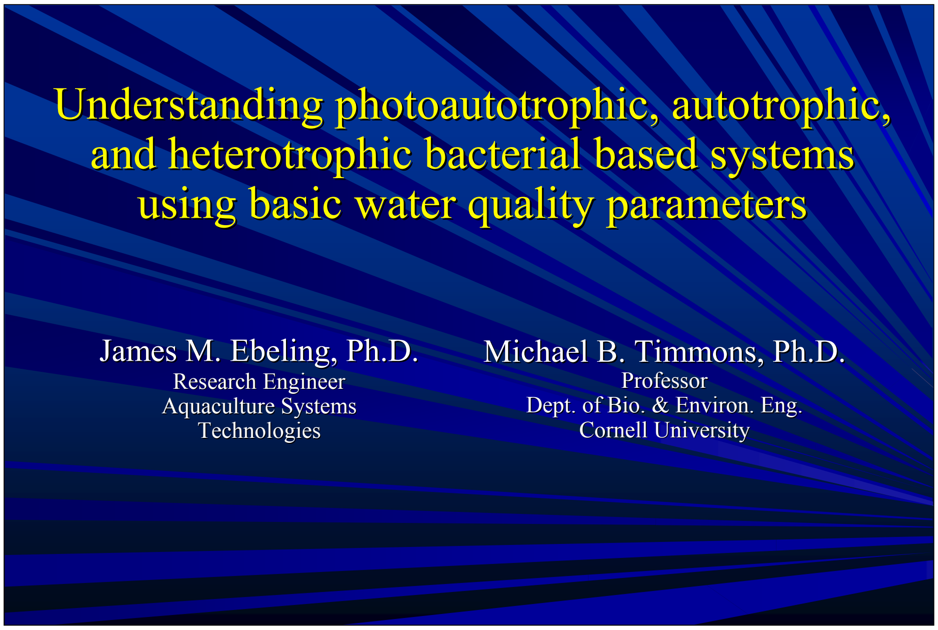


Abstract

Many of the current greenhouse based intensive marine shrimp production systems have used photoautotrophic algae systems to control inorganic nitrogen accumulation. A second strategy used in some indoor intensive recirculating production systems is using large fixed-film bioreactors. This option utilizes chemosynthetic autotrophic bacteria, *Nitrosomonas* and *Nitrobacter*, for the nitrification of ammonia-nitrogen to nitrite-nitrogen and finally to nitrate-nitrogen. More recently, zero-exchange management systems have been developed, which encourage heterotrophic bacterial growth and have been promoted for the intensive production of marine shrimp in both ponds and raceways. In this third pathway, heterotrophic bacterial growth is stimulated through the addition of carbonaceous substrate. At high carbon to nitrogen (C/N) feed ratios, heterotrophic bacteria will assimilate ammonia-nitrogen directly from the water and produce cellular protein.

In reviewing the literature on greenwater and zero-exchange systems, there appears to be confusion as to the pathway of ammonia removal being employed and whether it is photoautotrophic, autotrophic, or heterotrophic bacterial based, or in reality some mixture of the three. In this presentation, the fundamental stoichiometry of the three pathways is reviewed and the impact on water quality for each summarized. By keeping in mind the fundamentals of the three predominant pathways for ammonia-nitrogen assimilation in aquaculture production systems, it is possible to track which pathway is dominating at critical times during start-up and production phases. In addition, by manipulating key water quality parameters and ambient conditions, it becomes possible to guide a system towards a specific treatment process. For example, heterotrophic systems can be developed by adjusting the carbon/nitrogen ratio of the feed or through supplemental addition of carbon in the form of carbohydrates, e.g., sugar. These indicators are then examined for a series of research trials conducted at the Conservation Fund's Freshwater Institute examining water quality during the initial start-up and growout of juvenile marine shrimp, *Penaeus vannamei*.

1.0 Introduction

The economic success of an intensive production system for marine shrimp rests on the ability to produce shrimp with an acceptable feed conversion ratio and adequate growth rate. For numerous reasons, most importantly biosecurity, marine shrimp production is moving towards intensive recirculating systems and in the extreme towards indoor systems. Currently, the strategy most commonly used for controlling ammonia-nitrogen and nitrite-nitrogen in recirculating systems is by using large fixed-film bioreactors that rely on the nitrification of ammonia-nitrogen to nitrate-nitrogen by *Nitrosomonas* and *Nitrobacter* bacteria. Although a proven and accepted technology, such technology is economically unattractive for marine shrimp production due to the large upfront capital costs and ongoing operational costs for pumping and maintenance. More recently, zero-exchange management systems have been developed for large-scale pond production, where carbonaceous substrate is added to the systems to support microbial metabolism (Avnimelech, 1999, McIntosh, 1999). At high carbon to nitrogen (C/N) ratios, bacteria will take up nitrogen, i.e., ammonia, from the water and produce cellular protein, which can act as a supplemental feed to the shrimp (Moss et al., 1999, Burford et al., 2004). Due to the environmental impact of these nutrient rich discharges and the need for increased biosecurity, Waddell Mariculture Center, among others, researched the concept of reduced or zero-water exchange culture. Waddell Mariculture Center demonstrated that it was possible to obtain high shrimp yields from ponds using minimal exchange of water with high aeration rates. In the mid-90's, this concept with minor modifications was demonstrated at a commercial farm, BAL in Belize, Central America, hence the reference to the 'Belize zero-exchange system'.

This system was based on the following concepts:

- Shrimp – high health, selectively bred Specific Pathogen Free stock
- Feed – low protein feeds in combination with traditional high protein feeds
- Water management – zero water exchange, recycling water between crops
- Pond design – square shapes, depth of 1.0 to 1.8 m at center, HDPE liner
- Pond aeration – 30 to 50 hp/ha, completely mixed
- Pond management – C/N ratio maintained by feed protein and addition of additional carbon as needed (molasses, sorghum, sugar, cassava or wheat meal)
- Sludge management – frequent removal from center of pond or by settling between crops in holding ponds.

To prevent the introduction of disease, only disease resistant Specific Pathogen Free (SPR) PL's are stocked in the production pond at densities up to 120 to 200 m2. Recent strains of faster growing shrimp have become available through selective breeding. Feed protein content plays a critical role in maintaining a healthy bacterial population by balancing the carbon to nitrogen ratio. A carbon/nitrogen ratio of 16:1 was found to yield a very health heterotrophic community. This was accomplished by using a grain based feed with a high C/N ratio of 20 to balance the high protein diet used. In addition, molasses (> 40% carbon) was added during initial pond development to stimulate heterotrophic bacterial growth.

During the production cycle, there was no exchange of water except to make-up water loss to evaporation seepage and solids discharge. At harvest, pond water was routed to a settling basin, where the solids quickly settled out and excess nutrients were removed. After one week, the water was recycled back to the production pond and three days later, shrimp stocked out. This was possible because the recycled water had sufficient nutrients and bacterial population that no extensive pond preparations were needed. Ponds at Belize Aquaculture were square in shape with an average area of 1.6 ha (4 acres) and deeper compared to traditional shrimp ponds (1.8 m). In addition, each production pond was lined with a 40 ml HDPE liner. The liner was critical to allow the high mixing velocities created by the paddlewheel and aspirator aerators. This mixing action maintained floc in suspension and concentrated sludge in the center of the pond. At production levels of 1.8 to 1.9 kg/m2, approximately 1 hp of paddlewheel aeration was required to maintain dissolved oxygen for the production of 500 – 650 kg of shrimp. Ponds at Belize Aquaculture required 50 hp/ha of aeration. The aerators created a circular motion in the pond with water velocities ranging from 23 cm/s at the outside to 5 cm/s at the center.

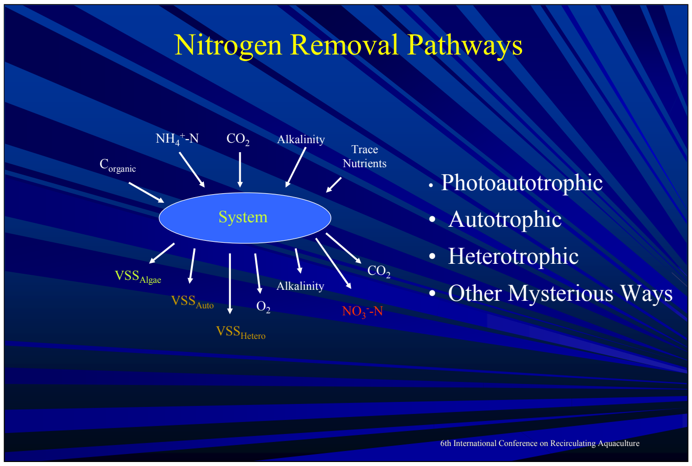
These high rates of aeration and mixing are the first major component of a zero-exchange production system. The second is the maintenance of an active heterotrophic bacterial community by controlling the amount of organic loading and the carbon/nitrogen ratio. At sufficiently high stocking densities, there is normally adequate inorganic material (predominately ammonia-nitrogen) to maintain a robust heterotrophic bacterial community. At these high stocking densities, there can be a problem with too high a nitrogen concentration for the available carbon due to the use of high (>30%) protein levels. Belize Aquaculture found that by increasing the carbon/nitrogen ratio in the feed to 16:1 by mixing in a grain based feed (20:1 ratio) the heterotrophic community appeared to be more in balance.

Finally, sludge management is important and consists of primarily removing sludge concentrated in the center of the pond by mixing action. Sludge is very different from the bacterial floc in suspension, consisting of fecal matter and uneaten feed particles. Sludge in the center of the pond is either drained out or pumped out to the drainage canals and eventually to the solids settling pond for treatment and ultimate disposal.

The Belize system, which is a solution to conventional shrimp pond constraints, also suggests the possibility that a recirculating system approach might be used to raise shrimp in an intensive manner. Using an indoor approach would also provide more control over water temperatures and heating costs that might permit a zero-exchange system to produce several crops per year in a moderate climate such as Atlanta GA, where pond systems would be limited by outdoor water temperatures for essentially 6 months of the year. Since maximum shrimp growth rates occur near 86 F (30 C), there are essentially no outdoor sites that have such temperatures year round. Thus, the ability to control water temperatures to optimal temperatures on a year-round basis is a distinct advantage.

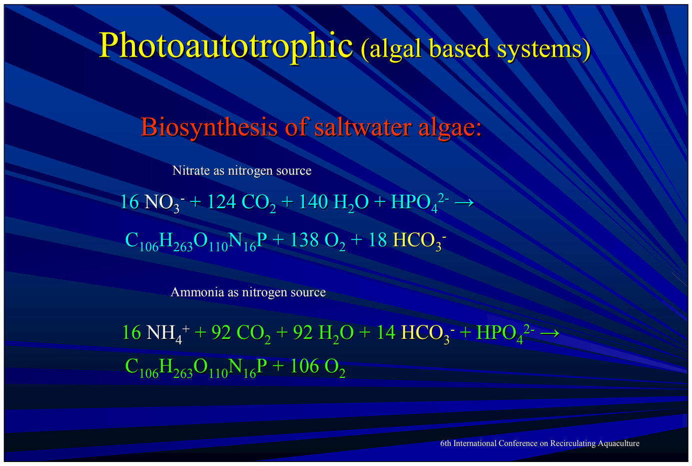
Over the past few years, zero-exchange management systems have been developed for large-scale pond production, where carbonaceous substrate is added to the systems to support microbial metabolism (Avnimelech, 1999; McIntosh, 1999). At high carbon to nitrogen (C/N) ratios, heterotrophic bacteria will assimilate ammonia-nitrogen directly from the water and metabolize the ammonia directly into cellular biomass.

In reviewing the literature on greenwater and zero-exchange systems, there appears to be confusion at times as to the type of ammonia removal pathway being employed and whether it is based upon photoautotrophic, autotrophic, or heterotrophic bacteria, or in reality some mixture of the three. The three pathways for ammonia removal are briefly reviewed here and their impact on easily measurable water quality indicators summarized. These indicators are then examined for a series of research trials conducted at the Conservation Fund's Freshwater Institute examining water quality during the initial start-up and growout of juvenile marine shrimp, *Penaeus vannamei*, at two salinities (4 and 12 ppt) and in either a greenwater or a zero exchange system using carbon supplementation.



2. Background

The three nitrogen conversion pathways traditionally used for the removal of ammonia-nitrogen in marine shrimp aquaculture systems are through the conversion of ammonia-nitrogen via photoautotrophic algae directly to algal biomass, autotrophic bacterial to nitrate nitrogen, and heterotrophic bacterial directly to microbial biomass. In order to fully understand these three conversion process, it is helpful to look at the stoichiometry involved, which helps conceptualize the impact of each pathway on specific water quality parameters.



2.1 Stoichiometry – Photoautotrophic systems

One of the most commonly used pathways for ammonia removal is through the use of "greenwater" systems, which use the high nutrient content of ponds, tanks, or raceways; outside or inside a greenhouse to grow algae at high density. The algae use either the ammonia-nitrogen or the nitrate-nitrogen and carbon dioxide as its carbon source to biosynthesize biomass. The biosynthesis of saltwater algae can be described in general by the following stoichiometric relationships (Stumm and Morgan, 1996):

for ammonia as the nitrogen source,

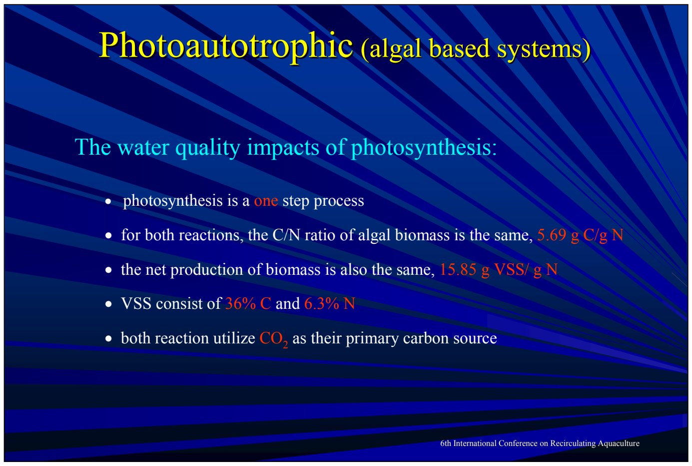
$$16NH_4^+ + 92\ CO_2 + HPO_4^{2-} + 92\ H_2O + 14\ HCO_3^- \rightarrow C_{106}H_{263}O_{110}N_{16}P + 106\ O_2 \quad (1)$$

or, for nitrate as the nitrogen source,

$$16\ NO_3^- + 124\ CO_2 + HPO_4^{2-} + 140\ H_2O \rightarrow C_{106}H_{263}O_{110}N_{16}P + 138\ O_2 + 18\ HCO_3^- \quad (2)$$

where $C_{106}H_{263}O_{110}N_{16}P$ represents the stoichiometric formula for algae in seawater.

Both reactions are endothermic, i.e., requires energy input, which for algae would be supplied by solar energy. Given a choice, ammonia is preferentially taken up by algal phytoplankton over nitrate. Respiration is just the opposite of the above equations.

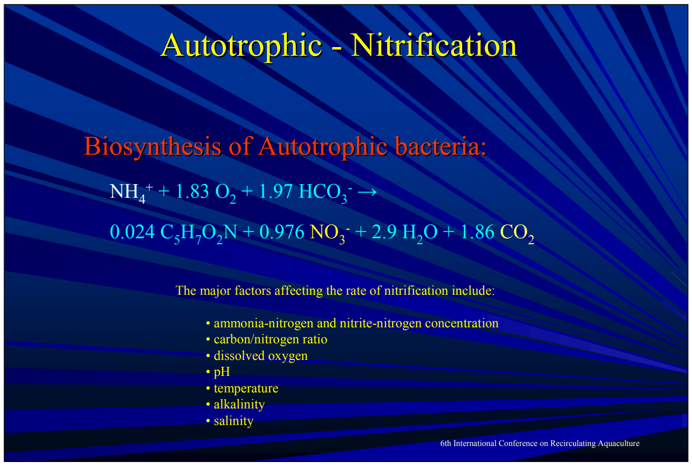


Obviously one of the most important requirements for photoautotrophic systems is sunlight. In outdoor ponds, this would seem not to be a problem. But depending upon location, weather conditions can yield extended periods with low light levels due to heavy overcast (Brune et al., 2003). In addition, high levels of suspended solids in the water column or high turbidity are often created in shrimp growout ponds by the activity of the shrimp on the bottom searching for food particles, i.e., bioturbation (Ritvo et al., 1997). Indoor systems have also experienced problems related to a lack of adequate light energy due to improperly selected greenhouse covers, which significantly reduce photoactive radiation levels, excessive foam that shade the water surface (Brune et al., 2004), and high turbidity due to excessive heterotrophic bacterial growth, which prevents sunlight penetration into the water column.

Photoautotrophic (algal based systems)

The water quality impacts of photosynthesis:

- both reactions metabolize only a small quantity of phosphorus, 0.14 g P / g N
- alkalinity is *consumed* when ammonia is the nitrogen source, 3.13 g Alk/ g N
- alkalinity is *produced* when nitrate is the nitrogen source, 4.02 g Alk/ g N
- pH increases due to consumption of carbon dioxide
- both reactions generate oxygen
- generation times for algal biomass range from 1 to 2 days
- at night or during low light levels, respiration dominates and the stoichiometric relations are reversed, i.e., pH decreases, oxygen is consumed, carbon dioxide is released.



Stoichiometry – Autotrophic Bacteria

There are two phylogenetically distinct groups of bacteria that collectively perform nitrification. These two groups of bacteria are generally categorized as chemosynthetic autotrophic bacteria because they derive their energy from inorganic compounds as opposed to heterotrophic bacteria that derive energy from organic compounds (Hagopian and Riley, 1998). Ammonia oxidizing bacteria obtain their energy by catabolizing un-ionized ammonia to nitrite and include bacteria of the genera Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus, and Nitrosovibrio. Nitrite oxidizing bacteria oxidize nitrite to nitrate, and include bacteria of the genera Nitrobacter, Nitrococcus, Nitrospira, and Nitrospina. Nitrifying bacteria are primarily obligate autotrophs, which consume carbon dioxide as their primary carbon source, and obligate aerobes, which require oxygen to grow (Hagopian and Riley, 1998).

Nitrification is a two step process, where ammonia is first oxidized to nitrite and then nitrite is oxidized to nitrate. The two steps are normally carried out sequentially, and since the first step has a lower kinetic reaction rate than the second step, the overall kinetics is usually controlled by ammonia oxidation. Thus normally there is no appreciable amount of nitrite accumulation. Equations 4, and 5 show the basic chemical conversions occurring during oxidation by Nitrosomonas and Nitrobacter (Metcalf & Eddy, 1991), where $C_5H_7O_2N$ represents the chemical formulation for bacterial biomass.

Nitrosomonas:

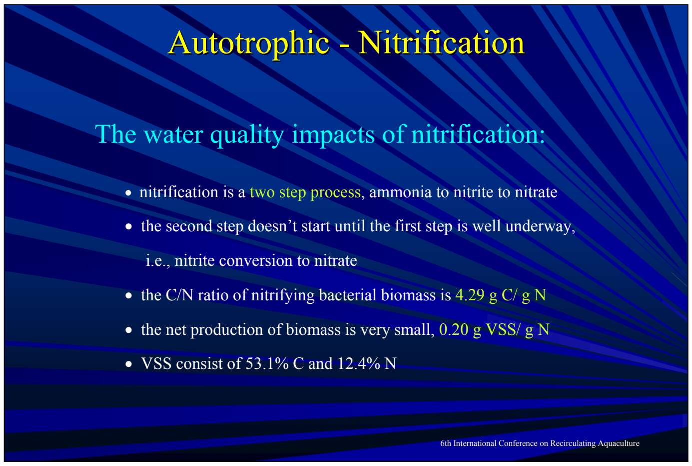
$$55\ NH_4+ + 76\ O_2 + 109\ HCO_3- \rightarrow 54\ NO_2- + C_5H_7O_2N + 57\ H_2O + 104\ H_2CO_3 \qquad (4)$$

Nitrobacter:

$$400\ NO_2- + NH_4- + 195\ O_2 + 4\ H_2CO_3 + HCO_3- \rightarrow 400\ NO_3- + C_5H_7O_2N + 3\ H_2O \qquad (5)$$

The overall removal process can be described as (Ebeling et al., 2005):

$$NH_4+ + 1.83\ O_2 + 1.97\ HCO_3- \rightarrow 0.0244\ C_5H_7O_2N + 0.976\ NO_3- + 2.90\ H_2O + 1.86\ CO_2 \qquad (6)$$

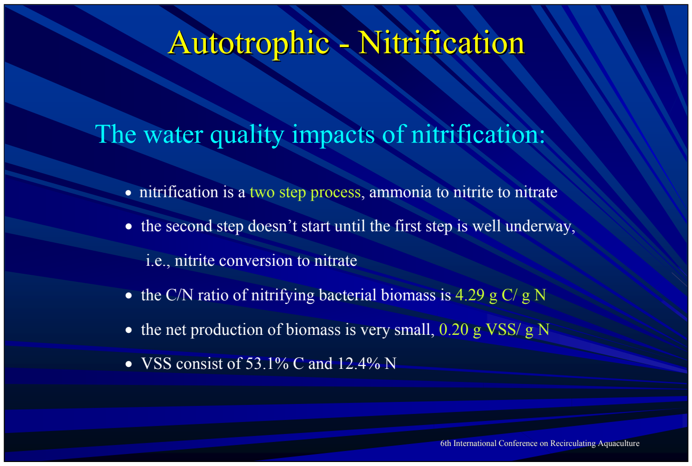


It should be noted that both the consumption of oxygen and alkalinity is less than that which normally reported, 4.57 g of O2 and 7.14 g of alkalinity for every g of ammonia-nitrogen converted (Timmons, et al., 2002), because in this equation some of the ammonia-nitrogen is converted to biomass. Traditionally, this biomass has not been included in the stoichiometric relationship because it is minor in comparison to the other factors.

One of the most important characteristics of autotrophic nitrification process is the extremely small amounts of bacterial biomass generated. And second because of the relatively slow maximum growth for the nitrifiers, it becomes very easy to 'wash-out' the nitrifying bacteria in a suspended-growth process, e.g., for example when suspended solids are removed to maintain water quality. This is particularly true if there is no sludge recycling that returns some of the bacteria back into the culture system. Finally, these nitrification processes result in a large amount of alkalinity consumed (7.05 g (as CaCO3)/g TAN) and high levels of carbon dioxide produced (5.85 g/g TAN). For water with low initial alkalinity this can be a significant problem requiring the addition of alkalinity, usually in the form of sodium bicarbonate, to maintain an adequate source of alkalinity (100 to 200 mg/L as CaCO3) for systems with limited water exchange. If alkalinity consumption is not compensated for by supplementation, pH will drop. Lowering pH will result in an inorganic carbon species shift in the water carbon balance from bicarbonate to dissolved carbon dioxide. This increase in dissolved carbon dioxide could affect some aquaculture species and in some cases be lethal. Although CO2 concentration can be controlled with gas stripping towers, significant energy is required for pumping both the water and air through these systems.

Autotrophic Bacteria – Impact on water quality

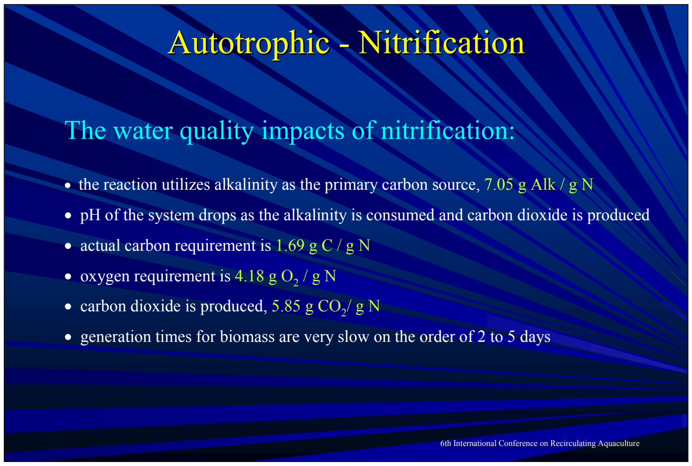
In the autotrophic nitrification process as opposed to heterotrophic processes, very small amounts of bacterial biomass are produced. And because of the relatively slow maximum growth rate for the nitrifiers in a suspended-growth process, it becomes very easy to 'wash-out' the nitrifying bacteria as opposed to a fixed-film system. This is particularly true if there is no sludge recycling that returns the bacteria back into the culture system. Also there is a significant amount of alkalinity consumed (7.05 g (as CaCO3)/g N) and high levels of carbon dioxide produced (5.85 g CO2 /g TAN). For water with low initial alkalinity this can be a significant problem, requiring the addition of alkalinity, in the form of sodium bicarbonate, lime, sodium hydroxide, to maintain an adequate concentration (100 to 150 mg/L as CaCO3), especially for systems with limited water exchange. If alkalinity consumption is not compensated for by supplementation, the system pH will drop. Lowering pH will result in an inorganic carbon species shift from bicarbonate to dissolved carbon dioxide, and this increase in dissolved carbon dioxide could affect some aquaculture species. Although CO2 concentration can be controlled with gas stripping towers, significant energy is required for pumping both the water and air through these systems. The end product of the reaction is nitrate-nitrogen, which is not normally toxic at moderate levels in aquaculture production systems, e.g., several hundred mg/L.



Autotrophic Bacteria – Impact of C/N ratio

The ratio of the biodegradable organic carbon to the nitrogen available for nitrification is argued to be one of the critical factors affecting the design and operation of a nitrification system (U.S. EPA, 1993). Heterotrophic bacteria have a maximum growth rate significantly higher than nitrifiers, 5 day-1 compared to 1 day-1 (U.S. EPA, 1993), thus in systems with even relatively modest C/N ratios, the heterotrophs are capable of out performing and significantly inhibiting nitrification. Zhu and Chen (2001) demonstrated the effect of sucrose on the nitrification rate of biofilters under steady-state conditions. They determined that at carbon/nitrogen ratios from 1.0 to 2.0, there was a 70% reduction of total ammonia-nitrogen removal rate as compared to C/N = 0. The data suggested that the nitrification rate decreased with an increase in the organic concentration, but the impact became less pronounced when the carbon concentration became sufficiently high.

Additionally in suspended-growth process with high C/N ratios, the increased production of heterotrophic bacteria requires that they be removed from the production system, i.e., using clarifiers. Since the yield of heterotrophic bacteria is greater than the yield of autotrophic nitrifying bacteria there is the potential, when attempting to control the TSS levels in the production system, that the nitrifiers will be washed out of the system.



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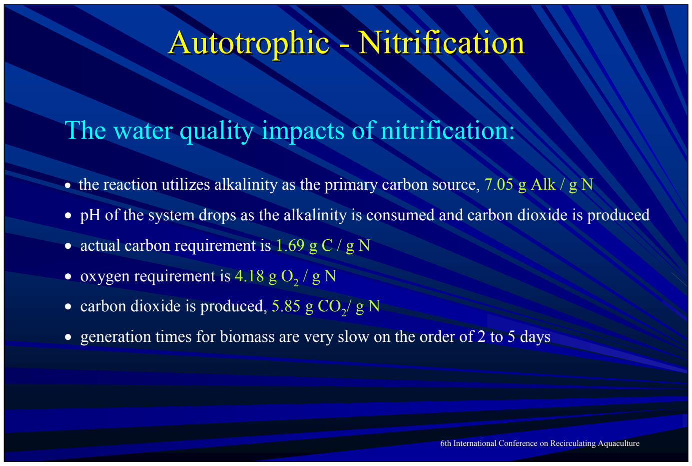

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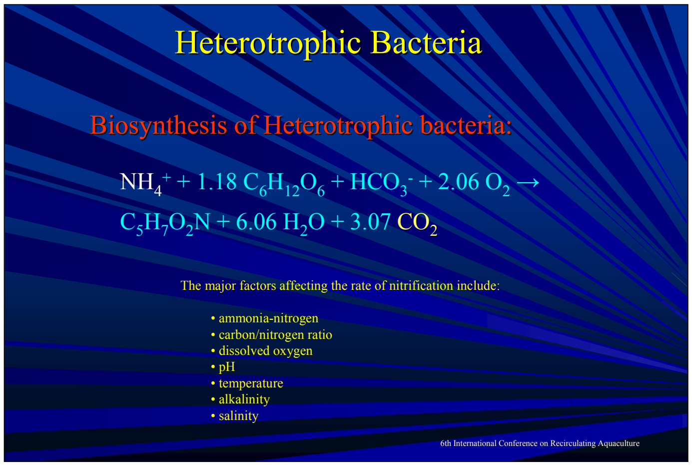
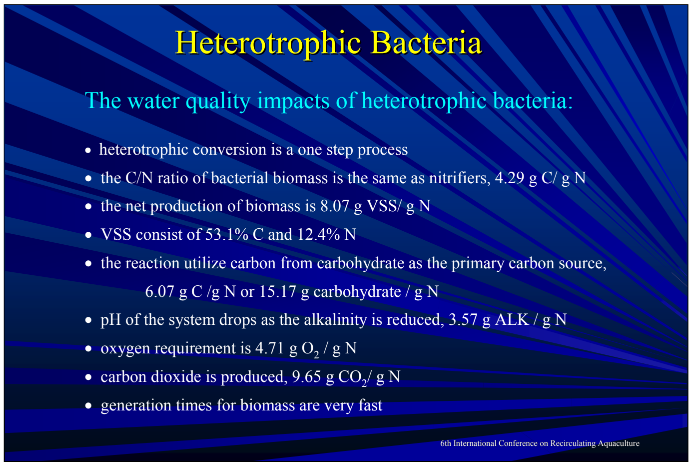


Heterotrophic Bacteria

Recently, a new shrimp production strategy has emerged called intensive zero exchange systems. In these systems, the ammonia build-up is controlled by the manipulation of the carbon/nitrogen ratio in such a way to promote the growth of heterotrophic bacteria (Avnimelech, 1999, McIntosh, 1999). As a result, the ammonia-nitrogen is removed from the system through assimilation into microbial biomass. It is not as easy to classify the heterotrophic bacteria that predominate in zero-exchange systems, since each system is unique and highly dynamic with frequent algal blooms and crashes of individual species. During a size fractionation experiment in which selected particle sizes were removed from growout tank's water, Moss, et al. (1999) reported shrimp growth rates increased 53% in water with suspended particles between 0.5 and 5.0 µm, as compared to clean water systems. Water with particles greater than 5.0 µm improved growth by an additional 36%. Half of the particulate organic carbon in the water was in the form of pinnate and centric diatoms. In contrast, Burford, et al. (2002) examined microbial and phytoplankton processes in several high-intensity shrimp ponds at Belize Aquaculture Ltd. in Central America. She found high concentrations (3.35 to 5.42 * 107 / ml) of autotrophic and heterotrophic dinoflagellates, nanoflagellates and cyanobacteria as well as protozoa, ciliates and rotifers. Diatoms were absent; except for a small number in only one of the five ponds studied. More importantly, Burford found that species dominance varied substantially over the time frames of day, which also has been the authors' experience in marine shrimp ponds in Hawaii. In addition, over 40% of the bacteria were associated with flocculated matter. McIntosh (1999) considered the heterotrophic bacteria and associated detritus as the dominant source of microbial nutrition for shrimp. Both Burford and Moss state that the current understanding of heterotrophic bacteria and the microbial loop in zero-exchange systems is poorly understood and additional research is needed to identify and characterize the microbial community structure and to develop best management strategies. (Burford, et al., 2003, Moss, 2002).

Removal of ammonia-nitrogen by heterotrophic bacteria is a single step process, where the ammonia-nitrogen is assimilated into bacterial biomass (Ebeling et al., 2006a). The stoichiometry is shown in Equation 7, where $C_5H_7O_2N$ again represents the chemical formulation for bacterial biomass and the carbon source is represented as a simple carbohydrate, i.e., sugar, $C_6H_{12}O_6$.

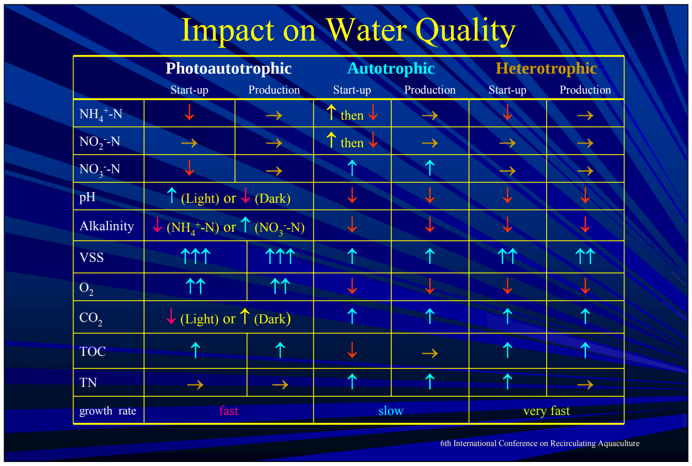
$$NH_4+ + 1.18\ C_6H_{12}O_6 + HCO_3- + 2.06\ O_2 \rightarrow C_5H_7O_2N + 6.06\ H_2O + 3.07\ CO_2$$



Heterotrophic Bacteria

Several aspects are important in the overall heterotrophic bacterial reaction. Paramount is the extremely large amount of bacterial biomass produced by this reaction, compared to the autotrophic reaction, 8.07 g VSS /g N versus 0.20 g VSS/g N. This translates into extremely high growth rates and substantial quantities of total solids produced. Some form of solids management is required to remove excess TSS production. A second issue is the modest amount of alkalinity consumed as the carbon source (3.57 g/g TAN) and the resulting high levels of carbon dioxide produced (9.65 g/g TAN). As pointed out above, for water with low initial alkalinity, this could be a problem requiring the addition of carbonate, usually in the form of sodium bicarbonate to maintain reasonable alkalinity (100 to 200 mg/L as CaCO3), especially for systems with limited water exchange. As a result, zero-exchange production systems that rely on suspended or attached heterotrophic bacteria should see a slight decrease in alkalinity, large suspended solids production, and high CO2 levels. Most importantly, theoretically there should be no production of nitrite-nitrogen, or nitrate-nitrogen in a pure heterotrophic system.

Generally, some form of carbon supplementation is required to develop a pure heterotrophic system. This is because there is usually insufficient carbon available from just the feed. For example a feed with 35% protein has sufficient available carbon to sequester only about 36% of the ammonia-nitrogen by the heterotrophic bacteria (Ebeling, et al., 2005b), and the remaining nitrogen is available to the autotrophic bacterial population. Thus for systems without carbon supplementation, a combination of heterotrophic and autotrophic systems will develop at low feed rates. At higher feed rates, the harvesting of suspended solids can actually ‘wash-out’ the autotrophic bacteria due to their slow growth rates.



Impact on Water Quality

	Photoautotrophic		Autotrophic		Heterotrophic	
	Start-up	Production	Start-up	Production	Start-up	Production
NH_4^+-N	↓	→	↑ then ↓	→	↓	→
NO_2^--N	→	→	↑ then ↓	→	→	→
NO_3^--N	↓	→	↑	↑	→	→
pH	↑ (Light) or ↓ (Dark)		↓	↓	↓	↓
Alkalinity	↓ (NH_4^+-N) or ↑ (NO_3^--N)		↓	↓	↓	↓
VSS	↑↑↑	↑↑↑	↑	↑	↑↑	↑↑
O_2	↑↑	↑↑	↓	↓	↓	↓
CO_2	↓ (Light) or ↑ (Dark)		↑	↑	↑	↑
TOC	↑	↑	↓	→	↑	↑
TN	→	→	↑	↑	↑	→
growth rate	fast		slow		very fast	

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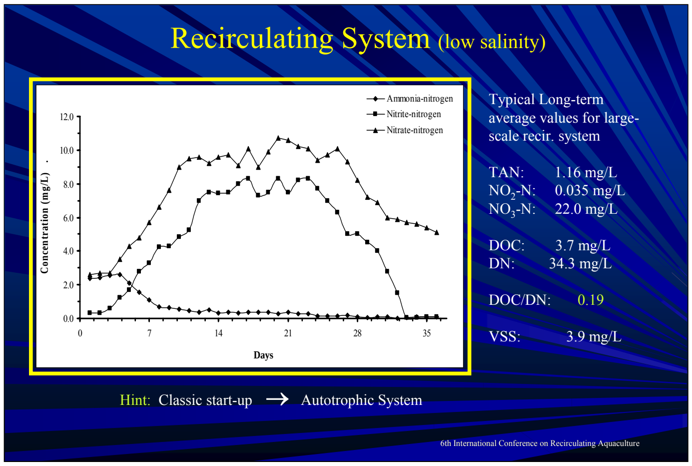
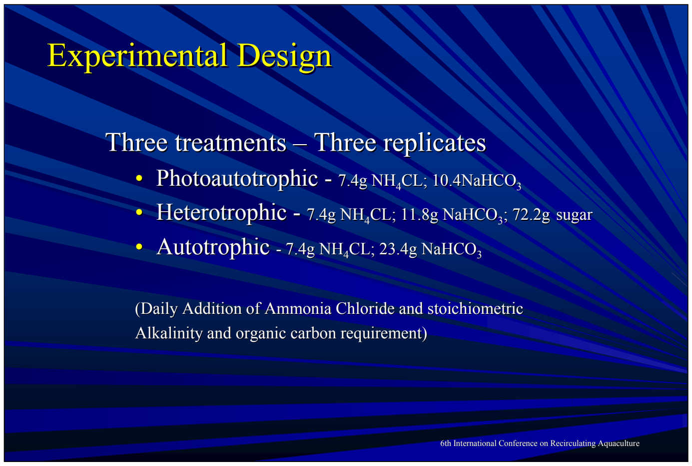
Nitrogen Removal Pathways

Table 1 presents a summary of water quality characteristics for the three pathways, photoautotrophic, autotrophic and heterotrophic bacteria. The arrows indicate in which direction the concentration of the water quality indicator will normally move during the initial system start-up and during long-term operation. For example, ammonia-nitrogen will usually decrease as it is consumed by algal biomass in a photoautotrophic process. In contrast, a system based on autotrophic bacteria might initially start with increasing ammonia-nitrogen concentrations and then see a dynamic drop as the *Nitrosomonas* bacteria "kick-in". At that time, the nitrite-nitrogen concentration will increase until it too drops off when the *Nitrobacter* bacteria "kick-in". Finally, the nitrate-nitrogen concentration will slowly increase until it reaches equilibrium with either flushing rates or incidental denitrification in anoxic pockets in the system. As another example, for heterotrophic systems, since ammonia-nitrogen is converted directly into bacterial biomass, there should be no increase in nitrite- or nitrate-nitrogen.

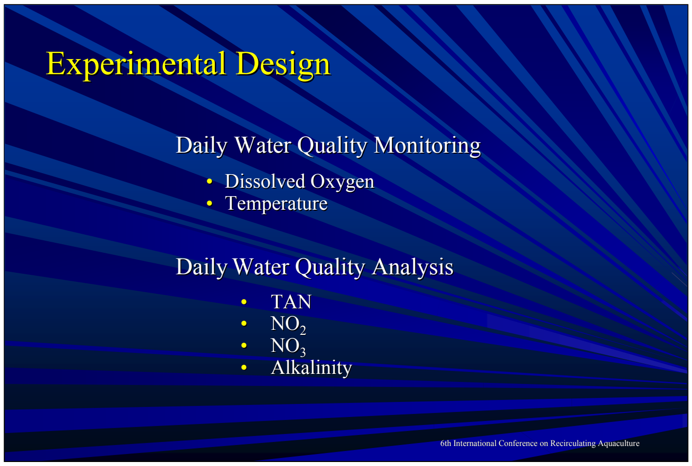


Water quality data for TAN (low), nitrite (moderate and falling) and nitrate (high) suggest a strong and robust autotrophic nitrification of TAN. Low C/N ratio would limit the heterotrophic bacteria growth.

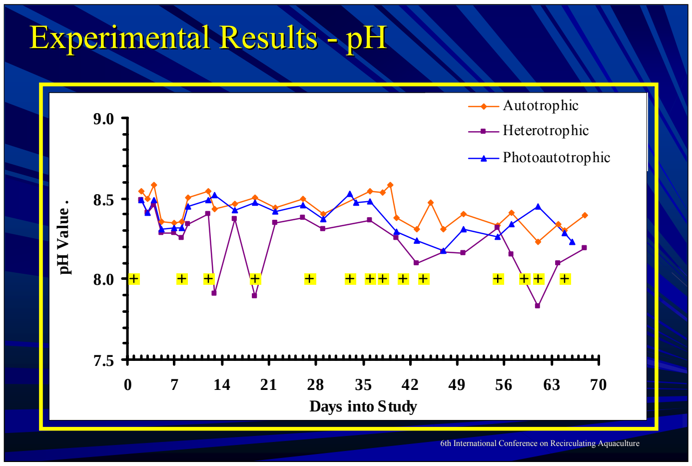


The research design was based on the availability of nine, 1 m research tanks. Thus three treatments were chosen with three replicates of each. Water quality parameters were monitoring on a daily or weekly bases as needed.

In this two part study, several concentrations of artificial sea salt in spring water were investigated using nine circular, flat bottom tanks of approximately 250 L (66 gal). During the first trial, triplicate replicate tanks were stocked with juvenile marine shrimp at a density of 25 juveniles/m2 and at salinities of 5.0, 2.5, and 0.5 ppt. The low stocking density was chosen to minimize water quality problems as a mitigating factor in the study.



Water quality parameters measured daily (8 – 9:00 am) were dissolved oxygen, temperature salinity and conductivity using YSI meters and probes. Bi-weekly, ammonia-nitrogen, nitrite-nitrogen, nitrate-nitrogen and alkalinity were measured using a Hach spectrophotometer and Hach proceedures based on standard methods. In addition, chlorides levels were measured weekly.



Experimental Results - pH
Autotrophic
Heterotrophic
Photoautotrophic
pH Value
Days into Study
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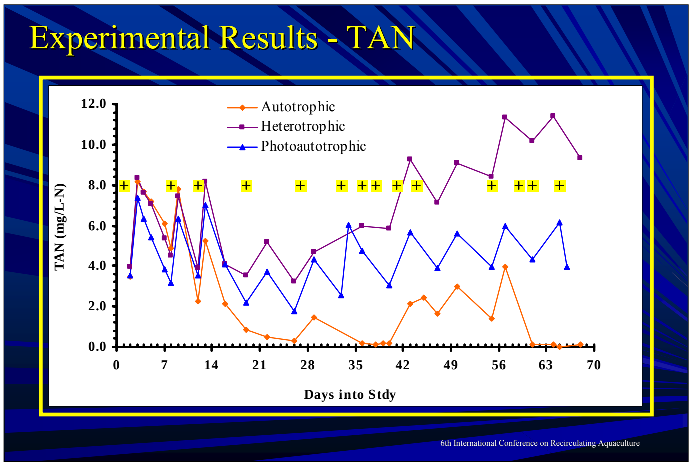
Experimental Results - TAN
Autotrophic
Heterotrophic
Photoautotrophic
TAN (mg/L-N)
12.0
10.0
8.0
6.0
4.0
2.0
0.0
0
7
14
21
28
35
42
49
56
63
70
Days into Stdy
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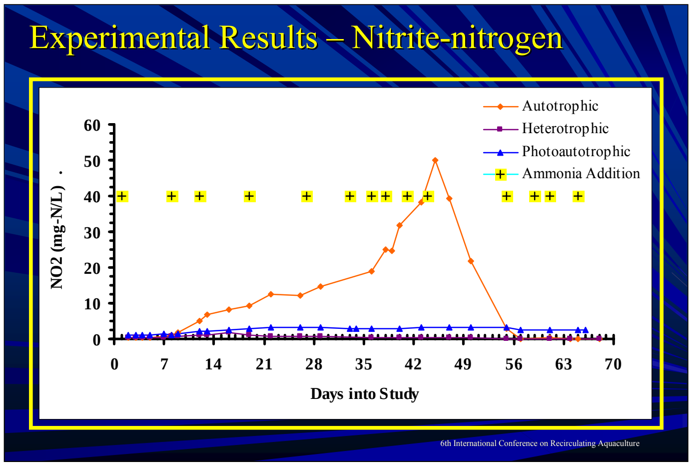
Experimental Results – Nitrite-nitrogen
Autotrophic
Heterotrophic
Photoautotrophic
Ammonia Addition
NO2 (mg-N/L)
Days into Study
0
10
20
30
40
50
60
0
7
14
21
28
35
42
49
56
63
70
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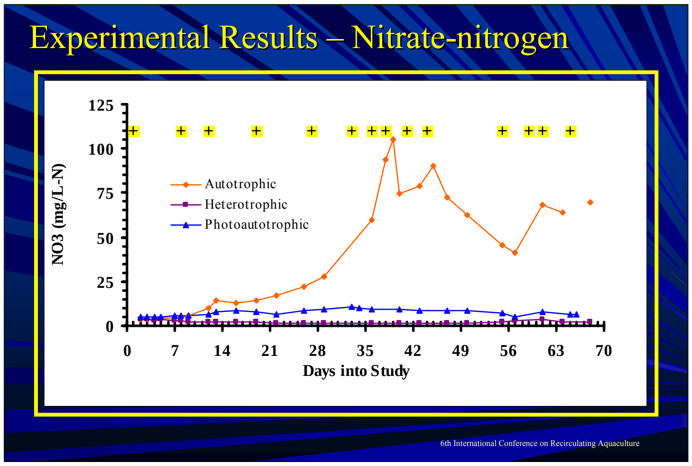

Experimental Results – Nitrate-nitrogen
NO3 (mg/L-N)
125
100
75
50
25
0
Autotrophic
Heterotrophic
Photoautotrophic
0
7
14
21
28
35
42
49
56
63
70
Days into Study
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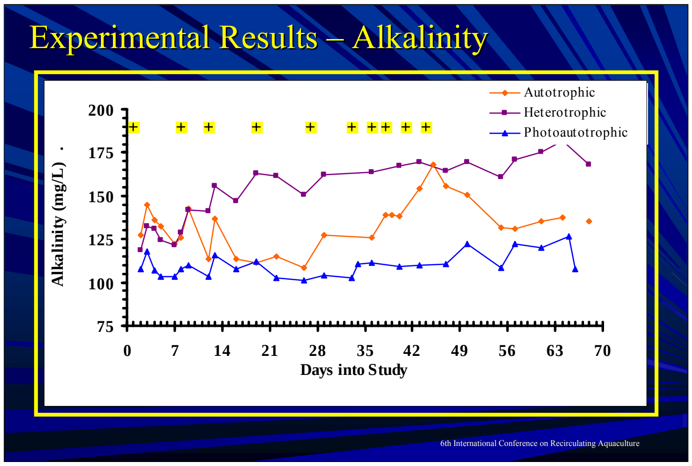
Experimental Results – Alkalinity
Autotrophic
Heterotrophic
Photoautotrophic
Alkalinity (mg/L)
200
175
150
125
100
75
0
7
14
21
28
35
42
49
56
63
70
Days into Study
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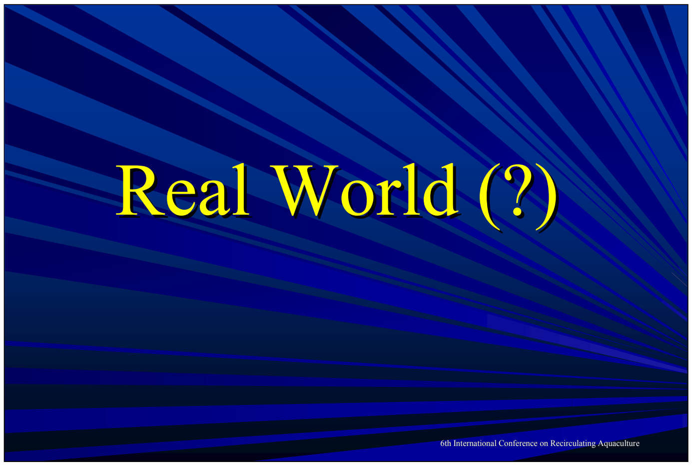

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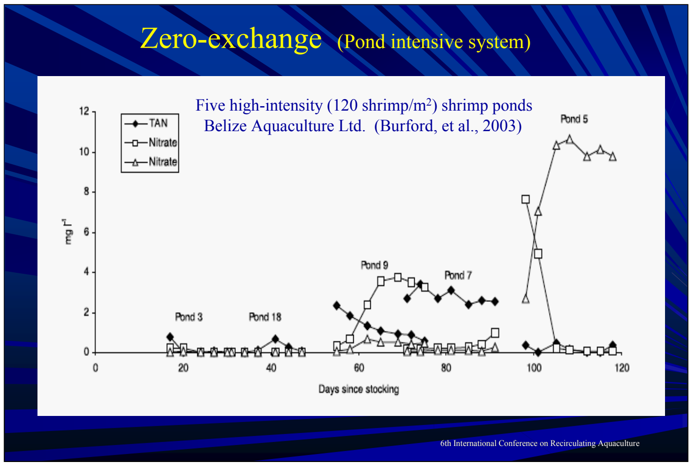
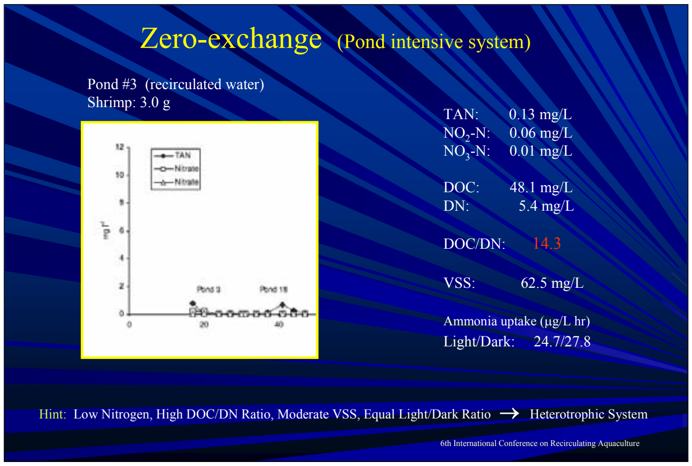
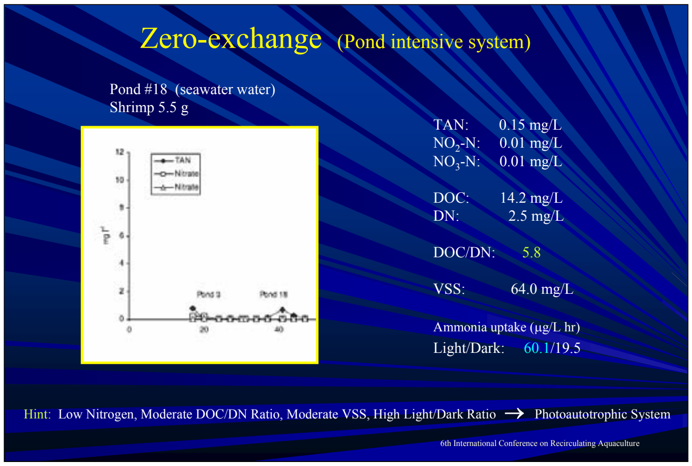


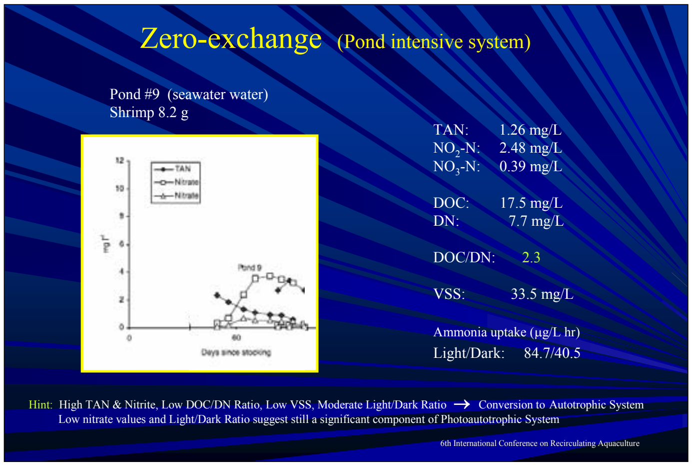
Water quality data from five research ponds over 3 week interval. Stocking density 120 shrimp/m^2, grain based 21.9% and 35% protein feeds.



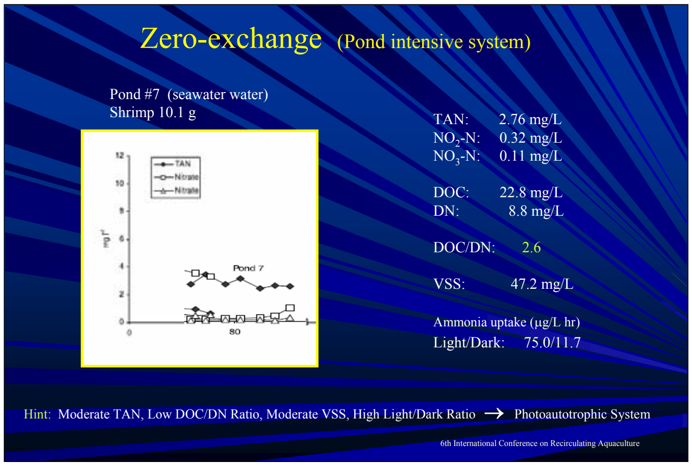
Low values of TAN, Nitrite and Nitrate-nitrogen suggest a dominate heterotrophic process with conversion of TAN directly into bacterial biomass. This would be due to the high C/N ratio of the feed, primarily grain based (14.7 C/N ratio), plus the addition of molasses, resulting in a pond C/N ratio of 14.3, significantly higher than the C/N ratio of bacteria (4.28 C/N). Total suspended solids are the second highest of the five ponds with the highest percent organics (83%), suggesting a young, log-growth stage of bacterial biomass. Pond 3 also has the highest DOC concentration (48.1 mg/L). Light/Dark bottle ammonia uptake suggests very little phytoplankton removal of TAN. All this is consistent with a pond using recirculated water containing a rich supply of bacterial seed and high C/N ratio.



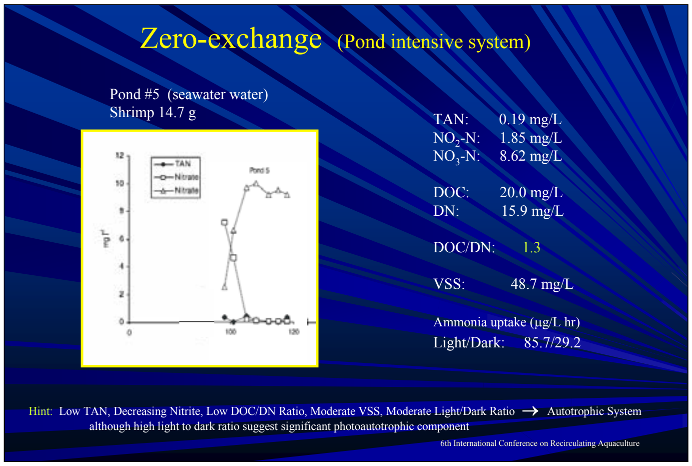
The high ammonia removal rate by the light bottle compared to dark bottle suggests a photoautotrophic system, which is supported by the low DOC/DN ratio. Also the pond was filled with fresh seawater, thus the low DOC compared to the previous pond filled with recirculated water. The moderate VSS concentration fits with the rapid growth and high yield of algae. The slight increase in TAN also is characteristic of the bloom and crash sequence seen in algae based systems.



Shrimp diet has slowly been changing from a grain based feed with a C/N ratio of 14.7 to a high protein feed (31%) with a C/N ratio significantly lower at 10.5. Ratio of DOC/DN in the water column has fallen to 2.3, far less than the C/N ratio of bacteria (4.28). Pond dynamics appear to be converting over to an autotrophic bacterial phase, reflected in the high TAN and significantly high nitrite-nitrogen. The can also be seen in the drop in TAN and the increase in nitrate and small increase in nitrate concentration suggesting that the second step in nitrification has not yet fully engaged. This is not unusual, due to the slow growth rates of NOB and the inhibition by high TAN concentrations. Total Suspended Solids has dropped to 48.5 mg/L, with a very low organic fraction (69%), suggesting an older aged biomass. This is reasonable, considering the significantly lower biomass yield of autotrophic bacteria (0.20 g/gN) compared to heterotrophic or photoautotrophic bacterial yields (8.07 g/gN). The lower than expected nitrate levels are possible due to small fraction of remaining algal biomass.



The high TAN concentrations and low nitrite and nitrate concentrations suggest that this pond has not generated sufficient AOB to begin the autotrophic process even with low C/N ratio. Light bottle/dark bottle ammonia uptake data indicates that TAN uptake by phytoplankton is the dominate pathway for TAN removal.



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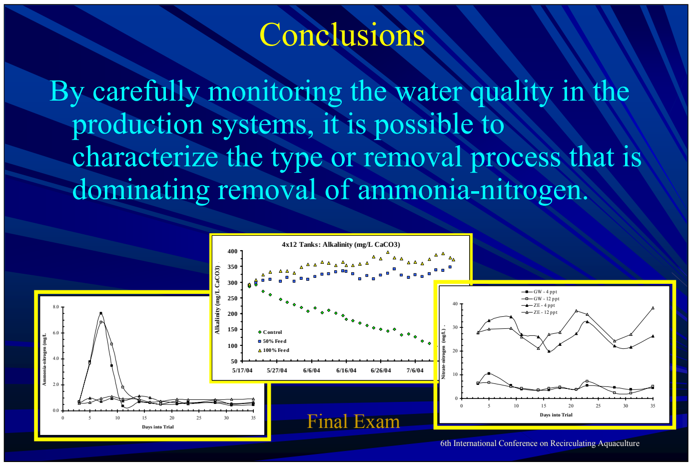


Conclusions

By carefully monitoring the water quality in the production systems, it is possible to characterize the type or removal process that is dominating removal of ammonia-nitrogen.

Things to look for during the start-up and production phase of an autotrophic nitrification process:

- •initial increase, peak and the falloff in ammonia-nitrogen concentration as nitrification by autotrophic *Nitrosomonas* bacteria begins to convert ammonia-nitrogen to nitrite-nitrogen,
- •rapid increase in nitrite-nitrogen concentration and then falloff as autotrophic *Nitrobacter* convert nitrite-nitrogen to nitrate-nitrogen.
- •finally, increase in nitrate-nitrogen,
- •over time a decrease in pH, and alkalinity, and
- •very slow bacterial growth rate, thus little increase in TSS.

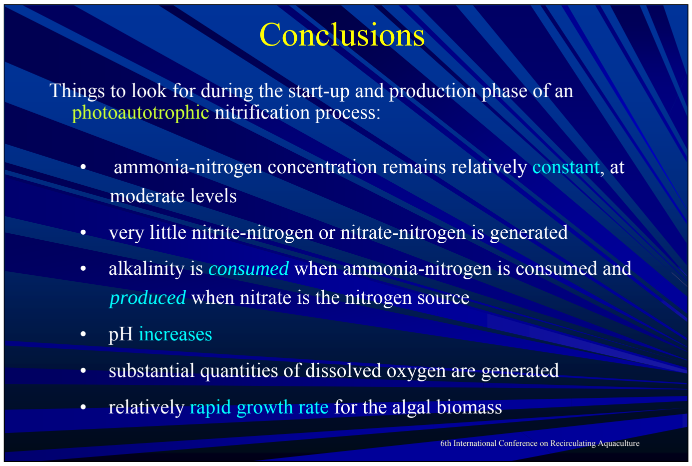
Things to look for in a photoautotrophic nitrification process:

- •ammonia-nitrogen concentration remains relatively constant, at moderate levels,
- •very little nitrite-nitrogen or nitrate-nitrogen is generated,
- •alkalinity is *consumed* when ammonia-nitrogen is consumed and
- •*produced* when nitrate is the nitrogen source
- •pH remains relatively constant,
- •substantial quantities of dissolved oxygen are generated, and
- •relatively rapid growth rate for the algal biomass.

Things to look for in a heterotrophic nitrification process:

- •ammonia-nitrogen concentration remains relatively constant, at moderate levels,
- •very little nitrite-nitrogen or nitrate-nitrogen is generated,
- •heterotrophic bacteria utilize carbon from the feed or supplemental sources,
- •pH of the system remains relatively constant,
- •carbon dioxide is produced, and
- •heterotrophic bacteria out compete autotrophic bacteria.

Thus similar to reading a medical chart, one can determine the overall state of health of a nitrification system, determine what pathway is dominate and if necessary take corrective action if the systems strays from the anointed path.



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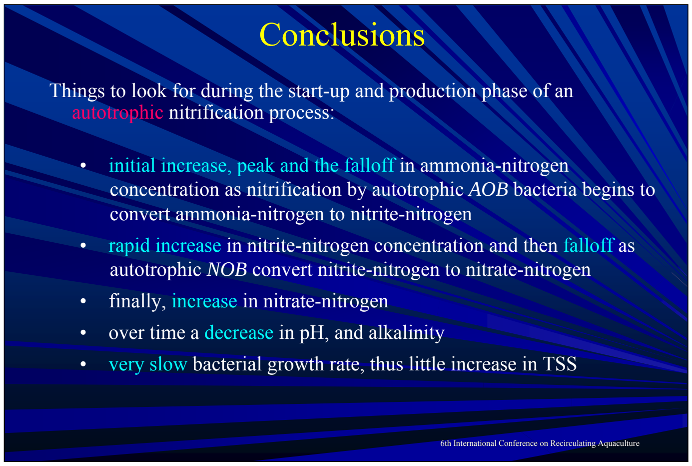

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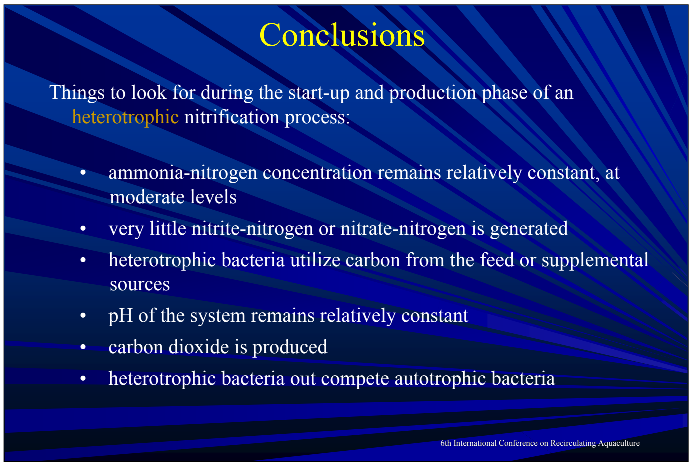

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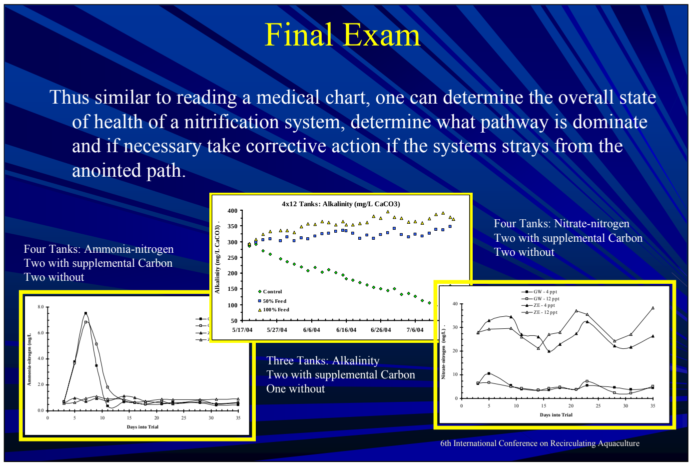

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Left graph: Ammonia-nitrogen, two bottom curves are with carbon supplementation, two top are classic acclimation curves for autotrophic nitrification.

Middle graph: Three tanks, two receiving supplemental carbon, bottom curve classic consumption curve for autotrophic nitrification, top two are heterotrophic – alkalinity?????

Right graph: Four tanks bottom are the two with supplemental carbon showing relative stable nitrate-nitrogen, top two are photoautotrophic showing the bloom and crash scenario seen in photoautotrophic systems.

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Opinions, conclusions, and recommendations are of the authors
and do not necessarily reflect the view of the USDA.

Questions?

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