL affected by ozonation from 2 g O_3/l/hr Ozone Generator using compressed air. Number of dead PL observed, and total residual ozone (TRO) for different exposure times are shown.

TRO (ppm)	0.173	0.382	0.556	0.278	0.382	0.573	0.382	0.486	0.434	0.503	0.503	0.313
Rep	2 hr	4 hr	6 hr	8 hr	10 hr	12 hr	14 hr	16 hr	18 hr	20 hr	22 hr	24 hr
1	0	0	0	1	26	11	6	1	6	-	-	-
2	0	0	0	1	20	5	4	4	11	1	2	-
3	0	0	0	1	24	0	5	1	6	2	3	-

Table 3. Total residual ozone (TRO) in respiratory rate trials with 2 g O_3/l/hr Ozone Generator using compressed air compressor.

Hours	TRO (ppm)
4	0.354
6	0.265
8	0.375
10	0.386
12	0.306

Table 4. Shrimp pond waste water treatment by ozonation (2 g/h/l running for one hour) compared to aeration. Data are average value of three trials.

	NH4	NO2	PO4	BOD
before	59.5	0.27	1.3	4.1
ozone	52.2	2.79	1.3	3.3
air	56.0	1.41	1.3	3.7
	%difference from control			
ozone	-12.2	+932	+1.2	-18.6
air	-5.86	+422	+5.6	-10.5

Table 5. Review of effective ozone dosages with different pathogens.

Pathogens	**TRO (ppm)**	**Time**	**Amount reduction**	**Reference**
V. harveyi	1	30 s	1.5 log units	Matsumura et al, 1998
V. Cholera	0.95	17 min	Total	Chen et al, 1992
V. parahaemolyticus	0.81	13	Total	Chen et al, 1992
V. vulnificus	1.0	2 min	Total	Wongchinda, 1994
	0.98	> 2 min	Total	Wongchinda, 1994
Bacteria	0.56		2-3 log units	
4 bacteria: *Aeromonas salmonicida* *V. anguillarum* *V. salmonicida* *Yersini ruckeri*	0.15-02	180 s	4 log units	Liltved et al, 1995
Pseudomonas *Flavobacterium* *Achomobacter*	0.56		2-3 log units	Blogoslawski et al, 1978
A salmonicida	1	1 min	Total	Colberg and Lingg, 1978
3 bacteria: *Aeromonas salmonicida* *Renibacterium salmoninarum* *V. anguillarum*	0.1	4 min		Austin, 1983 (cited after Liltved et al, 1995)
Marine bacteria Marine phytoplankton Arthropods Fish	0.56 0.08-1		Total	Blogo1awski and Stewart, 1977
SJNNV	0.1 ug/ml	2.5 min		Arimoto et al, 1996
IPNV	0.1-0.2	60 s	Total	Liltved et al, 1995
IPNV IHNV	0.01-0.02 0.01	60 s 30 s	Total	Wedemeyer and Nelson, 1977
V. harveyi *Bacillus*	0.34 2.5 0.14 0.26	30 s	3 log for 9 hr 3 log for 24 hr 1.75 log for 6 hr 2.2 log for 2 .hr	This study

SOME RECENT INNOVATIONS IN MARINE SHRIMP POND CULTURE

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ABSTRACT

World cultured shrimp production increased from 0.4 million MT in 1990 to about 0.8 million MT in 1999, or about 25% of total shrimp supply. Increased production was well below 1.2 million MT predicted 10 years earlier. The primary reason for this shortfall was shrimp disease, which effected shrimp yields worldwide. The most serious diseases were viral, for which there are still few solutions. As a result of shrimp disease problems, pond culture practices changed to reduce disease incidence. These changes included: use of specific pathogen free (SPF) and specific pathogen resistant (SPR) shrimp seed; reduced or zero water exchange during pond growout; shrimp culture at inland locations away from coastal influences; use of water recycling and reuse growout systems; development of biosecure systems to prevent disease access during shrimp's entire culture cycle; development of probiotics to reduce disease susceptibility; and genetic selection and improvements through closed, life-cycle culture. In addition, environmental awareness and concerns about shrimp culture sustainability became increasingly important with the informed public during the 1990s. This included concerns about habitat degradation and destruction, reduced biodiversity, and exotic shrimp introductions. We review herein developments with these culture innovations and environmental issues that have occurred during the past 10 years.

Key words : marine shrimp aquaculture, ponds, genetics, probiotics, environmental protection, best management plan, re-circulation aquaculture

I. INTRODUCTION

Before abundant marine shrimp seed availability from hatcheries and from wild caught seed, shrimp pond growout techniques changed very little over the centuries. Shrimp were mostly cultured incidentally with fish and other crustaceans in large, extensive pond systems characterized by low productivity (Table 1; Fast 1991, 1992a). These polyculture systems were basically catch, hold and harvest systems with little or no energy (other than tidal), feed, or material inputs. Shrimp yields were at best perhaps a few hundred kg/ha/yr. Growout was low-tech by any standards.

After breakthroughs in large-scale, shrimp larviculture technologies during the 1970's, all aspects of shrimp culture underwent rapid and innovative changes. These changes were motivated by abundant shrimp seed available for pond culture, static supplies of ocean caught shrimp, and high profits from pond cultured shrimp. Abundant, low-cost seed availability resulted in numerous growout innovations and intensification. Pond yields with the more intensive systems increased to >10,000 kg/ha/yr during the mid-1980s.

These innovations in all aspects of marine shrimp culture led to dramatic and substantial increases in pond cultured shrimp production during the 1980s. During 1980, only 2% of the world's shrimp production came from ponds, but by 1989 pond cultured shrimp accounted for 26% of total world production (Rosenberry 1990). Marine shrimp harvest from the world's oceans increased only slightly from 1.3 million metric tons (MT) during 1975 to 1.9 million MT in 1985 (Csavas 1988). Since 1985, ocean harvest of shrimp has been relatively stable at about 2 million MT, and has most likely reached or exceeded maximum sustainable yield (National Research Council 1992). Exponential increases in cultured shrimp production between 1970 and 1990 led to speculations and projections that by the year 2000 perhaps 45% or more of the world shrimp harvest would be from ponds (Fast and Lester 1992; Fig. 1). Estimated total culture shrimp production by the year 2000 was almost 1.2 million (MT). However realistic these estimates seemed 10 years ago, they were soon proven inaccurate. A combination of disease and pollution, mostly the former led to a series of cultured shrimp industry collapses in leading shrimp culture countries, including Taiwan, China, Thailand and Ecuador. During 1997 and 1998, total culture shrimp production was 0.66 and 0.75 million MT respectively (Jory 1998, 1999; Table 2), up from 0.4 million MT in 1990, but well below the 1.2 million MT forecast earlier. During 1997, cultured shrimp were about 25% of total world shrimp supplies (New 1999).

The single most important factor limiting continued expansion of the cultured shrimp industry during the 1990's was shrimp diseases (Lightner et al. 1997). While shrimp diseases have always been present, they became epidemic during large-scale, intensive shrimp monoculture. Viral diseases are the most devastating since they are often difficult to detect and impossible to treat in ponds (Brock et al. 1997). Although more than 20 known penaeid viruses, five viral species have caused greatest economic losses. These five are; yellow head virus (YHV), white spot syndrome virus (WSSV) and monodon baculovirus (MB) with black tiger shrimp (*Penaeus monodon*) in Asia, and infectious hypodermal and hematopoietic necrosis virus (IHHNV) and taura syndrome virus (TSV) with Mexican white shrimp (*P. vannamei*) and Pacific blue shrimp (*P. stylirostris*) in the Americas. In most cases, the only viable solution to viral diseases is to keep diseases from entering the culture system, and if infected, to clean up and disinfect.

Shrimp diseases can easily enter a shrimp pond by one or more means, including:

a. *Seed.* Both wild-caught and hatchery produced seed are primary sources of infection (Lightner et al. 1997). High density, intensive culture and stress amplify viral infections and can lead to infection of all shrimp within a short time (Lightner 1999). Hatchery produced seed are especially susceptible to infection since viruses can be introduced through infected broodstock, and cross contaminated within the hatchery. Seed from a given infected hatchery may be shipped to many farms with further spread of disease between farms.

b. *Source Waters.* Shrimp disease can be introduced to a farm through source water inflows, especially if these waters are from surface sources (Browdy and Bratvold 1998). Diseases may be carried in with source waters on organic and/or inorganic particles, in shrimp larvae or PL, or with other crustaceans. Diseases are often spread throughout a farm, and between farms with effluent and influent waters.

c. *Pond Intruders.* Pond intruders such as crabs and small shrimp species, or insects such as water boatmen are possible sources of viral disease introductions into shrimp ponds. Perhaps 40 or more crustacean species can harbor WSSV (Jory 1999a). Crabs come in through influent waters or by crawling over the dikes, while insects enter with water or by flying. Small shrimp species such as *Acetes* sp. And *Palaemon styliferus* can easily enter the pond with surface, source waters (Flegel et al. 1995).

d. *Birds and Mammals.* Garza et al. (1997) have shown that viable Taura syndrome virus (TSV) can pass through the gastrointestinal tract of seagulls, thus providing a probable transport vector between ponds and between farms. In Thailand, shrimp farmers are often first aware of disease problems in a pond by the presence of large numbers of seagulls and other birds feeding on dead and dying shrimp. These gulls could easily fly to other ponds and spread disease. Although not verified, it is likely that mammals such as rodents or mongoose could also serve as similar vectors.

e. *Feeds.* Fresh feeds probably pose a potentially greater threat than prepared, pelletized feeds. Fresh feed, especially frozen or fresh "trash fish", bi-catch could contain or be contaminated by shrimp. Ogle and Lotz (1998) caution that, "Feed that is devoid of shrimp meal will be less likely to carry contamination than feeds that employ shrimp meal." The dangers of shrimp viral contamination from pelleted feeds has not been confirmed, and Chamberlain (1998) doubts that virus would survive shrimp feed manufacturing processes.

f. *People and Equipment.* People and equipment, especially those that move between farms and/or between farms and shrimp processing plants could spread disease.

g. *Frozen and Fresh Shrimp.* Imported, frozen shrimp can contain shrimp viruses (Nunam et al., unpublished). If these are used as bait by sport fisher-persons or others, exotic shrimp diseases could be introduced locally. Food processors that import contaminated, raw shrimp and then reprocess the shrimp are another source of disease transmittal (Jory 1999a). Likewise, infected live shrimp used as bait can spread diseases.

All of the above sources undoubtedly contributed to disease problems, magnified by widespread distributions of broodstock and seed, and by the tendency to site farms in clusters. Clustered farms often recycle each other's waste effluents, including disease organisms.

Shrimp disease problems are far from over. Many of the most recent innovations in shrimp pond culture, and many of those innovations still in progress are direct responses to this threat. We will attempt to draw this to your attention during our review as we discuss different pond culture innovations.

In our present paper, we will describe some innovations that mostly occurred during the past 10 years. Fast (1991, 1992b,c,d) described many innovations in pond growout techniques that occurred during the 1980s, 1970s, and earlier. Additionally, we will also speculate on certain other technologies that have not yet been widely recognized, but which we feel hold special promise and potential for further improvements in pond culture of marine shrimp.

II. ENVIRONMENTAL CONCERNS AND SUSTAINABILITY

Before the 1990's, the main concern or emphasis of shrimp culture was on technological advancements, production increases, and profitability. There was relatively little awareness or concern by the industry of environmental issues and sustainability. That all changed during the 1990s. What had formerly been a murmur of concern about environmental issues became a roar as environmental and other non-government organizations (NGOs) became increasingly vocal about environmental and social impacts of aquaculture in general, and marine shrimp culture in particular. These growing concerns were highlighted in what is known as the *Choluteca Declaration* by 21 NGOs in Choluteca, Honduras during October 1996 (Accion Ecologica et al. 1997).

The Choluteca Declaration clearly stated the NGOs' concerns and listed 18 demands. Their concerns centered on what the NGOs perceived of as increasing environmental destruction by unsustainable shrimp farming. This destruction included: mangrove forest and wetland losses; eutrophication and sedimentation of receiving waters; salination of soils and aquifers; disease transfers to wild stocks; exotic species introductions; discharge of toxic and/or bioreactive substances; reduced biodiversity in shrimp cultured areas; and creation of social inequities and problems. They called for, "...a global moratorium on any further expansion of shrimp aquaculture in coastal areas until the criteria for sustainable shrimp aquaculture are put into practice." They also demanded, "...the formation of an independent body of national, regional and international organizations, including non-government organizations, to monitor the implementation of this process at the global level."

The Choluteca Declaration and what followed awakened deep and searching interests in the shrimp culture industry about all aspects of shrimp culture (Hargreaves 1997). This interest was further stimulated by treats of consumer boycotts by NGOs. Lockwood (1997) cautioned the industry to take these threats seriously since, "Historically, actions by environmental groups directed at consumers have been successfully employed in environmental disputes. In Europe, the consumer boycott led by Greenpeace against Shell over the Brent-Spar issue realized the desired objective. In the U.S., boycotts and protests at retail stores against irradiated foods have succeeded. Laws regulating seafood harvests now protect turtle and mammal populations. Organized environmental protests are a proven tool for change." Lookwood also correctly stated that a successful marine shrimp boycott in the U.S., Europe and Japan would have devastating economic effects on the lives of perhaps millions of people around the world, and, "…would result in great social and economic harm to some of the most impoverished people in the world, causing more poverty, more pollution, and increased pressure on the harvest of wild shrimp from the ocean, a resource which has its own set of environmental problems."

A centerpiece of the NGOs' goals was to develop a certification program aimed at identifying farmed shrimp which complied with their ideals of environmentally safe and socially just shrimp culture. This was presumably modeled on earlier successes with "dolphin-safe" and "turtle-safe" tuna certifications. However well meaning, such a certification program for cultured marine shrimp is essentially unworkable. The first hurdle is distinguishing ocean-trawler caught shrimp (75% of world production), from farmed shrimp (25%). It should be kept in mind that trawler catches are perhaps even more ecologically destructive than farms in terms of by-catch wastes and under-utilization (Fast et al. 1995). Secondly, and more importantly is rating of individual farms as either "safe" or "unsafe" and tracking production from each farm through processing and world wide distribution. Presumably, certification would not distinguish farm production that was only partially safe. It would require safe or unsafe rating. In the vast majority of farms, there will be no clear-cut distinction between safe and unsafe. Who would fund this certification program? Who would do the rating and certification? What criteria would be used? Most recent estimates are that almost 200,000 shrimp farms exist worldwide (Table 2). Will each farm be visited and rated on some regular basis? Who would check to see if a given farm had upgraded or decreased its rating? Who will trace farm harvests from farm to consumer? Will there be an appeals process? This thicket of certification problems essentially renders any fair certification process unworkable. However, unfair certification could happen and is the most likely outcome if NGOs or others attempt to institute such a program.

The concerns raised by NGOs have not gone unheeded. One result has been the formation of another international NGO supported by aquaculture businesses and organizations. This NGO, the Global Aquaculture Alliance (GAA) has a stated mission of furthering, "...environmentally responsible aquaculture to meet world food needs", (Boyd 1999a). The GAA has drafted a *Code of Practice for Responsible Shrimp Farming*. This code of practice is founded on an earlier *Code of Conduct for Responsible Fisheries* adapted by the 28th Session of FAO during October 1995 (FAO 1995a, Anonymous 1997). GAA's and FAO's codes of practice clearly addresses most, if not all of the concerns listed in the Choluteca Declaration.

Implementation of any code, best management plan, or other set of rules for the world aquaculture industry is problematic. Almost certainly, most large shrimp farms will adhere to these practices. It makes good business sense in almost all cases. However, the industry is mostly composed of relatively small farms, especially in Asia with 73% of total shrimp farm, culture area (Table 2). Average farm size in Asia was 3.8 ha. In Thailand, the world leading producer of farmed shrimp, 70% to 80% of all intensive farms had one or two ponds ranging from 0.16 to 1.6 ha (Lin 1995, Anonymous 1996d). Small farms and farmers often lack sufficient land and/or capital to adopt all of the most desirable culture practices.

II.1. <u>The mangrove issue.</u> Mangrove forest destruction is a core issue with environmental NGOs. There is deep concern about widespread mangrove forest destruction. Shrimp farms are often blamed as the major culprit for this destruction.

There is no question about the value of mangroves as important sources of: biodiversity; nursery grounds for a wide variety of aquatic species; lumber, charcoal, tannin, dyes, food and income for artisanal communities; and as protection against storm damage (Macintosh and Phillips 1992, Hambrey 1996). There is also no question about significant mangrove losses, especially during the past 50 years. The question then is how much of these losses are due to shrimp farm construction, and what if anything can be done.

Shrimp farms have been built on mangrove areas. In some cases, these shrimp farms directly converted mangroves to their use. In other cases, perhaps the majority in recent times, shrimp farms were converted from existing fish ponds, salt evaporation operations, or from agricultural farms that were themselves built many years ago on former mangrove or other lands. In some cases such as Indonesia and the Philippines, these original conversions occurred many years or even centuries ago, mostly for extensive fish culture operations (Ling 1977).

Mangrove lands are some of the poorest sites for shrimp farms for a number of important reasons, including:

a. Mangrove soils typically have high organic content, which is not suitable for shrimp well being. Ideal soils for shrimp farming are mineral with at most 5-10% organic matter (Boyd 1995). Highly organic soils also provide poor dike construction.
b. Mangrove soils often contain jelocite (sulfur containing substances), which oxidizes in ponds when exposed to oxygen and forms strongly acid conditions. This is a major cause of new shrimp farm failures in acid soil locations.
c. Mangrove lands near the ocean often do not have adequate elevation to allow complete and rapid draining of shrimp ponds. This greatly hampers shrimp harvest and water exchange.
d. In mangrove areas, numerous fish and crustaceans enter shrimp ponds with influent waters, or by crawling over dikes. This leads to shrimp crop losses due to disease introductions, competition for food and other pond resources, and predation on shrimp.
e. In mangrove areas, shrimp pond source waters and pond effluents often co-mingle due to inefficient water circulation. This results in wastewater recycling and deteriorated water quality in ponds.

For the above and other reasons, including social-economic, shrimp farms are better sited outside the inter-tidal mangrove area. If they are sited close to, but not in mangroves, both the shrimp farms and the mangroves can benefit from farm effluent discharges into the mangroves. These discharges provide nutrients and settleable solids, which benefit mangroves (Robertson and Phillips 1995).

The contention that shrimp farms are the main cause of mangrove forest destruction is unsupported (Hambrey 1996). While shrimp farms have destroyed a high percentage of mangroves in some areas, worldwide less than 6% of total mangrove resources have been converted to shrimp farms (Macintosh and Phillips 1992).

Mangrove conversion was documented in Thailand, the world's leading cultured shrimp producer for the past seven years, by a joint working committee of the Thai government departments of forestry, fishing, land development, and the National Research Council. They found that 47% of total mangrove land existing before 1961 was destroyed between 1961 and 1986, before large increases occurred in marine shrimp production in Thailand (Menasveta 1997). From 1986 to 1993, Thai cultured shrimp production increased from 17,886 MT to 225,514 MT, while total mangrove reserves decreased by only another 7% during this period. Only a portion of this 7% decrease was due to shrimp farm construction. Overall, shrimp farms in 1993 accounted for 17.5% of total mangrove areas that existed before 1961 (Table 3). This means that about one-third the mangrove areas converted to other uses since 1961 were eventually used for shrimp farm construction by 1993, while two-thirds of the converted mangroves were used for other purposes.

Most mangrove destruction in Thailand occurred before 1986, and that destruction attributed to shrimp farms during this time was mostly for extensive farm types. These farms typically have large ponds, tidal water exchange, and very low yields (Table 1). During 1986, average yields were <400 kg/ha/yr (Menasveta 1997). Most new farms constructed after 1986 were intensive types with much greater yields. Average shrimp farm production increased to >3,100 kg/ha/yr by 1993. Intensive culture, shrimp farms in Thailand now account for about 85% of total shrimp culture area. The implications of this statistic are important. Since much greater yields are achieved on relatively small land areas with intensive culture, total land area devoted to shrimp culture can be reduced by conversion to intensive culture systems. Proper farm siting should reduce pressures on land use and reduce environmental impacts. With proper farm management, water and effluent use, and other environmentally acceptable practices, impacts of these farms should be greatly reduced compared with more extensive farm operations. Conversion to intensive farms may be not only desirable but necessary if we wish to reduce environmental impacts. Marsh (1998) cautioned that, "If farming on the existing area does not become more intensive, environmental degradation will be unstoppable."

Thai farmers soon learned that intensive farms sited in mangrove areas produced poor results, and most of these new farms were thus sited on higher ground, or in areas without mangroves (Menasveta and Fast 1998). Many older, unprofitable farms in mangrove areas have even reverted to mangroves, a trend, which could continue as marine shrimp culture intensifies and culture techniques improve.

Governments, farmers, and the general public in most shrimp growing areas are now aware of the mangrove destruction issue. There is strong public concern about further mangrove forest destruction, regardless of the reason. The shrimp culture industry is on record as opposing any further use of mangroves for shrimp farms (Boyd 1999a).

II.2. Sustainability. Current concepts of sustainable shrimp culture were not widely held 10 years ago. Although most of the elements associated with sustainability were understood and accepted by the industry, there was not as much interest focused on trying to understand all the ramifications and meanings of the term as there is now.

Shrimp culture sustainability is perhaps an outgrowth of the even larger issue of human society sustainability. Sustainability has at least partial origins in the environmental movement which gathered considerable momentum about 30 years ago. Bardach (1997) describes formulation of sustainable development concepts by a special United Nations commission beginning in 1983 and concluded that what sustainability really means depends, "...on whether one sees the world through ecological or economic glasses. As long as populations grow and economic conditions improve for many, the most sustainable development will be one that attains the best possible relationship of the forces active in local and regional dynamic cultural and economic systems as well as in larger dynamic, but normally slower-changing, ecological systems. To be sustainable these systems must allow (a) human life to continue indefinately, (b) human individuals to flourish, and (c) human cultures to develop; at the same time the effects of human activities must remain within bounds so as not to destroy the diversity, complexity, and function of the ecological life support system", (Costanza 1991, Bardach 1997). FAO (1991) further defined sustainable development as, "...the management and conservation of the natural resource base, and the orientation of technological and institutional change in such a manner as to ensure the attainment and continued satisfaction of human needs for present and future generations. Such sustainable development conserves land, water, plant and animal genetic resources, is environmentally non-degrading, technically appropriate, economically viable and socially acceptable."

These definitions of sustainable, with appropriate modifications could be adopted to marine shrimp culture sustainability. In particular, sustainability must encompass, "...the broad, overlapping dimensions or systems associated with sustainability: economic, environmental and social. Each of these broad domains provides unique perspective, or partial "truth", regarding sustainable aquaculture", (Hargreaves 1997).

However, if you are looking for a concise, completely unambiguous and encompassing definition of shrimp culture sustainability, you could be disappointed. "Sustainability is a worthless word in the environmental context for no one knows what it means. We should work hard to replace it with the term environmental management. What we need for aquaculture is sound systems of environmental management to prevent or reduce negative environmental effects", (Boyd 1999b). "It is currently popular to talk about sustainability of agricultural, industrial, and other activities that utilize the world's resources. In fact, sustainability is used so often and in such varied contexts that it is essentially meaningless unless defined in relation to a particular activity. In the most literal sense, nothing is sustainable forever, but through wise use of resources, most activities can be sustained for a long time and until an alternative activity or resource is found to be appropriate", (Boyd 1999a).

Shrimp culture sustainability perhaps does not need a precise definition. Rather, it should include the concepts of: best available, non-polluting shrimp culture practices; financial profitability; and social justice. These concepts and ideals have been discussed more fully by signers of the *Choluteca Declaration* (Accion Ecologica et al. 1997), the Global Aquaculture Alliance (Boyd 199a), and FAO (1995a, Anonymous 1997).

It is also relevant to mention the role of government in all of this. Declarations and statements by environmental and industry NGOs, and others are appropriate and necessary, but in the end it is government's role to sort out a clear legal path that protects the public's best interests, and which includes providing acceptable means of food production (New 1999). "There are so many diverse opinions and special interests and so much greed that without government intervention anarchy would exist. There are those who want to use all the resources without regard to environmental effects, and there are those who want to limit resource use beyond the point of reason or necessity. Governments can, if they will, encourage a middle ground so that human needs can be supplied, producers can profit, and the environment can be protected', (Boyd 1999b).

II.3. <u>Human population pressures.</u> Food demand and production, whether by aquaculture or terrestrial agriculture are driven by the world's human population size. The world's population recently exceeded 6 billion people, doubling from 3 billion less than 40 years ago. Population expansion continues with at least 12 billion predicted before the end of the 21^{st} century (Bailey 1997). These human population increases put demands on food production systems, and on the environment. So far, humanity has been able to meet food demands through increased production, as a result of increased yields per land area and through culture area increases. These increases were possible only through continued improvements in culture stocks, culture techniques, and through continued reliance on fossil fuels and other items produced with fossil fuels. Within about 40 years, the world's supply of petroleum fossil fuel will be exhausted. It is now unclear what alternative energies will take the place of petroleum, and at what economic, environmental and social cost.

Human population increases have put extra-ordinary demands on aquatic animals for food. The world's fishery production has increased from 4 million MT (MMT) in 1900 (Borgstrom 1962) to about 100 MMT in 1995 (FAO 1995b) or more than 20 times increase. At the same time, human population increased from 1.6 to about 6 billion people, a 4 times increase. Capture fishery products from the world's oceans and freshwaters have reached, or in some cases exceeded sustainable supplies (FAO 1995b,c). Any further net increase in fisheries products must therefore come from aquaculture production. Indeed, aquaculture production has increased in response to this need. Between 1987 and 1996, capture fisheries production increased from 85 MMT to 95 MMT, while aquaculture production increased from 10.6 MMT to 26.4 MMT (New 1999). This represents a 249% increase in total aquaculture production, and an increase in aquaculture production from 11.1% to 21.8% of total fishery production. Aquaculture production is likely to continue its increase, while captive fisheries should at best remain nearly static.

Along with increased aquaculture production, production costs of some high valued species have also increased relative to agricultural products such as poultry and pork. This trend is likely to continue since many aquaculture feeds require higher levels of animal protein, mostly from fishmeal. By 1992, about 15% of the worlds fishmeal was used for aquaculture feed (Tacon 1994); including feeds for salmonids, marine shrimp and most other fishes that will accept pelleted feed. Fishmeal and fish oil consumption for aqua-feeds continues to increase along with aquaculture production, but at a greater rate than production. Fishmeal and fish oil consumption for aqua-feeds is are projected to be 25% and 80% of world production respectively for these commodities by the year 2010 (Pike 1997/8). Tacon (1997/8) forecasts even greater usage, and predicts 25% of the worlds fishmeal will be used in aqua-feeds by the year 2000. Aquaculture must compete against other agricultural animal crops for fishmeal and fish oil. This competition, plus periodic collapses of the forage fish stocks (Glantz and Thompson 1981) could not only reduce aquaculture growth, but it could also drastically alter economics and sustainability of high valued crops such as marine shrimp that rely on fishmeal and oil (Bailey 1997). Unless alternatives are developed to these high cost protein ingredients, aquaculture is more and more likely to provide food mostly for middle and upper income people. Aquaculture products are likely to become less and less a source of protein for the poor. Marine shrimp exemplify this trend.

II.4. **Summary:** Substantial, rapid and visible aquaculture expansion in many areas of the world during the past 10 to 20 years resulted in certain real and perceived deleterious environmental impacts. This has drawn the attention of a number of environmental NGOs, which pose threats to the aquaculture industry. The threats include forced certification programs and other restrictive legislation. The industry has responded by developing sets of best management plans, codes of conduct, and other guidelines intended to foster environmentally and socially conscientious aquaculture production methods, while at the same time promoting profitability. The marine shrimp culture industry was a primary target of thee environmental groups, and one that has responded most forcefully to the threats. So far, this forced scrutiny has been intense but rewarding. While the process is still on going, thee most likely outcome at this time seems to be a more environmentally and socially aware industry, especially by large corporate entities. It should also result in social and regulatory pressures towards more cost efficient and sustainable production systems. Intensive culture systems could benefit from this scrutiny since they require relatively small land areas, and their environmental impacts are more easily managed and mitigated by proper siting and operation. Ultimately, thee source of these problems relate to human world population growth which continues largely unabated. Planet earth's human population has increased from 1.6 to 6 billion people during thee last century, and is forecast to exceed 12 billion sometime during the 21st century. This population increase will put major stress on all aspects of food production, environmental quality, and on other societal functions.

III. NO WATER EXCHANGE

Intensive monoculture of marine shrimp is potentially unstable and risky. It requires large applications of organic feed and mechanical energy per unit water volume. These applications focus productivity from much larger land and oceanic areas within a smaller area of shrimp growout pond (Folke and Kautsky 1992). The pond becomes the "tip of the funnel." As a result, large amounts of uneaten feed, feces and metabolic wastes accumulate in pond waters and pond soils. These wastes are further degraded through microbial and other decomposition processes to produce among other things; ammonia, nitrite, nitrate and phosphate. These mineralized nutrients stimulate algal growth and lead to dense blooms in the pond. In addition to toxicity from some of these degradation products, algal population collapses can also cause shrimp stress and mortality through disease, oxygen depletion and increased metabolic toxicities. A conventional solution to this situation has been increased water exchange. Excess metabolites, suspended solids and algae are thus removed from the pond and replaced with water of lesser metabolite and algal concentrations and greater oxygen content. This water exchange or flushing solution is, however, not without potential perils of its own. In many cases, source waters for flushing contain disease organisms which infect the shrimp crop and cause massive mortalities. Industrial, domestic and agricultural pollution of source waters can likewise cause massive shrimp mortalities. In addition, source waters often contain high concentrations of suspended sediments which settle in the pond and cause shoaling of pond water depths which requires time consuming removal and disposal between crops. An alternative to high rates of water exchange includes systems with minimal, or no water exchange during crop culture.

Ten years ago water exchange rates of 10-20% or more per day were common with semi-intensive and intensive shrimp pond culture (Clifford

1985, Colvin 1985, Fast 1991). Semi-intensive culture ponds in Latin America typically pumped large volumes of estuarine water through very large ponds on a daily basis as a means of maintaining adequate dissolved oxygen (DO) concentrations in the ponds. Much smaller, intensive culture ponds in Asia combined high water exchange rates with electric paddlewheel aerators to achieve desirable DO and water quality (Fast et al. 1989). Water exchange and aeration also kept settleable solids in suspension where these materials could be flushed from the pond rather than accumulate on pond bottoms and contribute to toxic sediment conditions.

We now know that high water exchange rates through shrimp ponds are not always environmentally friendly, and do not always benefit shrimp culture. Water intakes can entrain and/or impinge biota, which are then lost. Pond influent/effluent waters also carry shrimp diseases into ponds, and discharge diseases from ponds into the environment. Nutrients and suspended solids in effluents can cause eutrophication and sedimentation in receiving waters. High sediment loads in source waters can lead to pond depth shoaling, increased operating costs, and lost culture time to remove sediments. Exotic shrimp species can also escape to the environment with waste waters and potentially become established, and/or cause disease transmittals. When genetic improvements occur in shrimp culture stocks and these stocks are more widely used, "improved" shrimp stocks could jeopardize population genetics of wild stocks through escapement and inter-breeding.

Water discharges from ponds occur for a variety of reasons. The most common reason is for water exchange as noted above, followed by drain harvest at the end of each shrimp crop cycle. Drain harvest typically contribute the highest concentrations of solid materials to receiving waters, especially in the last portion of the drain when sediments are resuspended and carried out with drain waters. The last 10-20% of pond drain waters can contain >60% of total settleable solids and >40% of suspended solids (Teichert-Coddington et al. 1999). Other discharges result from heavy rainfall, which overflows the outlet weir. In some cases in arid areas or during dry seasons, water must be exchanged to maintain pond salinity within acceptable ranges for shrimp (Hopkins et al. 1995). With extensive pond culture, water is often exchanged to provide seed, and to provide nutrients to stimulate in-pond productivity.

Ten years ago, common knowledge was that high rates of water exchange were necessary with intensive shrimp culture to remove nitrogenous and other potentially toxic metabolic waste products, and to prevent accumulations of potentially toxic organic sediments. With intensive culture, these wastes were thought to be one of the main limiting factors for shrimp production. Waste concentrations are related to feed input rates (Brune and Drapcho 1991); or more specifically to feed quantity and protein content (Westerman et al. 1993).

Before 1990, there was little of no systematic research on the relationships between water exchange, water quality, and shrimp yields from ponds. One of the few analyses of water exchange effects showed a dramatic increase in black tiger shrimp production with increased water exchange. Production increased from 10 MT/ha/yr with 20% maximum daily water exchange to 25 MT/ha/yr with 100% exchange (Hirasawa 1985, Fig. 2). More recent research, however, now casts doubts on the need for, or value of such high rates of water exchange. Indeed, there is now solid evidence that with proper pond management water exchange can be reduced to zero in many cases.

III.1. <u>Waddell Mariculture Center.</u> Shrimp pond and tank experiments with reduced water exchange began at the Waddell Mariculture Center, South Carolina during 1985-87 (Sandifer et al. 1988). During 1985, they intensively cultured *P. vannamei* using stocking densities, aeration and water exchange rates reported from Asia with *P. monodon* (Liu and Mancebo 1983, Chiang and Liao 1985). This included stocking 0.1 ha ponds with 40 PL/m^2, water exchange of 16-17%/day, and paddlewheel aeration. After five months culture, yields ranged from 6,010 to 7,503 kg/ha, which agreed with reported *P. monodon* results. The following year (1986), eight ponds were stocked with *P. vannamei* postlarvae ranging from 40 to 60 PL/m^2, water exchange was reduced to 8.3-8.5%/day, and aeration was reduced by 60%. Despite intense drought conditions during 1986, yields from these trials ranged form 4,390 to 6,881 kg/ha, with 77.3% average survival, and 2.5 average feed conversion ratio (FCR). They continued these trials during 1987 with a wider range of stocking densities (20-100 PL/m^2), and again with 8%/day water exchange. Yields ranged from a low of 2,487 kg/ha with 20 PL/m^2 to 12,680 kg/ha with 100 PL/m^2. Survival was 90%.

Following these successful pond trials with reduced water exchange of 8-9%/day during 1985-87, Hopkins et al. (1993) further evaluated water exchange and shrimp pond yields at the Waddell Mariculture Center during 1990. With these trials, they stocked 0.25 and 0.5 ha ponds with *P. vannamei* PL at 76/m^2 and with water exchange of either 14% or 4%/day. They observed no effects of reduced water exchange on yield, with average yields of 7,565 kg/ha in the 4%/day water exchange treatment, and 7,462 kg/ha with 14%/day water exchange.

Success with water exchange reduction to 4% with very high yields prompted Hopkins et al. (1993) to further reduce water exchange to zero during 1991. During 1991 they stocked five ponds with northern white shrimp (*P. setiferus*) PL at 22, 44 and 60 PL/m^2, water exchange of 25%, 2.5% and 0%/day, and aeration of 10-20 hp/ha. After 89 and 125 days culture, two ponds with zero water exchange and 44 and 60 PL/m^2 respectively, experienced mass shrimp mortality. Ponds with 2.5% and 25%/day water exchange yielded 6,375 and 5,718 kg/ha crops respectively, with corresponding 79.5% and 81.9% shrimp survival (Table 4). Only one pond with 0%/day water exchange yielded a credible crop. This pond was stocked at 22 PL/m^2 and yielded 3,219 kg/ha. Hopkins et al. (1993) could not relate mass mortality to any water quality parameters. They concluded that these shrimp mortalities were probably caused by gill fouling, since microscopic gill inspections revealed, "...some epicommensal bacteria and large amounts of trapped debris, similar to the filamentous gill disease described by Lightner et al. (1975) and Lightner (1983)." They associated this gill fouling in the 66 PL/m^2 and zero water exchange pond with abnormally high concentrations of suspended and organic solids in pond waters, although in the 44 Pl/m^2 and zero water exchange pond that had mass mortality, these solids were not excessive. Dissolved oxygen was well above critical levels at all times in all ponds. Hopkins et al. (1993) concluded that water exchange could be reduced to zero with *P. setiferus* provided that shrimp stocking densities were 44 PL/m^2 or less, and peak feed applications were 70-140 kg/ha/day. Further increases in feed applications would risk idiopathic mass mortality of shrimp due to gill fouling.

In a related set of trials during 1991, Hopkins et al. (1994) compared water quality and shrimp yields from three ponds with different sediment treatments. All three ponds had zero water exchange, *P. setiferus* stocked at 44 PL/m^2, and average feed rates of 97 kg/ha/day. All three ponds were aerated with one paddlewheel aerator (10 hp/ha) until day 55, when a second aerator was added (20 hp/ha). Pond sediments were handled very differently in each pond. In the REMAIN pond, sediments were allowed to settle and remain in place undisturbed (Fig. 3). In the REMOVE pond, sediments were removed using a pump, while in the RESUSPEND pond, the aerator was shifted 30° each day to resuspend bottom sediments and to keep these sediments from settling. As noted above, mass shrimp mortality occurred in the REMAIN pond on day 125 due to gill fouling, while survivals were not particularly high in the other two ponds with 32.8% and 54.1% (Table 5). Significant water quality and nutrient cycling differences existed between the three ponds. Nutrients in the REMPVE pond were lower and DO higher compared with the RESUSPEND pond. It also appeared that denitrification was inhibited in the RESUSPEND pond.

Undaunted by these mass shrimp mortalities during 1991, Hopkins et al. (1995) stocked four 0.25 ha ponds during 1993 with *P. vannamei* at 39 or 78 PL/m^2, fed prepared feeds with either 20% or 40% protein (2x2 factorial design). All ponds were fed at a constant rate of either 68 or 136 kg/ha/day during the entire 131-day trials, with the high and low feed rates corresponding to high and low shrimp stocking densities. All ponds had zero water exchange, and either 20 or 40 kWh/hr/ha paddlewheel aeration. The two high feed and high shrimp density ponds had aeration failures during the night on one occasion each, which resulted in observed DO of 0.8 and 0.5 mg/l respectively. Survival in these ponds correspondingly decreased to 57.5% and 63.3% (Table 5). Yet, yields were still very respectable, averaging 6,001 kg/ha for the low-feed/density ponds to 6,863 kg/ha for the high-feed/density ponds. Water quality reflects these differences in feed applications and shrimp densities. Average dissolved oxygen, turbidity, total suspended solids, organic suspended solids, total phosphorus and nitrate were all much higher in the high-feed/density ponds, and there was no apparent difference in shrimp survival, growth or yield between feeds with either 20% or 40% protein. FCR at high-feed/density do not reflect actual feed conversions due to DO induced mortalities. With intensive culture of *P. vannamei*, FCR typically average >1.8, and more typically >2.0 (Sandifer et al. 1988, Reid and Arnold 1992, Wyban et al. 1988). Almost as importantly, there were no incidents of mass shrimp mortality with *P. vannamei* due to gill fouling in the higher stocking and feed application ponds. Zero water exchange and high aeration rates, taken together with low FCR, high shrimp yields and the observed water quality parameters suggested that the microbial food web played an important role with successful, intensive culture of *P. vannamei* under these culture conditions.

Hopkins et al. (1997) and Browdy and Bratvold (1997) conducted further studies on water exchange at the Waddell Mariculture Center during 1995-96. Six 0.1 ha ponds were stocked each year with *P. vannamei* at 38 and 78 PL/m^2 each year respectively. Three of the six ponds had a constant 15%/day water exchange, while the other three had no water exchange. Feed rates were constant at 57 kg/ha/day during 1995, and 116 kg/ha/day during 1996, corresponding to different shrimp stocking densities each year. There were no significant differences between water exchange treatments either year. During 1995, survival and yields averaged 93.4% and 5,890 kg/ha for ponds with water exchange, and 91.2% and 5,443 kg/ha without water exchange (Table 6). Shrimp growth rates were much reduced during 1997 compared with 1996, but again survival and yields were similar for both water exchange treatments. Bacterial abundance and oxygen consumption were much higher in the ponds without water exchange (integrated ponds), during 1996 (Fig. 4).

III.2. <u>**Australia.**</u> Allan and Maquire (1993) cultured school prawn (*Metapenaeus macleayi*) in small pools in Australia with water exchanges ranging from 0-40%/day, and at stocking densities of 20 to 40 shrimp/m^2. They found no difference in shrimp survival, growth or FCR related to water exchange. High water exchange did reduce phosphorus and phytoplankton pigments, but had no effect on pH or nitrogen. They concluded that, "...simply increasing daily water exchange rates may not necessarily increase shrimp growth or survival. Water exchange can reduce nutrient concentrations and phytoplankton densities but most of the reduction occurs at water exchange rates of 0-5%/d."

III.3. <u>**Belize shrimp farm.**</u> Proof of zero water exchange benefits was clearly demonstrated on a commercial shrimp farm in Belize, Central America during 1997-present. The farm had sixteen 0.065 ha and eight 0.37 ha growout ponds, plus two 0.7 ha settling ponds and one 0.5 ha reservoir pond (McIntosh et al. 1999). Production ponds were all lined with HDPE plastic without substrate, while pond water depths were 1.4 and 2.3 m at shallow and deep ends respectively. The smaller ponds had 60 hp/ha of aeration with paddlewheel and aspirating-impeller aerators, while the larger ponds had 28 hp/ha. Aeration was provided to maintain adequate DO and to keep settleable solids suspended in the water column. Source waters were from the ocean (high salinity) and from a creek (low salinity). Creek water was used to lower salinity during the dry season. Pond drain waters were re-cycled through the settling basins and re-used in growout ponds. Sediments were removed by pumping every three weeks from pond areas where sediments collected. Sediments were drained into the settling basin. There were nearly zero water and sediment discharges from this farm.

McIntosh el al. (1999) cultured SPF *P. vannamei* (Mexican strain), SPF&R *P. vannamei* (resistant to TSV), and SPF *P. stylirostris* (Ecuadorian strain). Stocking densities ranged from 63 to 121 PL/m^2. Shrimp were fed a mixture of prepared feed (30% protein, complete diet), and a pelleted, organic mixture of soy meal, ground wheat and corn. The purpose of the organic mix was to stimulate growth of heterotrophic microbes in pond waters. During early crop cycles, feeds were applied at 70%/30% apportionment of organic mix and prepared diets respectively. This ratio was adjusted during growout such that by harvest the proportion was then 20%/80%. Peak feed applications often exceeded 350 kg/ha/day by harvest.

During the first year, 65,941 kg of shrimp were produced from 26 pond harvests, with average yields of 11,233 kg/ha/crop (McIntosh el al. 1999). Highest crop yield was 19,600 kg/ha. Average survival during the first two years of operation ranged from 56% for the TSV resistant *P. vannamei* to 82% for *P. vannamei* Mexican strain (Table 7). *Penaeus stylirostris* had intermediate survivals of 60%. Average yields were also greater with the Mexican strain, averaging 14,190 kg/ha/crop, compared with 10,340 kg/ha/crop for the TSV resistant strain and 7,450 kg/ha/crop for *P. stylirostris.* McIntosh and his colleagues felt that the Mexican strain of *P. vannamei* was much better suited for their high density and highly heterotrophic culture conditions, and that its superior performance was related to its having been bred in captivity for more than eight generations. This closed life-cycle culture over many genertions in intensive culture conditions undoubtedly resulted in selection for characteristics most suited for high-density culture. Small ponds produced higher yields than large ponds mostly because they were stocked at higher densities. There was also seasonal effects since the winters are cooler with higher salinity, and the summers have higher temperatures and more rain. Weekly average, high and low temperatures were 23°C and 32.5°C.

McIntosh et al. (1999) also observed profound changes in pond water quality and appearance during a growout cycle, "Ponds changed from a predominately autotrophic phytoplankton based pond ecology to a heterotrophic bacterial based pond ecology. Pond water coloration often changes from a green to a dark brown/black coloration with visible bacterial flocs present in suspension." These physical changes were accompanied by large changes in certain chemical parameters. Alkalinity, pH, DO, and transparency all decreased during culture, while carbon dioxide, nitrogen and phosphorus increased substantially (Table 8).

Substantial amounts of sludge were produced during zero water exchange shrimp culture. This sludge was pumped or drained to settling basins. For every kg of shrimp produced, 0.72 kg of dry sludge was produced (McIntosh et al. 1999). Overall water use was greatly reduced compared with conventional shrimp culture systems since only 2 m^3 of water were used to produce 1 kg of shrimp, and this water was re-used for subsequent crops. Semi-intensive shrimp culture in Central and South America typically used >100 m^3 of water per 1 kg shrimp (Clifford 1992). Energy consumption was increased with zero water exchange, however, since considerably more energy was consumed for aeration. With typical, high water exchange shrimp culture practices and 15%/day water exchange in semi-intensive culture (1,000-1,500 kg/ha), energy consumption for water pumps is 2.0 to 2.5 kWh/kg of shrimp produced. With zero water exchange, 3.47 and 4.62 kWh/kg of shrimp were used during the wet and dry seasons respectively. Added energy cost of zero water exchange in this case was thus $0.21/kg of shrimp at $0.12/kWh. This increased operating cost for aeration could be partially offset by lower capital and maintenance costs for the water pumping systems, and by more consistent production since reduced water exchange also decreases disease risks and chances of significant crop losses. As noted, this added aeration was essential to maintain adequate DO, to keep settleable solids in suspension, and to maintain healthy heterotrophic pond ecology.

McIntosh et al. (1999) had some disease problems, especially with the TSV resistant strain during high salinity (38‰) and temperatures (31.5°C). The other shrimp strains and species were relatively unaffected. Overall, though, their zero water exchange was highly successful. They attributed their success to four primary factors:

1. Use of virus free, SPF shrimp, which were adapted to high-density culture with no water exchange.
2. Pond management practices which promoted healthy, heterotrophic environments in the ponds.

3. Feeds, which promoted healthy, heterotrophic pond conditions.
4. Use of deep, lined ponds.

It is also worth noting that Belize is currently isolated from other shrimp farming areas. There are currently no shrimp farms in the Yucatan of Mexico, or on the Caribbean side of Guatemala or Honduras. Visits to their farm are discouraged to avoid disease imports. Belize is also currently free of TSV and most other serious shrimp viruses.

III.4. Summary and conclusions: Although our understanding of shrimp pond dynamics relative to water exchange is much less than perfect, it is now clear that shrimp yields in the range of 5,000 to 15,000 kg/ha/crop or more are possible without water exchange. This would have seemed unthinkable perhaps even 10 years ago.

Although intensive and semi-intensive shrimp culture has historically relied on high water exchange rates, some intensive fish culture has developed using little or no water exchange. In the U.S., channel catfish (*Ictalurus punctatus*) are commonly cultured in freshwater, earthen ponds with no water exchange and at yields greater than 7,500 kg/ha/yr (Tucker and Robinson 1990). These ponds use paddlewheel aerators and have prepared feed (30% protein) applications of 100 to 150 kg/ha/day, with average peak feed applications of about 112 kg/ha/day (Schwedler and English 1991).

Pond fish and shrimp yields in tropical areas, based solely on autotrophic production with solar radiation as the energy source and inorganic nutrients as the materials source, are limited to perhaps not more than 130 kg/ha/month (1,560 kg/ha/yr) of shrimp and/or fish crop biomass (Moriarty 1997). With a net 4.6 g C fixed/m^2/day by plants with primary production as the only organic carbon source, about 1% of net primary production is thus converted into shrimp and/or fish through a food web consisting of meiofauna, protozoa, zooplankton and macrofauna. Increased crop production above this level requires energy and material inputs from outside the pond. At high crop yields, the pond becomes a net consumer of energy and materials rather than a net producer. Usually, these inputs are animal manures, prepared feeds, and/or some other organic materials. Direct consumption of animal manures usually does not produce high crop yields, but manure fed ponds, which allow for manure digestion by a wide variety of microbes can produce very high yields of fish and other crops species. Microbes digest manures, thus converting this organic matter into more digestible and injestable forms, which are then consumed by fish and crustaceans. Fish yields of >7,000 kg/ha/yr in manure fed ponds with little or no water exchange are not uncommon (Tang 1970, Moav et al. 1977). Schroeder (1978) reported yields of >7,000 kg/ha during 220 days culture (>11,000 kg/ha/yr) with polyculture ponds in Israel (Fig. 5). Gut analyses of fishes from these manure fed ponds revealed predominance of detritus-like organic particles. Along with these particles were the same decomposer microorganisms found on organic detritus in the ponds. After passing through the fishes' guts, these particles were re-colonized by more decomposers and again re-cycled providing more food for fish.

Nutritional benefits of detritus fed systems to larger fish and crustaceans are widely recognized for other aquatic systems, such as freshwater streams and rivers (Cummins et al. 1995), where leaf litter and other organic debris from the watersheds are repeatedly passed through a wide variety of animals. With this mineralization process, energy and organic compounds benefit the decomposers and detritivores, who in turn provide food for fish and larger crustaceans.

Like certain pond fishes, marine shrimp have wide ranging food habits. In natural systems, shrimp consume detritus aggregates including bacteria, meiofauna including protozoa, micro-algae, zooplankton, macrobenthos and other items (Dall 1968, Gleason and Wellington 1988, Chong and Sasekumar 1997, Moriarty 1997). The importance of each food group or item in shrimp diets is unclear, but what is clear is that shrimp readily inject and presumably benefit nutritionally from the detritus food items.

Until recently, conventional thought was that with intensive shrimp culture, shrimp derived almost all of their nutrition from applied feeds, which were considered nutritionally complete. However, recent microcosm investigations at the Oceanic Institute in Hawaii using waters from intensive shrimp culture ponds clearly revealed that shrimp derive significant benefits from small suspended and settleable solids in these pond waters (Leber and Pruder 1988, Moss et al. 1992, Moss 1995). These microcosm trials included using unfiltered pond waters taken from a 337 m^2 round pond, which was stocked at high shrimp densities, fed a high quality prepared feed, and with peak water exchanges of 30%/day or more (Wyban and Sweeney 1989). In addition, clear well water, and pond water filtered using different filter mesh sizes were used in the microcosm trials. Shrimp

(*P. vannamei*) in each microcosm treatment were fed high quality, nutritionally complete, prepared feed. Trial results indicated that shrimp reared in clean well water and fed prepared feed only had the lowest growth rates (Fig. 6, Moss et al. 1992). Shrimp reared in pond waters filtered through 0.5 µm mesh with or without granulated activated charcoal (GAC) had growth rates statistically similar to the well water treatment. Shrimp in microcosms with pond water filtered through 5.0 µm mesh grew 53% larger than shrimp in the well water treatment, while shrimp receiving unfiltered pond water grew 89% larger than shrimp in the well water treatment. These results demonstrated that suspended pond water solids greater than 0.5 µm were making significant contributions to shrimp growth even in the presence of high quality shrimp diet. Pond water solids in the 0.5 to 5.0 µm were mostly small diatoms and microbial-detritus fragments, while solids >5.0 µm were mostly larger diatoms and large detritus aggregates. The diatoms were produced in the culture pond, while the detritus and other microbial materials were all produced from plankton, uneaten feed, and shrimp wastes through the detrital food web. Again, conventional wisdom has been that most penaeid shrimp obtained their food by probing the bottom with their chelated periopods and by transferring food items to their mouths (Hindley and Alexander 1978, Hill 1990). It is now clear that *P. vannamei* and presumably other penaeids can capture significant amounts of small, suspended particles from the water column and meet a substantial portion of their nutritional needs from these items. This work on detrital materials in pond waters helps explain improved feed conversions, successful use of low protein feeds, and other benefits of heterotrophic, aerobic pond culture experienced at Waddell Mariculture Center and in Belize.

Successful application of zero water exchange requires several conditions, or changes in normal pond management strategies. First and foremost, when water exchange is reduced to zero, then aeration and water turbulence must be increased to some suitable level to achieve continuous suspension, and/or resuspension of organic detritus and wastes, and to provide additional oxygen to compensate for increased BOD (Fig. 7). If aeration/turbulence are insufficient, suspended and organic solids will settle to the bottom in low turbulence areas of the pond and create anaerobic sediment accumulations. To prevent this occurrence, these sediment accumulations should either be removed from the pond, or resuspended before they produce substantial amounts of anaerobic decomposition products. Anaerobic conditions are generally considered undesirable, particularly when toxic hydrogen sulfide is produced in quantity. Large amounts of anaerobic sediment deposits may be most dangerous when disturbed since they could release substantial quantities of toxicants at one time. If adequate aeration/turbulence are provided, this will create an aerobic floc suspension with associated heterotrophic decomposers. This floc, and its associated microbes will provide valuable nutrition for the shrimp crop, increase shrimp growth rates, reduce protein requirements for the feed, reduce total feed requirements, and increase nitrogen conversion efficiencies from feed to shrimp. Low protein, prepared feeds are lower cost and use less fishmeal than high protein feeds. These cost savings will help offset higher energy costs for aeration.

There are still many important aspects of zero water exchange culture that need clarification. However, progress during the past 10 years has provided not only valuable insight into pond dynamic processes, but has also led to successful commercial applications using zero water exchange.

VI. BRINE BASED AND OTHER INLAND POND CULTURE

IV.1. Inland shrimp farming Thai-style. Intensive culture of *P. monodon* became increasingly popular in coastal areas of Thailand more than 10 years ago. Cultured shrimp production increased very rapidly as a result of existing hatchery, farm and feed infrastructure, and highly trained personnel for culture of freshwater prawn (*Macrobrachium rosenbergii*). Thailand has been the world's leading marine shrimp producer since 1991, and in 1998 produced 210,000 MT (Table 2). The Thai shrimp culture industry employs about 200,000 people in farms, hatcheries, processing plants, exporters, feed mills, equipment providers, and other aspects of the industry. Inland shrimp farming has now become a sizeable portion of total marine shrimp production in Thailand.

Brackishwater shrimp culture initially developed along some of the main rivers and estuaries in the upper Gulf of Thailand. During the dry season, with low volumes of river water outflows, seawater intruded up-river and provided adequate salinity for shrimp farms along the rivers. During the wet season, however, high volume outflows from rivers eliminated this source of saltwater. Some of these farmers soon discovered that they could truck concentrated brines from salt production works to their farms, mix brines with freshwater, and cultures *P. monodon* at low salinity (Flaherty and Vandergeest 1998, Miller et al. 1999). These successes, combined with serious disease problems in many coastal areas led to rapid expansion of shrimp culture into many inland areas, some hundred of kilometers from the coast. Traditional rice culture areas were often used for shrimp culture due to low land prices and availability. "In lieu of government regulation, the growth of inland shrimp culture is limited only by the availability of freshwater supplies and adequate road infra-structure to support saline water and post-larvae deliveries," (Miller et al. 1999).

A 1997 survey revealed 11,504 ha of inland shrimp farms in 12 central Thailand provinces (Musig and Boonnom 1998), while total estimated marine shrimp culture area in Thailand during 1997/98 was 70,000 ha (Table 2, Jory 1999, 1999). Some estimates indicate that as much as 27% of Thailand's total marine shrimp production came from these inland farms during 1998 (Pongthanapanich 1999).

In many ways, inland shrimp culture is similar to coastal shrimp culture, but there are some notable exceptions. With inland culture, shrimp ponds are prepared in the traditional way and filled to 30-50% of their volume with freshwater, usually from irrigation canals built for rice and other traditional crops. Concentrated brines (150-200‰) are trucked to the farm and added to this water, increasing pond salinity to 5-9‰ in most cases (Musig and Boonnom 1999), but ranging from 4-10‰ (Miller et al. 1999). Tanker trucks of 12-m^3 water capacity are used to transport brine to shrimp farms at $80 to $200/load depending on distance. *Penaeus monodon* PL are stocked at 30-65/m^2 or more, and pond water volumes are increased by freshwater additions, with further salinity decreases. Additional salinity decreases may occur during growout. Water may be added during the dry season to compensate for evaporation and seepage, although seepage is very low in most areas with thick clay soils. Rainfall can dilute salinity during the wet season. Typically, salinity decreases during crop growout to between 1 and 5% by harvest. Water is not normally discharged from ponds until harvest.

Most small farms, which constitute perhaps 80% of inland shrimp farms discharge pond waters and sediments into irrigation canals during harvest. Small farms typically have one hectare of ponds, or less. Large farms are much more likely to recycle their pond waters using dedicated reservoir ponds or by pumping between ponds. Many of these larger farms do not discharge any effluents, but use "closed-cycle" culture practices. This not only reduced environmental impacts, but it also saves money on salt purchase, reduces salt usage, and reduces overall disease risks not only to that farm, but to surrounding farms as well.

Especially with closed-cycle culture and use of SPF seed, disease risks are greatly reduced at inland shrimp farms. There is less risk of disease introductions through water exchange, and many of the common disease vectors such as seagulls and marine crustaceans are not present. There have been, however, incidents of yellow head and white spot virus outbreaks in these farms. The main source of infection appeared to be infected shrimp seed (PL), and freshwaters contaminated by discharges from other farms.

Inland farms have other problems not normally experienced by coastal shrimp farms. Two of the most common problems are off-flavor shrimp, and agricultural chemical problems. Off-flavor is most common in low salinity pond culture and is caused by blue-green algae (cyanobacteria) and microbes found in freshwater sediments. Off-flavor is common with intensive freshwater catfish culture, and in other low salinity, shrimp culture settings including those in Ecuador with *P. vannamei*. Off-flavor does not affect shrimp yield, but it does lower price.

Rice and other agricultural crops surround most inland shrimp farms. These crops are often sprayed with pesticides and insecticides, which can either drift into the shrimp ponds, or enter the pond with make-up water. Shrimp are especially sensitive to these agricultural chemicals since they are physiologically similar to the targeted pest organisms, which are usually insects. Other problems in inland shrimp farms include off-colored flesh, lower survival, soft exoskeleton, and smaller harvest size. Smaller harvest size and most other problems result in lower price.

Many rice and other traditional agriculturists became concerned by the rapid expansion of inland shrimp farms into their culture areas. They were most concerned about soil salinization caused by pond seepage, salinity increases in irrigation waters due to shrimp pond discharges, and sludge discharges from ponds into irrigation canals. Their concerns gained widespread public attention during April and May 1998 through a series of articles in Thai newspapers and on national TV. As a result, during the summer 1998 the Thai government banned all shrimp farming from freshwater inland areas. This resulted in protests by inland shrimp farmers and controversies between farmers, government departments, and among academicians. Attempts are now ongoing to find a fair and equitable middle ground.

The inland shrimp farming controversy involves both economic and social considerations (Table 9). Terrestrial agriculture in Thailand, especially rice farming is a revered tradition that supports and engages a large portion of the rural population. These rice farmers, "...often remain poor and usually in debt", (Pongthanapanich 1999). Shrimp farmers; on the other hand are seen as opportunists that have become rich through application of modern technology and access to investment money. The majority of inland shrimp farmers lease land for their farms, and some are not native to their farming district. Complicating the issue is that many rice and orchard farmers have seen a business opportunity and have converted their farms to shrimp.

IV.2. <u>Other inland shrimp culture</u>. Traditional marine shrimp culture is in coastal areas where seawater or brackishwater is exchanged with pond water. All shrimp culture intensities relied on this water exchange (Table 1). As noted above, zero water exchange and low water exchange systems have evolved, but even then, they rely on water brought from the coast by pipeline or as brine in tanker trucks. In addition to these systems, there are some mostly experimental shrimp culture farms well inland that use slightly saline or even freshwater to culture marine shrimp (Jory 1999).

Arizona shrimp farm. The Wood Bros. Shrimp Farm near Gila, Arizona uses slightly saline well waters (1-2‰) at 25°C to culture SPF *P. vannamei* (Jory 1999). Effluent water from the farm is used to irrigate olive trees and Durham wheat. The farm includes an intensive greenhouse nursery to produce shrimp pond seed. Nursery tanks were stocked with about 20,000 PL8/m^2 in 17‰ salinity water, which was reduced to 2‰ over 26 days culture. Nursery survival was nearly 100%, with FCR of 0.7. The farm had 10 growout ponds (0.15 to 0.9 ha), which were stocked at both low (5 shrimp/m^2) and high (44-55 shrimp/m^2) densities. Aeration was with paddlewheels and air diffusers at 20-40 hp/ha. Water was exchanged at 0-1.9%/day, plus an additional 1.34-8.33%/day (3% average) to compensate for pond seepage. Shrimp survival and yields were reduced by high mortality a few days before harvest due to low, pond temperatures of 15.8° C. Average yields ranged from 484 kg/ha/crop for the low-density ponds to 3,070 kg/ha/crop for high-density ponds. The study demonstrated that *P. vannamei* can be successfully cultured in essentially freshwater, but that