The Role of Nitrogen in Aquaculture

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Nitrogen

- Elemental symbol: N
- Atomic number: 7
- Atomic weight: 14.0067
- Gas (colorless, odorless, tasteless, unreactive)
- Density: 1.2506 g
- Common compounds: N_2 (dinitrogen gas); NH_3 (ammonia); NH_4^+ (ammonium); NO_2^- (nitrite); NO_3^- (nitrate); $R\text{-}NH_2$ or R-NH-R (amino nitrogen)

Atmospheric Nitrogen (N_2 gas)

- Comprises 78.08% of atmosphere
- It is greatest contributor of atmospheric gases to atmospheric pressure.
- It has a higher equilibrium concentration in water than do the other atmospheric gases.
- It is basically inert. But, some microorganisms can convert N_2 to ammonia, and it can be converted to ammonia by an industrial process.

Physiological and Nutritional Role of Nitrogen

- Component of protein. Tissues contain protein.
- Proteins in nucleic acids.
- Enzymes are protein.
- N is a component of heme (in hemoglobin).
- N is in chlorophyll.
- Ammonia is major nitrogenous waste of aquatic animals.

Role of Nitrogen in Ecological Systems

- Nutrient for plants. Nitrogen and phosphorus usually considered the nutrients most likely to limit plant growth.
- It is a component of amino acids which make up protein.
- Plant proteins are sources of amino acids for animals.
- Nitrogen is important in microbial decomposition of organic matter.
- There is a global N cycle.

Nitrogen

- Nitrogen can be either a gas or a solid. Also may be liquified for some agricultural and industrial uses.

- Nitrogen has nine possible valence states (-3 to +5).

- Thus, it is involved in many oxidation-reduction processes many of which are mediated by microorganisms.

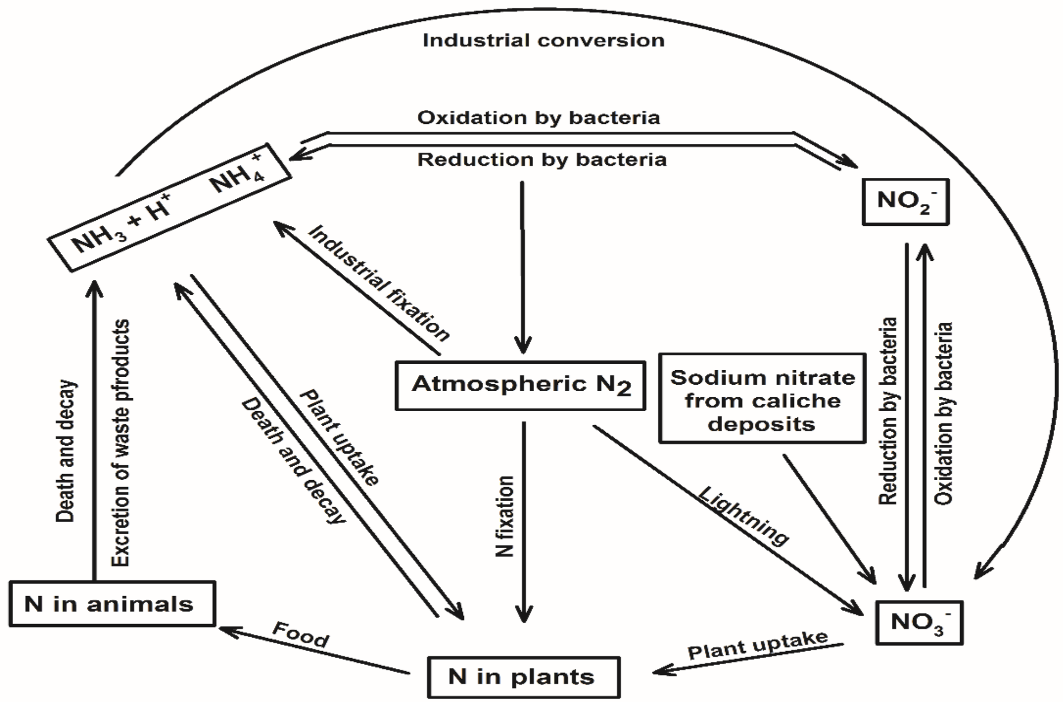
Valence states of forms of nitrogen.		
Compound or ion	Formula	Valence
Amino nitrogen	$R - NH_2$ or $R - NH - R$	-3
Ammonia (gas) and ammonium	NH_3 and NH_4^+	-3
Hydrazine	N_2H_4	-2
Hydroxylamine	H_2NOH	-1
Nitrogen (gas)	N_2	0
Nitrous oxide (gas)	N_2O	+1
Nitric oxide (gas)	NO	+2
Nitrite	NO_2^-	+3
Nitrogen dioxide (gas)	NO_2	+4
Nitrate	NO_3^-	+5

R = organic moiety

Importance of Nitrogen in Aquaculture

- Pond fertilizer
- Indicator of protein concentration in feed
- A potential toxin
- An indicator of rate of degradation of organic matter
- Indicator of food value of fisheries products

Global Nitrogen Cycle



The global N cycle is essentially reproduced in miniature in aquaculture ponds.

Nitrogen (N_2) solubility at equilibrium at 760 mm Hg (MSL pressure) in freshwater.			
°C	mg/L	°C	mg/L
0	23.04	25	13.64
5	20.33	30	12.58
10	18.14	35	11.68
15	16.36	40	10.89
20	14.88		

Solubility of N_2, O_2, and CO_2 in freshwater.

	Atmospheric concentration (%)	Equilibrium solubility at 25°C, MSL (mg/L)	Bunsen absorption coefficient for pure gas (mg/ L·atm)	Solubility relative to N_2
N_2	70.08	13.80	0.02401	---
O_2	20.95	8.26	0.05358	2.23X
CO_2	0.04	0.56	0.7562	31.5X

Nitrogen is the least soluble. It has greatest concentration, because of its high volume percentage of atmospheric gases.

Nitrogen Fixation

- Certain species of bacteria and blue-green algae can remove N_2 from water, convert it to ammonia nitrogen, and use ammonia in amino acid synthesis:

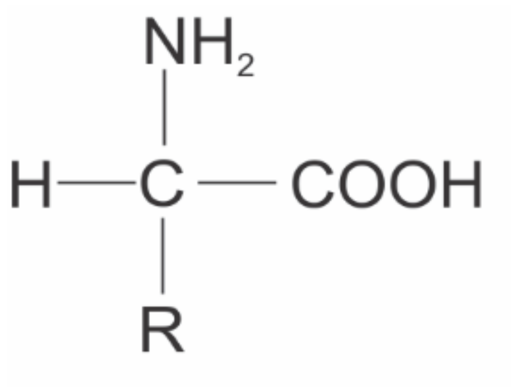
$$N_2 + 8H^+ + 8e^- + 16\ ATP \xrightarrow{Fe,\ Mo} 2NH_4^+ + 16ADP + 16\ PO_4^{3-}$$

Intermediate CH_2O compounds + $NH_4^+ \rightarrow$ amino acids

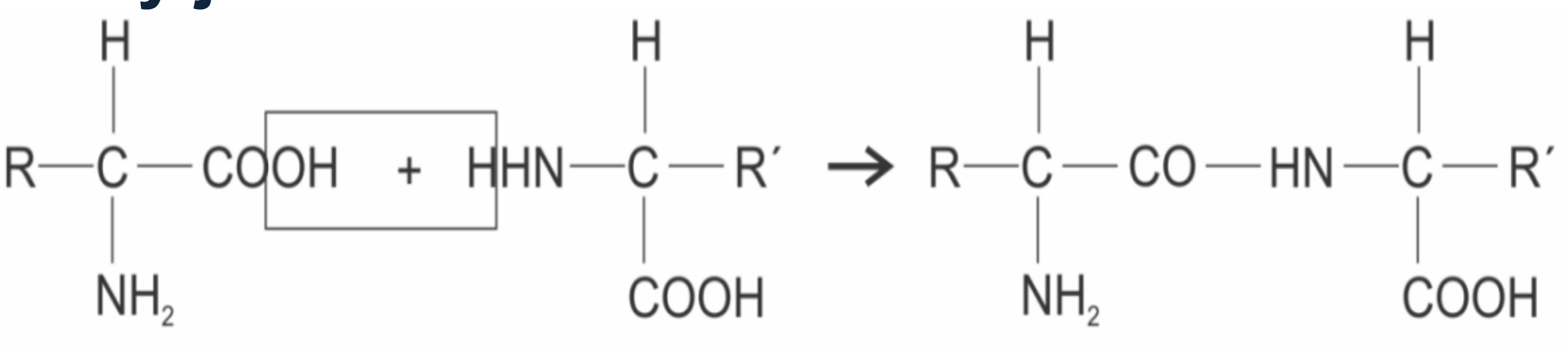
- Rates: 1-10 kg N/ha/yr. Rather small.

- Relationship to P: greater N/P ratio minimizes N_2 fixation. Reported to stop at N/P>13.

Amino Acids – There are about 20 kinds



They join as follows:



Dipeptides combine to make large polypeptides (proteins).

Nitrogen Fertilizer Production

Industrial fixation (Haber-Bosch process)

$$N_2 + 3H_2 = 2NH_3$$

Ammonia used to make fertilizers:

- $2NH_3 + CO_2 \rightarrow CO(NH_2)_2 + H_2O$
- $2NH_3 + H_2SO_4 \rightarrow (NH_4)_2SO_4$
- $4NH_3 + 5O_2 \rightarrow 4NO + 6H_2O$

  $2NO + O_2 \rightarrow 2NO_2$

  $3NO_2 + H_2O \rightarrow 2HNO_3 + NO$
- $HNO_3 + NH_3 \rightarrow NH_4NO_3$

Nitrogen Use by Aquatic Plants

- NO_3^- can be used, but it must be reduced to ammonia N through the nitrate reduction pathway to be useful to plants.

$$NO_3 \rightarrow NO_2 \rightarrow H_2N_2O_2 \rightarrow NH_2OH \rightarrow NH_3$$

$$CH_2O \text{ compounds} + NH_3 \rightarrow \text{amino acids}$$

- Some diatom species prefer NO_3^-.

- NH_4^+ preferred by most species, because it can be used directly in amino acid synthesis.

- Aquatic macrophytes are 1-4% N (dry weight basis); phytoplankton are 5-10% N (dry weight basis)

Typical Fate of Organic Matter during Decomposition in Soil

- Start with 100 g organic C in organic matter. After a period of 6-18 months, the following could be expected:

  3-8 g C in bacterial biomass

  60-80 g C released as carbon dioxide

  13-38 g C in organic matter continuing to decompose

- This suggests a conversion of new bacterial cells of 3.4% [(3 g bacteria/87 g organic matter decomposed) × 100] to 12.9% [(8 g bacteria/62 g organic matter decomposed) × 100)].

Effect of C/N Ratio on Organic Matter Decomposition

- **Bacteria: C/N ratio ≈5:1.**
- **Organic matter for fertilizer: C/N ratio 20:1-100:1.**
- **As bacteria degrade organic matter, they oxidize organic C to CO_2, but tend to retain N in microbial biomass.**
- **The C/N ratio of the decomposing organic matter declines – typically 8:1-12:1 in pond soil.**

Microbial Growth Efficiency (MGE)

- MGE is a ratio: Bacterial C/substrate C
- Summary of MGE estimates (Six et al. 2006. Soil Sci. Soc. Amer. J.) follows:
- 0.1 to 0.85 (ave. = 0.42 in laboratory studies)
- 0.14 to 0.77 (ave. = 0.53 in terrestrial soils)
- 0.01 to 0.70 (ave. = 0.33 in aquatic environments)

C, N, and MGE (mineralization of N)

- Bacteria are about 10% nitrogen and 50% carbon on dry matter basis.

- Suppose organic residue is 2.0% N and 40% C (dry matter basis) and it is entirely decomposed.

- 100 g residue contains = 40 g C and 2g N

- Assuming bacteria have MGE of 0.10:

  40 g C × 0.1 = 4 g bacterial C = 8 g bacteria

  8 g × 0.1 = 0.8 g N in bacteria

  2 g N in residue – 0.8 g N in bacteria = 1.2 g N released into water (mineralization).

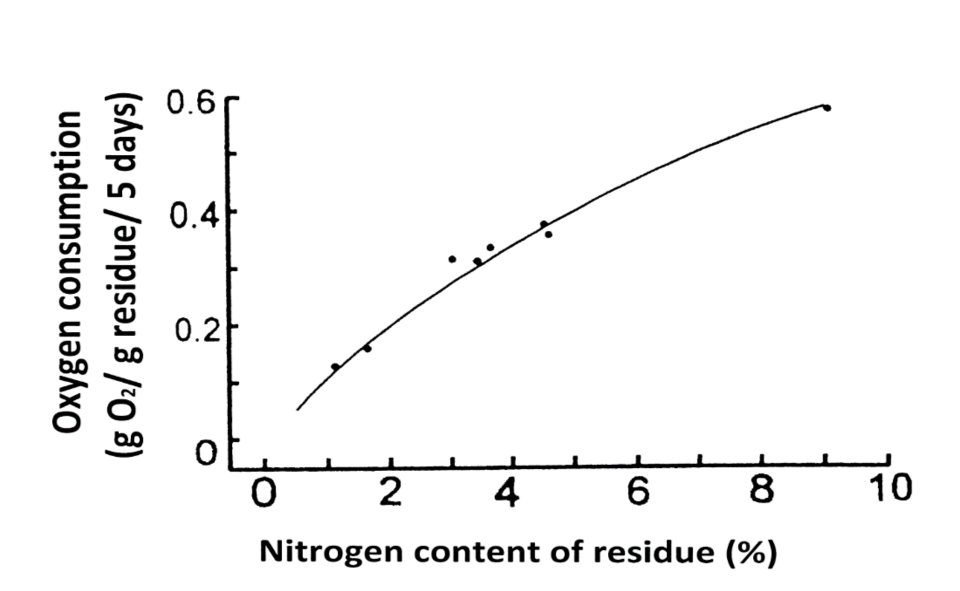
C, N, and MGE (immobilization of N)

- Residue with 42% C and 0.5% N
- 100 g residue contains 42 g C and 0.5 g N
- MGE of 0.1:

  42 g C $\times$ 0.1 = 4 g bacterial C = 8.4 g bacteria

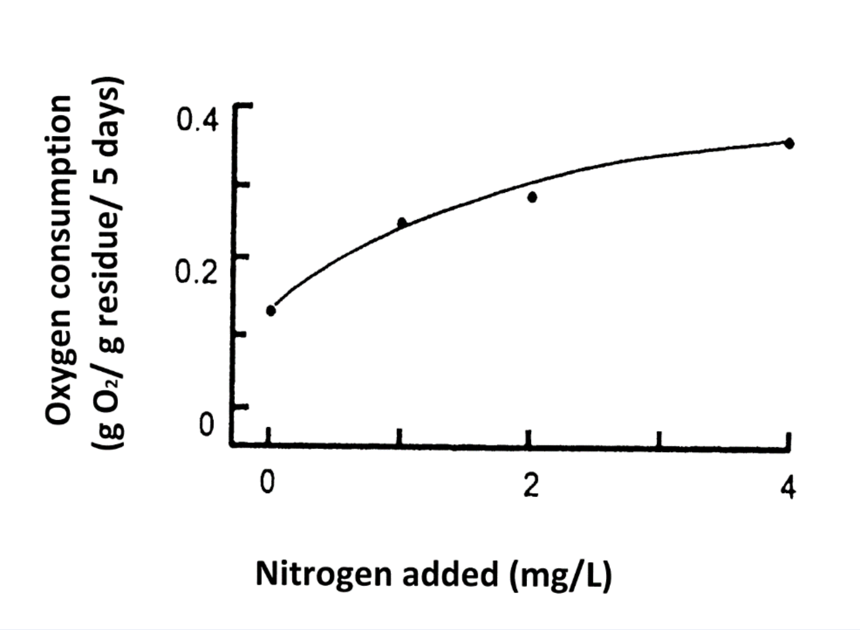
  8.4 g $\times$ 0.1 = 0.84 g N in bacteria

  Only 0.5 g N in residue
- Decomposition slow because N must be recycled.
- Bacteria can remove ammonia or nitrate from water for use in decomposition (immobilization).



Effect of nitrogen content of aquatic plant residues on their rate of decomposition, as evident from oxygen consumption in the culture medium.

Effect of dissolved inorganic nitrogen concentration in the culture medium on the decomposition of aquatic plant residue with a 1% nitrogen concentration.



Nitrification

Carried out by nitrifying bacteria.

Two-step process:

(1) $NH_4 + 1\frac{1}{2}O_2 \xrightarrow[\text{Nitrosomonas}]{} NO_2^- + 2H^+ + H_2O$

(2) $NO_2^- + \frac{1}{2}O_2 \xrightarrow[\text{Nitrobacter}]{} NO_3^-$

Summary: $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$

The energy is used by the bacteria to reduce CO_2 to organic C.

Nitrification and Water Quality

- **The process oxidizes NH_4^+ to NO_3^- to lower ammonia concentration.**

- **The process $NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$ demands oxygen and produces acidity:**

Denitrification

- Anaerobic process in which bacteria use oxygen from nitrate to oxidize organic carbon in respiration. The nitrogen in nitrate is converted usually to N_2 (recycled).

- Example:

$$6NO_3^- + 5CH_3OH \rightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH^-$$

- Other pathways also. Denitrification can release NH_3 or N_2O, but N_2 is common end product.

Pond Fertilization

- Used in extensive aquaculture to increase phytoplankton productivity the base of food web for fish and shrimp.

- Used in ponds to start phytoplankton bloom before stocking culture species.

- Sometimes used until feeding rate is sufficient to maintain bloom.

Nitrogen concentration and potential acidity of common nitrogen fertilizers.		
Fertilizer	Nitrogen (%)	Potential acidity (kg calcium carbonate/100 kg fertilizer)
Urea	45	161
Ammonium nitrate	34	118
Ammonium sulfate	20	151
Diammonium phosphate	18	97
Ammonium polyphosphate	13	72
Monoammonium phosphate	11	79

Average composition of livestock manures and plant wastes.

	Concentration (% as is basis)		
Organic matter	Dry matter	N	P
Swine	30.8	0.93	0.49
Dairy	24.1	0.72	0.20
Beef	31.4	0.92	0.33
Horse	37.4	0.50	0.15
Sheep	32.2	0.87	0.34
Chicken	60.6	2.71	1.32
Chicken litter	30.8	3.10	1.31
Mixed grass	22.0	0.80	0.08
Processing waste from plant crops	18.0	0.26	0.09

Pond Fertilizer Rates

- Common commercial fertilizers usually are applied to ponds at 5-15 kg N/ha/application. Applications usually are made at 1- to 2-week intervals.

- Organic fertilizers often are applied in amounts averaging 50-150 kg dry organic matter per hectare per day. Organic fertilizers vary from around 0.5 to 3.1% in nitrogen concentration. Weekly rates are typically 5-30 kg/ha/week.

Organic Fertilizers, Urea, and Ammonium Fertilizers are Acidic

Organic matter $\rightarrow CO_2 + NH_3$

$CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3$

$(NH_4)_2SO_4 \rightarrow 2NH_4^+$

The ammonia nitrogen is nitrified to nitrate by nitrifying bacteria.

Typical Recovery of Fertilizer N in fish or Shrimp Biomass

- 2,000 kg fish; 150 kg N in fertilizer during crop period.

- Fish contain about 56 kg N.

- Efficiency of recovery is $56/150 \times 100 =$ 37.3%.

Nitrogen in Feed

- **Nitrogen in feed is from plant and animal meals and in organic form.**
- **These are high quality products that are easily digested by culture animals and uneaten feed and feces decompose readily. Both actions release NH_3.**
- **C/N ratio usually about 6-9.**
- **More N released to water in NH_3 than recovered in fish or shrimp biomass.**

Nitrogen Efficiency of Feed

FNE = feed nitrogen efficiency (%)

FCR = feed conversion ratio (%)

$\%N_a$ = percentage nitrogen in live animals

$\%N_r$ = percentage nitrogen in ration (feed)

Example: Shrimp: 2.8% N

Feed: 6% N; FCR = 1.3

FNE% = 2.8% N/(1.3)(6% N) = 35.9%.

Nitrogen content of whole fish and shrimp.

Species	% N (live weight)
Litopenaeus vannamei (white-leg shrimp)	2.86
L. Stylirostris (blue shrimp)	2.75
Penaeus monodon (black tiger shrimp)	3.08
Oreochromis aureus (blue tilapia)	2.22
O. Niloticus (Nile tilapia)	2.25
Ictalurus punctatus (channel catfish)	2.38
Oncorhynchus mykiss (rainbow trout)	2.50
Salmo salar (Atlantic salmon)	2.96
Lates calcarifer (Barramandi)	2.91
Pangasius bocourti (Vietnamese catfish)	2.75
Catla catla (Indian carp)	2.59
Labeo rohita (rohu)	2.54
Cirrhinus mrigala (white carp)	2.48
Clarius batrachus (African catfish)	2.62
Cynprinus carpio (common carp)	2.75

Nitrogen Waste from Feed

- **The percentage nitrogen waste is 100% – % FNE, e.g., %FNE = 35.9%, then 64.1% of feed nitrogen becomes waste – mainly NH_3.**

- **In above example, 1,000 kg feed containing 6% N (total of 60 kg N) will result in 21.54 kg N in shrimp and 38.46 kg NH_3-N.**

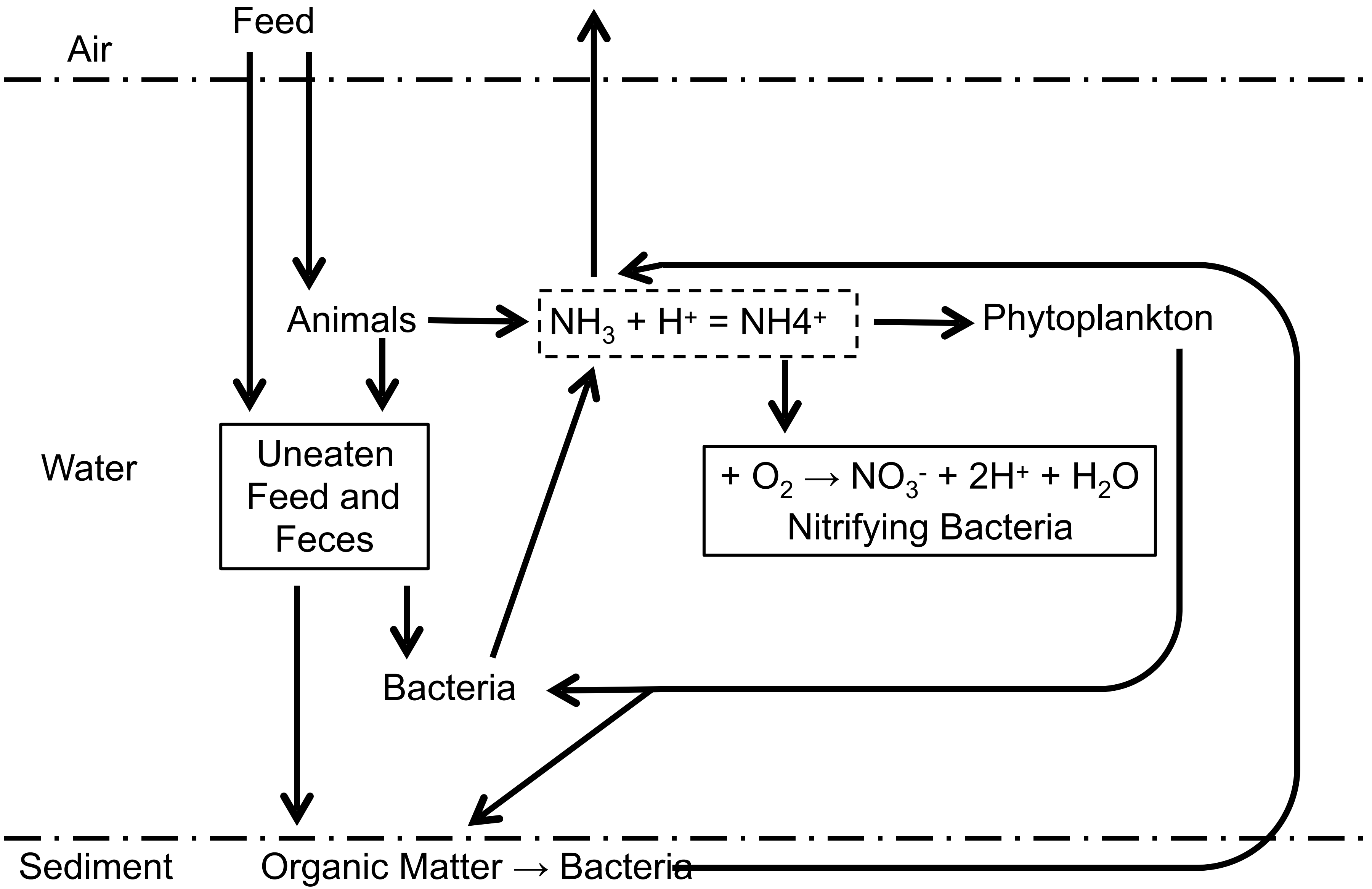
- **Alternatively, 1,000 kg shrimp requires 1,300 kg feed containing 78 kg N – 28 kg N in shrimp and 50 kg in NH_3-N (50 kg N waste/t shrimp).**

- **Nitrogen enters culture systems in source water, fertilizers, and feeds. It also is fixed by nitrogen-fixing microorganisms.**

- **Considerable ammonia usually is nitrified.**

- **Nitrogen is lost from culture systems in effluent, harvested animals, ammonia diffusion, denitrification, and seepage.**

- **The important issue is the efficiency of nitrogen use, and factors controlling toxic forms (NH_3 and NO_2^-).**


Feed
Air
Animals
$NH_3 + H^+ = NH4^+$
Phytoplankton
Water
Uneaten Feed and Feces
$+ O_2 \rightarrow NO_3^- + 2H^+ + H_2O$
Nitrifying Bacteria
Bacteria
Sediment
Organic Matter → Bacteria

Feed Is Acidic Because of Nitrification

Use feed with 6% N; 1 kg contains 0.06 kg N; 20-40% included in harvest biomass.

Waste load: 0.036-0.048 kg N enters water as ammonia nitrogen.

Acidification potential:

0.036 kg N/kg feed $\times$ 7.14 kg $CaCO_3$/kg N =

0.26 kg $CaCO_3$/kg feed

$0.048 \times 7.14 = 0.34$ kg $CaCO_3$/kg feed.

Toxic Ammonia

- The NH_3 form is toxic; NH_4^+ is not toxic at concentrations in aquaculture systems.

- The proportion of NH_3 relative to the total ammonia nitrogen (TAN) concentration depends on temperature and especially pH.

$$NH_3 + H_2O = NH_4^+ + OH^- \qquad K = 10^{-4.74}$$

- Ammonia toxicity is more likely at elevated pH.

Decimal fractions (proportions) of total ammonia existing as un-ionized ammonia in freshwater at various pH values and temperatures.

pH	Temperature (°C)						
	20	22	24	26	28	30	32
7.0	0.004	0.004	0.005	0.006	0.007	0.008	0.009
7.4	0.009	0.011	0.013	0.015	0.017	0.020	0.023
7.8	0.024	0.028	0.032	0.036	0.042	0.048	0.057
8.2	0.059	0.067	0.076	0.087	0.100	0.114	0.132
8.6	0.136	0.154	0.172	0.194	0.218	0.244	0.276
9.0	0.284	0.313	0.344	0.377	0.412	0.448	0.490
9.4	0.500	0.534	0.568	0.603	0.637	0.671	0.707

Examples of 96-hour LC50s for NH3-N to common aquaculture species.	
Species	96-hour LC50
Freshwater	
Channel catfish	0.74-3.10
Tilapia	2.88
Rainbow trout	0.32-0.93
Fathead minnows	0.20-3.4
Marine	
Striped bass	0.64-1.10
Spotted sea trout	1.72
Pacific white shrimp	1.20-2.95
Black tiger prawns	1.04-1.69

- **The 96-hr LC50 for NH_3 to fish and shrimp varies from <0.3 mg/L to >3 mg/L depending upon species. Coldwater species usually have 96-hr LC50 <1 mg/L.**

- **A factor of 0.05 $\times$ 96 hr LC50 often is considered a safe concentration for long-term exposure. Thus, maybe 0.01-0.015 mg/L is safe for most coldwater species and 0.02-0.03 mg/L is safe for most warmwater species.**

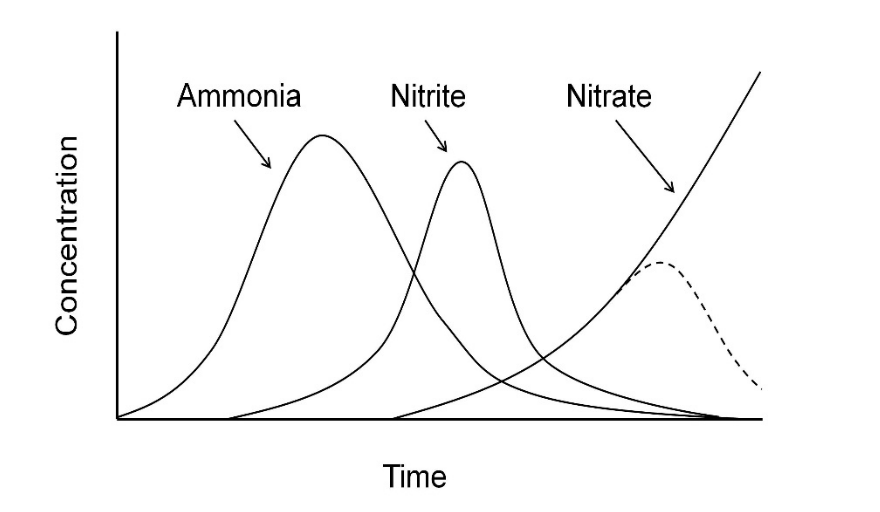
- **The pH often fluctuates in aquaculture systems making it difficult to estimate a reliable maximum acceptable NH_3 or TAN concentration.**

Ammonia Management

- Use ammonium fertilizers and urea conservatively.
- Strive to minimize FCR.
- Maintain adequate DO concentration – this encourages nitrification.
- Maintain adequate phytoplankton bloom.
- Lime low alkalinity water – pH buffer.
- Remove dead fish.
- Zeolite ineffective.

Source of Nitrite

- **Nitrite concentration may increase because of a lag between ammonia oxidation and nitrite oxidation in nitrification.**



- **Also, nitrite can enter water from denitrification under certain conditions.**

Published 96-hour LC50s for nitrite-nitrogen in several species of aquatic animals.	
Species	**96-hour LC50**
Freshwater	
Common carp	88.0 mg/L
Catla	117.0 mg/L
Freshwater prawn	8.6 mg/L
Channel catfish	7.1-44.0 mg/L
Fathead minnow	45.0-70.0 mg/L
Blue tilapia	16.0 mg/L
Rainbow trout	0.24-11.0 mg/L
Marine	
Mud crab	41.6-69.9 mg/L
Sea bass	154.0-274.0 mg/l
Pacific white shrimp	9.0-332.0 mg/L
Black tiger prawns	13.6 mg/L
Sea trout	980.0 mg/L

Nitrite Management

- Use feed with no more crude protein than necessary, and feed conservatively to obtain good FCR.
- Maintain a chloride concentration 20 times greater than NO_2^--N concentration by NaCl (sodium chloride) treatment.
- Maintain DO concentration by applying adequate aeration.

Carbohydrate Additions in Biofloc Systems

J. Hargreaves:

- **Ideal C/N = 12:1 to 15:1**
- **Add 0.5-1.0 kg sugar or other carbohydrate source for each kg feed**

Calculation:

35% CP (5.6% N); 400 kg/day; 10,000 m^3, FCR = 1.3; ammonia N load = 14 kg (1.4 mg/L)

1.4 mg/L N ÷ 0.1 mg N/mg bacteria = 14 mg/L bacteria

14 mg/L bacteria ÷ 0.5 mg C.mg bacteria = 7 mg/L bacterial C.

Carbohydrate Additions in Biofloc Systems (continued)

Pure sugar ($C_6H_{12}O_6$) is 40% C.

Assuming MGE = 0.5, organic C requirement is 14 mg/L (7 mg/L bacterial C ÷ 0.5 MGE).

Sugar is 40% C, so 35 mg/L/day of sugar required (14 mg/L C ÷ 0.4 mg C/mg sugar).

350 kg sugar (40% C) + 400 kg feed (42% C; 5.6% N results in C/N = 13.81 (Hargreaves: 12:1-15:1).

Sugar input of 0.875 kg sugar/kg feed (Hargreaves: 0.5-1.0).

Conversion of protein by five aquaculture species groups. The feed conversion ratio (FCR) is based on results with high quality feed and good feed management.

	FCR[a]	Feed crude protein (%)[a]	Dressout as fillets (%)[b]	Crude protein (%)			Protein recovery (%)	
				Fillet[c]	Whole fish[a]		Fillet	Whole
Catfish	1.8	32	45	15.55	14.9		12.1	25.9
Tilapia	1.7	32	35	18.50	14.0		11.9	25.7
Shrimp	1.5	38	37	20.31	17.8		13.0	31.2
Trout	1.2	45	69	20.87	15.6		26.7	28.9
Salmon	1.0	42	72	19.90	18.5		33.3	43.0

Terminology and Conversions

Ammonia (NH_3); Ammonia-nitrogen (NH_3-N); Ammonium (NH_4^+); Ammonium nitrogen (NH_4^+-N); Total ammonia nitrogen (NH_3-N + NH_4-N); Nitrite (NO_2^-); Nitrite-nitrogen (NO_2-N);Nitrate (NO_3^-); Nitrate-nitrogen (NO_3—N).

$$\left(\begin{array}{l} NH_2\text{-}N = NH_3 \times 0.823;\ NH_4^+\text{-}N = NH_4 \times 0.778; \\ NO_2\text{-}N = NO_2^- \times 0.304;\ NO_3\text{-}N = NO_3^- \times 0.226. \end{array} \right)$$

% Organic N $\times$ 6.25 = % crude protein.