THE REVIEW OF THE LITERATURE
2. THE REVIEW OF THE LITERATURE

It has been fairly well established that the intensification of fish culture through the use of high stocking and feeding rates can lead to severe water quality problems and major microbial changes. The review of the literature on the subject revealed a lack of balance in the volume of published work between the tropical and temperate systems, though the former was in more favour for aquafarming because of high temperatures and relatively little seasonal variation in temperature or daylength compared with temperate regions. Practically there is little information about the microbiological changes and water quality as affected by the farming of air-breathing catfish and herbivorous carps under high density culture systems.

2.1 HETEROTROPHIC ACTIVITY

Heterotrophic bacteria plays an important role in altering water quality and leads to the transformation of organic matter in the water column in an intensively cultivated fish pond. Tanaka et al., (1977) found the population density of heterotrophic bacteria in Lake Biwa to increase up to a certain limit in parallel with the concentration of organic matter in water. Schroeder (1978)
deduced the same results in the intensive fish ponds where the activity of heterotrophic organisms lead to the transformation of organic matter in the water column, thus playing an important role in affecting the water quality. The microbial density and heterotrophic activity were about 10 to 50 times higher at the sediment water interface (Rimon and Shilo, 1982). Furthermore, the heterotrophic bacteria adhering to suspended debris particles may be of direct importance as components of food for the fish (Martyshev, 1983).

2.2 NITROGEN FIXATION

Nitrogen fixation in aquatic environments is one of the important process of nitrogen cycle since the process is responsible for the recovery of N loss caused by denitrification (Kawai et al., 1971).

The fixation of molecular nitrogen in aquatic environment is brought about by many bacteria and cyanobacteria. Bacterial nitrogen fixation is usually attributed to the aerobic Azotobacters (Azotobacter agilis, A. chroococcum), facultatively anaerobic Klebsiellas (Klebsiella pneumoniae), facultatively anaerobic Bacilli group (Bacillus polymyxa and B. macerans), most of the anaerobic saccharolytic

In some Polish lakes, Niewolak (1970) observed maximum number of N-fixing bacteria in bottom sediments of muddy type than were in sandy bottom deposits. Dugdale and Dugdale (1962), Goering and Neess (1964) observed the seasonal pattern of N-fixation in temperate lake and showed N-fixation to be primarily a late summer phenomenon while Kuznetsov (1970) found Clostridium to increase in spring decreased to a low value in summer and increased again in autumn in an eutrophic lake. Similar observations were also made by Sysueva (1963), Niewolak (1970, 1972) and Simic (1975) and Jones (1982). According to Brezonik (1972), seasonal cycles are less pronounced in tropical and sub-tropical lakes and N-fixation is quite possible throughout the year. While studying the seasonal distribution pattern of nitrogen fixing bacteria in fish ponds Jana and Roy (1986) showed distinct peak in summer and ebb in winter.

Hutchinson (1941) recognized the role of blue-green
algae in N-fixation of aquatic environments, and the first quantitative in situ measurement of N-fixation rates was provided by Dugdale et al., (1959). Since then N-fixation rates have frequently been measured with $^{15}\text{N}$ technique in a variety of aquatic environments (Dugdale and Dugdale, 1962; Neess et al., 1962; Goering and Neess, 1964; Dugdale et al., 1964; Stewart, 1965 and Billaud, 1968). Rates of nitrogen fixation in natural lakes range from 0 to 0.125 mg/l per day (Dugdale and Dugdale, 1962). Nitrogen fixation rates are usually higher in eutrophic waters than in oligotrophic ones (Rusness and Burris, 1970; Horne and Fogg, 1970). Using $^{15}\text{NO}_3$ tracer technique Chen et al., (1972b) showed that the amount of N-fixation in sediment was significant ranging from 40 to 70% of the added $^{15}\text{NO}_3$. Hardy et al., (1973) has given an excellent comparative review of measurements of in situ N-fixation rates of lakes. A number of investigators (Billaud, 1968; Kuznetsov, 1968; Dugdale, 1969; Jones and Stewart, 1969; Stewart, 1968 and 1970 and Fogg, 1971a and b) found a good correlation between the occurrence of N-fixation in open water lakes and the presence of blue-green algae that possess heterocysts.

According to Fogg (1971a) and Fogg et al., (1973), rates of nitrogen fixation are generally inversely related to the concentration of inorganic nitrogen because both
nitrate and ammonia are known to suppress the synthesis of nitrogenase, the key enzyme in nitrogen fixation. In catfish ponds which usually contain high concentrations of inorganic nitrogen, rates of nitrogen fixation would be expected to be low. Unfortunately, no studies have been conducted to quantify rates of nitrogen fixation in catfish ponds (Tucker and Boyd, 1985). The same is true for tropical fish ponds with carps or catfish culture.

2.3 PROTEIN MINERALIZATION AND AMMONIFICATION

Hutchinson (1957) pointed out that some methylamines produced by algae in fresh waters may be decomposed by bacterial action resulting in ammonia formation. Tsuru et al. (1970) reported that amino acids are deaminized with the liberation of ammonia in the following way:

Proteins + H₂O = amino acids; Amino acids + H₂O = ammonia + carbon residue.

Ram et al. (1980) reported ammonia to be produced by bacterial ammonification process of the organic nitrogenous matter in the pond water under aerobic conditions in the water column, as well as anaerobically in the sediment. Kruger (1978) suggested that the decomposition of protein was initiated by proteolytic bacteria hydrolysing proteins to amino acids which,
in turn, acted by ammonifying bacteria liberate ammonia or ammonium sulphate. According to Jones and Simon (1960), release of ammonia into the water column may be divided into two phases, the initial phase when nitrate is still present in the overlying water column, and a second phase when the nitrate has been utilized.

A number of microorganisms (Escherichia coli, Proteus vulgaris, Bacillus subtilis, Aerobacter cloacai, Pseudomonas sp., and Flavobacterium sp.) are capable of hydrolizing proteins into simpler compounds such as peptides, urea, amino acids etc., which, in turn, are metabolized by ammonifying bacteria to liberate ammonia or ammonium sulphate (Morris and Koffron, 1967; Grant and Patel, 1969; Little et al., 1969; Kruger, 1978). Even some bacteria capable of carrying out denitrification (Achromobacter sp. and Micrococcus sp.) can actively take part in proteolysis (Alexander, 1971). Of the total 169 bacterial strains 68 strains were found to utilize 41 organic compounds as the sole carbon and energy source (Sepers, 1981).

2.3.1 Measurement

A number of techniques have been developed to measure rates of ammonification. Dugdale and Dugdale (1965), using
the isotope dilution method, found rapid ammonification of 25 µg N/l/hr in Smith Lake. Zilversmit et al. (1943) and Brezonik (1968) observed that decrease in $^{15}$N enrichment in the ammonia can be quantified and related to the rate of ammonification. Roy (1983) found the rates of ammonification to vary from 1.18 to 1.33 mg l$^{-1}$ (72 h)$^{-1}$ in some Indian fish farming ponds.

Maximal concentration of ammonia was found in autumn in some lakes (Yoshimura, 1932) while minimal in spring in others (Hutchinson, 1957). Niewolak (1965), on the other hand, reported maximal values of ammonification in summer and early autumn with a decline in winter. Further, Niewolak et al. (1978) observed in some Polish Lake, the occurrence of maximum rates of potential ammonification during May through July. Rheinheimer (1959, 1965), on the other side, reported most intense ammonification accompanied by greater abundance of proteolytic bacteria in the winter than in summer. Despite a large number of investigations on the rates of ammonification and the abundance of bacteria in a temperate water bodies, such information are extremely meagre with respect to tropical waters especially in intensive culture system where fish production is often limited by the surplus of nitrogen.
2.4 NITRIFICATION

Nitrification is best defined as the biological conversion of organic and inorganic nitrogenous compounds from a reduced to a more oxidized state (Alexander, 1965). A number of micro-organisms are found to be responsible for the nitrification process. As early as 1890 Winogradsky showed that these nitrifying bacteria were able to derive their energy from inorganic compounds. These bacteria are generally regarded as mesophilic type with a wide range of temperature tolerance of 1 to 37°C (Frederick, 1956), and grow optimally at a pH near neutrality.

Alexander (1965) stated that the nitrifying bacteria capable of the oxidation of ammonia to nitrite are largely confined to *Nitrosomonas* (Nitrobacteriaceae, Order-Pseudomonadales), although several other genera such as *Nitrosococcus*, *Nitrosospira* and *Nitrosolobus* are also able to carry out this process. Painter (1970) proposed nitrification to be performed by two groups of autotrophic bacteria. The first group, *Nitrosomonas*, oxidize $\text{NH}_4^+$ to $\text{NO}_2^-$, whereas the other, *Nitrobacter*, oxidize $\text{NO}_2^-$ to $\text{NO}_3^-$. Certain heterotrophic bacteria are also able to oxidize $\text{N}$ but they probably play a less important role in lakes. The autotrophic bacteria, besides, $\text{NH}_4^+$ or $\text{NO}_2^-$, require inorganic $\text{C}$ and a minimum
concentration of 0.5 mg O$_2$ l$^{-1}$ to perform the oxidations.
Furthermore, for optimal activity micronutrients such as Mg, Fe and Cu (Painter, 1975) and a weak alkaline pH (Wong-Chong and Loehr, 1978) are necessary.

Some workers (Silver, 1961; Funk and Kruhwich, 1964; Ghosh, 1967; Morrill and Dawson, 1967; Sommers and Haris, 1968 and Tsien et al., 1968; Jones, 1982) have isolated Nitrosomonas and Nitrobacter as agents responsible for two nitrification process. Clark and Schmidt (1967a and 1967b) and Hooper (1969) confirmed the lack of heterotrophic properties in Nitrosomonas europaea. A large number of microorganisms such as Nitrosocystis oceanus, Nitrobacter and Nitrocytis (Williams and Watson, 1968) and Nitrosococcus, Nitrosocystis, Nitrosoqloea, Nitrosomonas, Nitrosospira and Nitrobacter agilis (Webb and Wiebe, 1975) are capable of nitrification. Niewolak (1970) obtained 750 strains of bacteria from Ilawa Lake, Poland.

2.4.1 Heterotrophic Nitrification

Numerous heterotrophs including bacteria, algae and fungi are known to oxidize nitrogen compounds. Cutler and Mukherji (1931) first demonstrated heterotrophic nitrification and observed that several bacteria generated small
amount of nitrite from ammonium salt in media low in sugar. A large number of heterotrophic nitrifying organisms, *Pseudomonas* sp., *Corynebacterium simplex*, *Nocardia* sp., *Aspergillus* sp., *Streptomycetes* sp., *Mycobacterium rubrum*, *Bacillus* sp., *Vibrio* sp. isolated by Delwiche (1965) and Alexander (1978) were found responsible for nitrification. The rate of nitrogen oxidation by these heterotrophic microorganisms is generally accepted as negligible in nature compared to the autotrophs. Keeney (1972) concluded that the importance of heterotrophic nitrification cannot be assayed until more definitive data were available. For example, nitrification occurs readily in acid soils below the pH normally considered essential for the autotrophs, which would indicate fungi might be important in acid environment.

2.4.2 Measurement of Nitrification

Some workers (Neass et al., 1962; Bremner, 1965; Brezonik, 1968 and Billen, 1976) have measured the rate of nitrification in natural water using tracer technique. The rates of nitrification were highly variable both spatially and seasonally. In Lake Mendota, the rates were very low in surface water during summer (0.07 µg N/l/hr) but increased with depth in spring (0.62 µg N/l/hr).
(Brezonik, 1968). The rates varied from 100 μg N/l/hr at 10°C to 270 μg N/l/hr at 25°C in some Wisconsin Lakes (Chen et al., 1972a). Henriksen et al., (1981) determined the nitrification rate in different sediment from Danish Lakes and the rates were found to vary from 0.3 to 1.4 μmol NO₃-N m⁻² d⁻¹.

Nitrification process in water and surface sediments was found to be oxygen dependent (Stephenson, 1939; Porter, 1946). In quiescent lake sediments where oxygen is very low or absent nitrification was greatly reduced (Chen et al., 1972a). Vincent and Downes (1981) discussed the relative importance of benthic and planktonic nitrifiers in an oligotrophic lake; the fastest rates were recorded for planktonic nitrifiers in the epilimnion and benthic species in the superficial 2.5 mm of the sediments.

The rate of nitrification was also dependent on the trace amount of copper (Lees, 1948), TiO and ZnO (Waksman, 1952) and P, Fe, S and Mo (Aleem and Alexander, 1960 and Kiensow, 1962). Waksman (1952) found that the nitrate formation promote with the presence of CO₃ or other buffering agent and absence of large quantities of soluble organic matter. Olson (1981) observed the nitrification rate to be inhibited by light factor. Despite the importance of
nitrogen in the productivity of fish ponds, data on nitrification rates are extremely meagre for fish farming ponds.

2.5 DENITRIFYING BACTERIAL POPULATION

Denitrifying bacteria, found practically in all waters (Rheinheimer, 1980), are facultative anaerobes capable of utilizing nitrate or nitrite as hydrogen acceptor under anaerobic conditions. Nevertheless, few species occur in environments devoid of both oxygen and nitrate (Alexander, 1978). Niewolak (1972) had isolated more than 350 strains of facultative aerobic bacteria capable of denitrification from Lake Ilawa in Poland. *Pseudomonas* and *Vibrio* were common denitrifiers in three Scottish lochs (Stewart et al., 1977), while Alexander (1978) added few more genera (*Achromobacter*, *Bacillus* and *Micrococcus*) to the list of denitrifiers. According to Horsley (1979), the principal nitrate reducers were: *Pseudomonas*, *Moraxella/Acinetobacter*, *Flavobacterium/Cytophaga*, *Aeromonas* and *Enterobacteriaceae*.

Relatively little information is available on the spatial distribution of denitrifying bacteria in fish farming ponds, especially in tropical waters. Patel (1982) and Jana and Patel (1985) have, however, observed the total counts of denitrifying bacteria which ranged from 141.6 to
to $350.9 \times 10^3$ cells ml$^{-1}$ in different fish ponds.

A number of investigators (Nikitina, 1955; Niewolak, 1965, 1970, 1972) found the maximum abundance of denitrifying bacteria and most vigorous denitrification in the summer months and decline in the winter. Horsley (1979) observed the seasonal variation of nitrate reducers related to nitrate and dissolved oxygen content of lake water.

2.5.1 Denitrification and Nitrate Reduction

That the reduction of $\text{NO}_3^-$ to $\text{N}_2$ is operated in biochemical denitrification with the production of nitrous oxide ($\text{N}_2\text{O}$) as an intermediate has been confirmed by many investigators (Wijler and Delwiche, 1954; Cady and Bartholomew, 1960; Cooper and Smith, 1962; Van Cleemput et al., 1975, Cho and Sakdinan, 1978). There is evidence that *Nitrosomonas europaea* as well as several heterotrophs can produce $\text{N}_2\text{O}$ from $\text{NH}_4^-$ or $\text{NH}_2\text{OH}$ (Yoshida and Alexander, 1970). Further, Payne (1973), Haddock and Jones (1977), Coleman et al., (1978) and Cole and Brown (1980) suggested that certain heterotrophic bacteria can reduce $\text{NO}_3^-$ aerobically by assimilatory reduction, and anaerobically by either denitrification or reduction to $\text{NH}_4^+$. Anaerobically, $\text{NO}_3^-$ is reduced by *Pseudomonas denitrificans* stepwise to
NO$_2^-$, N$_2$O and ultimately N$_2$; whereas *Escherichia coli*, *Enterobacter aerogenes* and *Aeromonas hydrophila*, reduce it via NO$_2^-$ to NH$_4^+$ (Horsley *et al.*, 1982). *Pseudomonas* spp. are invariably reported as the principal nitrate-reducing organisms in aquatic systems (Horsley, 1978), but *Aeromonas* and members of the Enterobacteriaceae have been implicated as the predominant nitrate-reducing organisms in lake water (Horsley, 1979) and estuarine sediments (Cole and Brown, 1980). These heterotrophs may compete for nitrate *in vivo*, especially in river water receiving effluents from sewage treatment units. The nitrate could confer an ecological advantage and enable a group of organisms to dominate the bacterial flora.

2.5.2 Measurement of Denitrification Rates

Jannasch (1960) stated that denitrification in oxygenated water only takes place to anoxic parts of particles. It has been shown that nitrogen loss by denitrification would be larger if aeration was discontinued at the point of maximum nitrate accumulation (Chen *et al.*, 1979).

Brezonik and Lee (1968), using $^{15}$N tracer techniques, reported that in the transformation of NO$_3^-$-N in Lake Mendota, Wisconsin, about 11% of N$_2$ supplied in the annual budget
was lost by denitrification. In some Swiss Lakes the denitrification rate varied between 57% (Ahl, 1973) and 41-61% for different years (Ahlgren, 1972). Bouldin et al., (1974) found the nitrate losses ranging from 7-15% per day in six ponds at Ithaca, USA. Again, denitrification rate ranged from 0.0 - 54.0% of total N in six shallow Danish Lakes (Andersen, 1974). According to Isirimah et al., (1976), about 80% of the added $^{15}$N was denitrified with the remaining being immobilized in Lake Wingra (U.S.A.). Tiren et al., (1976), in situ measurements of denitrification, using $^{15}$N technique, found 80% to 90% of the labelled NO$_3$-N in the form of N$_2$ gas, about 4% in the ammonium fraction in the free water and less than 1% in the form of organic N. Data on denitrification rates are extremely meagre for fish ponds especially for tropical fish ponds. Recently, Patel (1982) made a detailed study on the abundance of denitrifying bacterial populations and natural and potential denitrifying activities in fish ponds managed under monoculture, polyculture and traditional systems.

A number of studies revealed that nitrification and denitrification can occur simultaneously. Patnaik (1965) noted nitrification in the oxidized surface layer with subsequent denitrification of NO$_3$-N in the reduced surface
zone of paddy soils in India. Chen et al., (1972a) have confirmed the same phenomenon in the quiescent lake sediment. Keeney et al., (1971) and Chen et al., (1972b) have further shown that assimilatory nitrate reduction and the denitrification can occur simultaneously in lake sediments. Knowles (1979) had experimentally proved that coupled nitrification and denitrification occurred due to existence of aerobic-anaerobic interface within the sediment.

Tan and Overbeck (1973) observed the seasonal and depth variation of denitrification rate in eutrophic lakes with high rates of nitrate reduction in the early summer. Niewolak (1970) observed high denitrification rate in July and August and minimal during winter in some Polish lakes. The rate of potential denitrification in Scottish lochs paralleled the seasonal trend of six Danish Lakes (Stewart et al., 1977). Measurement of denitrification rates in aquatic ecosystem has been extensively reviewed by Painter (1970), Keeney (1973), Brezonik (1975) and Kamp-Nielsen and Andersen (1977).

2.6 CHEMICAL BUDGET AND WATER QUALITY

2.6.1 Chemical Budget

In fish culture, water quality is often defined as
the suitability of water for the survival and growth of fishes, and it is normally governed by only a few variables. Information on the role of fish culture on the water quality and chemical budgets are extremely meagre for carp ponds especially in tropical conditions. Tucker and Boyd (1985) reviewed that most commercial catfish culture is conducted in ponds under essentially static conditions and at relatively high densities (8000 - 20,000 fish/ha) and fed a high quality diet at rates often exceeding 75 Kg/ha per day. Almost all feed is consumed by fish and feed conversion ratios of 1.2 - 2.0 Kg of feed to 1.0 Kg of fish are achieved (Prather and Lovell, 1974; Lovell et al., 1975). The metabolic wastes accumulating from the intensive feeding of the fish represents considerable nutrient enrichment of the water (Tucker and Boyd, 1985).

Because catfish feed is largely dry matter and catfish contain a high proportion of water, dry weight ratios for feed conversion are much larger and huge quantities of metabolic wastes reach the water. Inorganic nutrients in metabolic wastes stimulate photosynthetic production of organic matter by phytoplankton (Boyd, 1973). Since the organic matter from fish excrement and from photosynthesis stimulates respiration Boyd et al., (1979) concluded that feeding to
fish yield increases oxygen demand and causes low dissolved oxygen concentration.

Boyd (1982b, 1984) estimated water budgets for catfish ponds and with and without watersheds at Auburn, Alabama, USA. His calculations suggested that channel catfish farming consumes about 2 metres of water/ha/year. Clonts and Williams (1983) made crude estimates of the economics of water use in catfish production. Worsham (1975) determined nitrogen, phosphorus and water budgets for catfish culture in raceways. Water budgets had been calculated for catfish ponds (Boyd, 1982c), but only crude estimates are available for inputs and outputs of nitrogen, phosphorus and organic matter (Boyd, 1982b). Oxygen budgets for fish ponds have been determined for short periods (Schroeder, 1975; Boyd et al., 1978).

Boyd (1985) studied in detail the budget for organic matter, nitrogen and phosphorus in three experimental channel catfish culture ponds. He showed that the major additions of COD to the ponds were feeds (36%) and organic matter production by phytoplankton photosynthesis (62%).

Boyd (1974) measured changes in some water quality variables in nine channel catfish ponds where feeding
rates were gradually increased to a maximum of 45 Kg/ha. Concentrations of chlorophyll $a$, COD, BOD, organic nitrogen, total phosphorus, nitrate, total ammonia, and filtrable orthophosphate gradually increased during the growing season as feeding rates increased. Tucker et al., (1979) clearly demonstrated the relationships among channel catfish stocking rate, maximum daily feeding rate and water quality deteriorations.

Andrews and Matsuda (1975) showed that oxygen consumption rates of channel catfish became less as the dissolved oxygen concentration decreased. Respiratory dependence upon dissolved oxygen concentrations was evident to 7.0 mg/l, showing the 'incipient limiting level' (Basu, 1959). For the point where reduction in oxygen began to restrict the metabolic rates was above 7.0 mg/l (Tucker and Boyd, 1985). Moss and Scott (1964) reported that lean catfish consumed less oxygen than fat catfish. Prolonged exposure to sub-lethally low concentrations of dissolved oxygen is harmful to channel catfish (Andrews et al., 1973). Channel catfish in ponds reduced their food consumptions when dissolved oxygen concentrations are low (Tucker et al., 1979; Hollerman and Boyd, 1980). Swingle (1969) made a practical assessment of the dissolved oxygen requirement
for warm water pond fish. Unfortunately, no such data is available for carp culture ponds in tropical waters.

Although carbon dioxide is not appreciably toxic to most fish, it antagonizes the uptake of oxygen (Tucker and Boyd, 1985). Boyd (1979) presents a more complete discussion on the relationship among carbon dioxide, alkalinity and pH in fish ponds. Problems with pH, per se, are uncommon in most channel catfish ponds because the values were within the range desirable for fish culture. Swingle (1967) stated that waters having a pH range of 6.5 to 9.0 as recorded before day-break are most suitable for pond culture and those having pH values of more than 9.5 as unsuitable. Fish dies at about 11.0 and acid waters reduce the appetite of the fish (Jhingran, 1983).

2.7 SIGNIFICANCE OF NITROGEN IN PRODUCTIVITY OF WATER

A number of investigators (Gerloff and Skoog, 1954; 1957; Weiss, 1970) have indicated that nitrogen is the nutrient element limiting productivity in many water bodies including fish ponds. Goldman and Carter (1965) and Oglesby (1969) provided evidence of N limitation to algal growth in oligotrophic lakes. They showed that P might be more limiting to aquatic productivity in oligotrophic lakes, while
nitrogen was more significant in eutrophic lakes especially those with high content of phosphorus-P. Lueshow et al., (1970) used the nitrogen (organic and inorganic) content as a reliable index in describing the trophic status in Southern Wisconsin Lakes. Boyd (1982) pointed out that most of the nitrogen in a pond ecosystem is bound in living organism and decaying organic matter. Stewart et al., (1981) discussed the quantitative significance of nitrogen input from agriculture and urbanization in some aquatic habitats in the northern U.K.

2.8 FORMS AND AMOUNT OF NITROGEN

The dominant species of water are: NH$_4^+$-N, NO$_2^-$-N, NO$_3^-$-N, NH$_2$OH, dissolved organic nitrogen, particulate protein nitrogen, nitrogen gas, urea-N (Hutchinson, 1957; Brezonik, 1968; Packard and Dorth, 1975). Nevertheless, existence of NH$_2$OH is still uncertain (Vollenweider, 1968). A classification of lake on the basis of nitrogen levels had been proposed by Vollenweider (1968). According to him, the lakes having <200 mg inorganic-N mg/m$^3$ are considered as ultra-oligotrophic, 200-400 inorganic-N mg/m$^3$ - oligo-mesotrophic 300-650 inorganic-N mg/m$^3$ - meso-eutrophic, 500-1500 inorganic-N mg/m$^3$ - eu-polytrophic and >1500 inorganic-N mg/m$^3$ - polytrophic. Lueshow et al., (1970)
provided a similar classification but considered organic-N content of water as a better indicator to lake productivity; Keeney et al. (1970) on the other hand, found little correlation between the amount of total or organic form of N in sediments and trophic status of water bodies. They concluded, however, that the organic N in the hexoseamine decreased while the amino acid increased with increasing lake fertility. It has been further investigated that eutrophic lake sediments tended to have higher concentrations of ammonium-N in the interstitial water than did oligotrophic lakes.

Tucker and Boyd (1985) who carried out detailed investigations of the nitrogen budget in catfish culture ponds concluded that the cycling of nitrogen in catfish pond waters is basically similar to that occurring in other aquatic systems; the major difference is the role played by the fish because more than 90% of the total nitrogen that enters the catfish pond water is added in protein contained in fish feeds and is initially assimilated and transformed in ammonia by the fish. Because the rates of nitrogen fixation are generally related to the concentration of inorganic nitrogen (Fogg, 1971a) rates of nitrogen-fixation would be expected to be low in catfish ponds with high concentration of inorganic nitrogen (Tucker and Boyd, 1985).
2.8.1 Ammonia

The chief excretory product in freshwater teleosts is ammonia which is excreted primarily from their gills and forms between 80 and 98% of the amino-derived nitrogen in the excretory products of feeding fish (Black, 1957; Kleerekoper & Morgensen, 1959; Brett, 1962; Forster & Goldstein, 1969; Brafield & Solomon, 1972; Brett & Zala, 1975; Elliott, 1976; Guerin-Ancey, 1976). Urea is the major excretory product and occasionally forms a high proportion of the total nitrogenous excreta especially under hatchery conditions or in starving fish (Fromm, 1963; Burrows, 1964; Olson & Fromm, 1971; Brett & Zala, 1975). Other excretory products appearing in very small quantities in the urine are uric acid, creatine, creatinine, amines and amino-acids. Tucker and Boyd (1985) suggested the method of the amount of ammonia excretion by fish per weight protein added in feed. Lagler et al., (1962) provided evidence that fish urine accounts for about 7 to 25% of the total excretion, mainly creatinine, creatine, urea, ammonia and amino-acids. Dugdale and Goering (1967) suggested that nitrogen is excreted by aquatic animals chiefly as ammonium, free amino acids and other organic compounds which are then available for utilization by phytoplankton and bacteria. Johannes (1968) and Fenchel
(1970) indicated that nutrient release by autolysis and solubilization may account from 25 to 75% of the nutrients contained in animals. Tucker and Boyd (1985) showed that animals are quantitatively unimportant in the transformation of nitrogen in most natural waters.

Ammonia in water is present primarily as NH\textsubscript{4}\textsuperscript{+} and as un-dissociated NH\textsubscript{4}OH, the latter being highly toxic to many organisms, especially fish (Trussell, 1971). Un-ionized NH\textsubscript{3} is highly toxic to fish, but the NH\textsubscript{4}\textsuperscript{+} ion is relatively non-toxic. The proportion of the total ammonia nitrogen existing as un-ionized ammonia increases with increasing temperature and pH (Boyd, 1982a). The concentrations of various species of nitrogen in lake waters vary widely due to many transformations involved. Ammonium-N can range from 0.5 mg/l in surface water to several mg/l in bottom waters (Hutchinson, 1957; Greason and Meyers, 1969 and Armstrong et al., 1971). Un-ionized ammonia concentrations in channel catfish ponds range from 0 to 1.0 mg N/l or more. Because the concentration of un-ionized ammonia varies with pH, concentrations are usually highest in the late afternoon when pH is highest (Tucker and Boyd, 1985). The influence of pH on un-ionized ammonia concentration is greater than the effect of temperature. Tomasso et al., (1980) clearly
demonstrated the importance of pH on the toxicity of ammonia.

Acute toxic levels for total ammonia ($\text{NH}_3+\text{NH}_4^+$) vary not only between species of fish but also with changes of pH and temperature of water (Krüner and Rosenthal, 1983). According to Colt and Armstrong (1979), as the ammonia level increases in water ammonia excretion by fish decreases and levels of ammonia in blood and tissue increase. Exposure to sub-lethal concentration of ammonia probably increases susceptibility of fish diseases (Boyd, 1982). Lethal concentrations of total ammonia were found to be 4.0 mg NH$_4^+$ liter$^{-1}$ for tilapia at pH values varying between 7.3 and 7.5 and temperature above 20°C (Hora and Pillay, 1962).

The European Inland Fisheries Advisory Commission (1973) stated that toxic concentration of ammonia for short-term exposure are 0.6 and 2 mg/l of NH$_3$-N for most species. Ammonia is more toxic when dissolved oxygen concentration is low (Merkens and Downing, 1957). However, this effect is probably nullified in fish ponds since carbon dioxide concentrations are usually high when dissolved oxygen levels are low; Lloyd and Herbert (1960) showed that toxicity of ammonia decreases with increasing carbon dioxide concentration.

Poor growth of fish in culture tanks has been attributed
to the accumulation of ammonia (Smith and Piper, 1975; Andrews et al., 1971). Robinette (1976) reported that 0.12 mg/l of ammonia caused reduced growth and gill damage in channel catfish. Colt and Tchobanoglous (1978) found that un-ionized ammonia reduced the growth of juvenile channel catfish during a 31-day test. The effect was linear over the range of 0.058 - 0.99 mg/l of NH$_3$-N. A concentration of 0.52 mg/l of NH$_3$-N caused a 50% reduction in growth, and no growth occurred at 0.97 mg/l. They concluded that any measurable concentration of ammonia would adversely affect growth.

The distribution of ammonia in fresh waters varies regionally, seasonally and spatially within the lakes in relation to the level of productivity and the extent of pollution from organic matter. Pearsall (1930) and Juday et al., (1938) found no ammonia in some lakes. Batenko and Muratova (1970) stated that the distribution of all forms of nitrogen was dependent more on seasonal conditions of the year than on fertilizers added. The highest concentrations of total ammonia-N in catfish culture ponds usually occur in the autumn and winter months (Tucker, 1984). In some carp ponds, the concentration of ammonia ranged from 0.1 to 2.4 mg l$^{-1}$ with peak occurring in winter months (De, 1984).
2.8.2 Nitrite and Nitrate

Wetzel (1983) stated that nitrite levels of natural lake waters are generally very low in the range of 0 to 0.01 mg l\(^{-1}\), although up to 1.0 mg l\(^{-1}\) has been found in the interstitial waters of deep sediments of Lake Mendota (Konrad et al., 1970). Nitrite concentrations in commercial catfish ponds ranged from 0 to 4 mg/l or more (Boyd, 1982; Tucker and Schwedler, 1983). Nitrite is toxic to catfish at relatively low concentrations in waters of low chlorinity while nitrate is essentially non-toxic.

Concentrations of NO_2^-N as low as 0.5 mg l\(^{-1}\) were toxic to certain cold-water fish (Crawford and Allen, 1977). Addition of calcium (Wedemeyer and Yasutake, 1978) and chloride (Perrone and Meade, 1977; Tomasso et al., 1979) reduced the toxicity of nitrite to fish. However, the literature on the effect of nitrite toxicity in tropical fish ponds is extremely meagre. Sources of excessive nitrite in fish ponds have not been definitely identified. Hollerman and Boyd (1980) demonstrated that considerable nitrite can enter catfish pond waters from denitrification of nitrate in bottom muds. However, the common opinion is that an imbalance in nitrification reactions leads to the accumulation of nitrite.
Concentrations of NO$_3^-$-N ranged from 0 to nearly 10 mg l$^{-1}$ in unpolluted fresh waters, but are highly variable seasonally and spatially (Wetzel, 1983). The concentration of NO$_3^-$-N was somewhat higher in streams and rivers. Nitrate, in contrast to ammonia, phosphate and metal ions, moves freely through soils along with sub-surface waters. For example, if water rich in both nitrate and phosphate passes through soil, the outflowing water will become relatively richer in nitrate than phosphate (Goldman and Horne, 1983).

Nitrate is the most highly oxidized form of nitrogen and is usually the most abundant form of combined inorganic nitrogen in lakes and streams. In some carps ponds, nitrate-N concentrations of water varied from 1.3 to 1.98 mg l$^{-1}$ (De, 1984).