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-XIX Course-

WATER QUALITY CONTROL IN COASTAL AQUACULTURE SYSTEM: A TUSCANY FISH FARM AS A CASE STUDY

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1.INTRODUCTION TO THE STUDY:

1.1 Aquaculture in coastal wetland area:

Coastal ecosystems are ecotonal between marine, freshwater and terrestrial environments and may exhibit properties of these systems as well as unique characteristics of their own. Coastal water bodies are a particularly heterogeneous resource, typified by high spatial and temporal variability, which in part reflects their diverse origins. Some coastal water bodies are considered to be natural geomorphological features formed by sedimentary barriers, often shingle or sand damming river tributaries, or enclosing shallow bays or estuarine, as well as pools within natural longshore barriers (Barnes, 1989). Typically, these are known as lagoons and lie adjacent to the sea, which supplies the lagoon with saline water via percolation through the sedimentary barrier or from infiltration through a narrow channel. Coastal water bodies vary in size, depth, water dynamics (such as tidal exchange), and geomorphology. In particular, they exhibit a salinity gradient from freshwater (<0.5 g/L) to hypersalinity (>40 g/L) (Greenwood and Wood, 2003). They tend to be nutrient-rich and dynamic, subjected to extreme physical variables (e.g. storms, salinity, temperature) and more exposed to unfavourable conditions such as low oxygen content than marine or estuarine sites. Costal water bodies suffer from significant anthropogenic and natural pressures, which affects ecosystem functions and processes, and biotic communities. Threat include sea level rise and possible increases in storminess due to climate change, aggregate extraction, nutrient enrichment, succession and residential and leisure developments (Barnes, 1991; Bamber *et al.*, 2001; Sylaios and Teocharis, 2002). The wetland community is generally considered to be comprised of opportunistic and species from freshwater, marine and brackish sources (Barnes, 1988 and 1994). These areas often support filamentous green and brown algae, charophytes and several aquatic vascular plant. They have an abundance of molluscs and crustaceans, despite an often limited invertebrate diversity (Bramber *et al.*, 2001). Many species characterising wetlands are rare, legally protected, and of conservation importance; some species are apparently largely restricted to wetland area. It has been argued that specialist wetland species are better able to tolerate the large environmental variations (e.g. in salinity, hydrology) than their freshwater, estuarine and marine counterparts (Bramber *et al.*, 1992).

The aquaculture and environment are inextricably linked. The aquaculture is characterized

by the demand of high quality environment to ensure sound production; the spatial and temporal variability in wetland areas could influence the coastal productivity, so water inflow controls are required. The development of coastal aquaculture may produce multiple impacts on the costal area, some positive and some negative. The first one is related to the improvement of local hydrologic processes, while the second one is related to the potential release of nutrients and organic matter that can be source of pollution in receiving waters and consequently influence the biodiversity in the surrounding area.
Guidelines need to regard implementation of management scenarios by taking a multi level approach; in particular those regarding monitor fisheries resources productivity and exploitation, exploitable zones, and impacts of aquaculture on the water quality. The final result could lead to the establishment of sustainable fisheries and resource use, also focusing on environmental protection.

1.2 Introduction to the site:

The study was carried out in a fish farm located in *Ramsar Site* near the Orbetello Lagoon (Tuscany, Italy West coast, Fig. 1.1). This area, named *Diaccia Botrona*, is a Natural Reserve with total area of over 7,000 ha, and is considered the most important Italian wetland. This wetland area was acknowledged of international importance ever since 1991 and generated after a natural closing to marine gulf occurred about 3,000 years ago (Fig. 1.2). Diaccia Botrona (Fig. 1.3) wetland, located adjacent to the sea, is ecotonal between marine, freshwater and terrestrial environments. This area receive saline water from the sea by tidal cycles, and fresh water from the surrounding rivers. The area has several peculiar ecosystems such as the Salicornietum and Limonietum associations and the typical marshland habitats. This coastal ecosystem is a staging and breeding area for migratory and sedentary water bird species. More than 200 species have been counted in the Reserve of which 80 nesting in the area. In the recent years, because the increase in the water salinity, the wetland Diaccia-Botrona have undergone marked change in their vegetation. Vast areas have been replaced by halophilus plants that use to be limited only to the southern and more open areas of the wetlands.
The recent evolution of the area, with the loss of certain plant formations and the general "homogenization" of the habitat, caused by natural and human interventions, has brought

the wetlands to a high degree of degradation.

Fig. 1.1; Satellite image of Natural Reserve Diaccia-Botrona with "*Il Padule*" fish farm.

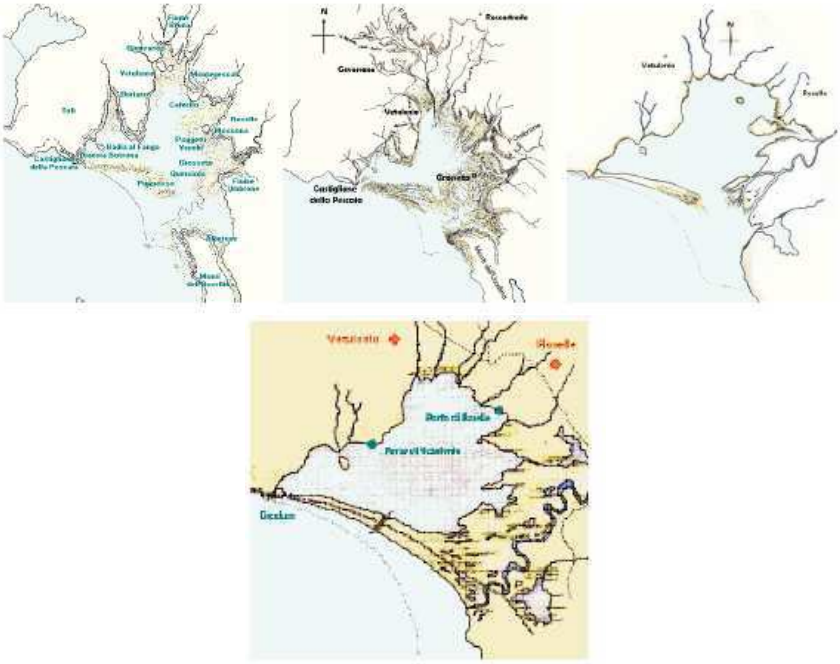


Fig. 1.2; Formation of Diaccia-Botrona wetland area (Internet source).

Fig. 1.3; Image of Natural Reserve Diaccia-Botrona and "*Ximenes House*".

2. ECOLOGY OF COASTAL AQUACULTURE:

2.1 Carbon in aquaculture systems:

Carbon is the basic building block of all organic matter, and fixation of inorganic carbon in plant photosynthesis is the ultimate source of organic carbon for almost all living organisms. The availability of carbon can be considered to be an important limiting factor for aquaculture production because animal growth is initially constrained by amount of food (which is mostly carbon) that can be supplied in autotrophic or heterotrophic food webs or by direct feeding of manufactured feed. Carbon is also important in fish farming because an excessive organic matter production by phytoplankton is at the root of many water quality problems encountered in aquaculture. Excessive primary production and accumulation of algal biomass may, for example, lead to imbalance in dissolved oxygen, production of carbon dioxide (which can be toxic to animals), and the accumulated organic matter may express an oxygen demand when pond water are discharged into receiving bodies (Boyd *et al.*, 1998).

2.1.1 Inorganic carbon:

Carbon compounds can be classified as inorganic and organic. Inorganic carbon compounds of importance in aquaculture include carbon dioxide (CO_2), carbonic acid (H_2CO_3), bicarbonate (HCO_3^-), carbonate (CO_3^{2-}), and carbonate-containing solids, such as limestone. These substance are linked through a complex set of chemical equilibria that determines some properties of water that are of fundamental importance in aquaculture.

Carbon dioxide:

Although carbon dioxide is highly soluble in water, it is only a minor constituent of the atmosphere and equilibrium concentrations of carbon dioxide in water are small. When carbon dioxide is dissolved in water, about 0.2% of the dissolved gas reacts with water to from carbonic acid:

$$H_2O + CO_2 \Leftrightarrow H_2CO_3 \qquad (1.1)$$

Carbon acid strongly dissociates:

$$H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \quad (1.2)$$

It is difficult to distinguish between dissolved carbon dioxide and carbonic acid by analytical procedures, so it is convenient to consider carbon dioxide plus carbonic acid as the hypothetical species "total carbon dioxide" and write:

$$\text{Total } CO_2 + H_2O \Leftrightarrow H^+ + HCO_3^- \quad (1.3)$$

The apparent equilibrium expression for this equilibrium at 25° is

$$[H^+][HCO_3^-] / [\text{Total } CO_2] = K_{a,1} = 10^{-6.35} \quad (1.4)$$

Bicarbonate, Carbonate and Alkalinity:

As indicate in Equation (1.3), some bicarbonate is formed when carbon dioxide dissolved in water and carbonic acid dissociates Bicarbonate deprotonate to from carbonate as the pH of water increases through the addition of base removal of acid:

$$HCO_3^- \Leftrightarrow H^+ + CO_3^{2-} \quad (1.5)$$

The sum of concentrations of bicarbonate and carbonate is defined as the total alkalinity of the water. Total alkalinity is also defined as the acid-neutralizing capacity of the water and includes all base tritatable down to a pH of about 4.5. In most waters, bicarbonate and carbonate are the principal bases contributing to alkalinity, although the alkalinity of some waters may include significant contributions from other compounds (i.e. borate in seawater). Total alkalinity is commonly expressed in the Unites States in units of equivalent calcium carbonate (mg L^{-1} as $CaCO_3$) or sometimes as millequivalentd/liter (1 meq/L = 50 mg L^{-1} as $CaCO_3$).

As evident from Equation (1.5), the distribution of total alkalinity between bicarbonate and

carbonate varies with pH. The equilibrium expressions for the pertinent reactions involving carbon dioxide, bicarbonate and carbonate can be used to calculate in relative proportions of the two anions at different pH values. Those calculations provide the information in Fig. 2.1. Some significant features of the distribution of various chemical species associated with total alkalinity at different pH values are as follows:

- Addition of carbon dioxide to water can reduce pH only to 4.5-4.8.
- At a pH of about 6.4 total carbon dioxide and bicarbonate are present at equimolar concentrations.
- At a pH of about 8.3 total carbon dioxide concentrations decrease to an analytically undetectable value and carbonate begins to appear in measurable concentrations.
- At a pH of about 10.2 bicarbonate and carbonate are present at equimolar concentrations.

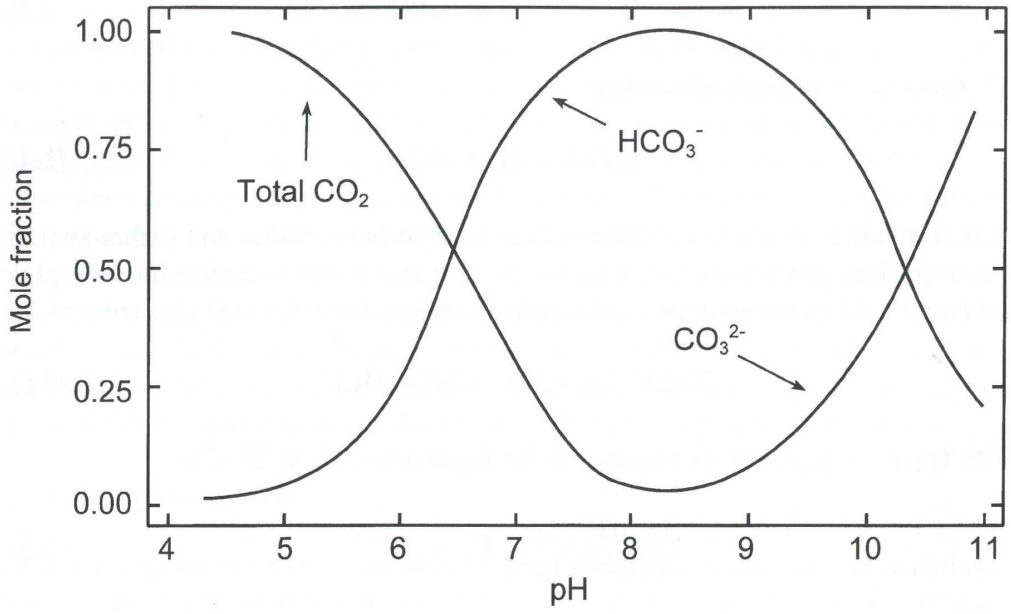


Fig. 2.1; Change in the relative concentrations of carbon dioxide, bicarbonate, and carbonate with pH. The mole fraction of a component is its decimal fraction contribution to the total moles of inorganic carbon ($CO_2 + HCO_3^- + CO_3^{2-}$) present (Boyd *et al.*, 1998).

Interactions of Carbon Dioxide and Alkalinity:

In most surface fresh waters, the pH is determined by the simultaneous equilibria existing between dissolved carbon dioxide, carbon acid, bicarbonate, carbonate and solid-phase carbonate-containing minerals. In the absence of biological activity that changes the dissolved carbon dioxide concentration, the buffer system can be simplified and the pH of waters at the equilibrium with atmospheric partial pressure of carbon dioxide can be approximately determined by using the equilibrium expression in Equation (1.4). First, assume that all of the alkalinity exists as bicarbonate, which is a fair assumption as an initial condition. Then, calculate the molar bicarbonate concentration from the concentration of total alkalinity (a total alkalinity of 50 mg L^{-1} as $CaCO_3$ = 10^3 mol of HCO_3^- L^{-1}) and insert the concentration into the numerator of Equation (1.4). The molar concentration of dissolved carbon dioxide in water at the equilibrium with the atmosphere is then inserted into the denominator and the equation is solved for $[H^+]$, which is then converted in pH (Tab. 2.1).

Tab. 2.1; Approximate pH values resulting by resolution of the equation (1.4) for a series of total alkalinity values (Boyd *et al.*, 1998).

Total Alkalinity (mg L^{-1} as $CaCO_3$)	Approximate pH of the water
0	5.6
1	6.6
5	7.3
10	7.6
50	8.3

This show that alkalinity determines the “initial” pH of water, before biological activity adds or removes dissolved carbon dioxide and causes pH to deviate from the equilibrium condition. Of course, other bases that may be present in the water (borate in seawater, for example) will change the “initial” pH of water. The carbonate alkalinity system not only sets the initial pH of water but also buffers the water against change in pH when either acids or bases are added.

For example if an acid is added, some of the H^+ is neutralized in this reaction:

$$H^+ + HCO_3^- \Leftrightarrow CO_2 + H_2O \quad (1.6)$$

And does not accumulate and depress the pH. Similarly, if a base is added, some of the OH^- is neutralized in this reaction

$$OH^- + HCO_3^- \Leftrightarrow CO_3^{2-} + H_2O \quad (1.7)$$

and the pH does not rise to the same extent it would if bicarbonate were not present. As such, waters of low total alkalinity have little capacity to resist changes in pH after additions of acids or bases. For natural waters, a significant consequence of this relationship is that waters of low alkalinity may be at risk of acidification by acid precipitation. Surface waters with total alkalinities below 10 mg L^{-1} as $CaCO_3$ are considered highly sensitive to acid precipitation because of their limited buffering capacity. Aquaculture systems should be limed to maintain an alkalinity of more than 20 mg L^{-1} as $CaCO_3$, and if this recommendation is followed, acid rain should not be a problem. The pH buffering capacity afforded by alkalinity is important in aquaculture systems because it reduces the extent to which pH is affected by diurnal changes in carbon dioxide concentrations. Dissolved carbon dioxide concentrations in tanks or ponds cycle diurnally because carbon dioxide is consumed in photosynthesis during daylight and produced at night. Removal of carbon dioxide during daylight causes pond pH to increase; addition of carbon dioxide at night causes pH to decrease. The extent of this daily fluctuation depends on the amount of carbon dioxide removed or added and total alkalinity. For a given change in carbon dioxide concentrations, the pH fluctuates less as total alkalinity increases. Generally, the pH in aquaculture system of high total alkalinity (>100 mg L^{-1} as $CaCO_3$) varies between 7.5 and 9.0; the pH in poorly buffered waters (total alkalinity < 20 mg L^{-1}) may vary over a wide range, from less than 7 to over 10 (Boyd *et al.*, 1998).

2.1.2 Organic Carbon:

There are thousand of organic carbon compounds found in water, and it is convenient to broadly classify organic carbon in aquatic ecosystems as either particulate organic carbon

or dissolved organic carbon. Particulate organic carbon consists of carbon in the animal crop, zooplankton, insects, phytoplankton and other plants, bacteria and detritus. The carbon in particulate organic matter exist in proteins, fats, carbohydrates and structural macromolecules such as cellulose and polymerized lignin. In many natural ecosystem, particulate organic carbon in the living biota constitutes a small fraction of the total particulate organic carbon, with dead detritus making up the largest fraction. Detritus is an important component of the particulate organic matter of aquaculture ponds, but the artificially high standing crops of animal and plants in aquaculture ponds makes organic matter in living organisms quantitatively more important than detritus. Dissolved organic carbons includes carbon in a tremendous variety of carbohydrates, proteins, peptides, amino acids, fats, pigments, tannins, lignins and other compounds dissolved in water or I a colloidal suspension. Most of the dissolved organic carbons is derived from the decomposition of particulate organic matter.

2.1.3 Carbon Cycling in Aquaculture:

The transformations of carbon in aquatic ecosystems are rather complex, and attempts to depict the flow of carbon through a lot of autotrophic and heterotrophic processes that occur in water and sediments usually results in illustrations that are so complicated as to be nearly useless. As such, a highly simplified diagram of carbon transformations is provided in Fig. 2.2 for a hypothetical system in which the food web has been reduced to a simple autotrophic food “chain” of inorganic nutrients => phytoplankton => zooplankton => fish. In the simple system, carbon flux can be considered to begin with the dissolution of carbon dioxide from the air into the water. As with any gas-transfer process, the rate of transfer across the air-water interface is proportional to the partial pressure differential between the gas dissolved in water and the gas in the atmosphere, the air-water interfacial area, and amount of turbulence within the system. Turbulence and surface area are especially important, and transfer is most rapid under breezy conditions that mix the water and produce waves. Movement of carbon dioxide from the atmosphere into the water is slow because dissolved carbon dioxide concentrations in the surface film are often above or near saturation.

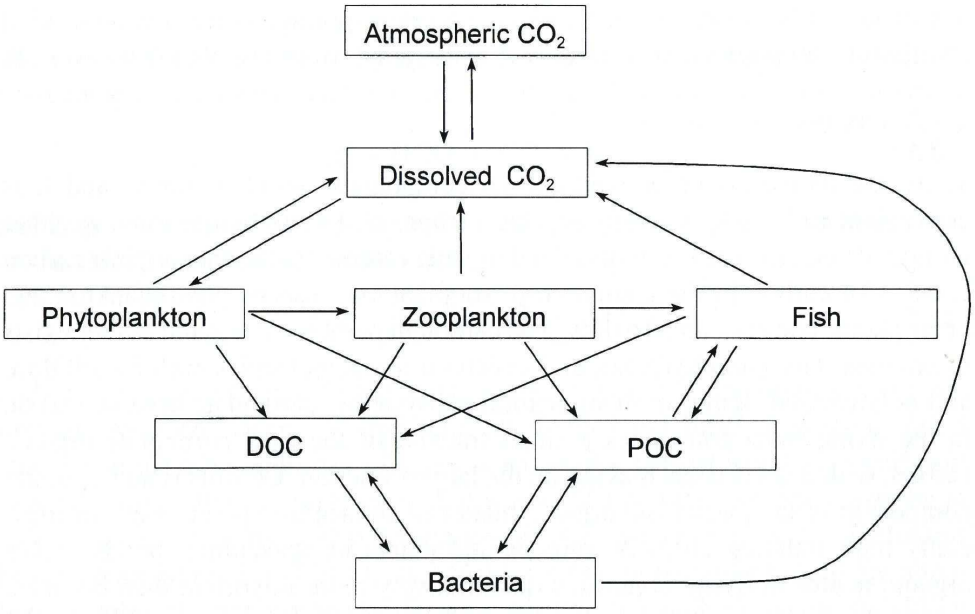


Fig. 2.2; A simplified illustration of the carbon cycle in fish culture system. DOC = dissolved organic carbon; POC = particulate (detrital) organic carbon (Boyd *et al.*, 1998).

One potential source of inorganic carbon to supplement atmospheric supplies is replenishment of dissolved carbon dioxide through its equilibrium with bicarbonate [Equation (1.3)]. As carbon dioxide is removal from the system, bicarbonate dehydrates to replenish the gas, with the associated removal of H^+ which causes the pH of the water to rise. Also many aquatic plants can use bicarbonate directly as a source of carbon, especially when dissolved carbon dioxide concentrations fall to very low levels. The ability to use bicarbonate is especially widespread among phytoplankton, and the blue-green algae in particular (Miller 1990; Raven 1991). Thus, inorganic carbon limitation of phytoplankton growth is less likely to occur in aquaculture waters of high total alkalinity that in ponds with poorly mineralized water with low bicarbonate concentrations. Although bicarbonate offers a significant reserve of inorganic carbon for plant growth in waters of high total alkalinity, the supply in the most water is still insufficient to meet the needs of plant for more than few days when rates of primary production are high. The third and, overall, most important source of inorganic carbon is carbon dioxide produced in respiratory processes.

Part of the inorganic carbon initially fixed into the organic matter by plants is lost as carbon dioxide in respiration and made available for the reassimilation. Another portion of the organic carbon is excreted by phytoplankton as various dissolved organic compounds

(Fogg 1971). Excretion of relatively large amounts of dissolved organic carbon by phytoplankton cells appears to be an energetically wasteful process, and the reason why cells excrete fixed carbon is not know, although the process may be involved in ridding the cell of excess carbon fixed during periods of rapid growth. The dissolved organic excreted by phytoplankton cells may serve as a substrate for bacteria growth, thereby producing particulate organic matter which can enter heterotrophic, detrital food webs. Bacterial decomposition of the dissolved organic matter excreted by algae also produces carbon dioxide that enter the pool of carbon dioxide available for reassimilation by plants.

In the simple model depicted in Fig 2.2, the organic carbon remaining in phytoplankton cells after respiratory losses and excretion of dissolved organic carbon may either be consumed by herbivorous zooplankton or be degraded after cells die. Carbon consumed by herbivorous zooplankton may than be assimilated into tissue, lost in respiration, or lost in particulate fecal matter or as excreted dissolved organic matter. The carbon lost in zooplankton respiration is then available for reassimilation by plants, and that lost in dissolved and particulate material is assimilated or decomposed by heterotrophic bacteria with the production of detritus and yet more carbon dioxide. The net organic carbon remaining in zooplankton may, in turn, be consumed by fish or degraded by bacteria when they die. Heterotrophic decomposition of dead zooplankton results in production of carbon dioxide and detritus. Organic carbon consumed by fish has the same fates as that consumed by zooplankton. It may assimilated into tissue, respired as carbon dioxide, or excreted as dissolved and particulate wastes. The fate of carbon lost from fish are also the same as that lost by zooplankton; carbon dioxide produced in fish respiration and in decomposition of wastes enters the pool of dissolved carbon dioxide available for additional plant growth and heterotrophic food chains are promoted by the dissolved and particulate organic wastes.

Some of the particulate organic matter from dead phytoplankton cells, dead zooplankton, fecal from fish and zooplankton, and detritus in microbial processing of particulate and dissolved organic matter is decomposed aerobically in the water column with the production of carbon dioxide and further processed detritus. However most aquaculture system are shallow and a large portion, if no most, of the particulate material settle to the bottom before it is completely decomposed. Depending on local oxygen conditions in the bottom muds, decomposition of the settled material may take place through aerobic or anaerobic respiration and fermentation. Aerobic or anaerobic decomposition of material in

the pond bottom result in a release of carbon dioxide that may either slowly diffuse into the water or be released into the water by ebullition of bubbles. Organic detritus in bottom muds may accumulate and may be resuspended into the water column due to "bioturbolence" caused by activities of fish.

The description of carbon fluxes in Fig. 2.2 is a coarse oversimplification of the processes actually occurring in a pond. The food web used in this example is again simplified and some processes, such as non-photosynthetic autotrophic carbon fixation (as by nitrifying bacteria) and conversion of carbon dioxide to methane by anaerobic bacteria, were not reported.

2.2 Nitrogen in aquaculture systems

The efficiency of nitrogen (N) assimilation by fish has important implication for water quality and profitability of pond aquaculture. Results from a variety of culture systems indicate that, on average, about 25% (range: 11 to 36%) of N added as feed or other nutrient input is recovered by the target organism (Tab. 2.2). Protein sources such as fish meal and soybean meal are the most expensive components of the formulated feeds and improvement in the efficiency of N assimilation and utilization will such thus improve the economics of fish production (Hargreaves, 1998).

Tab. 2.2; Estimates of the range (%) of nitrogen recovered by fish and released to the environment in various aquaculture production systems (Hargreaves, 1998).

Fish species	Production system[a]	Recovered Fish	Released			Refs.
			Total	Dissolved	Solid	
polyculture	P	11–16	84–89			Schroeder et al., 1990
Anguilla japonica	P	14–25	75–86			Chiba, 1986
Oreochromis niloticus	P	18–21	79–82			Green and Boyd, 1995
Oreochromis spp.	P	25–29	75–81			Avnimelech and Lacher, 1979
Morone saxatilis	P	22	78			Daniels and Boyd, 1989
Ictalurus punctatus	P	27	73			Boyd, 1985
Sparus aurata	P	36	64			Krom et al., 1985
S. aurata	P	26	74			Neori and Krom, 1991
S. aurata	T	27		66	7	Neori and Krom, 1991
S. aurata	T	30		60	10	Porter et al., 1987
Oncorhynchus mykiss	C	21		49	30	Phillips and Beveridge, 1986
O. mykiss	C	25		60	15	Pillay, 1992
O. mykiss	C	25–29	71–75			Penczak et al., 1982
Salmo salar	C	25		62	13	Folke and Kautsky, 1989
Salmo salar	C	25		65	10	Gowen and Bradbury, 1987
Clarias macrocephalus	C	24	76			Lin et al., 1993
I. punctatus	R	14	86			Worsham, 1975
O. mykiss	R	19		74	7	Foy and Rosell, 1991a,b

[a]Production system codes: P = earthen pond, T = tank, C = cage, R = raceway.

N-ammonia is excreted as the end product of protein catabolism, and may be toxic if allowed to accumulate. Ammonia toxicity is manifest by hyperactivity, convulsion, loss of equilibrium, lethargy and coma. However, ammonia toxicity in aquaculture is most likely expressed as the sublethal reduction of fish growth or suppression of immunocompetence, rather than as acute toxicity leading mortality. The toxicity of un-ionized ammonia is a function of pH, temperature, alkalinity and total ammonia concentration measured at

the gill surface (Szumski *et al.*, 1982). Ammonia is more toxic to fish at elevated pH and temperature, with shifts the ionization equilibrium toward the toxic, unionized gaseous form. The risk of elevate pH and unionized ammonia is greater in poorly buffered (low alkalinity) ponds in the late afternoon.

The contribution of ammonia excretion to N flow in aquaculture is substantial. If 25% of input N is retained by fish, then 75% of input N is excreted. Nitrogen excretion can be partitioned into dissolved (62%) and particulate (13%) fractions (Hargreaves, 1998).

Nitrite is another potentially-toxic nitrogenous compound that may accumulate in fish culture ponds. Nitrite is released as an intermediate product during nitrification and denitrification. The toxicity of nitrite is expressed through the competitive binding of nitrite to hemoglobin forming methemoglobin, which does not have capacity to carry oxygen.

2.2.1 Processes related to nitrogen flux in aquaculture ponds:

Feeding:

Application of formulated feeds constitutes the main (>90%) input of N to semi-intensive fish ponds. For example, at a feeding rate of 100 kg ha^{-1} d^{-1} (10 g m^{-2} d^{-1}) of 45% protein feed, more than 500 mg N m^{-2} d^{-1} are added to ponds.

Nitrogen fixation:

Nitrogen may be added to fish ponds by the reduction of atmospheric dinitrogen by heterocystous cyanobacteria. Nitrogen fixation ranged from 6 to 23 mg m^{-2} d^{-1} during the dry season and 21 to 57 mg N m^{-2} d^{-1} during the rainy season in tropical fish ponds (Lin *et al.*, 1988). Nitrogen fixation averaged 24 mg N m^{-2} d^{-1} in a tropical freshwater fish pond and accounted for 10% of estimate N input (Acosta-Nassar *et al.*, 1994). El Samra and Oláh (1979) measured an average nitrogen fixation rate of 4 mg N m^{-2} d^{-1} in a temperate aquaculture pond. The quantity of N added to aquaculture pond by fixation depends largely upon species composition of the phytoplankton community (significant proportion of heterocystous cyanobacteria) and ammonia concentration. The extend of inhibition of N

fixation is inversely related to ammonia concentration (Lin *et al.*, 1988). Nitrogen fixation is a minor, but occasionally important contributor to the N budget of aquaculture ponds receiving formulated feeds.

Phytoplankton uptake of inorganic nitrogen:

Phytoplankton uptake of dissolved inorganic nitrogen (DIN) from the water column of aquaculture ponds is the primary pathway of nitrogen removal. Semi-intensive aquaculture ponds often develop dense phytoplankton populations (chlorophyll *a* > 250 μg L^{-1}, Secchi disk visibility < 20 cm) in response to a high rate of nutrient input. Phytoplankton blooms in most fed aquaculture ponds are likely light-limited (Laws and Malecha, 1981; Smith and Pedrahita, 1988), suggesting nutrients are available at concentration exceeding those limiting uptake or are supplied in excess of cellular requirements.

Ammonia is the preferred N substrate for phytoplankton, and only after it has been depleted (< 0.03 mg N L^{-1}) will significant quantities of nitrate be assimilated (Syrett 1981; McCharthy, 1981). Nitrate assimilation and incorporation is an energetically less-favorable pathway of N nutrition for phytoplankton, as enzymatic-reduction to ammonia within the phytoplankton cell is necessary before incorporation into cellular amino acid.

In aquaculture ponds, the regulation of DIN concentration is mediated primarily by phytoplankton (Tucker *et al.*, 1984; Krom *et al.*, 1989). In these studies, short-term variation in ammonia concentration was inversely related to phytoplankton density. During phytoplankton die-off, ammonia concentration increased dramatically. As phytoplankton uptake density increased, ammonia concentration declined. In addition, seasonal changes in phytoplankton density affect DIN concentrations in aquaculture ponds (Tuker and van der Ploeg, 1993).

Ammonia volatilization:

The equilibrium between unionized ammonia (NH_3), considered gaseuous form, and ionized ammonium (NH_4^+), dissolved forms which has a pK_a of 9.24 at 25°C, is strongly affected by pH and much less strongly affected by temperature. Alkaline pH and higher temperature favors the unionized. As a crude approximation, at pH 9.3, about 50% of ammonia is unionized; at pH 8.3, about 10% is unionized; and, at pH 7.3 about 1% is

unionized. Volatilization is thus enhanced at elevated pH due to equilibrium relationships and the resultant increase in the partial pressure of ammonia gas. Ammonia volatilization is not important at pH < 7.5. Volatilization may be important as a mechanism of ammonia removal during the late afternoon in poorly-buffered (total alkalinity < 20 mg L^{-1} as $CaCO_3$) ponds, when pH may exceed 9 in response to the depletion of dissolved CO_2 from solution by phytoplankton (Hariyadi *et al.*, 1994).

Processes associated with organic matter:

Sedimentation and resuspension:

Aquaculture systems are generally in shallow waters, characterized in part by minimal organic matter decomposition within truncated water columns. Organic input, senescent phytoplankton, fish fecal and uneaten feed settle from the water column to the sediment. In addition, as much as 50% of the algal standing crop (about 10 g algal dry weight m^{-2} d^{-1}) may settle to the sediment surface each day. Sediment traps in freshwater fish pond collected 200 to 500 g dry matter m^{-2} d^{-1}, most derived from previously deposited material that is resuspended by the foraging activity of fish (Schroeder *et al.*, 1991). The resuspension of pond sediment has rendered accurate estimation of organic deposition difficult. Greater mineralization of the easily-decomposed fraction of settled organic matter (e.g., phytoplankton) may occur in the water column during resuspension and in the sediment (Overnell *et al.*, 1995).

Regeneration (mineralization) and diffusion:

Settled particulate organic matter develops into a dynamic, flocculent layer at the sediment-water interface (Visscher and Duerr, 1991; Hopkins *et al.*, 1994). Schroeder (1978) demonstrated the maximum hetrotrophic activity occurring in the flocculent sediment layer extending 2 cm above the firm sediment surface by measuring the rate of weight loss of cotton cloth.

The extent of decomposition of organic matter in the water column of shallow water aquaculture ponds is minimal compared to that occurring at the sediment-water interface. Mineralization of organic matter and the consequent regeneration of nutrients at the

sediment-water interface of aquaculture ponds is important as a source of ammonia to the water column and a sink for dissolved oxygen. The rate of decomposition of organic matter deposited at the sediment-water interface is likely very rapid. The quality of recently-deposited organic matter is high (low C:N ratio) and the half-life of organic N deposited to sediment is likely in the order of 1 to 2 weeks (Nixon and Pilson, 1983). In addition, sediment ammonia flux increased rapidly (within days) in response to a pulsed input of plankton-derived particulate organic matter and returned to background rates only after 1-2 month (Kelly and Nixon, 1984; Jensen *et al.*, 1990).

Dissolved organic nitrogen (DON) is produced by autolysis of settled phytoplankton cells or the hydrolysis of other particulate organic N. DON is further mineralized by proteolytic, heterotrophic bacteria to dissolved inorganic substances (e.g. ammonia produced by deamination of DON). Jana and Roy (1985) measured seasonal variation in the abundance of mineralizing bacteria in fish pond sediment over three years. Abundance of protein mineralizing bacteria (10^4 to 10^5 cells g^{-1}) and ammonifying bacteria (10^5 to 10^6 cells g^{-1}) were maximum during winter (November to January) and minimum during March and September. Bacterial density was directly related to management intensity, although site-specific differences were also apparent.

Despite the relatively high efficiency of organic matter decomposition mediated by aerobic heterotrophic bacteria and the deposition and rapid mineralization of high quality organic matter at the sediment surface, most decomposition in sediment takes place in anaerobic layer where the quality of the accumulated organic matter is low (high C:N ratio), and therefore, relatively recalcitrant to decomposition (e.g. fulvic and humic acid). Anaerobic decomposition is characterized by (1) incomplete oxidation of organic matter, (2) reduced microbial cell yield per unit substrate, and (3) reduced assimilatory requirement for N by anaerobic microbes (Reddy and Patrick, 1984). Thus, in general, relatively more N is released from organic matter decomposition under anaerobic conditions.

Ammonia accumulates in the sediment layer because the biochemical pathway of ammonia transformation requires oxygen. Concentration of sediment porewater or interstitial ammonia may be an order of magnitude greater than those of the water column. The profile of porewater ammonia in sediment is typified by a low concentration at the sediment-water interface that increase rapidly with depth. In response to a concentration gradient, ammonia diffuses from the reduced sediment layer to the oxidized surface where it is subject to oxidation to nitrate or further diffusion to overlying water.

Ammonia flux from the sediment can be enhanced by burrowing activities of macrofauna (bioturbation). Macrofauna can increase the effective surface area of sediment by 125% (Hylleberg and Henriksen, 1980). Benthic invertebrates increased the flux of ammonia from marine sediment by 50%, primarily by the irrigation of burrows that may extend from 8 to 12 cm into the sediment (Henriksen, 1980; Blackburn and Henriksen, 1983). The concept of bioturbation has been extended to benthivorous fish, although the functional effects of burrow irrigation by benthos and foraging behavior by benthivorous fish are different. To summarize, sediments are a source of ammonia for the water column of aquaculture ponds (Tab. 2.3). Most of this ammonia is derived from regeneration of N from the mineralization of relatively high-quality, recently-settled organic matter at the sediment-water interface. A smaller and variable source of ammonia is derived from mineralization of organic matter in the reduced sediment layer. Ammonia into the water column in response to a concentration gradient extending from the reduced sediment layer to the sediment-water interface, a process that may be enhanced by macrofauna or sediment resuspension.

Tab. 2.3; Estimate of ammonia flux (mg N m^{-2} d^{-1}) from freshwater, estuarine and marine sediments (Hargreaves, 1998).

Ammonia flux	Refs.	Comments
Freshwater		
0.3–3.1	Acosta-Nassar et al., 1994	new tropical fish pond
2.5–3.6	Reddy et al., 1990	Lake Okeechobee, FL
4.2	Schroeder, 1987	manured polyculture pond—diffusive flux + benthic regeneration
5–15	Fillos and Swanson, 1975	eutrophic lake and river ediments
< 10 (aerobic) 75 (anaerobic)	Rysgaard et al., 1994	freshwater lake sediment
11.4	Avnimelech, 1984	intensive fish pond—mineralization kinetics model
23	Hesslien, 1977	ELA Lake 227, Canada
19.6–43.2, 31.2 (mean)	Erickson and Auer, in press	freshwater reservoir sediment; anaerobic release rate; 8°C
29–44	Freedman and Canale, 1977	White Lake, MI
65	Cerco, 1989	20°C
78	Wickman and Auer, in press	Onodaga Lake, NY
25 (winter) 150 (summer)	Hargreaves, 1997	simulation model, catfish ponds
11–159 85 (mean)	Smith and Fisher, 1986	Lake Calado, Brazil
36–168	Höhener and Gächter, 1994	Lake Sempach, Switzerland
42–140	Jellison et al., 1993	Mono Lake, CA
185	Riise and Roos, 1997	polyculture fish pond, Thailand
Estuarine		
0–252	Kemp and Boynton, 1984	Patuxent River estuary
10–231	Klump and Martens, 1981	Cape Lookout Bight, NC
-10–207 43 (annual mean)	Reay et al., 1995	Chesapeake Bay
70	Callender and Hammond, 1982	Potomac River estuary
77	Vidal and Morguí, 1995	Alfacs Bay, Spain
175	Phoel et al., 1981	York River estuary, VA
119–271, 197 (mean)	Smith and Fisher, 1986	Choptank River
29–882	Sumi and Koike, 1990	Japanese estuary
Marine		
8.8–11.7 10.5 (mean)	Blackburn et al., 1988	marine fish pond
11 (mean) 22 (maximum)	Hargrave et al., 1993	Atlantic salmon cage culture site
50–148	Blackburn and Henriksen, 1983	Danish marine sediments
0–144	Caffrey, 1995	North San Francisco Bay
6–172		South San Francisco Bay
182	Blackburn, 1979	Limfjorden, Denmark
370	Klump and Martens, 1989	Cape Lookout Bight, NC
70–672	Mackin and Swider, 1989	Flax Pond

Ammonium adsorption:

Ammonium (NH_4^+) may weakly adsorb to negatively-charged ions exchange sites on the surface of clay minerals or organic matter in the sediment. Adsorbed (exchangeable) ammonium is important as a source of ammonia to the overlying water and as a sink for ammonia produced from DON mineralization. Acosta-Nassar *et al.* (1994) estimated that about 2% of N added to a freshwater fish pond was stored in the adsorbed pool, although undoubtedly greater amounts of ammonium were derived from the mineralization of soil autochthonous or previously-deposited sediment organic matter.

Adsorbed NH_4^+ and porewater ammonia are in equilibrium, so profiles adsorbed NH_4^+ and porewater ammonia are similar. The ratio of adsorbed NH_4^+ to porewater ammonia (partition coefficient) is variable, but generally much greater than 1. Differences in the partition coefficient are related to the cation exchange capacity of soil, adsorbed and porewater ammonia concentration, season (temperature) and sediment depth.

The concentration of adsorbed ammonium is affected by sediment drying and re-wetting. The exchangeable ammonium pool declined rapidly to very low levels after 6 weeks of drying a fish pond sediment (Diab and Shilo, 1986). Following refilling, the adsorbed ammonium pool increase within 10 days to levels equivalent to about 50% of that before draining and continued to increases during the cropping cycle (Shilo and Rimon, 1982; Diab and Shilo, 1986). Presumably, nitrification is responsible for reduction in exchangeable ammonium concentration, although evidence that adsorbed ammonium can be utilized by nitrifying bacteria is equivocal. The loose adsorption of exchangeable ammonium to sediment and the association of nitrifying bacteria with particles are evidence in support of the importance of this process (Seitzinger, 1990).

The dynamic nature of the adsorbed ammonium pool is further illustrated by measurement of the complete and rapid desorption of ammonium from a sandy sediment after two hours following suspension by wind-driven water turbulence (Simon, 1989). Ammonium supplied to the water column by desorption from sediment solids was estimated to exceed that supplied by diffusive flux. Suspension of aquaculture pond sediments by aeration or wind-driven water turbulence may increase, at least temporarily, the concentration of ammonia in the water column.

Nitrification:

Nitrification is the sequential, two-step oxidation of ammonia to nitrate. The process is mediated by predominately two bacterial genera. The oxidation of ammonia is mediated by *Nitrosomonas* and the oxidation of nitrite is mediated by *Nitrobacter*. The organism are chemoautotrophic, gram-negative, motile rods with long generation times (20 to 40 h). The reactions proceed as follow:

$$NH_4^+ + 1\ ½\ O_2 => NO_2^- + 2H^+ + H_2O$$

and

$$NO_2^- + ½\ O_2 => NO_3^-$$

Thus, two moles of oxygen are required for each mole of NH_4^+ oxidized. These organisms derive energy from oxidation of NH_4^+ and NO_2^-. The free energy yield (ΔG) from oxidation of NH_4^+ is about -65kcal $mole^{-1}$, and that from the oxidation of NO_2^- is about -18 kcal $mole^{-1}$ (Focht and Verstraete, 1977). Thus, over three times as much NO_2^- must be oxidized to support an equivalent microbial growth to that derived fro the oxidation of NH_4^+.

Nitrification rates of estuarine sediments range from 15 to 25 mg N m^{-2} d^{-1} (Henriksen and Kemp, 1988) and are probably representative of those of aquaculture pond sediments, although no direct measurements have been made (Table 2.4). Assuming 5 to 10% of sediment oxygen demand is utilized for nitrification (Henriksen and Kemp, 1988) then about 25 to 50 mg N m^{-2} d^{-1} is oxidized, equivalent to 10 5 to 10% of daily N input. Thus, the magnitude of nitrification is a relatively small in relation to the rate of other N transformations during the production cycle. Nitrification rates are elevated only during periods between cropping cycle when pond soil are aerated as they dry.

Nitrification is affected by dissolved oxygen concentration, temperature, substrate concentration, pH, numbers of nitrifying bacteria, and availability of surfaces. Many of these factors are interrelated and their effect on nitrification is complex.

Nitrifying bacteria require oxygen to derive energy from reduced N. The half-saturation concentration (K_m) for oxygen ranges from 0.3 to 0.9 mg L^{-1} and is directly related to temperature (Painter, 1970). The K_m for oxygen is higher for *Nitrobacter* than for Nitrosomonas at 30°C suggesting that nitrite oxidation is more sensitive to low oxygen concentration at warm temperature. With all other conditions sufficient, nitrification rate is

constant at dissolved oxygen concentration above 2 mg L^{-1}. The K_m for oxygen of nitrifying bacteria is several orders of magnitude greater than that of heterotrophic aerobic bacteria, suggesting that heterotrophic bacteria may be competitively more successful than nitrifying bacteria at low oxygen concentration (Tab. 2.4).

Tab. 2.4; Nitrification rate (mg N m^{-2} d^{-1}) estimates in the sediments of marine and freshwater systems (Hargreaves, 1998).

Nitrification rate	Refs.	Location/Comments
0	Blackburn et al., 1988	tropical marine fish pond
0.4–0.9 (mean = 0.5)	Acosta-Nassar et al., 1994	tropical freshwater fish pond
1–35	Riise and Roos, 1997	polyculture fish pond, Thailand
0–42	Henriksen, 1980	Danish coast
4–18	Henriksen et al., 1981	Danish coast
3–48	Billen, 1978	Belgian coast (North Sea)
11	Blackburn and Henriksen, 1983	Danish coast
11	Lindau et al., 1988b	rice soil
13	DeLaune and Lindau, 1989	Lac des Allemands, LA
15		Little Lake, LA
	Henriksen et al., 1980	Danish coast
16		without fauna
28–35		with fauna
7–37 (mean = 20)	Hansen et al., 1981	Danish coast
7–45 (mean = 20)	MacFarlane and Herbert, 1984	Scottish estuary
8–34 (mean = 22)	Nishio et al., 1983	Japanese coast
24	Boynton et al., 1980	Patuxent River estuary
26–30	Jenkins and Kemp, 1984	Patuxent River estuary
30	DeLaune and Smith, 1987	Lake Verret, LA
7–45 (mean = 39)	Seitzinger et al., 1984	Narragansett Bay
27–67 (mean = 45)	Koike and Hattori, 1978	Japanese coast
59–76	Jensen et al., 1994	freshwater lake sediment
	Chaterpaul et al., 1980	freshwater stream sediment
29		without fauna
69		with fauna
63	Vanderborght et al., 1977	Belgian coast (North Sea)
67	Rysgaard et al., 1994	freshwater lake sediment
60–152	DeLaune et al., 1991	Calcasieu River, LA

Oxygen penetration into sediment is a key factor regulating nitrification (Reddy and Patrick, 1984; Rysgaard *et al.*, 1994). The depth of oxygen penetration into aquatic sediments is typically on the order of 1 to 5 mm depth and is inversely related to temperature (Revsbech *et al.*, 1980). Although nitrification increases with temperature, the volume of sediment involved in nitrification is restricted by the depth of oxygen penetration, which exerts control on overall nitrification rate. Nitrification potential was demonstrated in reduce sediment (6 to 8 cm depth) indicating the ability of nitrifying

bacteria to survive in anaerobic environments, although actual nitrification was restricted to the sediment surface (Hansen *et al.*, 1981; Henriksen *et al.*, 1981). Nitrification activity of dormant, nitrifying bacteria in anoxic sediment layers will increase rapidly (within few hours) in response to exposure to oxygen in overlying water (Jensen *et al.*, 1993). Nitrification potential was minimum during the summer, coincident with minimum sediment oxygen penetration. The depth of oxygen penetration, and consequently nitrification rate, is also inversely related to the sedimentation of organic matter, which is maximum in the warmer months.

The optimum temperature range for growth of pure cultures of nitrifying bacteria (25 to 35°C) is fairly narrow, although the scope for growth (3 to 45°C) is much wider (Focht and Verstraete, 1977). Evidence to differential sensitivity of the two principal nitrifying genera to temperature is equivocal, but tends to implicate the greater sensitivity of nitrite oxidizer to low temperature, particulary at pH values outside the optimum range (Focht and Verstraete, 1977). However, climatic and other environmental variables exert strong selection pressure on populations of nitrifying bacteria, suggesting that adaptation to local conditions is also likely.

Nitrification rate is also affected by substrate concentration. In aquaculture pond sediments, ammonia is supplied (1) by the mineralization of organic N at the sediment-water interface, (2) diffusion of ammonia from the reduced sediment layer to the sediment-water interface, and (3) the bulk water.

Nitrifying bacteria require slightly alkaline pH (7 to 8.5) for optimal growth. At pH > 8.5, *Nitrobacter* may be inhibited more than *Nitrosomonas*, resulting in an accumulation of nitrite (Fenchel and Blackburn, 1979). Increased nitrification at alkaline pH suggest than NH_3 may be the substrate for nitrification. Also, unionized ammonia can inhibit nitrite oxidation at 0.1 to 1.0 mg NH_3-N L^{-1} (Belser, 1979). However, these concentrations are rarely observed in fish ponds as they are also toxic to fish. Finally pH is important because two hydrogen ions are released for each mole of ammonia oxidized. Natural waters usually contain sufficient alkalinity to buffer an increase in hydrogen ion concentration from nitrification.

Nitrifiers are lithotrophic, requiring organic or mineral surfaces for attachment. Nitrifier density in the soil at the sediment surface (10^6 to 10^9 cm^{-3}) is about three orders of magnitude greater than that in the water column (10^3 to 10^4 mL^{-1}). The abundance of ammonia oxidizers (10^4 to 10^5 cell g^{-1}) in sediment is greater than that of nitrite oxidizer

(10^3 cells g^{-1}) (Ram *et al.*, 1981 and 1982).

The sediment surface is the locus for mineralization of particulate organic matter settling from the water column. In addition, ammonium may be concentrated on sediment mineral particles (clays) as part of the ions exchange complex. Competition for surfaces between heterotrophic and nitrifying bacteria may contribute to limitation of population density of the latter group.

Nitrification at the sediment-water interface is more important than nitrification in the water column in stratified or periodically-mixed fish ponds. Nitrification in the water column is restricted by the availability of surfaces and possibly by light inhibition. Nitrification may increase temporarily following phytoplankton die-off in response to elevated ammonia concentration. Water column nitrification is an important mechanism of ammonia transformation in high-intensity pond system in which particles suspended by mechanical aeration are sites of active mineralization and nitrification.

The pattern of nitrification following the establishment of conditions favourable for processes development is characterized by the rapid oxidation of ammonia, an accumulation of nitrite coincident with a decline in ammonia, and after a lag period, a decline in NO_2^-. This characteristic pattern explains, in part, the bimodal distribution of annual nitrite concentration maxima measured in warm and temperate commercial catfish ponds (Tucker and van der Ploeg, 1993). Interpretation of factor analysis of the data of Tucker and van der Ploeg (1993) suggested that the sediment oxygenation was an important regulator of nitrification in these ponds (Hargreaves and Tucker, 1996).

During the summer, nitrification in aquaculture pond sediment is more likely limited by the depth of oxygen penetration in the sediments, typically 1 to 5 mm. In estuarine sediments, the summer depression of nitrification rate has been attributed to limited oxygen diffusion into sediments (Hansen *et al.*, 1981; Jekins and Kempt, 1984). During the summer, the rate of input of organic matter to aquaculture pond sediment is maximum due to maximum feeding rates and standing crops pf phytoplankton, and are coincident with maximum seasonal temperatures. The decomposition of recently deposited organic matter by large and active populations of aerobic heterotrophic bacteria limits the diffusion of oxygen into sediment. Although operation of aerators may prevent near-sediment dissolved oxygen from declining to concentrations < 2 mg L^{-1}, it is likely that a laminar benthic boundary layer (≈ 100 μm) depleted of dissolved oxygen develops at the sediment-water interface in the summer.

Stirring of sediments during pond aeration may increase the depth of oxygen diffusion (Revsbech *et al.*, 1980). In the water column, nitrification rate is low because ammonia is present at substrate-limiting concentrations due rapid uptake by large and actively-growing phytoplankton populations.

As temperature declines during the fall, dissolved oxygen concentration of the water column increases due to reduced feeding rate and pond respiration and increased oxygen solubility in water. Sediment oxygen demand declines as metabolic activity of bacteria is depressed by cooler temperatures and reduced inputs of organic matter from the water column. Consequently, the depth of oxygen penetration into the sediment surface increases, thereby increasing the volume of sediment involved in the nitrification processes. In the water column, nitrification is stimulated by the increase of ammonia, resulting from the reduced uptake by phytoplankton.

Nitrification rate is reduced during the winter due seasonally minimal temperatures. In the spring, as temperature increases, organic N that accumulated is rapidly mineralized to ammonia and the rate of nitrification is once again stimulated, producing another sharp increase in water column nitrite concentration. As temperature increases further, the oxygen dissolved in the water column declines, therefore oxygen penetration in the sediment and nitrification processes are reduced.

In summary, the interaction between temperature and the depth of oxygen penetration in the sediment exerts a control on the nitrification processes in fish pond sediments. The interaction between temperature and substrate concentration exerts a control on nitrification processes in the water column. During summer nitrification in the ponds sediment is controlled (limited) by oxygen penetration into the sediment and low substrate concentrations in the water column, despite seasonally maximum temperatures. During the fall, control of nitrification moves from oxygen penetration (sediment) or substrate concentration (water column). During the winter, low temperature limits nitrification. During the spring, control of nitrification shifts from temperature to sediment oxygen penetration.

Nitrate reduction:

Nitrate may follow several biochemical pathways following production by nitrification. Plant and microbes may reduce nitrate to ammonia for incorporation into cellular amino acid (assimilatory nitrate reduction). Nitrate may function as a terminal electron acceptor during the oxidation of organic matter and thereby supply energy for microbial growth. Nitrate respiration results in the reduction of nitrate to dinitrogen (denitrification) or ammonia (dissimilatory nitrate reduction to ammonia).

$$NO_3^- \Rightarrow NO_2^- \Rightarrow NO \Rightarrow N_2O \Rightarrow N_2$$

Oxygen is the energetically preferred terminal electron acceptor for the oxidation of organic matter. However, when oxygen concentration becomes limiting (~ 0.1 to 0.2 mg L^{-1} or E_h < 220 mV), heterotrophic facultative anaerobic shift to nitrate as the terminal electron acceptor. The energetic yield from the oxidation of organic carbon (e.g. glucose) by nitrate (ΔG = - 649 kcal $mole^{-1}$) is only slightly less than that by oxygen (ΔG = - 686 kcal $mole^{-1}$).

Unlike the limited species diversity of bacteria mediating nitrification, at least 14 genera of bacteria can reduce nitrate, and *Pseudomonas*, *Bacillus*, *Alcaligines* are the most prominent numerically (Focht and Verstraete, 1977). Also, the growth, activity and population density of denitrifying bacteria exceed that of nitrifying bacteria. Most denitrifying bacteria are considered facultative anaerobes. Although denitrification is inhibited by oxygen, the reaction occurs primarily near the sediment surface, possibly in reduced (suboxic) microzones in the oxidized sediment surface layer.

The rate of denitrification depends on temperature, concentrations of nitrite, organic carbon, and oxygen, and the population density of denitrifying bacteria (Tab. 2.5).

Tab. 2.5; Denitrification rate (mg N m^{-2} d^{-1}) estimates in the sediments of marine and freshwater systems (Hargreaves, 1998).

Denitrification rate	Refs.	Location/Comments
1.4–3.6	Messer and Brezonik, 1983	Lake Okeechobee acetylene blockage
3.6–7.1		mass balance
0.1–7.4	Acosta-Nassar et al., 1994	tropical freshwater fish pond
2.3	Oren and Blackburn, 1979	Kysing Fjord, Denmark (~ 0.15 mg l^{-1} NO_3^-–N)
2.7–10.9	Kaspar, 1982	intertidal mud flat
3.6–7.2	Chan and Knowles, 1979	eutrophic ponds
5	Tirén, 1977	oligotrophic Swedish lake
3.4–13	Nishio et al., 1983	Japanese coast
3.6–18	Sweerts and de Beer, 1989	eutrophic lake (Vechten)
3.8	Smith and DeLaune, 1983	freshwater/estuarine eutrophic lake sediments
0–29	Billen, 1978	Belgian coast
4–55	Cerco, 1989	Potomac River (10–30°C, 8 mg l^{-1} DO, 0.21–0.63 mg l^{-1} NO_3^-–N)
4–71	Andersen et al., 1984	Danish estuary; seasonal variation
10–40	Henriksen et al., 1980	Danish coast
14	Sørensen, 1978	Danish coast
14–20	Chan and Campbell, 1980	eutrophic Canadian lake
18–35	Nielsen, 1992	eutrophic stream bed
17–34	Seitzinger et al., 1984	Narragansett Bay
< 25	Rysgaard et al., 1994	freshwater sediment
25–40	Tirén, 1977	3 eutrophic Swedish lakes
26–30	Jenkins and Kemp, 1984	Patuxent River estuary (spring)
29	Vanderborght et al., 1977	Belgian coast
1.5–57	Lindau et al., 1990	urea-treated rice plot
47–81	Roos and Eriksen, 1995	semi-intensive polyculture pond
	Blackburn et al., 1988	marine fish ponds
14–25		acetylene blockage
71–119		nitrite + nitrate reduction
56–69	Krom, unpublished (cited in Blackburn et al., 1988)	marine fish ponds
52	D'Angelo and D'Angelo, 1993	Lake Okeechobee
57	Riise and Roos, 1997	polyculture fish ponds, Thailand
58	Andersen, 1977	Byrup Langsø (lab cores)
110		Byrup Langsø (mass balance)
34		Kvind Sø (lab cores)
85		Kvind Sø (mass balance)
100–500		enriched lake sediment
45	DeLaune and Smith, 1987	Lake Verret, LA—nitrate reduction
34–52	DeLaune et al., 1991	Calcasieu River, LA
95–160	van Kessel, 1977	enriched ditch sediment
100–200	Nishio et al., 1982	polluted estuary, Japan
101–296	Seitzinger and Nixon, 1985	enriched marine mesocosm
367	Lindau et al., 1988a	enriched bottomland hard-wood forest swamp plot
33–342	Lindau et al., 1990	KNO_3-treated rice plots
420–490	Binnerup et al., 1992	enriched, bioturbated marine sediment

Denitrification rates increase with substrate concentration. However, denitrification rates in

most natural aquatic system are first order with respect to nitrate concentration, and can be considered substrate limited. In aquaculture ponds, nitrate is typically < 0.5 mg N L^{-1} (Ziemann *et al.*, 1992; Tuker and van der Ploeg, 1993), a concentration likely below reported half-saturation constant (K_m) for denitrification. Nitrate concentration in temperate aquaculture ponds are maximum during winter, when phytoplankton blooms are minimal.

Kinetic constants vary with available carbon (reductant). Reported K_m values range from 0.1 to 170 mg N L^{-1}, and increase in direct relation to carbon (Focht and Verstraete, 1977). Ina multiple (stepwise) regression model, dissolved organic carbon concentration was the most important predictor of denitrifying bacteria abundance in tropical fish ponds (Jana and Patel, 1985). The large quantity and low C:N ratio of settled organic matter in aquaculture ponds suggests that carbon limitation of denitrification is not likely.

Aquatic sediments consist of a very thin oxidized layer overlying a much thicker anoxic layer. Therefore, the potential for denitrification in fish ponds is very high.

Although denitrification is an anaerobic process, it is largely dependent on oxygen concentration for the production of nitrate through nitrification. Factors that stimulate nitrification (e.g. warm temperature, abundant oxygen) will also stimulate denitrification. In aquatic sediment in which the nitrate concentration is overlying water is low, the denitrification rate will be limited by nitrification rate, which in turn is regulated by the depth of sediment oxygen penetration (Rysgaard *et al.*, 1994; Jensen *et al.*, 1994). The presence of an oxidized sediment layer also increases the diffusion distance of nitrate from the overlying water to anoxic sediment thereby reducing the rate of denitrification of nitrate derived from the overlying water.

2.3 Phosphorus in aquaculture systems:

Phosphorus is a key metabolic nutrient; yet it is available in relatively small amounts in most unpolluted surface water. As such, the supply of phosphorus often regulates the productivity of natural waters. In fact, Boyd (1998) indicated that most natural waters respond to additions of phosphorus with greater plant production. Phosphorus is also important because aquaculture pond waters are often enriched with phosphorus relative to natural waters, and the discharge of pond waters may pollute receiving waters with phosphorus compounds and lead to excessive plant growth.

Forms of Phosphorus in water:

Plant assimilate phosphorus as orthophosphate ions, which may be considered as ionization products of orthophosphoric acid (H_3PO_4):

$$H_3PO_4 \Leftrightarrow H^+ + H_2PO_4^- \qquad pK_{a,1} = 2.1$$
$$H_2PO_4^- \Leftrightarrow H^+ + HPO_4^{2-} \qquad pK_{a,2} = 7.2$$
$$HPO_4^{2-} \Leftrightarrow H^+ + PO_4^{3-} \qquad pK_{a,3} = 12.3$$

The ionic species present in water depend on pH because $[H^+]$ appears in all expressions. At common environmental values of 7-9, most of the orthophosphate exists as a mixture of $H_2PO_4^-$, which can be considered equally available to phytoplankton.

Many other forms of phosphorus occur in water. Inorganic polyphosphates often reach waters in various effluents (especially those containing synthetic detergents). Polyphosphates are formed of two or more orthophosphate groups linked together in a linear molecule through P-O-P linkages. Polyphosphate are rapidly hydrolized by microorganisms in water to from orthophosphate. Most of phosphorus in natural waters is present in various organic compounds, such as nucleic acids, adenosine phosphates (ATP and ADP), and phosphate esters of enzymes, vitamins, and lipids. Organic phosphorus may be contained in particular matter, colloids, or dissolved molecules. The phosphorus in most organic phosphate is not directly available to phytoplankton, but may forms of organic phosphorus are readily hydrolyzed by extracellular phosphatase enzymes produced by phytoplankton and heterotrophic microorganisms. The orthophosphate released by

enzymatic hydrolysis is then available for uptake by the plants (Boyd *et al*., 1998).

It is difficult to analytically discriminate between orthophosphate (the form available to plants) and certain other forms of phosphorus in natural waters. In the common analytical method for dissolved phosphorus, a water sample is filtered to remove particulate matter and a molybdenum-containing reagent is added to the filtrate. The molybdenum reagent reacts with orthophosphate under acidic conditions to form an intensely colored compound that can be measured spectrophotometrically. Under the acid conditions needed for the reaction, a wide variety of easily hydrolyzed organic phosphate compounds and some phosphorus in polyphosphates are partially hydrolyzed to release orthophosphate, which then reacts with the molybdenum reagent and appears as orthophosphate in the analysis. The phosphorus measured using this method is correctly called "soluble reactive phosphorus", rather than orthophosphate. Soluble reactive phosphate is not a direct measure of the orthophosphate available for plant growth, but it is considered to be highly correlated with phosphorus that is readily available for plant uptake in most waters (Nurnberg and Peters 1984). "Total phosphorus" is the other common analytical value used in studies of phosphorus in aquatic system. Total phosphorus includes all forms of soluble and particulate phosphorus in the water sample. In aquaculture ponds, most of the total phosphorus is the particulate fraction as phytoplankton cells or detritus of algal origin. Concentration of soluble reactive phosphorus are quite low in most natural waters; soluble reactive phosphorus concentrations are usually not greater than 5-20 μg/L and seldom exceed 100 μg/L even in highly eutrophic waters. Concentrations of total phosphorus are usually less than 1000 μg/L (Boyd *et al.*, 1998).

Soluble reactive phosphorus concentrations are low in most water because of available phosphorus being rapidly removed from the water by plant uptake. Pond bottom muds also remove orthophosphate from water and, in the most water, concentrations of soluble reactive phosphorus will be low even in the absence of plant uptake. All muds adsorb orthophosphate, but adsorption is particularly great in either highly acidic muds or muds with a pH ranging from neutral to alkaline and an high concentration of calcium. In waters with high calcium concentrations and elevate pH, calcium phosphate may precipitate directly from the water without involvement of the muds.

The phosphorus cycle:

The various transformations of phosphorus in aquaculture ponds are illustrated in Fig. 2.3. Previous studies on phosphorus dynamics in aquaculture systems have shown that a relatively large fraction of the feed phosphorus (80–90%) is released within fish culture systems due to low phosphorus uptake by fish (Avnimelech and Lacher, 1979; Boyd, 1985; Schroeder *et al.*, 1991). Modern diets, however, combine lower phosphorus content and improved bioavailability to reduce phosphorus losses. In conventional, earthen-bottom fish culture systems, much of the phosphorus is retained within the sediment (Boyd, 1995) and phosphorus is discharged, either with the sludge or with effluent water. Sludge from these systems contains most of the phosphorus.

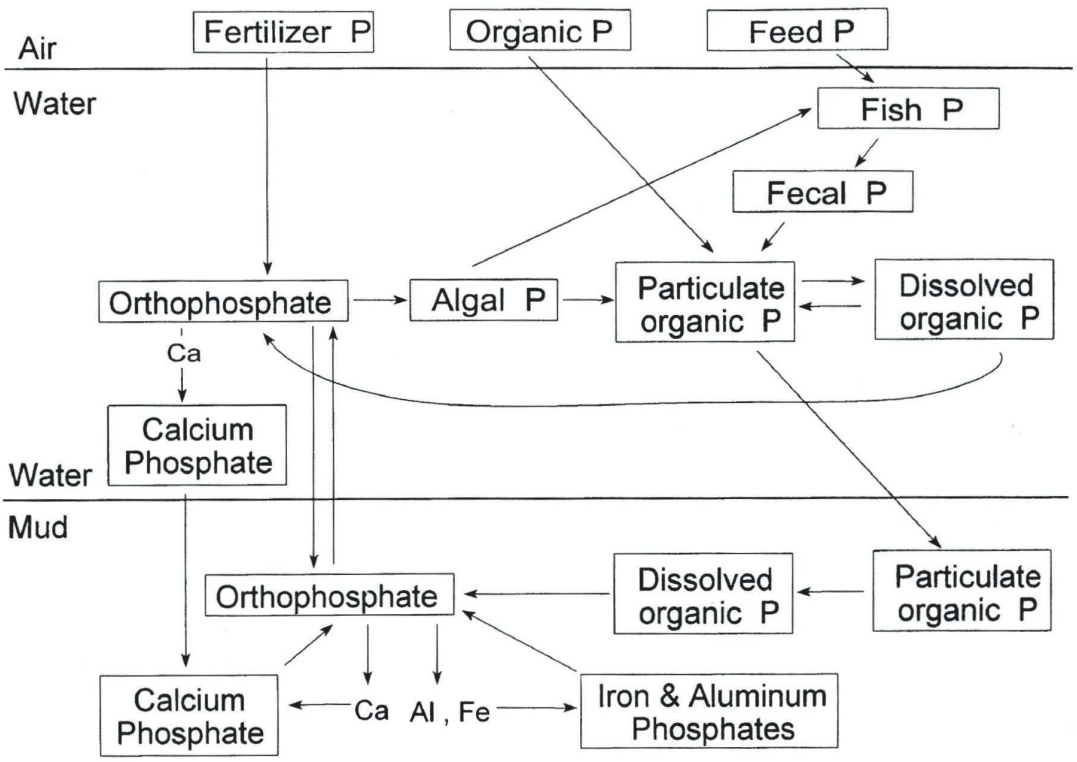


Fig. 2.3; The phosphorus cycle in aquaculture systems. The illustration is simplified by omitting details of the food chain between algae and fish. Particulate organic P includes phosphorus in zooplankton and detritus (Boyd *et al.*, 1998).

Phytoplankton is efficient in removing orthophosphate down to concentrations as low as 1 μg P/L, or less (Rigler 1966). Some plant can adsorb more phosphorus than they need for growth and stored it possible future use (Boyd *et al.*, 1998). The absorption of phosphorus or other nutrient in excess of the amount required for growth is termed luxury consumption. Blue-green algae are particulary adapt acquiring and storing intracellular phosphorus when the nutrient is abundant. Obviously, the ability to assimilate and store more nutrients than needed is a competitive advantage because it allows plants to survive brief periods when ambient nutrient are low (Kilham and Hecky 1988). Rapid recycling of phosphorus within the pond ecosystem is essential to maintain high rates of primary productivity in the water column. Considerable quantities of soluble organic phosphorus may be released from actively growing phytoplankton cells. Those compounds are rapidly hydrolyzed by aquatic bacteria or phytoplankton with the release of orthophosphate which is then rapidly reassimilated by other phytoplankton cells. Orthophosphate is also produced when dead phytoplankton cells undergo bacterial decomposition. The soluble organic phosphorus that is produced in the decomposition process is subject to hydrolysis with the release of orthophosphate, which is then available for reassimilation by phytoplankton. Phosphorus in phytoplankton cells and algal-based detritus nay also be assimilated and subsequently excreted by zooplankton. Regeneration of orthophosphate through zooplankton grazing is believed to be an important pathway for phosphorus recycling (Boyd *et al.*, 1998). Of course, phosphorus in phytoplankton, detritus, and zooplankton may be consumed by the fish or crustacean under culture; part of the phosphorus consumed will be excreted and re-enter the cycle.

At each step in the process of phosphorus recycling, orthophosphate is produced and becomes available for assimilation by the plants. The orthophosphate produced may also enter into various chemical reactions that ultimately result in phosphorus being lost to the pond bottom muds. Also, much of the phosphorus in phytoplankton cells and organic detritus settles to the pond bottom before the opportunity for bacterial degradation and release of orthophosphate into the water column. Although particulate organic matter associated with bottom muds is decomposed with the release of orthophosphate, the orthophosphate that is produced during decomposition is rapidly made unavailable through chemical reactions in the mud. The exchange of phosphorus between bottom muds and water is, in fact, the major component of the phosphorus cycle in aquaculture ponds.

Mud-Water Phosphorus Exchange:

Pond muds strongly adsorb phosphate (Boyd *et al.*, 1998), and muds are eventually the recipient of most of the phosphorus added to aquaculture ponds. Boyd and Musig (1981) measured uptake of dissolved orthophosphate added at 3.0 mg L^{-1} to laboratory mud-water system. Mud pH ranged from 4.4 to 7.3. System were kept in the dark to prevent phytoplankton growth, but bacteria were present in muds. Phosphorus decline from the water in all system was logarithmic, and within 30 days, 70-90% of added phosphorus had disappeared from the water. Phosphorus is removed from water by muds in the form of inorganic compounds which have limited solubility. The major compounds affecting mud-water phosphorus exchange are aluminium, calcium and iron compounds. The solubility of aluminium and calcium phosphate are strongly affected by the mud pH; the solubility of iron phosphate are affected by the oxygen level, pH, and the mud redox potential (Eh). The relative roles of aluminium, calcium, and iron phosphate in regulating mud water phosphorus fluxes depend mainly on the type of soil in the bottom of the pond and the pH and Eh of the mud. Generally, phosphorus availability is controlled by iron and aluminium phosphates in acidic muds (Masuada and Boyd 1994a) and by calcium and iron phosphate in near-neutral to basic muds (Moore and Reddy 1995a).

Aluminium phosphate generates in muds according to the general reaction:

$$Al^{3+} + H_2PO_4^- \Leftrightarrow AlPO_4 + 2H^+$$

The solubility of aluminium phosphate is low. Adams (1971) reported the solubility of a representative compound, $Al(OH)_2H_2PO_4$, as $10^{-30.5}$. The formation of aluminium phosphates from phosphate added to muds depends on the concentration of aluminium ions, which, in turn, is regulated by pH as shown by the following equation for representative aluminium mineral, gibbsite, which is a major aluminium compound in mud and soil:

$$Al(OH)_3 \Leftrightarrow Al^{3+} + 3\ OH^-$$

This equation shows that the concentration of Al^{3+} increases as the concentration of OH^- decreases (pH decreases). Therefore, as the pH decreases, more aluminium ions are in solution to precipitate added phosphorus as aluminium phosphate.

As the pH of muds and soils increases, concentrations of aluminium ion decrease so that less aluminium phosphate precipitates. Between pH 5.5 and 6.0, the precipitation of aluminium phosphate ceases to be in an important factor regulating the phosphorus mud-water flux. However, as muds or soils increase in pH, the concentration of calcium tends to

increase, and calcium phosphate will precipitate in the mud if phosphorus is added. The primary forms of calcium phosphate in muds and soils are dicalcium phosphate ($CaHPO_4 \cdot 2H_2O$) and the mineral apatite, one form of which has formula $Ca(OH)_2 \cdot 3Ca_3(PO_4)_2$. The solubility pf apatite is exceedingly small ($K_{sp} = 10^{-59}$), whereas dicalcium phosphate is more soluble ($K_{sp} = 10^{-6.56}$). Dicalcium phosphate is transformed over time to the more stable apatite, the rate of transformation being favoured by increasing pH and calcium concentration. Monocalcium phosphate, which has the formula $Ca(H_2PO_4)_2$, often is added to terrestrial and system as fertilizer. If phosphorus concentration are low, monocalcium phosphate is transformed directly to apatite. At higher phosphorus concentrations, monocalcium phosphate is first converted to dicalcium phosphate and then to apatite.

Several different compounds of iron phosphate occur in muds and soils. Under aerobic conditions, these compounds have solubility similar to those of aluminium phosphates, but iron phosphate are much more soluble under anaerobic conditions. Accordingly, the importance of iron phosphate in regulating phosphorus mobility varies with the oxygen status of the pond bottom muds. In aerobic muds, much of the phosphorus is associated with amorphous ferric (Fe^{3+}) oxyhyroxide gels or as phosphorus co-precipitated in coating of ferric oxide surrounding silt or clay particles. These compounds and co-precipitated very low solubility and, therefore, soluble reactive phosphorus concentrations in pore water is very low. Under reducing conditions in anaerobic muds, ferric iron is reduced to soluble ferrous (Fe^{2+}) iron and associated phosphorus is solubilised. So, in anaerobic muds, pore-water phosphorus concentrations will typically be much higher than in pore waters of aerobic muds (Fig. 2.4).

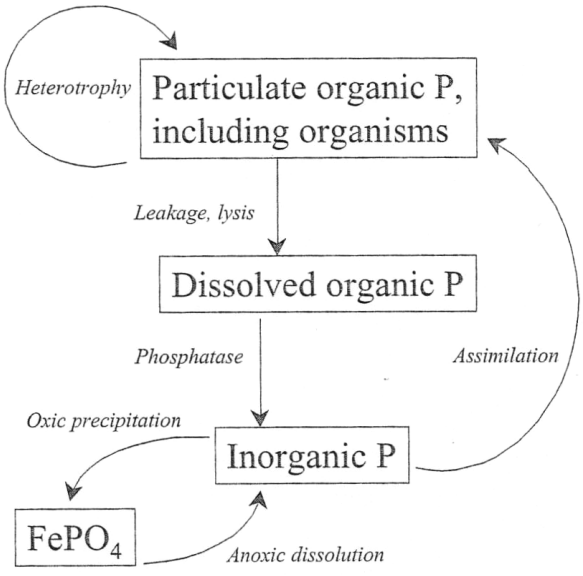


Fig. 2.4; A simple diagram of the phosphorus cycle (Dodds W. K., 2002).

In most aquatic sediment, a thin, oxidized surface layer of mud overlies a thicker, reduced (anaerobic) layer. As reduced substances diffuse to the surface, they are oxidized. For example, the oxidation of ferrous iron at the sediment surface will result in the adsorption or coprecipitation of phosphorus. Consequently, the oxidized mud surface functions as a barrier to the diffusion of phosphorus and reduced substances into the overlying water column. In the absence of an oxidized surface layer, soluble reactive phosphorus will be released from the sediment layers in response to a concentration gradient between sediment pore water and the overlying water column (Masuda and Boyd 1994b). This mechanism of soluble reactive phosphorus release from sediment pore water with an anaerobic sediment-water interface is supported by a study that evaluates the effects of mechanical aeration on phosphorus chemistry (Masuda and Boyd 1994c). In that study, the soluble reactive phosphorus concentration in pond water was higher in not aerated ponds that nevertheless was sufficiently oxidized to function as an effective barrier to the diffusion of pore-water soluble reactive phosphorus.

In summary, the tendency for phosphate to precipitate in muds as soluble aluminium phosphate increases with decreasing pH. The solubility of calcium phosphate increases with increasing pH. The overall result of those tendencies is that maximum solubility of added or native phosphates in mud or soil occurs at an intermediate pH, which has been found to be somewhere between pH 5.5 and 6.0. In this pH range, the concentration of aluminium ions is low so that there is less formation of aluminium phosphate. Calcium concentration in muds with a pH < 6 are less than at higher pH values, so there is less tendency for apatite formation. Formation of iron phosphate depends primarily on the oxygen status of the bottom muds and can be important in either acidic or basic soils. In the aerobic surface layer of muds are aerobic, iron will be present in the oxidized, ferric state and phosphorus will be strongly held as ferric phosphate solids of very low solubility. Orthophosphate concentrations can be much higher in pore waters of anaerobic sediments because iron is reduced to the ferrous state, and ferrous phosphate are much more soluble than ferric phosphates. Formation of ferric phosphates in the surface layers of sediment will tend to remove orthophosphate from the overlying water and will also prevent the release of orthophosphate from deeper, anaerobic layers of mud into the overlying water.

3 WATER QUALITY REQUIREMENT FOR AQUACULTURE:

Water quality is a somewhat nebulous and often used term that is seldom defined adequately. In fact, many dictionaries do not even have water quality as an entry. Probably as good definition as any follows: "Water quality includes all physical, chemical, biological and aesthetic characteristics of water that influence its beneficial use". Any characteristic of water in production system that affects survival, reproduction, growth and production of aquaculture species, influences management decisions, causes environmental impacts, or reduces product quality and safety can be considered a water quality variable. Other factors being equal, aquaculture species will be healthier, production will be more efficient, environmental impacts less and product quality better in culture systems with "good" water quality than in those with "poor" water quality. Knowledge of water quality principles will help aquacuturists to determine the potential of a water body to produce aquaculture species, to maintain or to improve water quality in the culture system, to minimize problems or fish stress and fish health, to produce higher-quality aquacultural products and to reduce the environmental impacts of fish farming.

Successful aquaculture depend on providing animals with an optimized environment for growth. Good initial conditions for aquaculture can be assured by selecting a site with suitable soils and high-quality water supply. An adequate environment must then be maintained through all the farming period so that animals will survive and grow fast. Seasonal water quality characteristics may affect the well-being of fish but only a few ones may play a decisive role. Some variables, such water salinity and temperature, are important when assessing the suitability of a site for the culture of a particular species. Other proprieties such as alkalinity, turbidity, phosphorus and nitrogen compounds are important because they effect plant productivity, which, in turn, may influence aquaculture production. Moreover nitrogen compounds as ammonia and nitrite, may exert a toxic activity on fish. Together, dissolved oxygen, carbon dioxide, ammonia and other factors come into play during the grow-out period because they are potential stressors of the farmer fish. As well as being necessary for life and welfare of fish, the aquaculture water is important for the dissolved oxygen transport and for the removal of wastes including excretions, feces, uneaten feed and dissolved CO_2. The oxygen level in aquaculture water could be maintained by the use of oxygenating system that dissolves pure oxygen in the water, while the removal of catabolite depends on the water exchanges. In facts the water

flow required of aquaculture system depends also to the levels of wastes that must be removed. For example, to maintain the NH_3 concentration under the toxicity level (0.07 mg L^{-1}) in a fish tank with a total biomass and density like to 1 t and 20 kg m^{-3} respectively, the water flows (Q) (L sec^{-1}) can be calculated through the pH unit, with application of formula 3.1 (Brambilla *et al*., 2006 unpublished data):

$$Q = 4 \cdot 10^{-10} \cdot e^{2.3026 \cdot pH} \qquad (3.1)$$

Temperature:

Water temperature is perhaps the most important variable affecting aquaculture production. Water temperature affects the natural productivity of aquatic ecosystems and directly or indirectly affects all other water quality variables. In aquaculture, it is seldom cost-effective to cool or heat large volumes of water, so the water temperature prevailing at a particular site determines which species can be cultured and the potential growth, health, and reproductive success of that species.

The temperature of surface water supplies is established by local temperature and solar radiation, except of course when the body of water is subject to the inflow of water of a different temperature. The temperature of surface waters therefore varies through the year with seasonal changes in air temperature, day length and solar radiation. The magnitude of annual variation in water temperatures and the rate at which temperatures change are less in deep lake and large river than in small streams and ponds in the same region because the large volume of water provides a “buffer” against changes temperature. For the same reason, shallow coastal plain estuaries have greater annual water temperature variation than deeper bays or offshore waters. For a given body of water, the average maximum and minimum water temperatures are fairly consistent year to year, and climatological data on air temperature or solar radiation can be used to model pond-water temperatures (Klemetson and Rogers 1985; Wax and Pote 1990).

Fish and crustaceans are poikilothermic or “cool-blooded”. This means that their body temperature is roughly the same as the temperature of the water surrounding them, and because water temperature changes daily and seasonally, the body temperature of fish also changes frequently. The rates of all biochemical processes are temperature dependent. Within the temperature range that normally occurs in the natural habitat of a particular

species, the rate of biochemical processes roughly doubles for every 10°C increase in temperature. Each aquatic species has a characteristic range of upper and lower lethal temperature limits and a range of temperatures considered “optimum” for growth and health.

Survival at or near the extreme of thermal tolerance depends strongly on the rate of temperature change and acclimation temperature. When temperatures change slowly (no more than about 2°C/day), physiological changes occur that allow aquatic animals to adapt and survive at the new temperature. Acclimation involves biochemical changes in enzyme and cell membrane structure that allow metabolic reactions to proceed more efficiently at the new temperature. Animal are stressed when temperatures change rapidly because there is not enough time for physiological adaptation. Very rapid temperature changes (more than about 0.5°C/min for changes of more than about 5°C) may cause thermal shock and possibly death. A temperature change of 0.2°C/min usually can be tolerated, provided the total change in temperature not exceed more than about 5°C (Boyd *et al.*, 1998).

Water temperature plays an important, indirect role in the health of aquatic animals because it strongly influences the occurrence and outcome of infectious diseases. The relationship between temperature and aquatic animal epizootic is complex because temperature affects both the host and pathogen, as well as other environmental factors that may influence host immunocompetence. These relationships vary greatly among species, specific pathogens, and type of culture system. The immune system of aquatic animals generally functions most effectively at temperatures roughly corresponding to the range for best growth rate. At higher and lower temperatures, immunocompetence is diminished and nonspecific mediators of immunity are the primary means of preventing disease. Rapid temperature changes may also impair immune function, even if changes occur within the range considered optimal. Each pathogen also has an optimal temperature range for growth (or replication) and virulence, and it is the interaction between the effects of temperature on pathogen and host that determine the outcome of the epizootic; this interaction can lead to a pronounced seasonality of epizootics of certain diseases.

Water temperature also may indirectly affect disease outbreaks through effects on other water quality variables. For example, exposure of fish to low dissolved oxygen concentrations can result in stress-mediated immunosuppression and subsequent predisposition to infectious diseases. Water temperature is a major factor affecting dissolved oxygen budgets in aquaculture system, and low dissolved oxygen concentrations

are most common at high water temperatures. So, high water temperatures may indirectly contribute to infectious disease outbreaks through the effect of temperature on dissolved oxygen budgets (Boyd *et al.*, 1998).

Salinity:

Salinity refers to the total concentrations of all ions in water. The major ions contributing to salinity are calcium, magnesium, sodium, potassium, bicarbonate, chloride and sulfate. The absolute and relative concentrations of these ions vary greatly among different waters. Salinity my reported in milligrams per liter (or its equivalent, part per million or ppm). Each species of aquatic animal has an optimum range of salinity for reproduction and growth; outside that range, performance is diminished and survival may be poor. Fortunately the salinity tolerance of most aquaculture species is rather wide and only large differences with other or sudden changes are likely to be important. Salinity also interacts with other water quality variables because the ionic strength of a solution affects equilibrium constants for all chemical reactions, and increasing the salt concentration of a solution decreases the solubility of dissolved gases through the "salting-out effect".

Of practical importance, as the salinity of water increases, the solubility of dissolved oxygen decreases and the percentage of total ammonia present as toxic un-ionized ammonia decreases (Boyd *et al.*, 1998).

The salinity of water supply determines the initial salinity of the ponds. Over time, the addition of substances in feed or the physical processes of dilution or concentration may alter salinity. Pond-water salinity changes during periods of high precipitation or evaporation. In climate with pronounced wet and dry seasons, the change can be significant. In brackish water system, the salinity of the water varied with the salinity of the water supply. Most brackish water are in areas where there is a pronounced wet and dry season. During the wet season, high supply of fresh water from rivers cause salinity values to decline, whereas low discharges of fresh water during the dry season result in higher salinities.

pH:

The pH value expresses the intensity of the acidic or basic character of water. Exposure of aquatic animals to extremes of pH can be stressful or lethal, but the indirect effects of pH and interaction of pH with other variables are usually more important in aquaculture than

the direct toxic effects. Important interactions include the effects of pH on certain aqueous equilibria involving ammonia, hydrogen sulfide, chlorine and methals. The fertility of aquatic ecosystem is also strongly influenced by environmental pH (Boyd *et al.*, 1998).

The pH of most surface waters varies diurnally as photosynthesis and respiration cause changes in dissolved carbon dioxide concentrations. During daylight, aquatic plants remove carbon dioxide from water for use in photosynthesis. Both plants and animal continually release carbon dioxide into the water by respiration. However, during daylight, aquatic plants usually remove carbon dioxide from the water faster then it can be replaced by respiration, and pH increase. During the night, carbon dioxide accumulates and pH declines. The magnitude of the daily fluctuation in pH depends on the buffering capacity (total alkalinity) of the water and the rates of photosynthesis and respiration.

The optimum pH for growth and health of most aquatic animals is in the range 6.5-9.0. The acid and alkaline death points are approximately pH 4 and pH 11, respectively. Fish living in brackish water are often exposed to a wide range of pH values as the relative amounts of fresh water and seawater change with variations in river discharge and tidal flow. As such, brackish water inhabitants are rather tolerant of extremes of pH.

The gills of aquatic animals are the primary target of elevated hydrogen ion concentrations in the environment, which is not surprising considering the structural delicacy of gills and their intimate contact with the external environment. It appears that there are seven major effects of low pH on gill structure or function. These effects are listed in approximate order of their occurrence as pH declines from a minimum “safe” level of about pH 6 to acutely toxic levels around pH 4 (Boyd *et al.*, 1998):

1. Inhibition of sodium and chloride uptake mechanism;
2. Increased ion permeability and diffusional ion efflux;
3. Increased hydrogen ion influx across the gills;
4. Increased mucus production;
5. Mucus coagulation and precipitation;
6. Inhibition of gas exchange across the gills;
7. Damage to gill epithelial layers.

Increased mucus production and gill structural damage at low pH also impairs respiratory efficiency. Respiration is further compromised by blood acidosis, which decrease the affinity of oxygen-binding blood pigments for oxygen. Under mill acid stress, animals

must expend extra metabolic energy for maintenance of gill function at the expense of other processessuch as growth and immune function. Under extreme acid stress, the animal is not capable of maintaining homeostasis and diets.

The gills of fish also are highly sensitive to alkaline solutions (high pH), mucus cells at the base of the gill filaments become hyperotrophic and the gill epithelium separates from the pilaster cells (Boyd *et al.*, 1998). Damage to lens and cornea also occurred. One also would expect gill damage to result in problems with osmoregulation, respiration and blood acid-base balance. High environmental pH also hinders excretion of waste nitrogen because at high water pH, a large fraction of the ammonia immediately outside the gill will be in the form of un-ionized ammonia, thereby reducing the diffusive gradient for passive excretion of ammonia.

Total Alkalinity:

Total alkalinity is the sum of titratable bases in water. In most waters, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) are the predominant bases that contribute to alkalinity. Total alkalinity is expressed equivalent calcium carbonate (mg L^{-1} as $CaCO_3$) or in milliequivalents/Liter (1 meq/L = 50 mg L^{-1} as $CaCO_3$). The total alkalinity of waters ranges from less than 5 to over 500 mg L^{-1} as $CaCO_3$ and is determined by the geology of the aquifer or watershed. Bicarbonate and carbonate are derived primarily from the dissolution of basic minerals.

Aquatic animals do not have a significant physiological requirement for waterborne bicarbonate or carbonate, but total alkalinity is an important environmental variable in aquaculture because it interact with other variables that affect the health of aquatic animals. The pH of most ponds waters is determined by interactions among dissolved carbon dioxide, carbonic acid, bicarbonate, carbonate and carbonate-containing minerals. The uncomplexed ions of certain metals (such copper, zinc, cadmium, nickel and aluminum) can be extremely toxic to aquatic animals. The solubilities of these metals and the concentrations of the free ions increase as pH decreases. Accordingly, the potential for metal toxicity decreases as total alkalinity increases because generally increases with total alkalinity.

Dissolved Oxygen:

The availability of dissolved oxygen frequently limits the activities and growth of aquatic animals. If dissolved concentration are consistently low, aquatic animals will no eat or grow well and will be susceptible to infectious diseases. If concentrations fall to very low levels, the animal may die.

At the same air pressure, the oxygen solubility in water is controlled by water temperature and salinity. It's solubility decreases as water temperature and salinity increases. Table 3.1 shows the solubility of oxygen in water at different temperatures and salinities.

Tab. 3.1; Solubility of oxygen (mg L^{-1}) in water at different temperatures and salinities from moist air with pressure of 760 mm Hg (Boyd *et al*., 1998).

Temperature (°C)	Salinity (ppt)								
	0	5	10	15	20	25	30	35	40
0	14.60	14.11	13.64	13.18	12.74	12.31	11.90	11.50	11.11
1	14.20	13.72	13.27	12.82	12.40	11.98	11.58	11.20	10.82
2	13.81	13.36	12.91	12.49	12.07	11.67	11.29	10.91	10.55
3	13.44	13.00	12.58	12.16	11.76	11.38	11.00	10.64	10.29
4	13.09	12.67	12.25	11.85	11.47	11.09	10.73	10.38	10.04
5	12.76	12.34	11.94	11.56	11.18	10.82	10.47	10.13	9.80
6	12.44	12.04	11.65	11.27	10.91	10.56	10.22	9.89	9.57
7	12.13	11.74	11.36	11.00	10.65	10.31	9.98	9.66	9.35
8	11.83	11.46	11.09	10.74	10.40	10.07	9.75	9.44	9.14
9	11.55	11.18	10.83	10.49	10.16	9.84	9.53	9.23	8.94
10	11.28	10.92	10.58	10.25	9.93	9.62	9.32	9.03	8.75
11	11.02	10.67	10.34	10.02	9.71	9.41	9.12	8.83	8.56
12	10.77	10.43	10.11	9.80	9.50	9.21	8.92	8.65	8.38
13	10.52	10.20	9.89	9.59	9.29	9.01	8.73	8.47	8.21
14	10.29	9.98	9.68	9.38	9.10	8.82	8.55	8.29	8.04
15	10.07	9.77	9.47	9.19	8.91	8.64	8.38	8.13	7.88
16	9.86	9.56	9.28	9.00	8.73	8.47	8.21	7.97	7.73
17	9.65	9.36	9.09	8.82	8.55	8.30	8.05	7.81	7.58
18	9.45	9.17	8.90	8.64	8.38	8.14	7.90	7.66	7.44
19	9.26	8.99	8.73	8.47	8.22	7.98	7.75	7.52	7.30
20	9.08	8.81	8.56	8.31	8.06	7.83	7.60	7.38	7.17
21	8.90	8.64	8.39	8.15	7.91	7.68	7.46	7.25	7.04
22	8.73	8.48	8.23	8.00	7.77	7.54	7.33	7.12	6.91
23	8.56	8.32	8.08	7.85	7.63	7.41	7.20	6.99	6.79
24	8.40	8.16	7.93	7.71	7.49	7.28	7.07	6.87	6.68
25	8.24	8.01	7.79	7.57	7.36	7.15	6.95	6.75	6.56
26	8.09	7.87	7.65	7.44	7.23	7.03	6.83	6.64	6.46
27	7.95	7.73	7.51	7.31	7.10	6.91	6.72	6.53	6.35
28	7.81	7.59	7.38	7.18	6.98	6.79	6.61	6.42	6.25
29	7.67	7.46	7.26	7.06	6.87	6.68	6.50	6.32	6.15
30	7.54	7.33	7.14	6.94	6.75	6.57	6.39	6.22	6.05
31	7.41	7.21	7.02	6.83	6.64	6.47	6.29	6.12	5.96
32	7.29	7.09	6.90	6.72	6.54	6.36	6.19	6.03	5.87
33	7.17	6.98	6.79	6.61	6.43	6.26	6.10	5.94	5.78
34	7.05	6.86	6.68	6.51	6.33	6.17	6.01	5.85	5.69
35	6.93	6.75	6.58	6.40	6.24	6.07	5.91	5.76	5.61
36	6.82	6.65	6.47	6.31	6.14	5.98	5.83	5.68	5.53
37	6.72	6.54	6.37	6.21	6.05	5.89	5.74	5.59	5.45
38	6.61	6.44	6.28	6.12	5.96	5.81	5.66	5.51	5.37
39	6.51	6.34	6.18	6.02	5.87	5.72	5.58	5.44	5.30
40	6.41	6.25	6.09	5.94	5.79	5.64	5.50	5.36	5.22

Dissolved oxygen concentrations in aquaculture system are affected by five major processes operating in waters: air-water gas transfer, sediment oxygen uptake, animal respiration, plankton respiration and photosynthesis. Although the budget appears quite simple because of the limited number of component processes, the rate of each process are affected by several physical and chemical factors (Tab. 3.2). Oxygen is transferred to and from the water depending on partial pressure of oxygen in the water relative to the partial pressure in the air, respiration and sediment uptake remove oxygen and photosynthesis adds oxygen. The high biological activity in some aquaculture systems can lead to pronounced daily cycles in concentrations of dissolved oxygen, with highly supersaturated conditions in the afternoon and highly undersaturated conditions at night. Quite often, mechanical aeration is needed to supplement natural supplies of dissolved oxygen so that concentration do not decline to levels that are overly stressful or lethal to the animals under culture.

Tab. 3.2; Component processes in dissolved oxygen budgets, including the estimate magnitude of the contribution to the oxygen budget and factor affecting the rate of the processes (Boyd, 1998).

Component	Approximate magnitude (mg O_2/L/day)	Factors affecting process rate
Oxygen sources		
Photosynthesis	0–40	Phytoplankton biomass Sunlight Water temperature Non-algal turbidity Phytoplankton species
Reaeration	0–6	Wind speed Dissolved oxygen level Aerator characteristics
Oxygen sinks		
Plankton respiration	0–40	Plankton biomass Water temperature Plankton species
Fish respiration	0–5	Fish biomass Fish size Water temperature
Sediment oxygen uptake	0–4	Benthos density Organic matter content Water temperature Sediment chemistry
Degassing	0–6	Wind speed Dissolved oxygen level

Freshwater fish often are classed as either salmonids (or cold-water fish) or nonsalmonids (or warm-water fish) with respect to oxygen requirements because it is generally thought that cold-water fish are more sensitive to hypoxia than are warm-water fish. When responses to hypoxia are based on concentrations (mg L^{-1}) of dissolved oxygen, that appears to be true. For instance, critical concentrations that reportedly affect growth range between 5 and 6 mg L^{-1} for trout and salmon and 3-4 mg L^{-1} for warm-water fish (Boyd *et al.*, 1998). It is difficult to specify critical dissolved oxygen concentrations because responses are not all-or-nothing, but rather a continuum of physiological effects as dissolved oxygen concentration change. Also effects are not due solely to environmental dissolved oxygen concentrations, but to the interaction among ambient concentrations and exposure time, size and health of the animal, water temperature and other environmental conditions. However for a practical purpose, warm-water fish feed best, grow fasted and are healthiest when dissolved oxygen concentrations are above about 5 mg L^{-1}. As dissolved oxygen concentration fall below about 5 mg L^{-1}, fish attempt to compensate for the decreased availability of oxygen through a series of behavioral and physiological changes. Ventilation volume increase because less oxygen is available in a given amount of water. Fish also minimize extraneous activity to reduce metabolic oxygen demand. Certain physiological responses to low dissolved oxygen concentrations also result in an increased capability for gas exchange at the gills. These adaptations and response allow healthy warm-water fish to survive for days even the dissolved oxygen concentrations are low as 1-2 mg L^{-1}. At some point, these compensatory response are no longer sufficient, and the oxygen demand of tissues exceeds the amount that can be supplied. At about that point, fish swim to the surface in an attempt to exploit oxygen in the surface film. Fish can live for a brief time under these conditions because metabolic energy demands are supplied in part by glycolysis or anaerobic metabolism.

Cold-water fish usually die at a slightly higher concentration than required to kill warm-water fish. For example, rainbow trout may die at dissolved oxygen concentrations of 2.5-3.5 mg L^{-1} (Boyd *et al.*, 1998).

Carbon Dioxide:

Carbon dioxide (CO_2) is a highly water-soluble, biologically active gas. Carbon dioxide is produced in respiration and consumed in photosynthesis. Thus, concentration of dissolved carbon dioxide usually vary inversely with dissolved oxygen. Dissolved carbon dioxide is of interest in aquaculture because it can be a stressor of aquatic animals and it influences the pH of waters. Carbon dioxide is highly soluble in water, but concentrations in pure water exposed to air are low because carbon dioxide is a minor constituent of the atmosphere (about 0.035% by volume). Biological activity may, however, cause significant variation in carbon dioxide concentrations.

The concentration of dissolved carbon dioxide in surface waters depends on the relative rate of respiration, photosynthesis and diffusion of the gas to and from atmosphere. Biological activity is relatively meager in most unpolluted stream, lakes estuarine and marine waters; thus, dissolved carbon dioxide concentrations in unpolluted surface waters are primarily controlled by diffusion. Concentrations are usually less than 5 mg L^{-1} and diurnal seasonal variations in concentration are relatively small (Boyd *et al.*, 1998).

The dynamics of carbon dioxide concentrations are, like those of dissolved oxygen, dominated by the activity of phytoplankton. Dissolved carbon dioxide concentrations cycle daily and the amplitude of those daily fluctuations depend on the relative rates of photosynthesis and respiration. Conditions that favor rapid rates of photosynthesis (abundant phytoplankton, bright sunlight and warm water) favor rapid removal of carbon dioxide. Dissolved carbon dioxide becomes essentially depleted from water on warm, sunny afternoons in ponds with moderate to dense phytoplankton blooms. At night, photosynthesis ceases and carbon dioxide from respiration accumulates.

High concentrations of dissolved carbon dioxide (more than about 60-80 mg L^{-1}) have a narcotic effect on aquatic animals and even higher concentrations may cause death. Exposure to lower concentrations may stress fish by interfering with respiration or by causing formation of calcareous deposit in the kidney (nephrocalcinosis). High environmental concentration of dissolved carbon dioxide (hypercapnia) reduce carbon dioxide excretion at fish gills, causing elevated levels of plasma carbon dioxide and uncompensated respiratory acidosis. These conditions decrease the affinity of hemoglobin for oxygen (Bohr effect), which reduce oxygen uptake by blood at the gills, even if environmental dissolved oxygen concentrations are high.

Ammonia:

Ammonia is the principal nitrogenous waste product excreted by crustaceans and most fishes. Some fish excrete significant quantities of urea, but it is rapidly hydrolyzed in the environment to ammonia and carbon dioxide. Ammonia is also produced when nitrogen-containing organic matter decomposes. Accumulation of ammonia in aquaculture system is undesirable because un-ionized ammonia is toxic to aquatic animals. Ammonia is also a source of combined inorganic nitrogen for plant growth and its availability may influence the productivity of aquatic ecosystems.

Most of the waste nitrogen in aquatic animals is carried in the blood as ammonia and is excreted primarily as un-ionized ammonia, which freely diffuses across the gill epithelium in response to the concentration gradient existing between blood ammonia and environmental ammonia (Fig. 3.1). When external un-ionized ammonia concentration are low, blood un-ionized ammonia quickly diffuses into the water. The gradient of un-ionized ammonia efflux is facilitated by the acidification of water at the external gill surface by expired carbon dioxide. The decrease in external pH at the gill boundary layer helps maintain low external concentrations of un-ionized ammonia by forcing the equilibrium between un-ionized ammonia and ammonia toward ammonium. As un-ionized ammonia is lost from the blood, equilibrium conditions are disturbed and ammonium ion in the blood dissociates to form more un-ionized ammonia, which then may diffuse outward. As long as the external un-ionized ammonia concentration remains low, this process provides a highly energy-efficient mechanism for removal of waste nitrogen. However, as external un-ionized ammonia concentrations increase, the diffusion gradient between blood and water decreases and ammonia may begin to accumulate in the blood and tissues with serious physiological consequences. If external un-ionized ammonia concentration become high enough, the direction of net ammonia movement can become reversed and ammonia will diffuse into the blood, and blood ammonia levels will rapidly increase (Boyd *et al.*, 1998).

Ammonia toxicity is related primarily to the concentration of waterborne un-ionized ammonia, as that determines the diffusive gradient for ammonia loss. As such, it is customary to report ammonia toxicity in terms of the concentration of un-ionized ammonia, rather than total ammonia. Note, however, that some ammonia is also excreted from aquatic animals as ammonium ions that are actively exchanged for some sodium ions from the external medium (Fig. 3.1). High concentrations of ammonia ions in the external medium can partially inhibit or reverse this reaction and contribute to accumulation of

ammonia in the blood.

The mechanisms of ammonia toxicity have not been firmly established. It appears that the primary mechanism of acute ammonia toxicosis is associated with the suppression of metabolic energy production in the central nervous system (Smart, 1978). This is brought about when high blood and tissue ammonia levels lead to the amination of α-ketoglutarate, which is an intermediate in the tricarboxylic acid cycle. Loss of α-ketoglutarate disrupts the cyclic nature of that metabolic pathway and can severely curtail metabolic energy production. Symptoms of acute ammonia toxicosis progress from hyperactivity and convulsions to lethargy, loss of equilibrium and finally coma. These symptoms support the notion that the primary effect of acute exposure is on the central nervous system. In most animals, acute toxicosis can occur within hours of exposure to more than about 1-2 mg L^{-1} of un-ionized ammonia-nitrogen. Several other dysfunctions and lesions have been linked to ammonia exposure and may contribute to toxicity, particularly under conditions of chronic, long-term exposure. Exposure to low concentration of un-ionized ammonia causes osmoregulatory disturbances, blood acidosis and reduced respiratory efficiency. The overall effect chronic exposure to ammonia is reduced growth and greater susceptibility to infectious diseases (Colt and Armstrong, 1979).

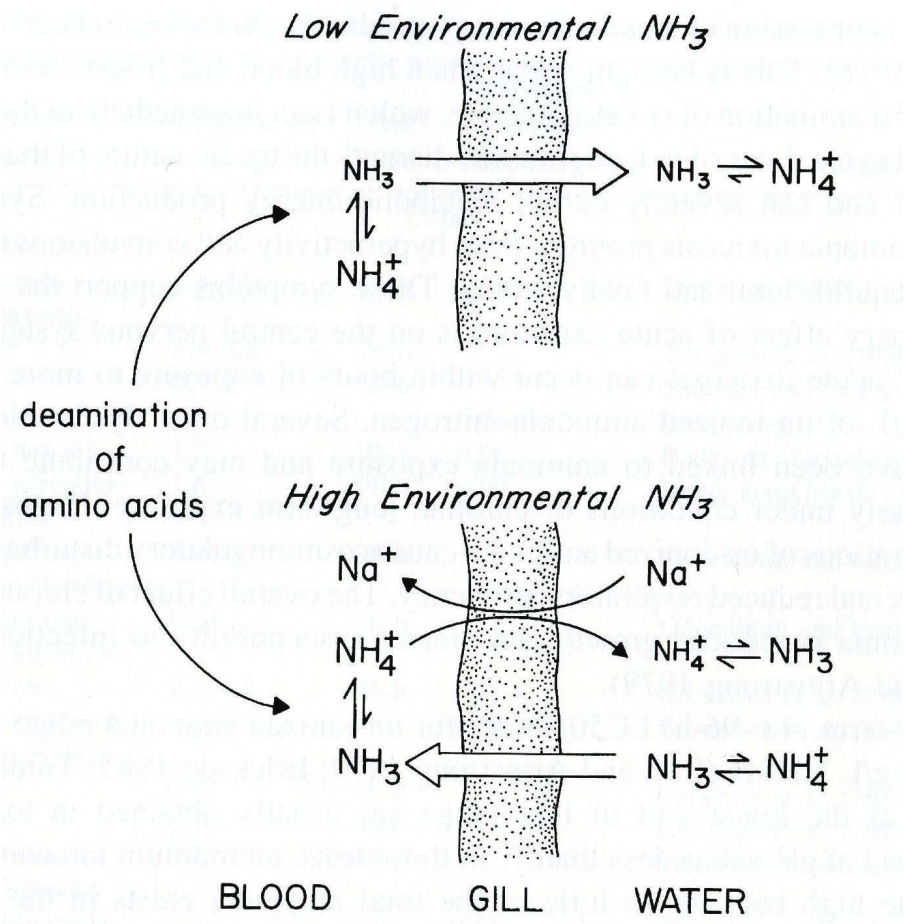


Fig. 3.1; Movement of ammonia across the gill membrane of a typical ammonotelic aquatic animal. Un-ionized ammonia can freely diffuse across the gills in response to concentration gradient. In the upper diagram, un-ionized ammonia is removal from the blood because the external concentrations are low. In the lower diagram un-ionized cannot diffuse across the gills but may be actively transported across the gill in exchange for sodium. This processes becomes increasingly important as the external concentration of un-ionized ammonia increases (Boyd *et al.*, 1998).

Nitrite:

Nitrite (NO_2^-) is a naturally occurring intermediate product in two bacteria-mediated processes involving transformations of nitrogen in water and soils. Nitrite occasionally accumulates in aquaculture system and can be toxic to aquatic animals. Nitrite is an intermediate in the process of nitrification, which is the two-step oxidation of ammonium to nitrate carried out by highly aerobic, gram-negative, chemoautotrophic bacteria. Nitrite normally does not accumulate in the environment because is converted to nitrate as quickly as it produced. Under certain conditions, however, the rate of ammonia oxidation can

exceed the rate of nitrite oxidation and nitrite will accumulate. Nitrite is also an intermediate in denitrification, which is the biological reduction of nitrate to dinitrogen gas (N_2) or nitrous oxide (N_2O). Denitrification occur under anaerobic condition when heterotrophic bacteria use nitrate instead of oxygen as a terminal electron acceptor in respiration. Nitrite is an intermediate in the process and may accumulate in anaerobic soils and bottom muds.

Nitrite may briefly accumulate in water after sudden increases in ammonia concentrations following phytoplankton die-off. Decomposition of the dead plant material releases large amounts of ammonia into the water. The increased availability of ammonia stimulates the activity of ammonia-oxidizing bacteria, and nitrite is produced. Growth and metabolism of nitrite-oxidizing bacteria lag behind until sufficient substrate (nitrite) accumulates, after which nitrite concentrations decrease as the nitrite is oxidized to nitrate. The lag time in development of active populations of the two groups of bacteria results in a characteristic temporal pattern of elevated total ammonia levels, followed by a transient increase in nitrite concentrations and finally an increase in nitrate levels (Fig. 3.2).

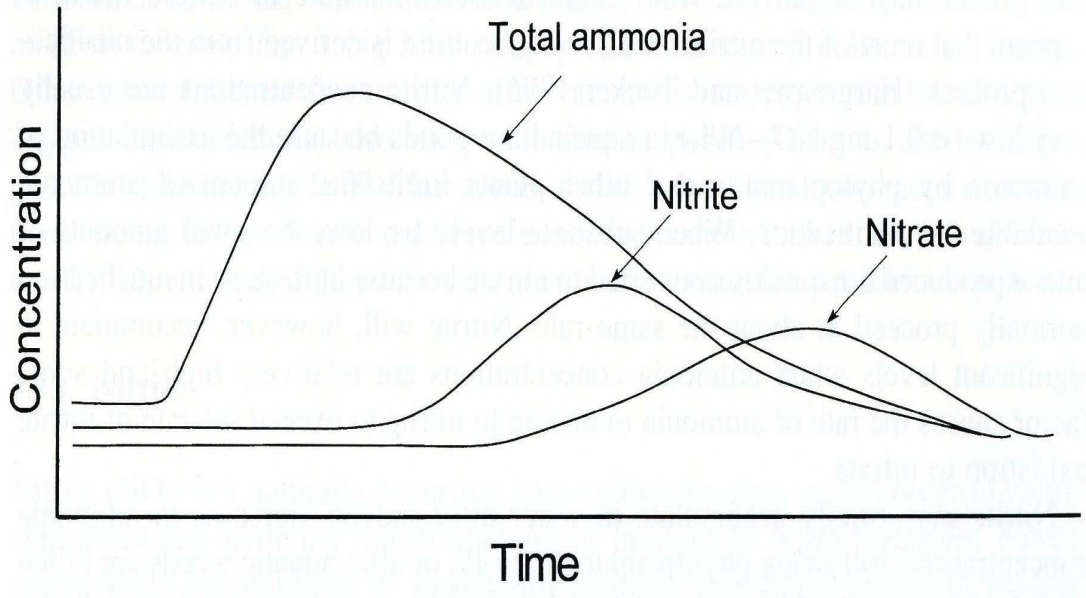


Fig 3.2; A typical series of events following a sudden increase in total ammonia concentrations in water. The increase in ammonia concentrations is followed by an increase in nitrate concentrations. This series of event occurs because there is a lag time between the development of populations of ammonia-oxidizing and nitrite-oxidizing bacteria (Boyd *et al.*, 1998).

Waterborne nitrite enters in the fish circulatory system through the gills. At very low pH values, nitrite may enter the bloodstream passively as nitrous acid (HNO_2), which freely diffuse across gill membranes because of its uncharged and lipophilic nature. However, very little nitrous acid exists in water at common environmental pH values and most of the nitrite that enters the circulatory system of aquatic animals does so as the nitrite anion. Nitrite crosses the gills by the mechanism that normally transport chloride into the bloodstream. Both nitrite and chloride are monovalent anions with similar hydrated ionic radii and, in certain fish species, the ion transport mechanism apparently does not discriminate between the two ions. After the entering the bloodstream, notrite oxidized the iron in the hemoglobin molecule from the ferrous state (Fe^{2+}) to the ferric state (Fe^{3+}). The resulting product, called methemoglobin, has a characteristic brown color that becomes grossly noticeable when the methemoglobin concentration of the blood exceeds about 20% of the total hemoglobin. This is the basis for the common name of the syndrome, "brown-blood disease". Methemoglobin is incapable of reversibly binding with oxygen, so exposure to nitrite may cause considerable respiratory distress because of the loss in blood oxygen-carrying capacity (Saroglia *et al.*, 1981).

The high chloride concentration in brackish water and seawater is generally assumed to afford great protection against nitrite exposure for fish inhabiting those waters, although the fish may be quite intolerant of nitrite when chloride concentrations are low.

Turbidity:

Turbidity refers to an optical property of water that causes light to be scattered or adsorbed rather than transmitted through the water in a straight time. Turbidity is caused by suspended material (such a soil particles, plankton and organic detritus) and soluble colored organic compounds. The relatively still, unmixed water in many aquaculture systems favors of solids, and suspended solid seldom exceed 100 or 200 mg L^{-1} for more than a few days (Boyd *et al.*, 1998). Even though turbidity caused by suspended soil particles will seldom have immediate direct effect on fish, in the long run it may harm fish populations. Turbidity will restrict light penetration, adversely affecting plant growth, and some of particles will settle to the bottom and smother fish eggs and destroy benthic communities.

Alabaster and Lloyd (1980) stated that there is no evidence that suspended solids

concentrations less than 25 mg L^{-1} have any harmful effects on fisheries, and that good to moderate fisheries can be maintained in waters with 25-80 mg L^{-1} suspended solids. Good fisheries cannot be expected in waters with suspended solids concentration above 80 mg L^{-1}. Water supplies often contain extremely turbid water because of erosion in drainage basins of rivers and because tidal action in estuaries maintain large amount of soil particles in suspension. When water is pumped from rivers or canals, sedimentation may be a major problem. Some sediment contains a large amount of organic matter and exerts a high oxygen demand.

4. WASTE MANAGEMENT:

4.1 Sources and fate of nutrient and organic matter

The discharge waters coming to the aquaculture systems contain nutrients, organic matter and suspended solids that can be sources of pollution in receiving waters. The primary source of nutrients and organic matter is feed and the secondary sources of organic matter originated by phytoplankton photosynthesis. The fate of feed added to aquaculture system is illustrated diagrammatically in Fig. 4.1 (Boyd and Tucker, 1995).

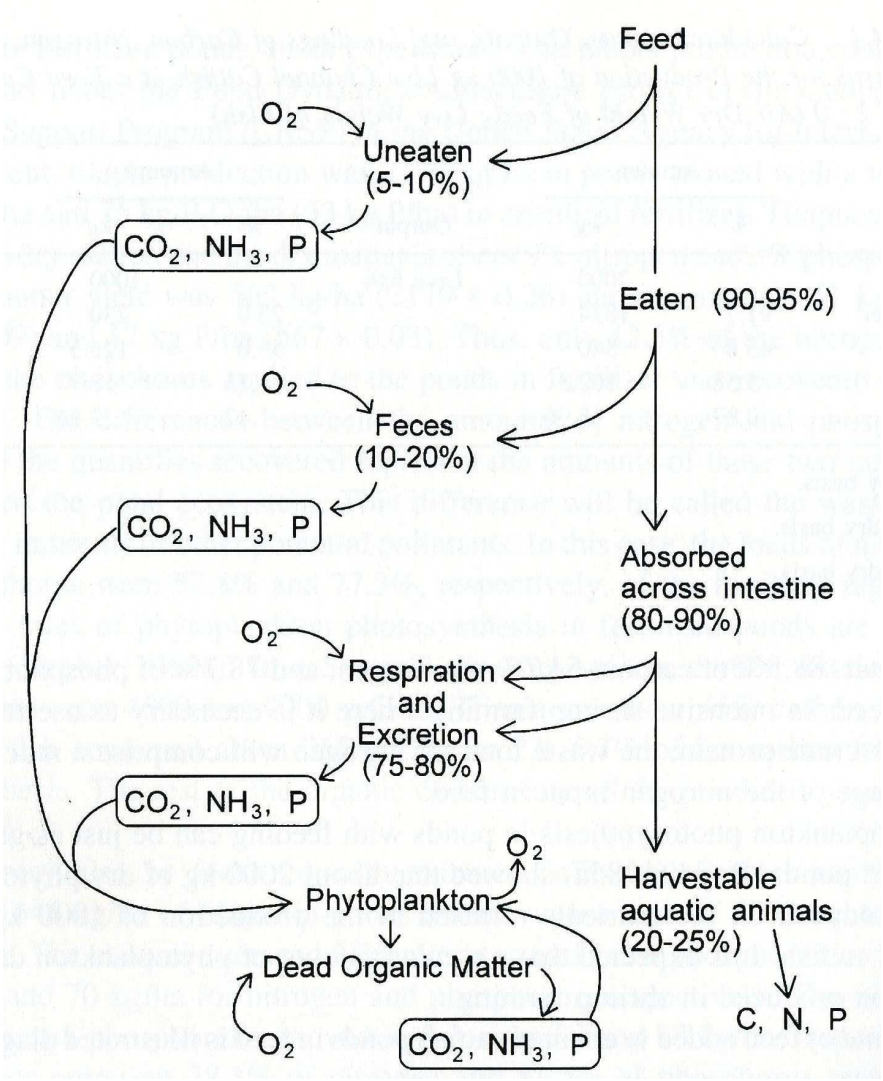


Fig 4.1; Fate of feed in aquaculture system (Boyd *et al.*, 1998).

The waste load resulting from feeding can be assimilated by some biological, chemical and physical processes occurred into aquaculture systems (Fig. 4.2). Principally, eaten feed will be excreted as feces, ammonia and carbon dioxide. Uneaten feed, feces and dead phytoplankton, will be decomposed by microorganisms in the water column or in the sediment. The organic matter will be converted to basic mineral components such as carbon dioxide, ammonia and phosphate.

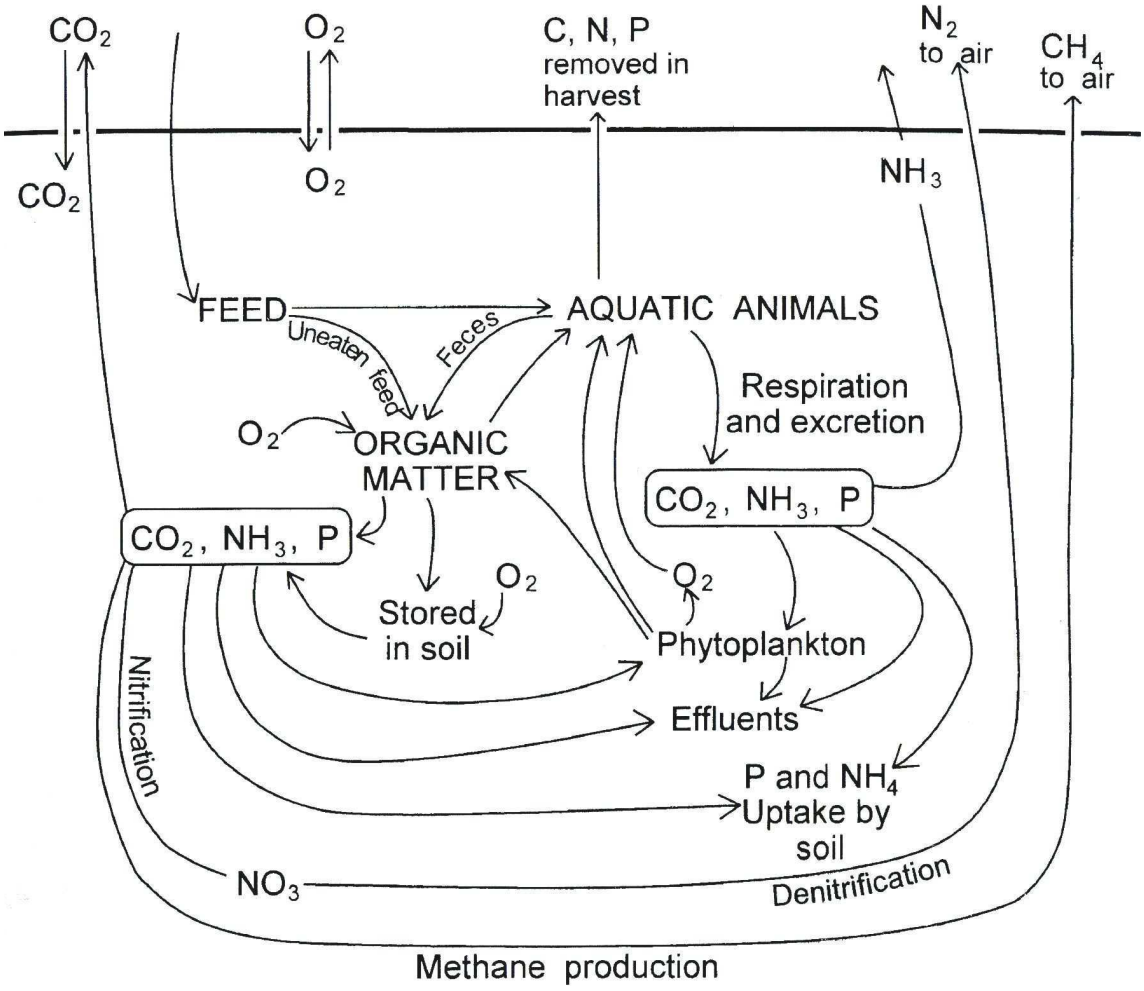


Fig. 4.2; Assimilation of organic matter and nutrients in aquaculture systems (Boyd *et al.*, 1998).

The two major sources of suspended solids in aquaculture effluents are suspended soil particles and particulate organic matter resulting from detritus and live phytoplankton. The organic detritus result from degradation of feces, uneaten feed and dead plankton. Solids enter into aquaculture systems from water inflow, but these solids tend to settle onto

bottoms. The aquaculture system have also an internal sediment load from bottoms by water currents generated by aerators, bioturbation caused bottom disturbance by fish and water current. Soil particles that are resuspended by these processes will settle again, but a portion of the particles will be contained in the effluents. Organic matter is usually a major constituent of the particles that settle to the bottom. Thus, the flocculent layer that extend a few centimetres above the soil-water interface and the highly fluid surface layer of bottom soil contains organic matter (Munsiri *et al.*, 1995).

In the last years, there have been many changes in feed and feeding technologies for reply to requirement of environmental protection, resulting in substantial improvements in feed conversion rations (FCR) and fish growth rates. In general, respect to the N and P contents in feed, nitrogen and phosphorous retention range between 10-49% and 17-40% respectively. Similarly N and P releases in faces range to 3.6-35% and 15-70% respectively, and dissolved N and P excretions range to 37-72% and 1-62% respectively. Although these numbers are highly variable, the tendency is to increase nutrient retention and reduce losses by improvement of feed quality (Piedrahita, 2003).

4.2 Environmental and natural resources effects

Aquaculture has become large enough to have significant impacts on the environment and natural resources, and a number of concerns have been expressed by both environmental activists and scientists (Boyd, 2003). The most serious concern are the following:

- Destruction of wetland and other sensitive aquatic habitat by aquaculture projects;
- Water pollution resulting from fish farm effluents;
- Excessive use of drugs, antibiotics, and other chemicals for aquatic animal disease control;
- Inefficient utilization of fish meal and other natural resources for fish (and shrimp) production;
- Salinization of land and water by effluents, seepage, and sediment from brackish water ponds;
- Excessive use of ground water and other freshwater supplies for filling ponds;
- Spread of aquatic animal diseases from culture of organisms to native populations;

- Negative effects on biodiversity caused by escape of non-native species introduced for aquaculture, destruction of birds and other predators;
- Conflicts with other resource users and disruptions of nearby communities.

Of these and other possible negative impacts, water pollution by fish farm effluents is probably the most common complaint, and this concern has attracted the greatest official attention in most nations (Boyd, 2003). Feed are applied to ponds or fish tanks to promote fish production, and normally no more than 25% to 30% of the nitrogen and phosphorus applied in feed is recovered in fish or shrimp at harvest (Boyd *et al.*, 1998). Ponds or fish thanks often have higher concentrations of nutrients, catabolites plankton, suspended solids and oxygen demand than the water bodies into which they discharge (Schwartz and Boyd 1994). Thus, this effluents are potential sources of pollution in receiving waters.

Water quality regulations development by governmental agencies usually have effluent standards and rules for issuing and enforcing permits for individual effluent outfalls (Goldesteen, 1999). Water quality are designed to prevent effluents from causing negative impacts on receiving waters. Standard normally specify limits for selected water quality variables and may contain other restrictions. A water quality standards contain the following water quality restrictions: pH, 5.5 to 9.5; dissolved oxygen, 5 mg L^{-1} or more; 5-day biochemical oxygen demand (BOD_5), 40 mg /L or less; total suspended solids, 80 mg L^{-1} or less. Sometimes, the standard also may place a restriction on discharge volume or restrict discharge volume indirectly by specifying a maximum daily load for a variable. To illustrate, the maximum daily volume might be 10,000 m^3/day, or the maximum BOD_5 load cannot exceed 100 kg/day. The load limit would restrict volume to 3333 m^3/day at a BOD_5 concentration limit of 30 mg L^{-1}, or restrict BOD_5 concentration to a maximum of only 10 mg L^{-1} at a maximum daily discharge At least two non-governmental organizations have made effluent standards for aquaculture. The Global Aquaculture Alliance (GAA) is an international aquaculture organization promoting environmentally responsible production. It suggest that members use environmentally responsible culture methods to comply with effluent standards (Tab. 4.1).

Variable	Initial standard	Target standard
pH (standard units)	6.0–9.5	6.0–9.0
Total suspended solids (mg/l)	100 or less	50 or less
Total phosphorus (mg/l)	0.5 or less	0.3 or less
Total ammonia nitrogen (mg/l)	5 or less	3 or less
5-Day biochemical oxygen demand (mg/l)	50 or less	30 or less
Dissolved oxygen (mg/l)	4 or more	5 or more

Tab. 4.1; Initial and target water quality standard for farm effluents recommended by the Global Aquaculture Alliance (Boyd, 2003).

The GAA standards consist of initial, rather lenient limits, and stricter target limits with which the member should comply within 5 years (Boyd and Gautier, 2000). The International Finance Corporation (IFC) sometimes provides low interest loans to aquaculture projects in developing countries. The IFC requires compliance with water quality standards (International Finance Corporation). The standards that have been required for farm effluents apparently are the same ones required for fish processing operations (Tab. 4.2).

Parameter/pollutant	Maximum value
pH	6 to 9
BOD_5	50 mg/l
Oil and grease	10 mg/l
Total suspended solids	50 mg/l
Coliforms	Less than 400 MPN/100 ml (MPN = Most Probable Number)
Temperature increase	Less than or equal to 3 °C[a]

[a] The effluent should result in a temperature increase of no more than 3 °C at the edge of the zone where initial mixing and dilution take place. Where the zone is not defined, use 100 m from the point of discharge.

Tab. 4.2; Liquid effluent standards suggested by International Finance corporation (Boyd, 2003) .

The actual environmental impacts of aquaculture effluents have seldom been established. Receiving water bodies have capacity to dilute and assimilate the pollutants entering them, and if that capacity is not exceeded, the discharges from aquaculture farms will not cause eutrophication or other environmental problems. In some places, the effluents from aquaculture ponds are more concentrated in nutrients, solids and organic matter then

receiving waters. The influence of pond effluents on receiving water bodies depends on the pollution load and the ability of the receiving water to dilute and assimilate the pollution. If the receiving water bodies is large and the pollution load is rather small, pond effluents will not cause adverse impacts. On the other hand, if the pollution load from pond effluents is large and the receiving water body is small or is already receiving pollution from other sources, adverse impacts may occur. Example of water pollution resulting from pond effluents have occurred in China where lakes are used to supply water to ponds and pond effluents are discharged back into the lake. Stream reaches downstream from areas with a high density of aquaculture ponds may be polluted by effluent. In coastal aquaculture, where there are strong marine currents, the tidal plume will carry water from ponds offshore, and the currents will cuts off the tidal plume and transport the water into the open sea where it will be greatly diluted. Where the currents are weak, a part of the tidal plume will flow back into the estuary on high tide, and nutrients and organic matter will be retained for a longer time in estuaries. Where shallow mud flats restrict water flow and where estuaries discharge into lagoons with narrow openings to the sea, flushing times for estuarine will be long, and there is a greater danger of eutrophication by aquaculture farm effluents.

4.3 Best Management Practices (BMPs)

In environmental management, practices used to prevent water pollution and other negative environmental impacts are called best management practices and usually are referred to as BMPs (Hairston *et al.*, 1995). A BMP is considered to be the best available and practical means of preventing a particular environment impact while “best” is not intended to imply that a particular BMP will always be the best practices. The “best” practices must be selected based on site characteristic, and as technology advances, BMPs must be revised to reflect new knowledge. Some workers do not like the term BMP and insist on referring to good management practices (GMPs) or better management practices. Nevertheless, the term BMP is acceptable terminology in environmental management, and its use should be encouraged over GMP or other acronyms. It should be emphasized thea a BMP is seldom applied alone. A system of several BMPs usually must be installed to prevent water

pollution and achieve resource management goals.

There are many BMPs available for use in aquaculture, so a simple listing of BMPs is not useful. It is customary to group BMPs according to specific operations or objectives.

Pond effluents contain nutrients that may cause eutrophication or receiving water bodies. Nutrient loads in effluents may be minimized through application of the following BMPs:

- Select stocking and feeding rates that do not exceed the assimilation capacity of ponds or fish thanks;
- Feed should be of high quality, water stable, and contain no more nitrogen and phosphorus than necessary;
- Apply feeds conservatively to avoid overfeeding to assure that as much of the feed is consumed as possible;
- Do not use water exchange or reduce water exchange rates as much possible;
- In intensive aquaculture, apply enough mechanical aeration to prevent chronically low dissolved oxygen concentration and to promote nitrification and other aerobic, natural water purification processes;
- Provide storage volume for heavy rainfall to minimize storm overflow;
- Where possible, discharge pond draining effluent through a settling basin or a vegetated ditch;
- Reuse water where possible.

4.4 Effluent treatment

Many schemes for using or improving effluents from ponds have been advanced over the years to include wetland, settling basins, biological filters, nutrient removal by water hyacinths or other floating macrophytes, fluidized-bed filters, and other. The most efficient procedures appear to be irrigation, settling basins, and wetlands. Effluents from ponds are not concentrated enough in nutrients for use in hydroponics unless nutrient concentrations are supplemented, which may defeat the purpose of using this procedure to "treat" effluents. Filter-feeding fish and molluscs and certain plants have been successfully cultured in effluents, but this practice has seldom been economical, and it does not greatly improve effluent quality. Complicated water-treatment procedures such as biological

filtration and activated-sludge processes are too expensive to use with pond effluents. Also nutrient and organic matter concentrations in most pond effluents are too low for these treatment procedures to be effective.

The treatment of aquaculture waste water, to control their quality, are many various but based on important mechanical and biological processes. The removal of solids, organic matter, ammonia, nitrite and phosphorus are critical for aquaculture system. Solids are usually removed using physical processes, including sand and mechanical filters (Kristiansen and Cripps, 1996). Biological processes such as submerged biofilters, trickling filters, rotating biological contactors and fluidized bed reactor are employed in the oxidation of organic matter, nitrification or denitrification (Van Rijn, 1996). These treatment methods have the disadvantages of producing sludge and requiring frequent maintenance.

Elimination of nitrogen forms is generally managed through biofiltrationin aerobic conditions, for the conversion of toxic ammonia produced by the fish to nitrate through nitrification. Nitrate is typically maintained at low levels by delay replacement of water. However, strict environmental regulations for nitrate levels in discharge water are motivating to aquaculture industry, above all re-circulating system, to integrate a denitrifying biofiltration stage, which result in the conversion of nitrate to nitrogen gas. Denitrification is performed mainly by facultative anaerobic bacteria that utilize organic (heterotrophic denitrification) or inorganic (autotrophic denitrification) compounds as electron sources to reduce nitrate. One option for closing the nitrogen cycle without exclusive reliance on denitrifacation is to integrate the anaerobic oxidation (anammox) processes in treatment systems. An important pathway of the nitrogen cycle (Fig. 4.3), anammox allows ammonia to be oxidized by nitrite under anoxic conditions and is performed by autotrophic bacteria that are member of the order Planctomycetales (i.e. *Brocadia anammoxidans*) (Fig. 4.4A):

$$NH_4^+ + NO_2^- \Rightarrow N_2 + 2\,H_2O \qquad \Delta G^{\circ\prime} = -357\ kJ$$

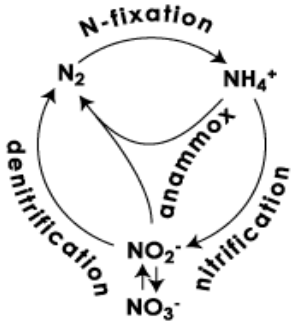


Fig. 4.3; Nitrogen cycle with anammox processes in anoxic conditions (Internet source).

The anammox reaction has been shown to occur an organelle-like compartment called the anammoxosome (Fig. 4.4 B), where ammonia is oxidized via hydrazine (N_2H_4) and hydroxylamine (NH_2OH) intermediates (Fig. 4.5). This process took place naturally in the sediment sampled but the bio-promoter addition improve the efficiency of NH_4-N removal.

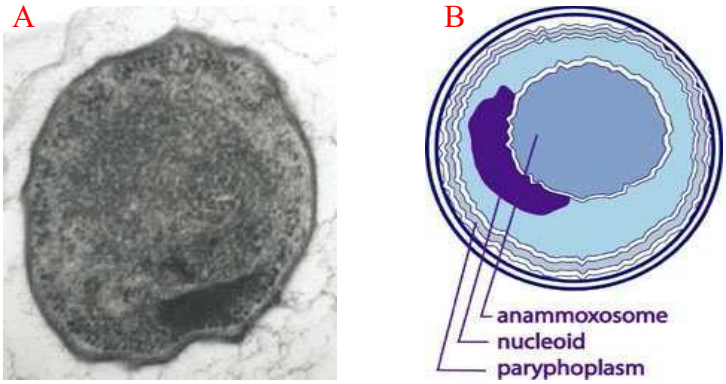


Fig. 4.4; TEM image of *Candidatus* "*Brocadia anammoxidans*" (photo John Fuerst/Rick Webb)(A) and scheme of anammox ultrastructure (B).

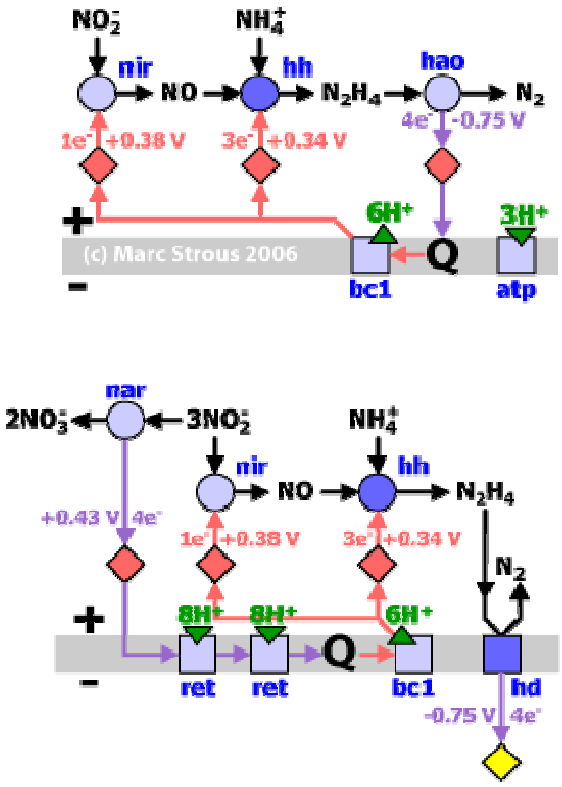


Fig. 4.5; Anammox mechanism with hydrazine and nitric oxides as intermediates(top); reversed electron transport to upgrade electrons from nitrite oxidation to drive anammox carbon fixation via the acetyl CoA pathway (bottom) (Internet source).

Utilization of the process for complete removal of nitrogen from contaminated water is economically viable since ammonia oxidation requires, at a minimum, 50% less oxygen than that required by the conventional nitrification-denitrification processes. (Tal *et al*., 2006).

Traditional methods for phosphorus removal from waste water are based on chemical precipitation of phosphorus with mainly iron and aluminium salts. However, due to the technical and economical constraints of these chemical processes, the use of alternative, biological treatment methods is steadily increasing. Enhanced biological phosphorus removal (EPBR) is the most common biological phosphorus-removal method. EPBR is a wastewater treatment based on the selective enrichment of bacteria accumulating inorganic polyphosphate as an ingredient of their cells (Tab. 4.3). It involves microbial metabolic cycling via several microbial-accumulated biopolymers (polyphosphate, PHA, and glycogen). This metabolic cycling is induced in microorganisms by alternating incubation condition of the wastewater between initially carbon-rich, strictly

anaerobic incubation (no oxygen or nitrate are present), followed by carbon-poor, aerobic incubation (Fig. 4.6). In essence, during the anaerobic phase, microbes residing in the sludge (apllied as an inoculum to the wastewater) deplete the organic matter and carbon sources from the wastewater, accumulate storage biopolymers (mainly PHAs and glycogen), and release soluble orthophosphate from the sludge. The energy source for these processes is derived mainly from polyphosphate. Polyphosphate is a high-energy storage molecule that, upon hydrolysis, can supply ample energy for biochemical reactions within the cells. This molecule is particularly useful during the anaerobic storage of EPBR, where it supplies the needed energy for uptake of the organic substrates. Polyphosphate is stored in the cells of the same bacteria residing in the applied sludge of previous wastewater treatment cycles. The other biopolymer, glycogen, which usually serves as regulators of redox balance in cells, also provides additional energy helping polyphosphate accumulating organisms (PAOs) to absorb organic substances under anaerobic conditions. When conditions in the bioreactor change to aerobic, PHA molecules serves as energy and carbon sources for uptake of even larger amount of orthophosphate than the amount originally released during the anaerobic process, and this enhanced uptake includes the phosphorus arriving with the new wastewater. The aerobic process reincorporates the orthophosphate into new intracellular microbial polyphosphate. This leaves the wastewater phosphate-poor, and in case of complete EPBR success, phosphate free. EPBR is attributed to PAOs, mainly bacteria. An obligatory requirement to achieve high and stable EPBR is maintaining PAOs in the system. While the appearance and disappearance of microbial biopolymers is known, the metabolic pathways involved biomass synthesis and energy production in these bacteria are far from being understood and were reviewed recently (de-Bashan *et al*., 2004). The activated sludge produced after the processes (containing organic matter and a large microbial population) is even richer in phosphate, usually discarded, and may be used for biogas production.

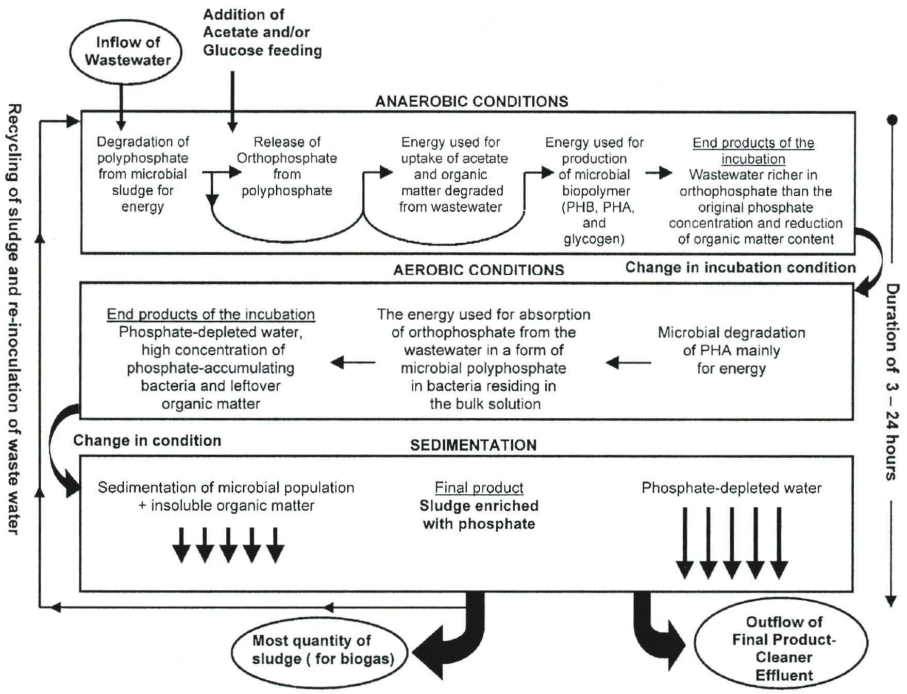


Fig. 4.6; Conceptual model for enhanced biological phosphorus removal (EBPR) (de-Balshan *et al*., 2004).

Tab. 4.3; Bacterial species and genera involved in induction of or participating in Enhanced Biological Phosphorus Removal (EPBR) (de-Balshan *et al.*, 2004).

Bacterial genera or species	Evidence for involvement in EBPR	Evidence against involvement in EBPR	Reference
Acinetobacter spp.	1. Predominance in EBPR, based on culture media growth. 2. Strains accumulate polyphosphate and PHAs under aerobic conditions.	1. Culture media detection method is selective for *Acinetobacter* spp. 2. Fluorescent antibody staining, quinone profile or fluorescent in situ hybridization with oligonucleotide probe specific for *Acinetobacter* showed that the species is not primarily responsible for EBPR and is present as a small percentage in the sludge (<10% of total bacteria). 3. No strain possesses the typical metabolic pathways of EBPR-like acetate uptake and its conversion to PHA and hydrolysis of polyphosphate and release of orthophosphate under anaerobic conditions. 4. As dominant respiratory mechanism, bacteria uses quinone Q-9 when PAO processes are Q-8 or MK-8(H_4). 5. Polyamine pattern show that *Acinetobacter* has the polyamine diaminopropane, where EBPR processes have almost no presence of the compound.	Mino et al. (1998), Christensson et al. (1998), Fuhs and Chen (1975)
Microlunatus phosphovorus	1. Bacteria accumulate large amounts of polyphosphate under aerobic conditions.	1. Bacteria do not take up acetate and do not accumulate PHA under anaerobic conditions.	Eschenhagen et al. (2003), Nakamura et al. (1995), Kawaharasaki et al. (1998), Santos et al. (1999)
	2. Capable of anaerobic uptake of glucose.	2. A 16s rRNA-targeted oligonucleotide probe, specific to the species, showed that its population is <3% of total bacteria in the sludge, when PAO are about 9% of the total population. 3. Contains Q-9 as a major quinone and not the common Q-8 and MK-8(H_4) of PAO-enriched-sludge.	
Lampropedia sp.	1. Bacteria possess key metabolic characteristics of PAO. Bacteria accumulate polyphosphate and poly β-hydroxybutyrate. 2. Bacteria take up acetate and store it as PHA with polyphosphate degradation and release of orthophosphate.	1. Has a unique sheet-like organization, which is uncommon in EBPR processes.	Stante et al. (1997)
Rodocyclus sp.	1. Bacteria possess key metabolism of PAO.	No available data.	Lee et al. (2003), Bond et al. (1999), Ahn et al. (2002), Eschenhagen et al. (2003)
	2. Fluorescent in situ hybridization show that the group dominates the EBPR process (>81% of the population).		
Tetrasphaera japonica, *Tetrasphaera australiensis*	Phosphate-accumulating coci.	Microscopically similar to glycogen-accumulating bacteria from activated sludge.	Eschenhagen et al. (2003), Maszenan et al. (2000)
Tessaracoccus bendigoensis	1. Contains intracellular polyphosphate granules. 2. Morphologically similar to dominant microorganisms in activated sludge	Resembles glycogen-accumulating bacteria.	Maszenan et al. (1999)
Paracoccus denitrificans	Polyphosphate-accumulating bacterium.	Does not need the alternating anaerobic/aerobic cycle for phosphate accumulation. Can accumulate PHB, but does not accumulate polyphosphate when cells are rich in PHBs.	Barak and van Rijn (2000)
Burkholderia cepacia	Contains intracellular polyphosphate granules.	No available data.	Mullan et al. (2002)
Agrobacterium sp. *Aquaspirillum* sp. *Micrococcus* sp. *Staphylococcus* sp. *Acidovorax* sp. *Microsphaera multipartite*, *Dechlorimonas* spp. Unidentified yeast, Cytophaga-Flavobacteria group	Found in large and dominate numbers in EBPR processes.	No available data.	Merzouki et al. (1999), Ahn et al. (2002), Melasniemi et al. (1998), Melasniemi and Hernesmaa (2000), Van Ommen Kloeke and Geesey (1999)

PHA = polyhydroxyalkanoate; PAO = Phosphate accumulating organisms.

4.4.1 Wetland systems:

Wetland technology has grown in popularity for wastewater treatment since early 1970s (Cole, 1998). Constructed and natural wetland are low-cost, low-tech process to control environmental pollution. Basically is a container (as small as a bucket or as big as a very large pond) planted with mainly aquatic, but sometimes with terrestrial plants. Inflow wastewater current slowly flows either end and, in the process, the outflow is cleaner. Afterwards, the treated wastewater is commonly discarded to natural bodies or used for irrigation of inedible plants without any further treatment. The wetland may be visualized as consisting of several compartments: water, plants, microbiota, litter and soil. Wetlands act as biological filters to remove pollutants from water, and natural and constructed wetlands sometimes are used for treatment of animal, agricultural, municipal, and industrial wastewaters (Hammer 1992; Mosshiri 1995). There are several advantages to wetland wastewater treatment. Wetlands are relatively inexpensive to build and operate, chemical treatment of wastewater is eliminated, wetlands contribute stability to local hydrologic processes, and plant communities in wetlands are excellent wildlife habitat. The main problem with wetlands for treating aquaculture effluents is that large areas of land may be necessary. More frequently, wetland system are constructed, and these may be classified as either free water surface (FWS) (Fig. 4.7) or subsurface-flow (SSF) (Fig. 4.8) wetlands according in their hydraulic loading rates and hydraulic residence time (Crites, 1994).

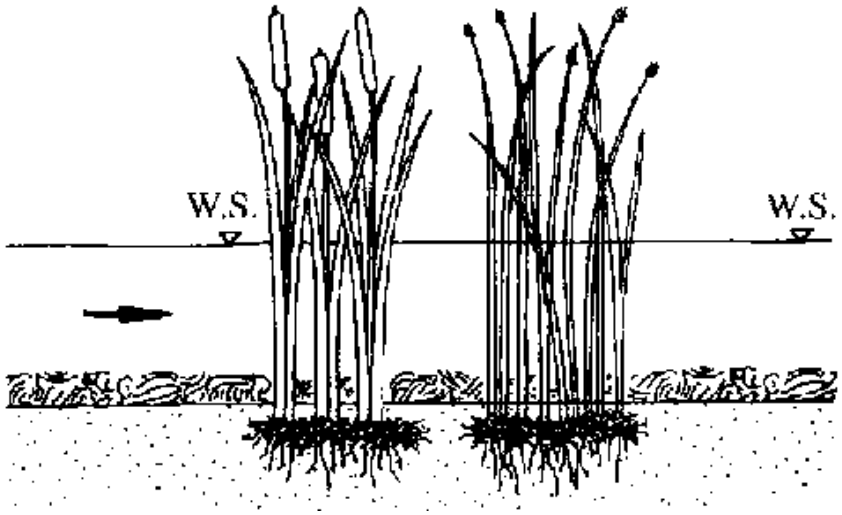


Fig. 4.7; Scheme of free water surface (FWS) wetland system (W.S. = water surface) (Internet sources).

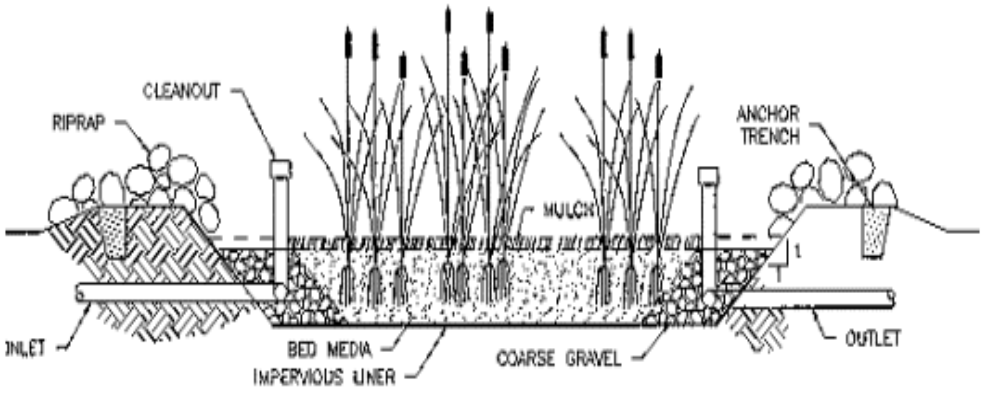


Fig. 4.8; Scheme of sub-surface flow (SSF) wetland system (Internet sources).

The substrate of the wetland and the plant present are essential components of the wetland concept and significantly affect performance. Removal of substances from water by a wetland involves a various biotic and abiotic processes including sedimentation of suspended particle, filtration of suspended particles by plant materials, uptake of nutrients by plant and bacteria, decomposition of organic matter, denitrification, nitrification, and adsorption of ions by the soil (Kadlec and Knight, 1996; Reddy and D'Angelo, 1997). Wetland are generally sediment traps. Input-output data miss most of the important phenomena interior to a wetland. Internal measurement of vertical sediment fluxes show a very large scale of deposition and resuspension (Fennessey *et al.*, 1994). Wetland remove incoming solids by settling and trapping. These finely divided materials are capable of resuspension by a variety of mechanisms: bioturbation, gas floation by oxygen or methane, or water shear. The result of the combination of processes is a very slow lateral transport of generated solids in the water flow direction. The sequence of resuspension, lateral movement, and resettling is repeated over and over for a given particle. Thus, the solids exiting the wetland are not contemporaneous with those entering, and are likely to be of entirely different character. Treatment wetlands frequently receive large external supplies of carbon in the added wastewater. Kadlec and Knight (1996) identified microbial respiration of carbon components to CO_2 in the aerobic zones of wetland to be a major process of the carbon cycle. In anaerobic zones, nitrate, iron, and sulphate reduction and methanogenesis occurred under evaporation of CO_2 or CH_4 (methanogenesis), respectively. Wetland are efficient users of external carbon sources, manifested by

excellent reduction in BOD and COD. Degradable carbon compounds are rapidly utilized in wetland metabolic processes. At the same time, a variety of wetland processes produce available carbon. The balance with uptake and production provides the carbon exports. BOD removal includes several chemical and biochemical processes: aerobic respiration, fermentation, anaerobic reduction of nitrate, manganese and iron, methanogenesis. BOD production is due to the wetland biota. The cycle of growth, death and partial decomposition uses atmospheric carbon, and produces gases, dissolved organics and solids. Decomposition involves sugar, starches and low molecular weight celluloses in the plant material. Gaseous products include methane and regenerated carbon dioxide. A spectrum of soluble large organic molecules, collectively termed humic substances, are released into the water. The solid residual of decomposition is organic sediment or peat.

Nitrogen is speciated in several forms in wetlands, and partitioned into water, sorbed and biomass phases (Fig. 4.9). Nitrogen elimination in wetlands begins with microbial ammonification of organic bound nitrogen, which can be either aerobic or anaerobic (Kadlec, 1995). Nitrification or oxidation of ammonia (ammonificated and excreted ammonia) to nitrate, as an oxygen-demanding process, occurs in two steps involving microbial species such *Nitrosomonas* and *Nitrobacter*. The decrease in quantity of inflowing NH_4-N can be attributed to high microbial ammonification and nitrification within each wetland, promoted by mean oxygen levels of 3.5-5.6 mg L^{-1}. Nitrogen is released to the atmosphere as ammonia and as nitrous oxide and dinitrogen produced by denitrification.

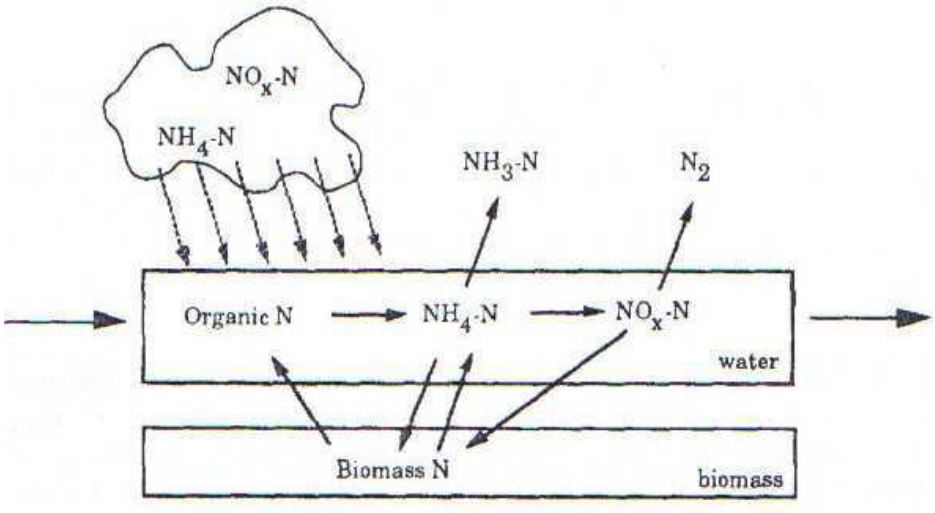


Fig. 4.9; A minimal representation of nitrogen process in FWS wetlands (Kadlec, 1995).

The removal capacity of phosphorus by a wetland can be substantial and comprise sedimentation, precipitation and adsorption reaction through ligand exchange of inorganic dissolved phosphorus in aluminium-, iron-, manganese hydroxidies/oxidise, calcium and clay minerals, and biochemical nutrient removal by plants. The composition of substrate is very important with respect to P removal by sorption process. Most soils do have sorptive capacity for phosphorus, but this storage is soon filled under any increase in phosphorus loading. Assessment of the contribution of aquatic plants and its associated microorganisms (algae and bacteria forming an attached biofilm) to remove nutrients showed that the biological floating mat complex (plants and microbes) is responsible for removing up to 75% of the nutrients in the wastewater. The aquatic plants contribute up to 52% of phosphorus removal by its own growth; the associated organisms and microorganisms removed the rest (Korner and Vermaat, 1998).

The larger aquatic plants growing in wetlands are usually called macrophytes. These include aquatic vascular plant (angiosperms and ferns), aquatic mosses and some large algae that have tissues that are easily visible. The presence of vegetation in wetland distributes and reduces the current velocities of the water (Pettcrew and Kalff, 1992; Somes *et al.*, 1996). This creates better condition for sedimentation of suspended solids, reduce the risk of erosion and resuspension and increase the contact time between the water and the plant surface areas. The macrophytes are also important for stabilizing the soil surface in treatment wetlands, as their dense root system impede the formation of erosion channels. The stems and leaves of macrophytes that are submerged in the water column provide a huge surface area for biofilms (Gumbrichth, 1993; Chappell and Goulder, 1994). The plant tissue are colonized by dense communities of photosynthetic algae as well as by bacteria and protozoa. Likewise, the roots and rhizomes that are buried in the wetland soil provide a substrate for attached growth of microorganisms. These biofilms – as well as the biofilms on all other immersed solid surfaces in the wetland system, including dead macrophyte tissues – are responsible for the majority of the microbial processing that occurs in wetlands. Wetland plants require nutrients for growth and reproduction and the rooted macrophytes take up nutrients primarily through their root system. Some uptake occurs also through immersed stem leaves from the surrounding water. As wetland plants are very productive, considerable amounts of nutrient can be bound in the biomass.

Natural wetlands and constructed wetlands can be effective in reducing nutrient and

organic matter concentrations in aquaculture wastewater. Aquaculture wastewater is a good candidate for treatment using constructed or natural wetlands due to its low strength in pollutants. However there is a concern about the feasibility of wetland s to become a cost effective method because wetlands typically require a low hydraulic loading rate (HLR) and a long hydraulic retention time (HRT) to achieve efficient pollutant removal. That means wetland method may need a large land area when a great amount of aquaculture wastewater needs to be treated. The area of wetland necessary for treating a given volume of water can be calculated as follow (Boyd, 1998):

$$A = V / (HLR)*(T_d)*(10^{-3})$$

Where T_d is the time of pond draining (days), V is the pond volume (m^3) and HLR is the hydraulic loading rate (L/m^2 per day). For example assuming a 1-ha pond of 1.5 m average depth, a drained time of 7 days, and HLR = 80 L/m^2 per day, 2.68 ha of wetland would be required. Even a 1-day HRT would require 0.67 ha of wetland.

Integration with other pond effluent management procedures could reduce the area of wetland necessary for treating farm effluents. Hollerman and Boyd (1985) and Seok *et al.* (1995) showed that water deterioration was not a major factor in seine-harvested catfish ponds which were not drained for 3 years. However, after 5-10 years, ponds must be drained to repair levees and adjust fish inventories. When a pond must be drained, about 80% of the water could be pumped into adjacent ponds for reuse, and remaining 20% of water could be discharged through a wetland. Draining time would not be a critical factor after fish have been removed by seining. If a 15-day draining time is used, a 1-ha x 1.5-m-deep pond would require a 0.25-ha wetland (25% of pond area) to provide a 4-day HRT (hydraulic retention time) and HLT of 80 L/m^2/day.

5. OBJECTIVE OF THE STUDY

The aim of this study was to assess the inflow and outflow water quality in a coastal aquaculture commercial farm to improve the production processes and minimized the potential impacts of this activity on the receiving aquatic environment.
The three main objective were:

- To optimize the inflow water quality control to maintain an adequate environment in which fish can grow minimizing stress, and outflow water quality control to minimize the level of potential environment pollutants discharged into the surrounding basin.

- To improve the effluent treatment optimizing wetland system depuration which include biotic and abiotic processes such as sedimentation, filtration, decomposition, and uptake of nutrients by plant and bacteria.

- To study the interaction between fish farm and surrounding environment by the development of a “global monitoring system” that consists in a satellite optical remote sensing approach to assess the quality in the water bodies influenced by the control of land-based aquaculture releases.

6. THE FISH FARM:

The "*Il Padule*" fish farm produces about 400 t y^{-1} of large size (1.2-2.0 kg) European sea bass (*Dicentrarchus labrax* L.) in brackish water, obtained by mixing marine waters with waters coming from the surrounding marsh and inflow rivers. For the entire production cycle at this site, four summer seasons are required. The farm occupied a total surface of about 65 ha and comprised two head lagoon ponds, 15 on-growing fish ponds and 11 final discharge lagoon ponds (Figs. 6.3 and 6.4). The head lagoon system (HLS) (Fig. 6.5) consisted of two lagoon ponds 1.5 m depth with surfaces of 5 and 10 ha respectively, receiving the water from three pumps with a maximum total flow of 3 m^3 s^{-1}. After this systems, the most of water enters the fish on-growing ponds by an internal channel, while the excess of water returned in the HLS (Fig. 6.6 and 6.7). The volume of the fish ponds varied increasing from 4,500 m^3 to 27,500 m^3, water supply ranges from 0.1 to 0.2 m^3 s^{-1} and the retention time of the wider ponds ranges in 1.6-3.2 day^{-1}. Stocked fish density varied renges 2.6 -4.8 kg fish m^{-3}, fishes are fed with a commercial pellets diet (43-47% d.m. protein, 18% d.m. crude fat, 8-9.3% ash, 1.6-1.8% d.m. crude fibre and 1.05-1.25% d.m. phosphorus) produced by Hendrix Skretting®. All the rearing ponds are supplied with pure oxygen delivered by SON®, through a distribution network, manually regulated to avoid PO_2 dropping below 6 mg L^{-1} and to avoid excess of oxygen consumption. To ensure it, up to 10 AquaEco Forza 7® oxygen delivery machines (1.5 HP each), are operative in each rearing pond.

About 70% of the water released by the rearing ponds is discharges in a lagoon basins system (DLS) (Fig. 6.8), while about 30% is return in the HLS, realizing an internal water recirculation. The DLS consists of 11 free water surface wetland system (FWS) placed in parallel. Each wetland pond has a range area of 9,000-9,200 m^2 (about 20 x 460 m), with a depth of 0.7 m at the head while 1.2 meters at the end. The hydraulic retention times (HRT) of the whole final wetland unit is 0.30 and 0.25 (day) for spring and summer periods respectively. The vegetable community in the DLS established naturally (1.14 kg m^{-2} ww) and included Hydrophytes (*Potamogeton pectinatus*) and macroalgae (*Chaetomorpha linum* and *Gracilaria verrucosa*), being dominated by *C. linum.* After the final wetland system the water is discharge into the near marsh canal and return to the external system.

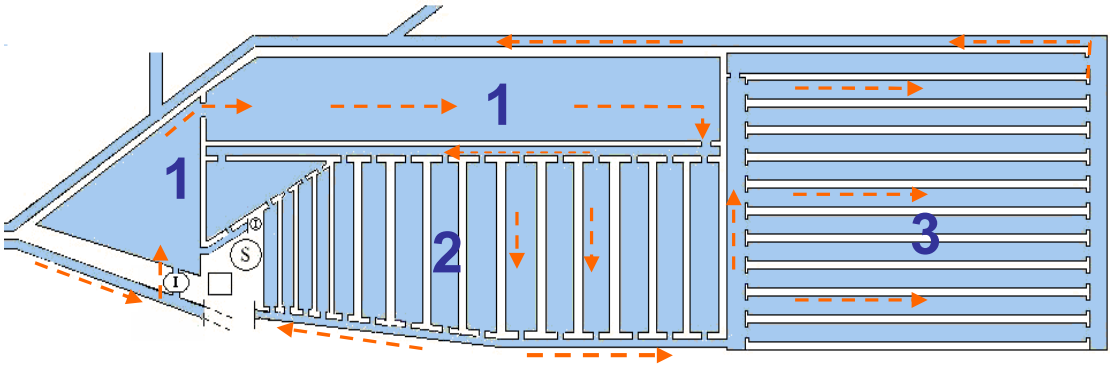


Fig. 6.3; General scheme of "*Il Padule*" fish farm; the arrows indicate the principal internal water flow (1-3 = head lagoon system; 2 = fish on-growing pond; 3 = discharges in a lagoon system; I = water pump; S = stalling).

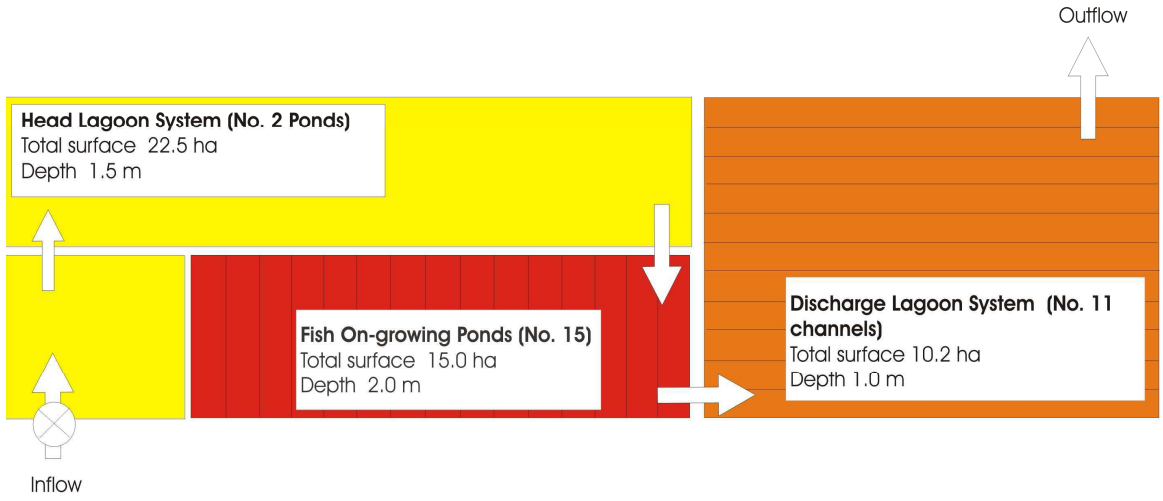


Fig. 6.4; A simplified scheme of "*Il Padule*" fish farm.

Fig. 6.5; "*Il Padule*" fish farm: head lagoon pond (HLS).

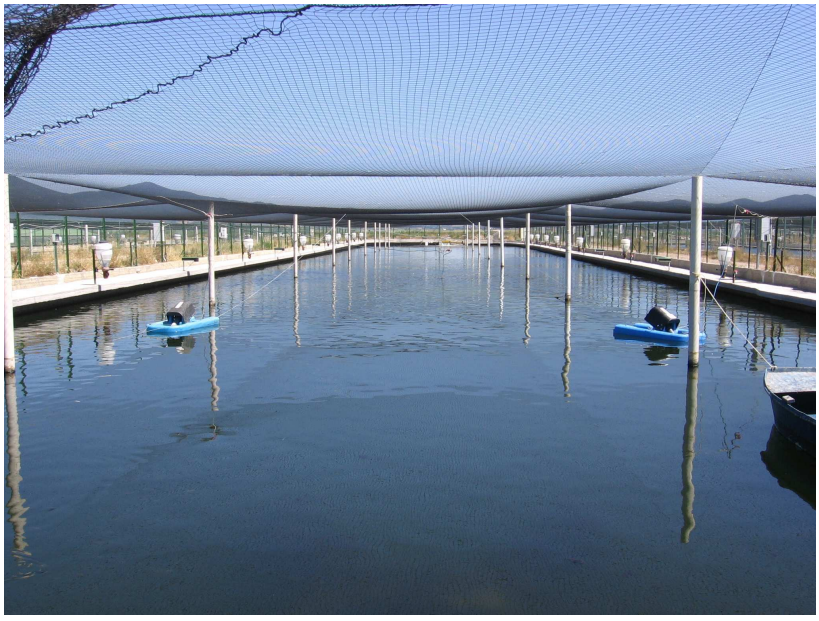


Fig. 6.6; "*Il Padule*" fish farm: fish on-growing pond.

Fig. 6.7; "*Il Padule*" fish farm: fish on-growing pond.

Fig. 6.8 "*Il Padule*" fish farm: discharge lagoon pond.

7. MATERIALS AND METHODS

The study has been carried out on field during different analytical season and under laboratory conditions. Because autumn and winter seasons being reported by a little biological activity, the on field study have been done in spring and summer seasons (2004-2005), when water temperature are higher.

In general, the study consists in a fish farm monitoring for assess the inflow and outflow loading and the efficiency of wetland systems in the control of water used and produced by aquaculture activity. More, some field and laboratory trials were carried out for improve these wetland systems by study the effect of water flow change in the discharge lagoon compartment and the applications of a macroalga *G. verrucosa* and bacterial-enzyme bio-promoter, respectively.

For these objectives, the following physical and chemical parameters were measured: dissolved oxygen, temperature, pH, salinity, total suspended solids (TSS), settlable suspended solids (SSS), un-settleable suspended solid, COD, ammonia nitrogen (NH_4-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), soluble reactive phosphorus (SRP), transparency and chlorophyll. The analysis of nitrogen and phosphorus forms and COD were carried out by photometric methods (Nanocolor, Macherely-Nagel) after 0.45μm pore size filtration (Schleicher & Schuell) and dilution with deionised water (1:1 or 1:2) in according to APHA Standard Methods (Eaton *et al.*, 2006). Dissolved oxygen, pH, temperature and salinity were measured with Handy Gamma (Oxiguard), pH-meter 250 Aplus (Orion), manual refractometer (Mod.106 ACT) respectively. TSS concentrations were measured by portable suspended solid analyser (Insite Instrument Group). In according to APHA Standard Methods (Eaton *et al*., 2006), the relative fraction of settleable and un-settleable suspended solids were evaluated by gravimetric methods while the chlorophyll levels and transparency were evaluated by photometric methods and Secchi disk respectively (Eaton *et al.*, 2006).

7.1 Monitoring of loaded nutrients and wetland retention efficiency:

7.1.1 Fish farm monitoring:

Key physical and chemical parameters were monitored daily and during nictemeral cycles in spring (May) and summer (July) 2004. Dissolved oxygen, temperature, pH, total suspended solids (TSS) and salinity were daily measured at 7:00, 13:00 a.m. and 17:00 p.m. at the monitoring points showed in Fig. 7.1.1, for a total of 30 days in each season. During the nictemeral monitoring period the samples were taken at 2 h interval for a 24 h period at the same monitoring points for 1 day in each season. The following parameters were measured: dissolved oxygen, temperature, pH, total suspended solids (TSS), COD, ammonia nitrogen (NH_4-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N) and soluble reactive phosphorus (SRP). Mean concentrations and relative standard deviations were calculated for all physic and chemical parameters. Tests for significant difference in water quality between influent and effluent of the wetland units were determined by ANOVA test (Statistica '98) for each set of data.

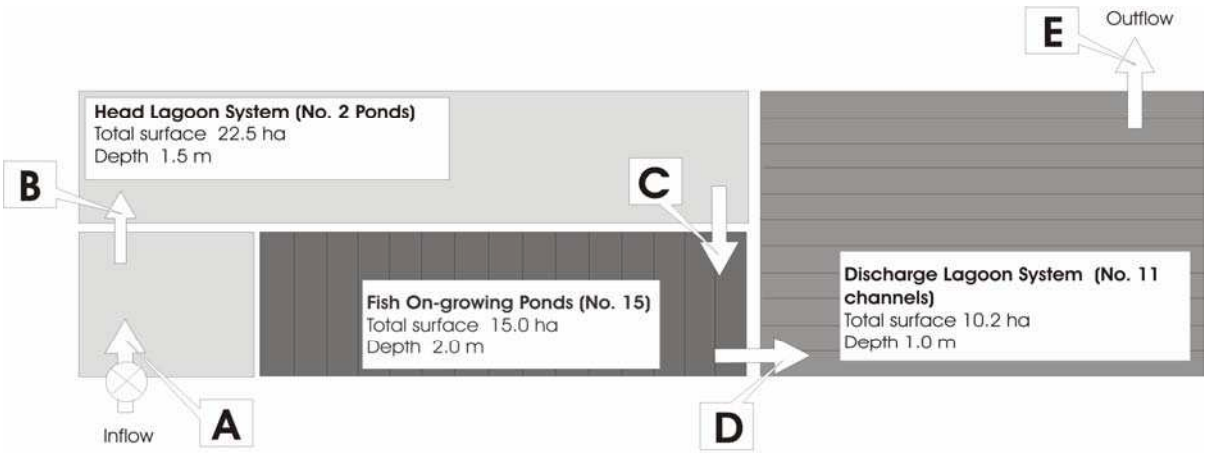


Fig. 7.1.1; Schematic diagram of the "*Il Padule*" fish farm and sites of sampling of daily and nictemeral cycles. (Site A = inlet ; Site B = end of first head lagoon pond; Site C = end of second head lagoon pond; Site D = outflow of fish on-growing ponds; Site E = end of discharge lagoon system).

7.1.2 Toward a model for nitrogen:

A modelling approach was applied into the discharge lagoon ponds to better evaluated the dynamics that occurred in the wetland system. The model (Eq. *1*) was extracted from a modelling nitrogen cycling in a French mariculture system (Lefebvre, 2001), and represents the total ammonia nitrogen mass balance of the lagoon system itself (gN h^{-1}). Table 7.1.1 shows the definitions and formulae of the variables and parameters used in the model that could be divided in the following principal components: water flow, ammonia concentration, ammonia flux from the sediment, ammonia supply from ammonification processes, ammonia removal from nitrification processes and other ammonia losses (i.e. volatilization). Two sampling periods in Spring and Summer 2004, were carried out to validate the model. Water was sampled at the inlet and at the outlet of two discharge lagoon ponds (E and F) during the nictemeral cycles (Fig. 7.3). To compare the analytical data with the model forecast, the mass balance values were converted in concentration accounting for the water flow (L h^{-1}).

$$dNH_4lg/dt = W_{lg}([NH_4]_{vp} - [NH_4]_{lg}) + JNH_4S_{lg} + am(DON_{lg} + UREA_{lg}) - ni[NH_4]_{lg} - nloss[NH_4]_{lg} \qquad (1)$$

Tab 7.1.1; Definition and formulae of the variables and parameters used in the ammonia nitrogen mass balance Lefebvre model.

Variable	Definition	Formula
W_{lg}	Water inflow (m^3/h)	
$[NH_4]_{vp}$	Concentration of N-NH_4 (gN/m^3) in fish pond(s)	
$[NH_4]_{lg}$	Concentration of N-NH_4 (gN/m^3) in lagooning basin(s)	
JNH_4	Ammonia diffusive flux from the sediment (gN/m^2/h)	α_{NH4} exp (βNH_4*T)
S_{lg}	Surface of the lagooning basin(s) (m^2)	
am	Ammonification rate (h-1)	$\alpha_{ammonification}$ exp(Kt*T)
DON_{lg}	Dissolved organic nitrogen in the lagooning basin(s) (gN/m^3)	
$UREA_{lg}$	UREA concentration in the lagooning basin(s) (gN/m^3)	
ni	Nitrition rate (h-1)	$\alpha_{nitrition}$ exp(Kt*T)
nloss	Ammonia loss rate (h-1)	α_{nloss} exp(Kt*T)
T	Temperature (°C)	

Parameter	Definition	Value and unit
K_t	Temperature increasing rate	0.07 $°C^{-1}$
$\alpha_{ammonification}$	Ammonification rate (at 0°C)	0.002 h^{-1}
$\alpha_{nitrition}$	Nitrition rate (at 0°C)	0.0014 h^{-1}
α_{nloss}	Ammonia loss rate (at 0°C)	0,005 h^{-1}
α_{NH4}	Sediment ammonia diffusion rate (at 0°C)	0.00028 (N/m^2/h)
β_{NH4}	Sediment diffusion increasing rate	0.1 C^{o-1}

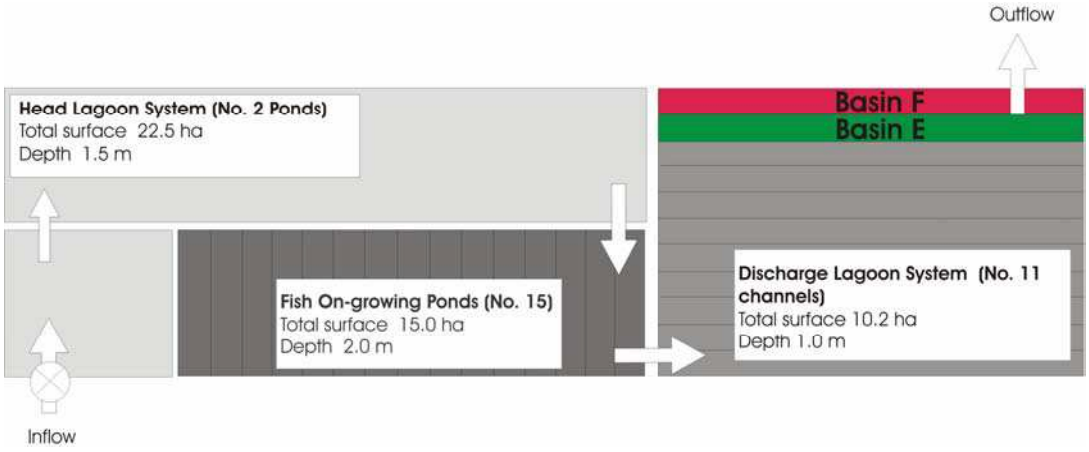


Fig. 7.1.2; Schematic diagram of the "*Il Padule*" fish farm with indicate the two discharge lagoon ponds (E and F) used for the application to Lefebvre model.

To improve the Lefebvre's model, some laboratory experimental trials were carried out to evaluate the N-NH_4 release (24 hours) during the algal decomposition under partial anoxic conditions. These experimental trials, in triplicatele, were carried out in an isolated system without air exchange where the level of dissolved oxygen after 24 hours dropped from 7.0 to 1.5 mg L^{-1}. A 50:50 blend of *Gracilaria verrucosa* and *Chaetomorpha linum*, collected in the discharge lagooning ponds, were utilized in three different quantities (30, 120 and 240 g ww); these algae were minced and maintained at 26°C for 24 hours in the brackish water (22 g L^{-1}) of a water recirculate aquaculture system to ensure a substrate (Fig. 7.1.3 A). After this time, NH_4-N levels were monitored for each different alga biomass.

To understand the real NH_4-N supply from the sediment, some experimental trials, replaced in triple, were carried out triplicate to evaluate the NH_4-N sediment release under hypoxic condition (4.1 mg L^{-1}), obtained by isolate system without air exchange. About 3 L of brackish water (22 g L^{-1}) collected from a water recirculation aquaculture system were added to 2.5 kg of the discharge lagoon sediment, and water temperature was maintained at 26 °C. NH_4-N concentrations plus form of dissolved nitrogen NO_2-N and NO_3-N were monitored at the starting time, after 24 and 48 hours in the added water (Fig. 7.1.3 B).

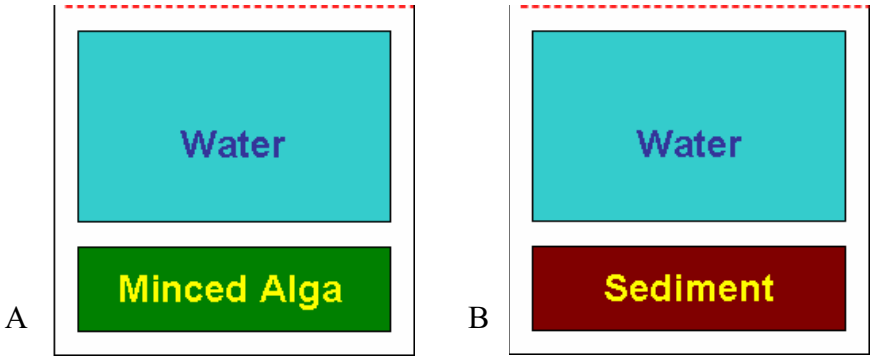


Fig 7.1.3; Schematic representation of laboratory experimental trials for the evaluate NH_4-N released from algae decomposition (A) and from the sediment (B).

6.2 Remote sensing monitoring :

A remote-sensing approach has been carried on in order to monitoring qualitatively some of the environmental indicators that may describe aquaculture originating polluting elements. The basis of this kind of remote-sensing monitoring is that water colour (chromaticity) is a function of the aquatic component concentrations. Study have shown the relationship to redness and suspended solid concentrations, to greenness and chlorophyll concentrations, and to blueness and transparency (Zilioli *et al*., 1995) . The use of multispectral scanning spectrometer (MSS) mounted aboard the satellites allows the chromaticity studies of natural water bodies. In this way, chromaticity analysis were conducted on chlorophyll levels, transparency and on suspended solids of the water basins. These coordinates are associated to a chromatic interval between 0 and 1, expressed in the form of chromatic scale in which chromatics response close to 0 indicate a lower level, while chromatics close to 1 a higher level of chlorophyll, suspended solids and transparency respectively. In addition without making any multi-temporal and/or multisensorial analysis and since the chromaticity range map is purely qualitative one can disregard the atmospheric effect without invalidating the chromaticity analysis.

Images obtained from Quick Bird Satellite Image were elaborated with a specific software (ENVI®program), containing algorithms to define chromatic coordinates in function of different levels of radiation. The elaboration was made, in the form of first approach, on a single QuickBird Satellite images collected on the 3rd September 2004 (Fig. 7.2.1). Following the Commission Internationale de l'Éclairage (CIE) standard colorimetric system, the

chromaticity coordinate method used, is radiance-derived information that allows to provide direct information about the quality of waters in quality term of tristimulus values of an upwelling irradiance spectrum of water colour. The tristimulus values of an upwelling irradiance spectrum E(λ) ere given by:

$$X' = \int E(\lambda)x(\lambda)d\lambda$$

$$Y' = \int E(\lambda)y(\lambda)d\lambda$$

$$Z' = \int E(\lambda)z(\lambda)d\lambda$$

where x(λ), y(λ) and z(λ) represent CIE colour mixture data for the red, green and blue regions of the spectrum, respectively (Bukata *et al*., 1995). These colour mixture data are wavelength-dependent hypothetical standard values selected in such manner that y(λ) identically corresponds to the standard luminosity curve for the photopic vision. The numeral values of x(λ), y(λ) and z(λ) are those appropriate to an equal energy incident spectrum and are listed in table 7.2.1.

The chromaticity coordinates X, Y and Z (for red, green and blue respectively) are obtained from:

X = X'/(X'+Y'+Z')	***redness*** X
Y = Y'/(X'+Y'+Z')	***greenness*** Y
Z = Z'/(X'+Y'+Z')	***blueness*** Z

Where X' is the radiation measured in the red band, Y' is the radiation measured in the green-yellow and Z' the radiation measured in the blue-green. Thus, using the CIE colour mixture values x(λ), y(λ) and z(λ) and assuming monochromatic light of a given wavelength as the spectrum E(λ), the CIE chromaticity coordinates may be obtained for that particular wavelength. In this way, the three coordinates X,Y and Z appear as a normalization of the radiations measured in red, in green and in blue, with respect to the sum of the same three radiations made equal to 1 (Bukata *et al*., 1995).

Fig. 7.2.1; QuickBird Satellite image to Natural Reserve "*Diaccia Botrona*" and "*Il Padule*" fish farm, collected on the 3rd September 2004 (NW Lat: 42°78'82'' Long: 10°89'30''; SE Lat: 42°74'27'' Long: 10°95'44'').

Tab. 7.2.1; CIE Color Mixture Data for Equal Energy Spectrum (Bukata *et al*., 1995).

Wavelength (λ) (nm)	$x(\lambda)$	$y(\lambda)$	$z(\lambda)$
380	0.0023	0.0000	0.0106
390	0.0082	0.0002	0.0391
400	0.0283	0.0007	0.1343
410	0.0840	0.0023	0.4005
420	0.2740	0.0082	1.3164
430	0.5667	0.0232	2.7663
440	0.6965	0.0458	3.4939
450	0.6730	0.0761	3.5470
460	0.5824	0.1197	3.3426
470	0.3935	0.1824	2.5895
480	0.1897	0.2772	1.6193
490	0.0642	0.4162	0.9313
500	0.0097	0.6473	0.5455
510	0.0187	1.0077	0.3160
520	0.1264	1.4172	0.1569
530	0.3304	1.7243	0.0841
540	0.5810	1.9077	0.0408
550	0.8670	1.9906	0.0174
560	1.1887	1.9896	0.0077
570	1.5243	1.9041	0.0042
580	1.8320	1.7396	0.0032
590	2.0535	1.5144	0.0023
600	2.1255	1.2619	0.0016
610	2.0064	1.0066	0.0007
620	1.7065	0.7610	0.0003
630	1.2876	0.5311	0.0000
640	0.8945	0.3495	0.0000
650	0.5681	0.2143	0.0000
660	0.3292	0.1218	0.0000
670	0.1755	0.0643	0.0000
680	0.0927	0.0337	0.0000
690	0.0457	0.0165	0.0000
700	0.0225	0.0081	0.0000
710	0.0117	0.0042	0.0000
720	0.0057	0.0020	0.0000
730	0.0028	0.0010	0.0000
740	0.0014	0.0006	0.0000
750	0.0006	0.0002	0.0000
760	0.0003	0.0001	0.0000
770	0.0001	0.0000	0.0000
Total	21.3713	21.3714	21.3715

7.3 Improvement of the wetland efficiency:

7.3.1 Effect of water flow:

In Summer 2005 the water flows of three discharge lagoon basins named I, E and L were modified to study the relationship with the removal efficiency of the wetland system and the hydraulic retention time. Water flows were fixed to 0.3 m^3 sec^{-1} in the basin I, 0.2 m^3 sec^{-1} in the basin E and 0.1 m^3 sec^{-1} in the basin L, and the corresponding hydraulic retention times were, 0.35, 0.53 and 1.06 day respectively (Fig. 7.3.1). The following parameters were measured at the inflow and at the outflow of these basins, during 5 days: dissolved oxygen, temperature, pH, total suspended solids (TSS), settleable suspended solids (SSS), un-settleable suspended solids (USS), COD, ammonia nitrogen (NH_4-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), soluble reactive phosphorus (SRP). Chlorophyll concentration and water transparency were recorded in the site D (outlet of farming ponds collector) and in the I, E and L outlet (Fig. 7.3.1).

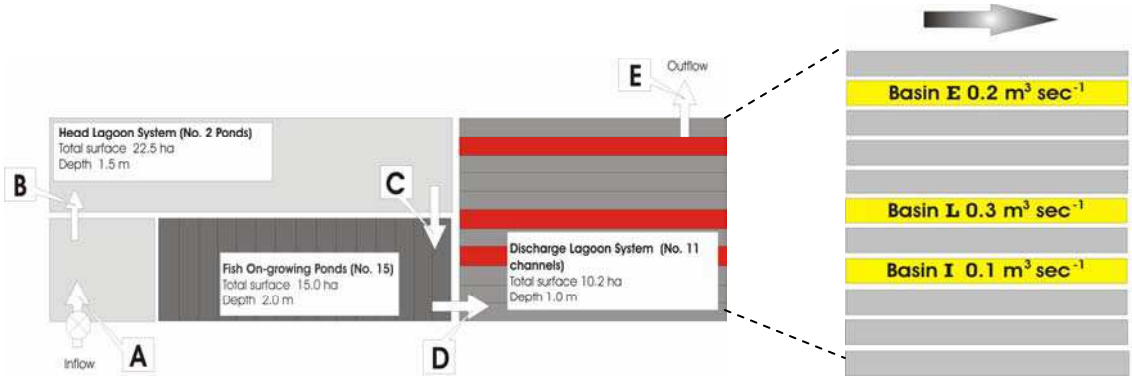


Fig. 7.3.1; Schematic diagram of the "*Il Padule*" fish farm the discharge lagoon ponds. In red and yellow are highlight the basins named I, E and L with a water flow set up to 0.1, 0.2, 0.3 m^3 sec^{-1} respectively.

7.3.2 Gracilaria verrucosa:

Some experimental trials were carried out to study the improvement of the ammonia removal rate in the water column using a commercial macroalga *G. verrucosa*. About 30 g w.w. of *G. verrucosa* (2.8 g d.w.), taken in the discharge lagoon system, were placed in a container with about 3.5 L of brackish water (22 g L^{-1}) collected from a water recirculation aquaculture system, and were subjected to artificial constant light by a lighthouse (150 Watt) for 96 hours at 21 °C (Fig. 7.3.2) like to $51 \cdot 10^3$ Lux. The same trials was conducted under dark and a control with only water was carried out to evaluate the real effect of algae assimilation; each experimental trial was run in triplicate.

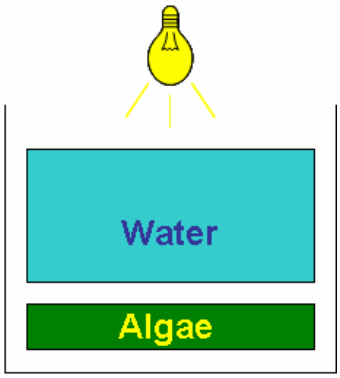


Fig 7.3.2; Schematic representation of experimental trials with application of *G. verrucosa*.

7.3.3 Bacterial-enzyme bio-promoter

Experimental trials were carried out to study the improvement of nitrification processes using bacterial-enzyme bio-promoter. The product was supplied by Eurovix S.r.l. in a lyophilized form (Aqua-Clear®). The composition of Aqua-clear® is considered confidential nevertheless the Company provides a list with the composition in enzymes as lipases, proteases, cellulases, amylases, hemicellulases, all extracted from natural products, plus selected bacteria genes Bacillus spp. (GMO free) (Table 7.3.1). About 2.0 kg of sediment and 100 g w.w. of minced algae taken in the discharge lagoon system, were placed in a container with 2 L of brackish water (22 g L^{-1}) collected from a water recirculation aquaculture system. About 20 g of bacterial-enzime bio-promoter were resuspended in 1L of brackish water, for the re-activation processes, and after 24h in aerobic conditions, the suspension was added in the container with algae and sediments. A control trial with only addition of brackish water (3 L) was carried

out to evaluate the real effect of the bio-promoters. Each experimental trial was run in triplicate. The removal efficiency of NH_4-N and SRP was evaluated in partially anoxic conditions, obtained without air exchange, and in aerobic conditions, obtained with aeration (Fig. 7.3.4 A and B). In the anoxic experiments, after 72 h, air was insufflated to recreate aerobic conditions. Only in aerobic trials NH_4Cl and $Na_2H_2PO_4H_2O$ were added for increase the NH_4-N and SRP level.

Tab 7.3.1; General information about bacterial-enzyme bio-promoter Aquaclear® (Eurovix S.r.l.).

Enzimi alfa-amilasi	Enzimi emicellulasi
Enzimi beta-amilasi	Enzimi pectinasi
Enzimi pentosanasi	Microrganismi utili selezionati da fermentazione controllata
Enzimi lipasi	Estratti vegetali
Enzimi gluco-amilasi	Carboidrati
Enzimi beta-glucanasi	Fattori di crescita naturali
Enzimi cellulasi	Principi attivi di focus laminariae
Enzimi proteasi	Terreno colturale Agar
Enzimi fosforilasi	Alghe *Lithothamnium calcareum*
Enzimi pullulanasi	Biocatalizzatori minerali ricchi di oligoelementi
Non contengono OGM	
Stato fisico: polvere	Infiammabilità: -----
pH in soluzione: 6-7	Schiuma: nulla
Umidità: 4-6	Colore: noce

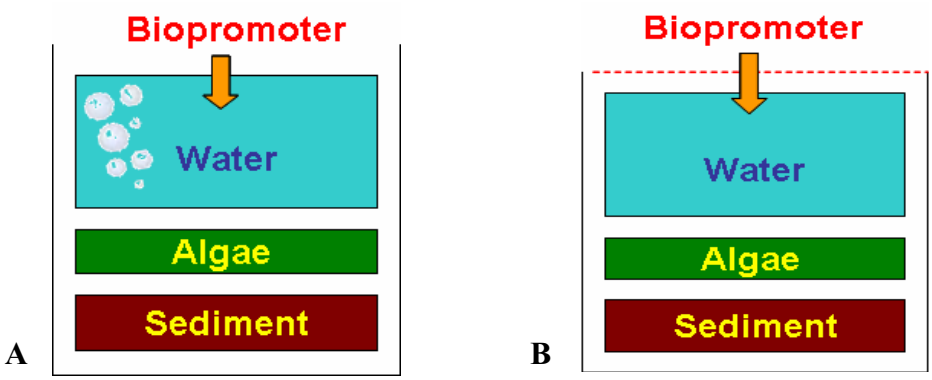


Fig 7.3.4; Schematic representation of experimental trials with application of bacterial-enzyme bio-promoter in aerobic (A) and partial anoxic (B) conditions.

8. RESULTS

8.1 Monitoring of loaded nutrients and wetland retention efficiency:

8.1.1 Fish farm monitoring:

Tables 8.1.1, 8.1.2 and figures 8.1.1, 8.1.2, 8.1.3, 8.1.4, 8.1.5, 8.1.6 and 8.1.7 show the results obtained from daily (Temperature, Oxygen, % Oxygen Saturation, pH, Salinity, TSS) and nictemeral sampling (Temperature, Oxygen, % Oxygen Saturation, pH, Salinity, TSS, COD, NH_4-N, NO_2-N, NO_3-N and SRP). Table 8.1.3 and 8.1.4 shows the ANOVA results of the comparison of the data measured in the sites A, B, C, D and E in the daily and nictemeral sampling respectively.

In spring 2004 the analytical measures varied within the following rangers: temperature 16.7-17.2 °C, pH 7.35-7.79 unit, oxygen 9.3-11.8 mg L^{-1}, oxygen saturation 98.4-126.6 %, and salinity 10.0-10.9 g L^{-1}. The highest values of TSS were monitored in site A (79.35±23.17 mg L^{-1}), which results directly influenced by water inflow). After water flows through the HLS the TSS mean value decrease to 31.45±9.23 mg L^{-1} (A<C; *P<0.05*), than increased again after the fish on-growing ponds, assuming the mean value of 55.00±8.39 mg L^{-1} (C<D; *P<0.05*). At the end of the DLS the mean value monitored was 45.90±14.66 mg L^{-1} (D>E and A>E; *P*<0.05) (Fig. 8.1.1 A and 8.1.2). A similar pattern was monitored for the nitrogen forms and COD (Fig. 8.1.4 A-C and 8.1.5). The level of SRP decreased after the passage into the HLS with mean values ranging from 0.17±0.07 to 0.14±0.05 mg L^{-1}. In the water flowing out from the fish on-growing ponds, the SRP concentration increased again assuming a mean value of 0.25±0.08 mg L^{-1} (C<D, *P<0.05*), but the mean concentrations at the end of the DLS resulted higher (0.30±0.10 mg L^{-1}) compared to those detected in the water inflow (A<E; *P<0.05*) (Fig. 8.1.4 B).

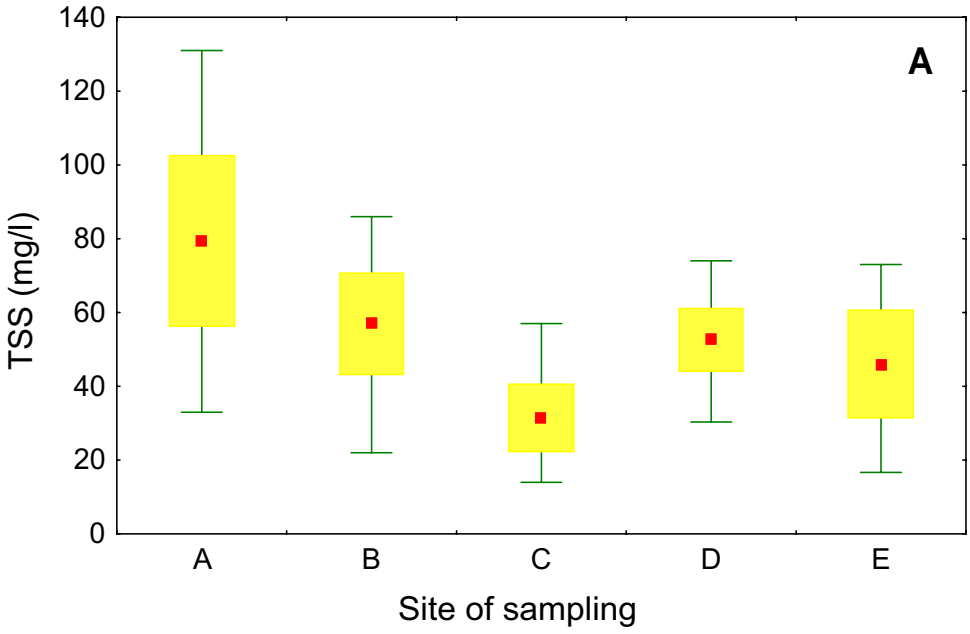
In the summer 2004 the following ranges were detected in all the sampling sites: temperature 25.8-27.6 °C, pH 7.03-7.55 unit, dissolved oxygen 6.0-8.8 mg L^{-1}, oxygen saturation 77.0-115.1 %, and salinity 35.2-37.2 g L^{-1} (Tab. 8.1.1). The pattern of TSS concentration was similar to the Spring data with means of concentration in mg L^{-1} 47.36±17.30 (site A), 33.91±9.48 (site B), 26.00±9.02 (site C) and 39.65±8.34 (site D); the comparison resulted respectively A>C, C<D and D<E (*P<0.05*) but the highest mean value monitored was at the end of DLS (61.56±28.68 mg L^{-1}, A<E; *P<0.05*) (Fig. 8.1.1 B and 8.1.3). The levels of COD, NH_4-N at the end of DLS (104.1±20.2 and 0.74±0.11 mg L^{-1})

were higher than those detected at the inflow levels in the same system (97.31±41.56 and 0.72±0.12 mg L^{-1}) (Fig. 8.1.6 A-C and 8.1.7) while the levels of SRP decreased after the passage into the DLS (Fig. 8.1.6 B). For the NH_4-N, NO_2-N and SRF levels, the values detected in the fish farm water effluent in the summer season (0.74±0.11, 0.28±0.02 and 0.34±0.05 mg L^{-1} respectively) were higher than the values detected in the inflow water (0.37±0.19, 0.15±0.09 and 0.25±0.13 mg L^{-1} respectively; A<E *P<0.05*).

Tab. 8.1.1; Water qualities parameters monitored in daily sampling (mean ± s.d.) at sampling location for spring and summer period.

Daily Sampling

		Spring					Summer				
		A	B	C	D	E	A	B	C	D	E
Temperature (°C)	mean	16.66	16.75	16.75	16.87	17.15	25.81	25.92	26.32	26.74	27.61
	d.s.	2.01	1.81	1.74	1.63	2.51	1.70	1.55	1.51	1.26	2.34
Oxygen (mg L^{-1})	mean	9.32	10.14	11.39	9.96	11.81	6.04	6.91	8.82	6.09	7.20
	d.s.	2.16	2.16	2.35	2.35	4.82	1.77	2.13	3.19	1.08	3.46
% Saturation O_2	mean	98.36	107.19	121.41	104.94	126.61	77.03	88.64	115.06	78.98	96.46
	d.s.	24.67	23.81	26.35	23.68	55.37	24.54	30.41	44.63	15.82	49.23
pH	mean	7.49	7.65	7.79	7.35	7.67	7.51	7.55	7.72	7.03	7.40
	d.s.	0.49	0.50	0.51	0.32	0.69	0.31	0.20	0.21	0.10	0.21
Salinity (g L^{-1})	mean	10.44	10.02	10.39	10.78	10.91	35.24	35.33	36.00	36.89	37.18
	d.s.	2.40	1.58	2.03	1.62	1.59	4.56	4.05	2.90	2.12	1.94
TSS (mg L^{-1})	mean	79.35	56.93	31.45	52.59	45.90	47.36	33.91	26.00	39.65	61.56
	d.s.	23.17	13.92	9.23	8.39	14.66	17.30	9.48	9.02	8.34	28.68



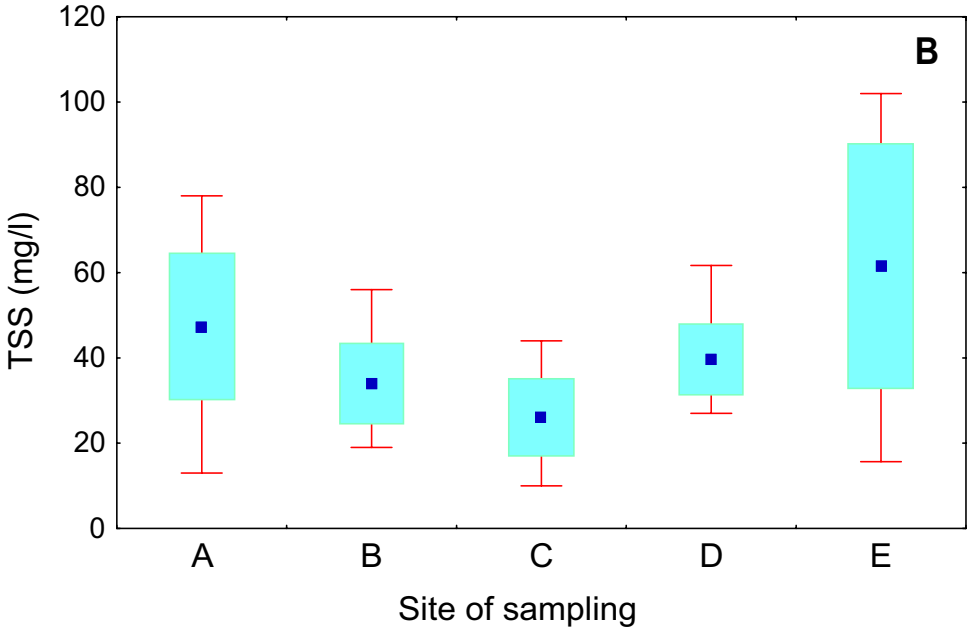


Fig. 8.1.1; TSS levels monitored during nictemeral sampling at sampling locations in spring (A) and in summer (B) period. The square, box and whiskers indicated the mean value, mean ± d.s. and minimum-maximum value respectively.

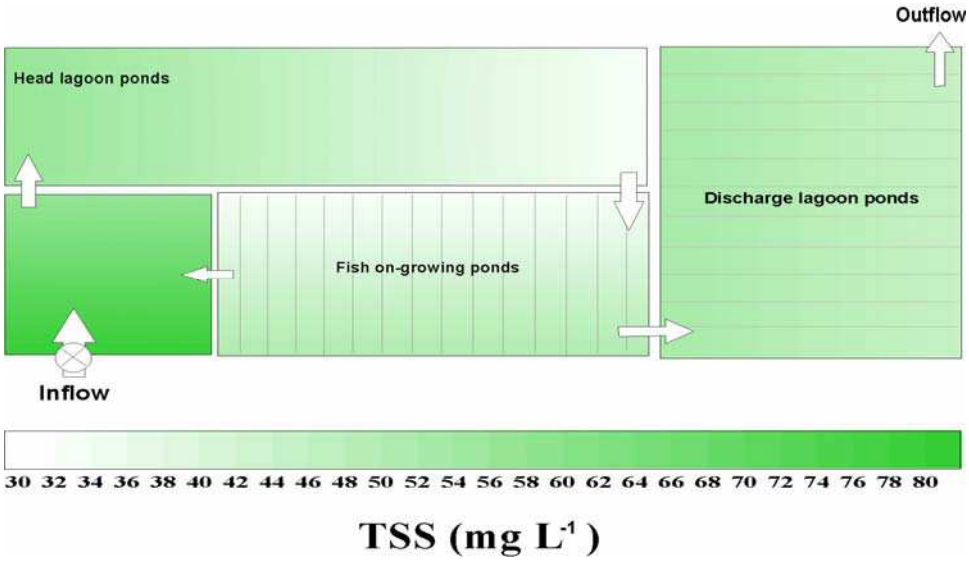


Fig. 8.1.2; Variation to the TSS levels in the "*Il Padule*" fish farm monitored during spring daily sampling.

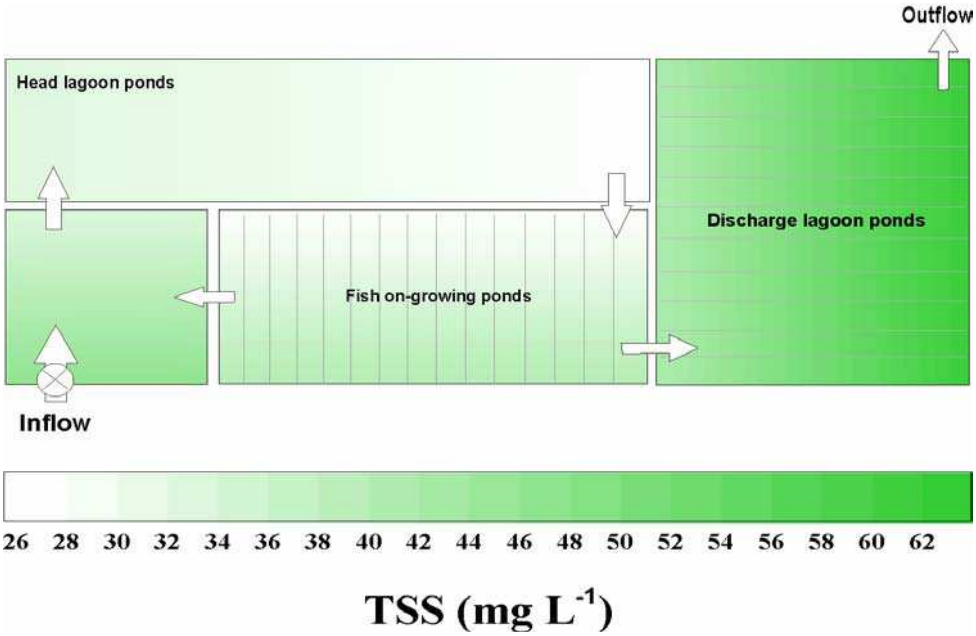


Fig. 8.1.3; Variation to the TSS levels in the "*Il Padule*" fish farm monitored during summer daily sampling.

Tab. 8.1.2; Water quality parameters monitored during nictemeral sampling (mean ± s.d.) at sampling location for spring and summer period.

Nictemeral Sampling

		Spring					Summer				
		A	B	C	D	E	A	B	C	D	E
Temperature (°C)	mean	15.08	15.18	15.20	14.99	14.69	23.82	24.35	24.51	25.32	25.94
	d.s.	0.62	0.57	0.37	0.79	1.03	2.06	1.28	0.85	0.70	1.78
Oxygen (mg L^{-1})	mean	10.23	10.62	12.51	11.68	10.71	5.32	5.47	6.45	5.33	4.58
	d.s.	1.46	1.09	1.47	1.43	3.00	0.48	0.43	1.29	0.43	2.06
% Saturation O_2	mean	103.63	110.35	129.42	119.12	108.46	64.69	67.38	80.15	66.69	58.74
	d.s.	14.34	11.93	15.44	15.92	33.33	6.12	7.10	18.16	5.86	28.93
pH	mean	7.19	7.06	6.94	7.24	6.95	7.54	7.48	7.73	6.95	7.14
	d.s.	0.60	0.73	0.89	0.22	0.90	0.39	0.15	0.11	0.14	0.09
Salinity (g L^{-1})	mean	10.15	10.31	11.00	11.46	11.67	35.46	34.92	35.54	35.31	35.54
	d.s.	0.55	0.48	1.53	1.90	1.73	2.67	1.85	0.78	1.25	1.44
TSS (mg L^{-1})	mean	93.08	66.46	34.00	54.92	46.46	38.77	30.31	22.23	36.00	53.03
	d.s.	19.35	9.47	6.68	9.38	10.23	20.43	10.48	7.66	6.82	29.90
COD (mg L^{-1})	mean	28.15	22.00	24.00	24.54	23.42	105.38	93.23	96.92	97.31	104.13
	d.s.	12.54	12.96	14.89	9.55	6.32	23.19	32.34	21.19	41.56	20.19
NH_4-N (mg L^{-1})	mean	0.26	0.15	0.09	0.41	0.25	0.37	0.44	0.25	0.72	0.74
	d.s.	0.19	0.04	0.04	0.05	0.06	0.19	0.11	0.11	0.12	0.11
NO_2-N (mg L^{-1})	mean	0.09	0.15	0.15	0.12	0.12	0.15	0.19	0.16	0.33	0.28
	d.s.	0.01	0.23	0.21	0.01	0.01	0.09	0.04	0.01	0.01	0.02
NO_3-N (mg L^{-1})	mean	0.80	0.75	0.67	0.66	0.66	0.29	0.30	0.31	0.40	0.30
	d.s.	0.68	0.48	0.62	0.60	0.49	0.01	0.03	0.06	0.19	0.03
SRP (mg L^{-1})	mean	0.17	0.15	0.14	0.25	0.30	0.25	0.25	0.23	0.42	0.34
	d.s.	0.07	0.07	0.05	0.08	0.10	0.13	0.05	0.04	0.02	0.05

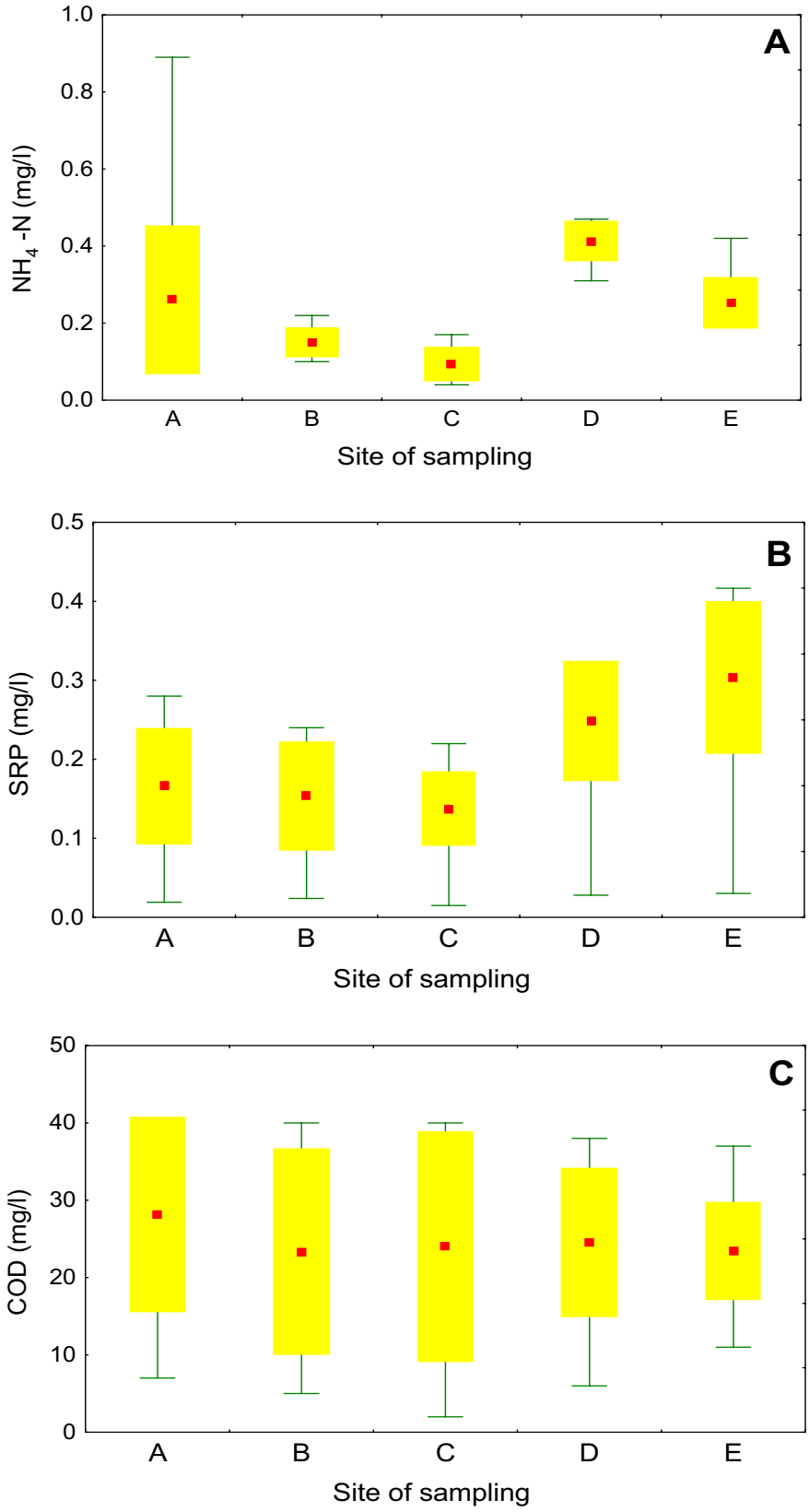


Fig. 8.1.4; Concentrations of NH_4-N (A), SRP (B) and COD (C) monitored during in spring nictemeral sampling at sampling locations. The square, box and whiskers indicated the mean value, mean ± d.s. and minimum-maximum value respectively.

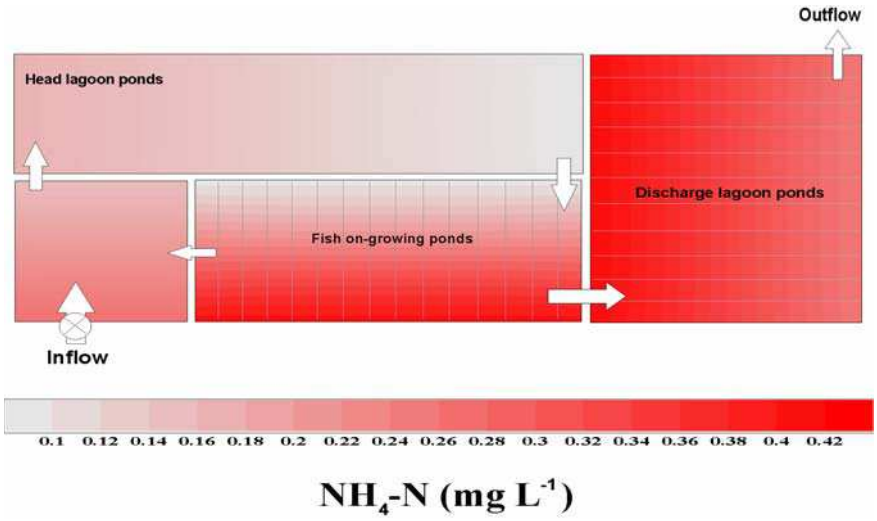


Fig. 8.1.5; Variation to the NH_4-N levels in the "*Il Padule*" fish farm monitored during spring nictemeral sampling.

Tab. 8.1.3; Comparison (*ANOVA*) to site A versus site B plus site C, site C versus site D, site D versus site E and site A versus site E. In the table the asterisks indicate the comparison with significative difference ($P < 0.05$).

Daily Sampling

	Spring				Summer			
Temperature	A < C	C < D	D < E	A < E	A < C	C < D	D < E *	A < E *
Oxygen	A < C *	C > D *	D < E *	A < E *	A < C *	C > D *	D < E	A < E
% Saturation O_2	A < C *	C > D *	D < E *	A < E *	A < C *	C > D *	D < E *	A < E *
pH	A < C*	C > D *	D < E *	A < E	A < C *	C > D *	D < E *	A < E *
Salinity	A > C	C < D	D < E	A < E	A < C	C < D	D < E	A < E *
TSS	A > C *	C < D *	D > E *	A > E *	A > C *	C < D *	D < E *	A < E *

* = P < 0.05

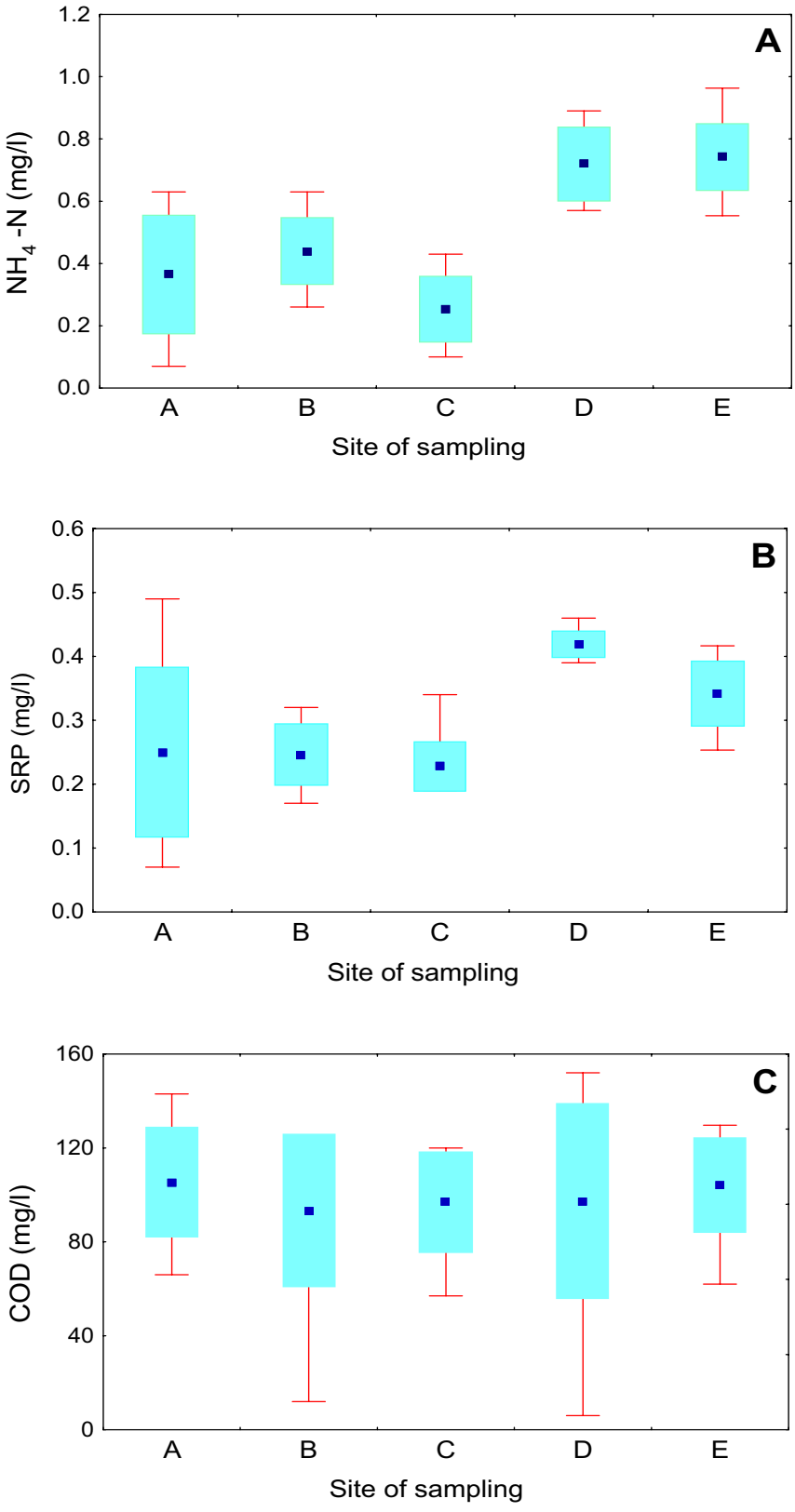


Fig. 8.1.6; Concentrations of NH_4-N (A), SRP (B) and COD (C) monitored during summer nictemeral sampling at sampling locations. The square, box and whiskers indicated the mean value, mean ± d.s. and min-max value respectively.

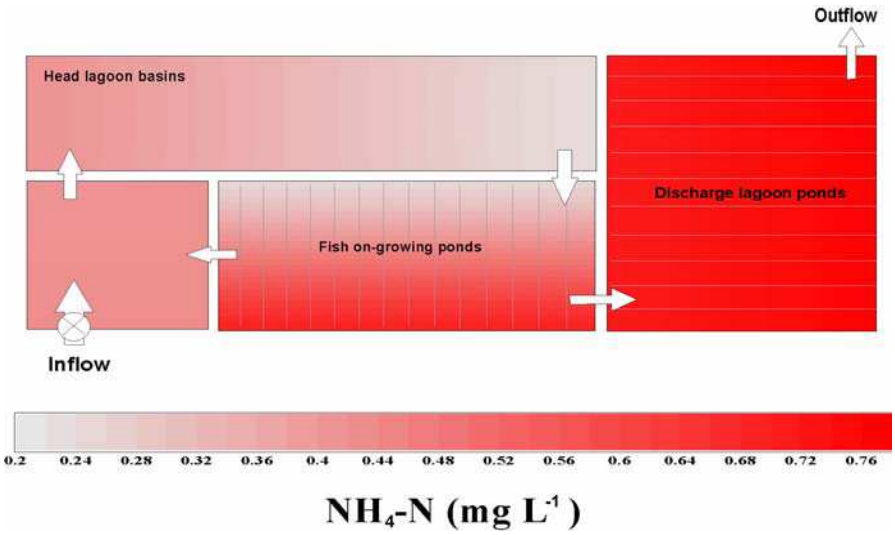


Fig. 8.1.7; Variation to the NH_4-N levels in the "*Il Padule*" fish farm monitored during summer nictemeral sampling.

Tab. 8.1.4; Comparison (*ANOVA*) to site A versus site B plus site C, site C versus site D, site D versus site E and site A versus site E. In the table the asterisks indicated the comparison with significative difference ($P < 0.05$).

Nictemeral Sampling

	Spring				Summer			
Temperature	A < C	C > D	D > E	A > E*	A < C	C < D	D < E	A < E *
Oxygen	A < C *	C > D	D > E	A < E	A < C	C > D *	D > E	A > E
% Saturation O_2	A < C *	C > D	D > E	A < E	A < C	C > D *	D > E	A > E
pH	A > C	C < D	D < E	A < E	A < C	C > D *	D < E *	A > E *
Salinity	A < C	C < D	D < E	A < E *	A < C	C > D	D < E	A < E
TSS	A > C *	C < D *	D > E	A > E *	A > C *	C < D *	D < E *	A < E *
COD	A > C	C < D	D > E	A > E	A < C	C < D	D < E	A > E
NH_4-N	A > C *	C < D *	D > E *	A > E	A > C	C < D *	D < E	A < E *
NO_2-N	A < C	C > D	D > E	A < E	A < C	C < D *	D > E *	A < E *
NO_3-N	A > C	C > D	D < E	A > E	A < C	C < D *	D > E *	A < E
SRP	A > C	C < D *	D < E	A < E *	A > C	C < D *	D > E *	A < E *

* = P < 0.05

The percent reduced of concentration and the removal rate (g m^{-2} d^{-1}) for the key parameters of water quality, in head and discharge lagoon system, are showed in Table 8.1.5. The removal rates were defined as hydraulic loading rate (HLR) multiplied the difference in concentration between the inflow and outflow water. The HLR was calculated dividing the average flow rate (m^3 d^{-1}) by the surface area of the wetland(s). In the head lagoon system, the HLR values resulted 1.05 m day^{-1} during the spring period, and 1.30 m day^{-1} during the summer period, while in discharge lagoon system, the HLR values were 2.49 and 2.98 (m day^{-1}), in spring and summer period respectively.

For the head lagoon system, higher percentages of reduction were monitored during spring with values up to 60.37, 14.89, 65.38 and 17.65% for the TSS, COD, NH_4-N and SRP respectively. Similarly, the removal rates were higher during the spring period with the highest values for TSS (57.29 g m^{-2} d^{-1}). In summer the percentage of reduction for TSS, COD, NH_4-N and SRP were 45.10, 8.06, 32.43 and 8.00% respectively and the highest removal rate was monitored for TSS (27.68 g m^{-2} d^{-1}).

In the discharge lagoon system, the percentage of reductions of TSS, COD and NH_4-N during spring were 12.72, 4.56 and 39.02 % respectively, while the difference between inflow and outflow SRP concentrations in DLS resulted negative. Similar pattern was monitored during the summer period for TSS COD and NH_4-N, but the reduced concentrations in percent of SRP was higher, with a value of 19.05 %. The higher removal rates were observed for TSS and COD in spring periods with values of 16.65 and 2.78 g m^{-2} d^{-1} respectively while in the summer period the higher values was observed for NO_3-N (0.30 g m^{-2} d^{-1}).

Tab 8.1.5; Percentage of reduction concentration and removal rate ($g/m^2/day$) for various parameters describing of water quality in head (HLS) and discharge lagoon system (DLS).

	Head Lagoon System				Discharge Lagoon System			
	Reduced Concentration (%)		Removal Rate ($g/m^2/day$)		Reduced Concentration (%)		Removal Rate ($g/m^2/day$)	
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer
TSS *	60.37	45.10	57.29	27.68	12.72	N	16.65	N
COD	14.89	8.06	4.41	11.02	4.56	N	2.78	N
NH_4-N	65.38	32.43	0.17	0.15	39.02	N	0.40	N
NO_2-N	N	N	N	N	1.06	15.15	0.003	0.15
NO_3-N	16.25	N	0.14	N	0.27	25.00	0.045	0.30
SRP	17.65	8.00	0.03	0.02	N	19.05	N	0.24

N = Negative values

* = Dailyng sampling

Table 8.1.6 reports the constants of removal rate constants for key pollutants determined for the constructed wetland system studied and provides a comparison with reference data from literature. These constant rates were determined using the basic equation of the first-order plug kinetic model:

$$C_o / C_i = \exp(-Kt)$$

where C_i = influent pollutant concentration (mg L^{-1}); C_o = effluent pollutant concentration (mg L^{-1}); t = nominal hydraulic retention time (HRT) of the whole wetland unit (day); K = first-order removal rate constant (day^{-1}). Nominal retention time (HRT) was computed as surface area times water depth times porosity of wetland(s) divided by average flow rate. The porosity or fraction of the space available for water to flow through the wetland in this study was assumed to be 0.75 in according with Lin *et al.* 2002. Because average temperature of the influent and effluent of the final wetland system in

each season ranged from 14.69-17.15 and 25.32-27.61 °C respectively (Tab. 8.1.1) we omitted the temperature effect on each seasonal *K* and considered the *K* determined as an apparent reaction rate constant. All the values of the rate constant estimated resulted comparable to those reported in other studies (Tab. 8.1.6).

Tab. 8.1.6; A summary of average first-order removal rate constant (*K*) from this study and literature for treating aquaculture water and wastewater; FWS = water surface wetland; SW = subsurface flow wetland; t = nominal retention time (surface area times water depth times porosity of wetlands divided by flow rate); HLR = hydraulic loading rate (flow rate divided by total surface wetland area).

Literature source	Wetland type	t (day)	HLR (m/day)	*K* for TSS (day^{-1})	*K* for NH_4-N (day^{-1})	*K* for NO_2-N (day^{-1})	*K* for SRP (day^{-1})
This study Spring	FWS	0.300	2.490	0.453	1.648	0.035	N
This study Summer	FWS	0.250	2.980	N	N	0.657	0.845
Lin et al. (2005)	FWS-SF	0.090	1.540	5.950	7.580	17.320	-
Lin et al. (2005)	FWS-SF	0.060	1.950	7.050	11.370	19.600	-
Lin et al. (2003)	FWS-SF	1.31	0.300	1.685	1.115	3.323	0.470
Lin et al. (2002)	FWS-SF	6.50	0.023	0.195	0.372	0.468	-
Lin et al. (2002)	FWS-SF	4.40	0.034	0.291	0.410	0.790	-
Lin et al. (2002)	FWS-SF	2.20	0.068	0.294	0.893	1.489	-
Lin et al. (2002)	FWS-SF	1.10	0.135	0.382	1.167	2.911	-
Tilley et al. (2002)	FWS	1.00	0.177	1.050	-	-	-

N = negative value

8.1.2 Toward a model for nitrogen:

The application of the Lefebvre model (Eq. 1) in the discharge lagoon ponds (E and F) (Fig. 8.1.8) resulted in data reported on table 8.1.7. For this application were used the NH_4-N concentrations measured in the fish ponds and in lagoon basins E and F in both seasons with corresponding values like to 0.55 and 0.42 g m^{-3} in spring period and 0.90 and 0.70 g m^{-3} in summer period respectively. The mean values of temperature used were 15.0 °C and 25.0 °C for spring and summer application. Water flow in the discharge lagoon system was 0.2 m^3 sec^{-1} in both season, and the surface of each basins was considered like to 9000 m^2. The Urea and DON concentrations were estimated in according to Lefebvre (2001), and represented 13% of the total dissolved nitrogen excretion (1.45 gN m^{-2} d^{-1} in spring and 2.71 gN m^{-2} d^{-1} in summer period) and 9% of the

total nitrogen consumed (estimated about 2.13 gN m^{-2} d^{-1} in spring and 3.98 gN m^{-2} d^{-1} in summer period) in each on-growing pond respectively. Because DON in the model represented the dissolved organic nitrogen exclude Urea, the effective DON was calculated by difference to total DON and Urea. As well as, the Urea and DON discharge lagoon pond concentrations assumed the values of 0.81, 1.51 mg L^{-1} in Spring period, and 0.01 and 0.03 mg L^{-1} in summer period respectively.

The values of N-Ammonia forecasted by the model were 0.11 and 0.14 g m^{-3} in spring and summer period respectively while the spring analytical data were 0.25 ± 0.06 g m^{-3} and 0.16 ± 0.10 g m^{-3} in basin E and F respectively, and the summer data were 0.82 ± 0.13 g m^{-3} and 0.67 ± 0.14 g m^{-3} in the same basins (Tab. 8.1.7). The values observed for each model's component were 93.6 and 144.0 g h^{-1} for $W_{lg}([NH_4]_{vp} - [NH_4]_{lg})$, 11.4 and 30.7 g h^{-1} for JNH_4S_{lg} , 42.48 and 159.52 g h^{-1} for $am(DON_{lg}+UREA_{lg})$, 15.2 and 50.7 g h^{-1} for $ni[NH_4]_{lg}$ and 54.4 and 181.2 g h^{-1} for $nloss[NH_4]_{lg}$ in spring and summer application respectively.

$$dNH_4lg/dt = W_{lg}([NH_4]_{vp} - [NH_4]_{lg}) + JNH_4S_{lg} + am(DON_{lg}+UREA_{lg}) - ni[NH_4]_{lg} - nloss[NH_4]_{lg} \quad (1)$$

These results suggest an improvement to the algorithm with a new additional component, representing the anoxic algal decomposition. Figure 8.1.9 shows the relationship between algae biomass and nitrogen ammonia release (g NH_4-N / h) in an partial anoxic conditions.

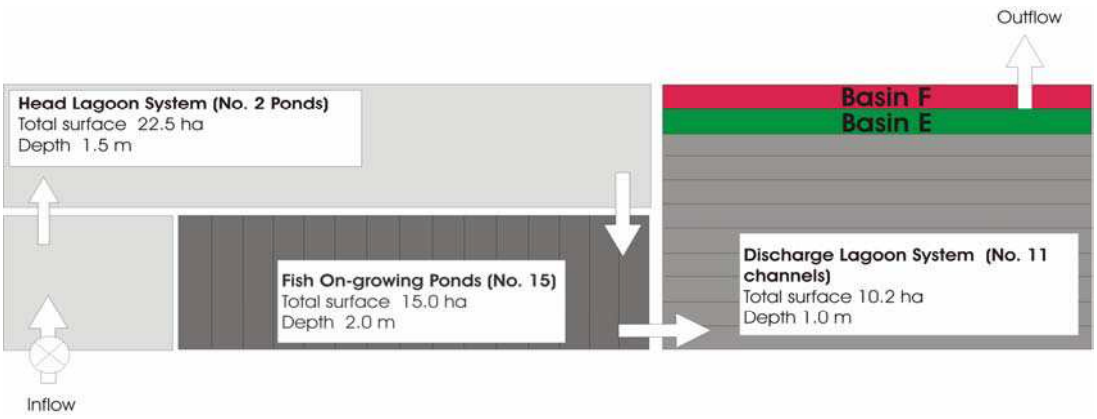


Fig. 8.1.8; Schematic diagram of the "*Il Padule*" fish farm with indicate the two discharge lagoon ponds (E and F) used for the application to Lefebvre model.

Tab. 8.1.7; Results of Lefebvre model application, in comparison with sampling periods (Spring and Summer) at the end of two final lagoon ponds (E and F).

	Eu			Fu			
NH_4-N	mean	d.s.	min-max	mean	d.s.	min-max	Teoric (*Lefebvre*)
Spring ($g\ m^{-3}$)	0.25	0.06	0.17-0.39	0.16	0.10	0.06-0.43	**0.11**
Summer ($g\ m^{-3}$)	0.82	0.13	0.55-1.07	0.67	0.14	0.37-0.94	**0.14**

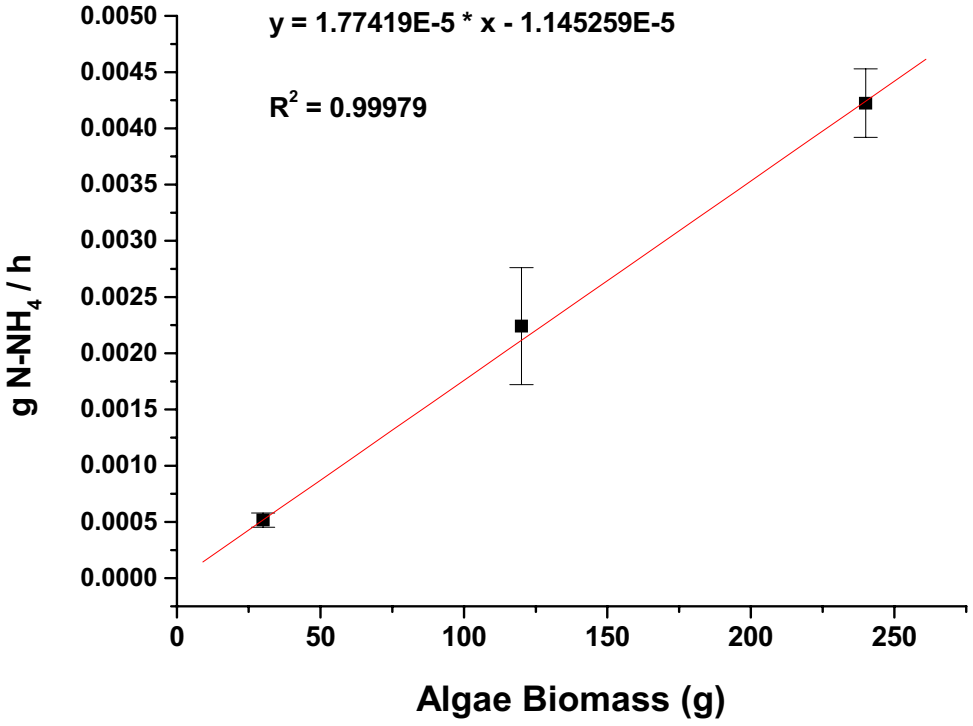


Fig. 8.1.9; Relationship to algae biomass and experimental ammonia nitrogen release (g NH_4-N h^{-1}) after partial anoxic algae decomposition.

The algorithm is so improved with a new equation obtained from the interpolation to experimental trials (Fig. 8.1.9), where *Algae* represents the total algae biomass present in each basin:

$$dNH_4lg/dt = Lefebvre\ Model + [(1.77419E\text{-}5 * Algae) - 1.45259E\text{-}5] \quad (2)$$

With the max algae density observed in the farm lagoon ponds (1.81 kg m^{-2} ww), the nitrogen ammonia level obtained after application of the equation (*2)* at the end of discharge lagoon basins was 0.39 g m^{-3} (Tab. 8.1.8).

Tab. 8.1.8; Results of the Lefebvre model application and new improved model, in comparison with sampling periods (Spring and Summer) at the end of two final lagoon ponds (E and F).

	Eu			Fu				
NH_4-N	mean	d.s.	min-max	mean	d.s.	min-max	Teoric (*Lefebvre*)	New Teoric
April (g m^{-3})	0.25	0.06	0.17-0.39	0.16	0.10	0.06-0.43	**0.11**	-
July (g m^{-3})	0.82	0.13	0.55-1.07	0.67	0.14	0.37-0.94	**0.14**	**0.39**

Figure 8.1.10 shows the results of the experimental trials to evaluate the NH_4-N supply from the sediment in partial anoxic concentrations (4.1 mg L^{-1}). The initial mean NH_4-N concentration was 0.2 ± 0.01 mg L^{-1} and after 24 hours these concentration increased up to 0.61 ± 0.32 mg L^{-1}. The mean release in the first 24 hours was 1.1 x 10^{-6} kg NH_4-N/m^2/h , and by considering the total surface of each lagoon basin (9000 m^2), the theoretic NH_4-N sediment supply in the discharge lagoon pond was to 9.9 gNH_4-N/h while the value predicted by the model for this component was 11.4 g NH_4-N h^{-1}. After 48 hours, the mean level of NO_2-N increased from 0.18 ± 0.01 to 0.43 ± 0.1 mg L^{-1} while the mean NO_3-N concentration decreased from 178.00 ± 0.01 to 119.0 ± 9.1 mg L^{-1} (Fig. 8.1.10).

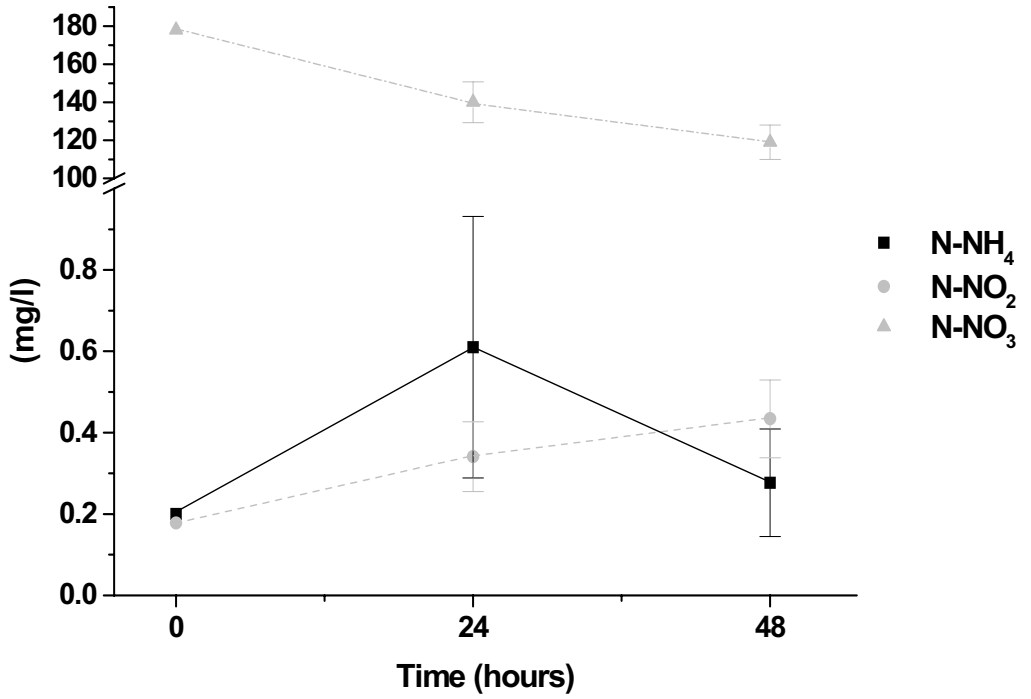


Fig. 8.1.10; Concentrations of NH_4-N, NO_2-N and NO_3-N in the experimental trials to evaluate the NH_4-N supply from the sediment in a partially anoxic condition: the symbols indicate the mean concentrations while the whiskers the relative standard deviation.

8.2 Remote sensing monitoring:

Figures 8.2.1, 8.2.2, 8.2.3 and 8.2.4 show the results obtained by the chromaticity analysis on the level of chlorophyll of the marsh land and the levels of transparency, total suspended solids and chlorophyll in to the fish farm, respectively. In the marsh land (Fig. 8.2.1) there are larger concentrations of chlorophyll in some of it areas and within the Bruna river which flows into the sea and which can condition the water in both the marsh land and in the fish farm intake.

Inside the farm (Fig. 8.2.2, 8.2.3 and 8.2.4) higher levels of chlorophyll are to be found at the level of the lagoon head basins and inside some of the production tanks, in general those with larger dimensions characterized by a higher concentration of nutrient. At the level of the final basins of the farm one observes higher variability with, in some cases, levels of chlorophyll concentration lower at outflow with respect to inflow sample, due to different hydrodynamics between the basins. With regard the levels of transparency and suspended solids at the fish farm one denotes a decrease and an increase respectively within the production tanks.

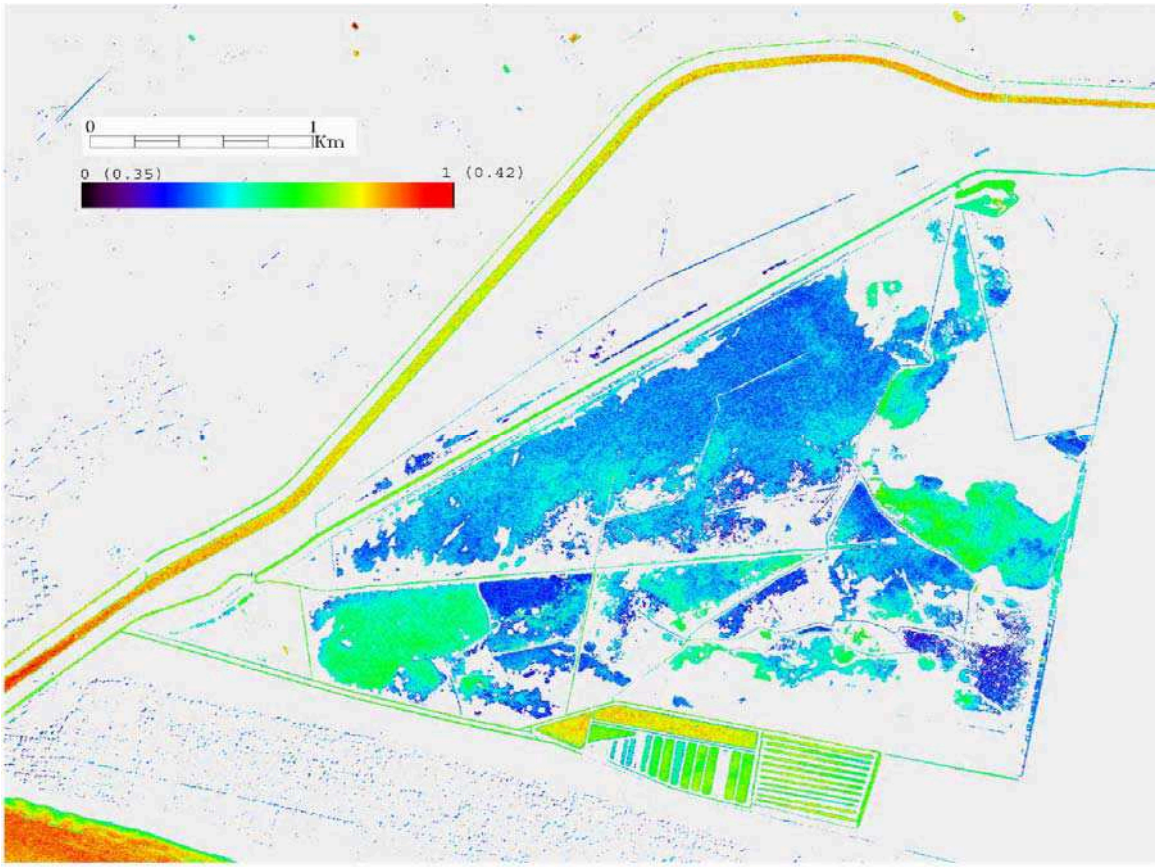


Fig. 8.2.1; Chromaticity analysis (ENVI®) on the Quick Bird Satellite image showing the chlorophyll levels in the wetland "*Diaccia Botrona*" and in "*Il Padule*" fish farm. Dark blue indicates low concentration, light green moderate concentration and dark red high concentration.

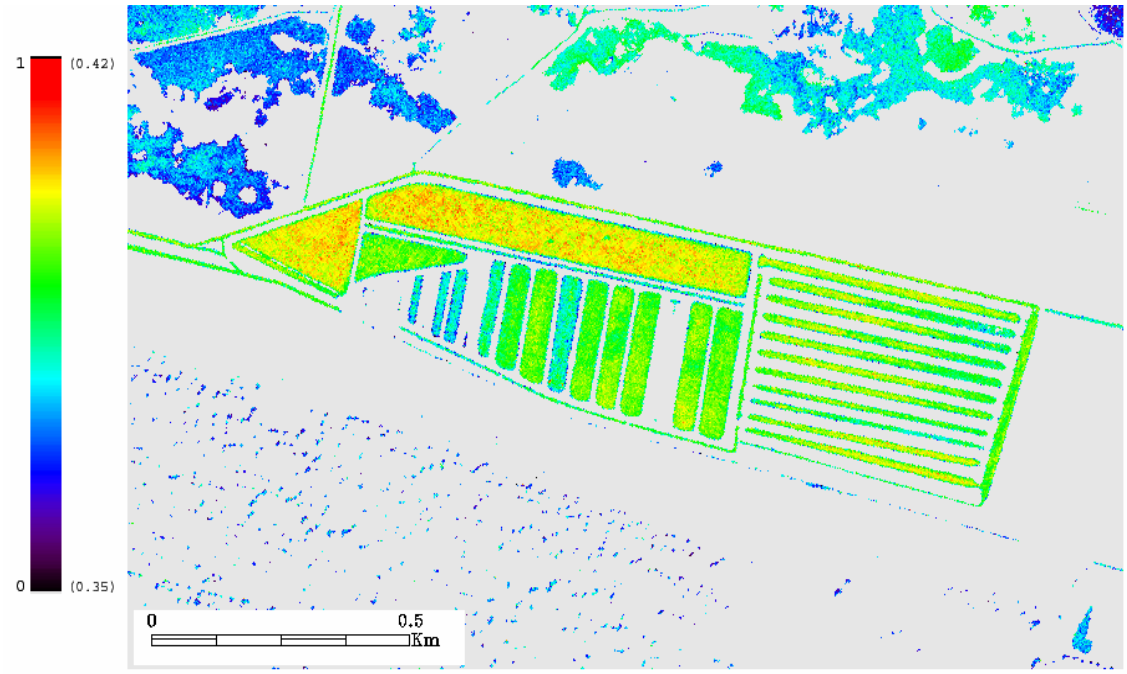


Fig. 8.2.2; Chromaticity analysis (ENVI®) on the Quick Bird Satellite image showing the variation of the chlorophyll levels in "*Il Padule*" fish farm: dark blue indicates low concentration, light green moderate concentration and dark red high concentration (0 = lower concentration; 1 = higher concentrations).

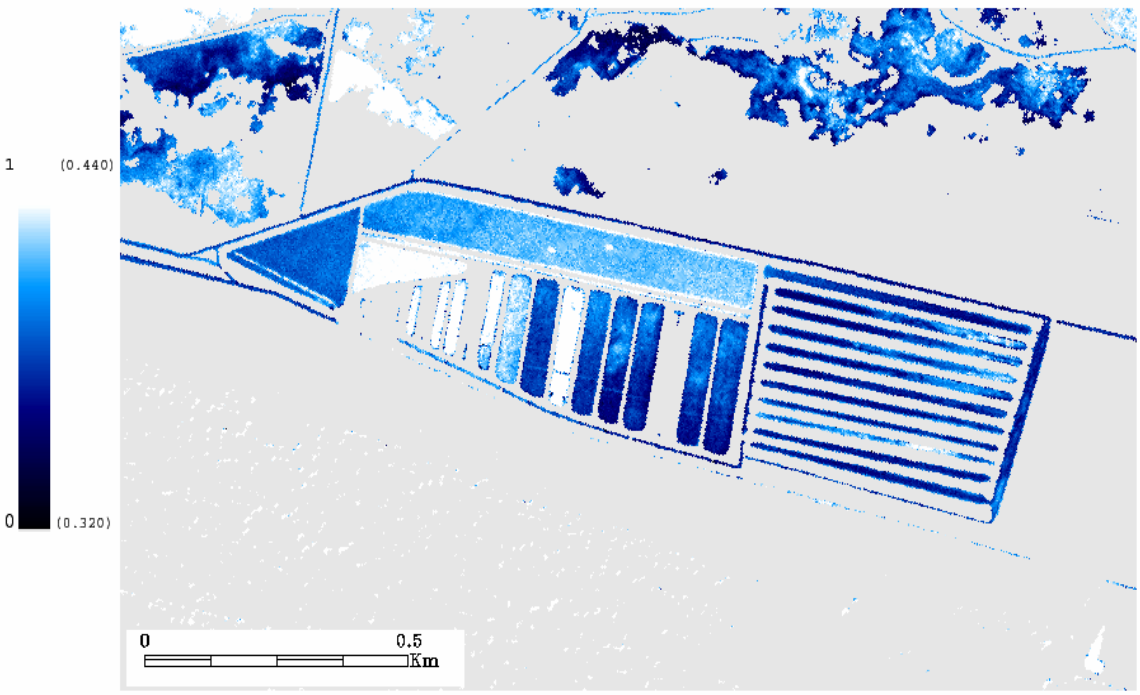


Fig. 8.2.3; Chromaticity analysis (ENVI®) on the Quick Bird Satellite image showing the variation of the transparency in "*Il Padule*" fish farm: dark blue indicates low transparency and white high transparency (0 = lower transparency; 1 = higher transparency).

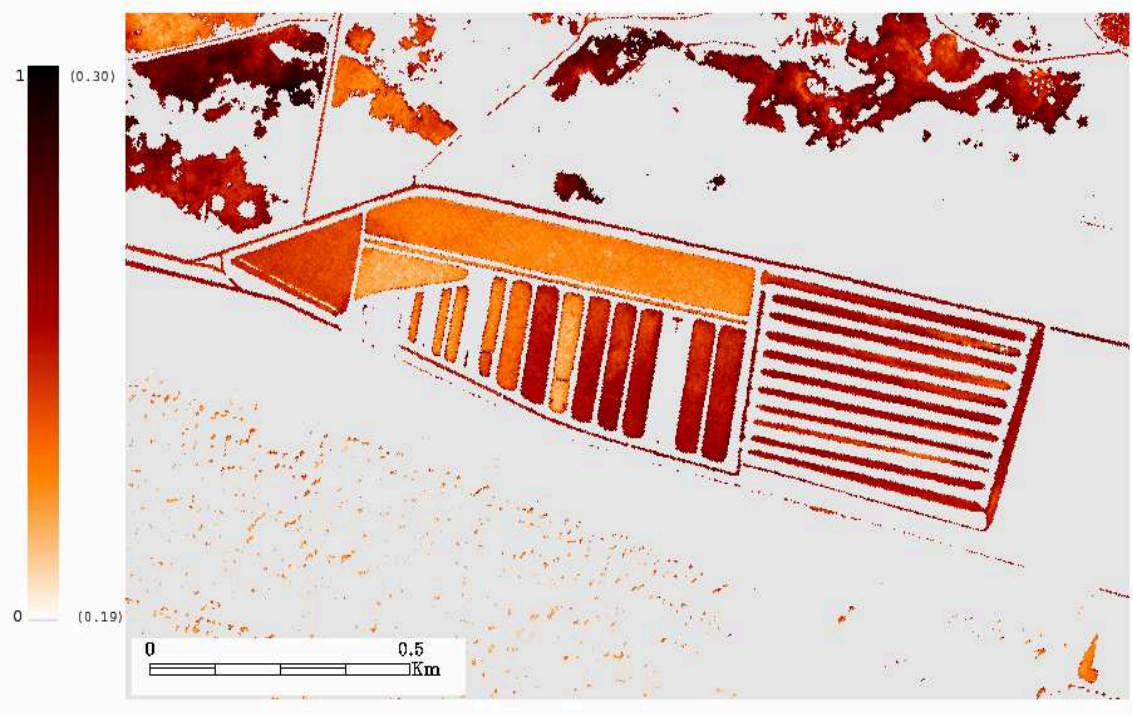


Fig. 8.2.4; Chromaticity analysis (ENVI®) on the Quick Bird Satellite image showing the variation of the total suspended solids (TSS) in "*Il Padule*" fish farm: dark red indicate high concentration white low concentration (0 = lower concentration; 1 = higher concentrations).

8.3 Improvement of wetland efficiency:

8.3.1 Effect of water flow:

Table 8.3.1 and figure 8.3.2 and 8.3.3 show the levels of chemical and physical parameters monitored in the inflow and outflow of three discharge lagoon basins (I, E and L) after water flows alterations (Fig. 8.3.1), and the *ANOVA* results of the comparison of the data measured in these sites.

The analytical measures varied within the following ranges: temperature 26.76-28.02 °C, oxygen 7.44-12.96 mg L^{-1}, oxygen saturation 98.4-171.6 %, and salinity 35.20-35.33 g L^{-1}. The mean levels (units) of pH at the inflow and outflow water were 7.26±0.15 and 7.42±0.15 in the basin I, 7.28±0.11 and 7.57±0.18 (*P<0.05*) in the basin E and 7.31±0.15 and 7.93±0.35 (*P<0.05*) in basin L, respectively. The levels of TSS decreased after the passage in the basin I while increased in the basins E and L. The mean TSS inflow and outflow concentrations were 0.24±0.04 and 0.20±0.07 mg L^{-1} in the basin I, 0.21±0.01 and 0.33±0.05 (*P<0.05*) mg L^{-1} in the basin E and 0.26±0.04 and 0.39±0.16 mg L^{-1} in the basin L respectively (Fig. 8.3.2 A). A similar pattern was recorder for the settleable suspended solid (SSS) with inflow and outflow mean concentrations like to 0.20±0.03 and 0.28±0.07 (*P<0.05*) mg L^{-1} in the basin E and 0.23±0.04 and 0.32±0.17 mg L^{-1} in the basin L (Fig. 8.3.5 B). The levels of unsettleable suspended solids (USS) increased after the passage into the all basins. The means USS concentrations in the inflow and outflow water were 0.02±0.01 and 0.04±0.02 mg L^{-1} in the basin I, 0.02±0.01 and 0.04±0.03 mg L^{-1} in the basin E and 0.02±0.01 and 0.05±0.02 (*P<0.05*) mg L^{-1} in the basin L (Fig. 8.3.2 C). The mean concentrations of COD increased only after the passage into the basin E with values of 49.75±20.17 mg L^{-1} in the water inflow and 68.25±45.51 mg L^{-1} in the water outflow. The concentrations of NH_4-N decreased after the passage into basin E and L. The mean concentrations in the E and L inflow water were 0.94±0.20 mg L^{-1} and 1.04±0.18 mg L^{-1} while in the E and L outflow water were 0.85±0.23 mg L^{-1} and 0.76±0.28 mg L^{-1} respectively (Fig. 8.3.3 A). The SRP decreased after the passage into the basin I, E and L with inflow and outflow mean concentrations like to 0.49±0.01 and 0.42±0.07 (*P<0.05*) mg L^{-1} in the basin I, 0.50±0.03 and 0.42±0.08 mg L^{-1} in the basin E and 0.52±0.07 and 0.47±0.15 mg L^{-1} in the basin L respectively (Fig. 8.3.3 B).

Table 8.3.2 shows the levels of chlorophyll and transparency monitored in the site D and in the outflow water of lagoon basin I, E and L. The chlorophyll levels increased after the

passage into the basin I, L and E respect to the site D (25.36 ± 12.01 mg m^{-3}), with means outflow water concentrations like to 26.7±1.7, 29.37±8.01 and 49.84±2.16 mg m^{-3} respectively. The levels of transparency in the outflow of basin I (0.62±0.02 m) were similar to the values monitored in the site D (0.61±0.02 m) while in the E and L outflow water the mean levels of transparency, 0.40±0.01 and 0.37±0.02 m respectively, were lower than these sites.

The vegetation community in the discharge lagoon system was composed to about 30% of aquatic plant (*P. pectinatus*) and 70% of macroalgae (60% *C.linum,* 10% *G. verrucosa*) and the mean density in the basin I, E and L was about to 0.87±0.08, 0.73±0.06, and 1.81±0.42 kg/m^2 (w.w.) respectively.

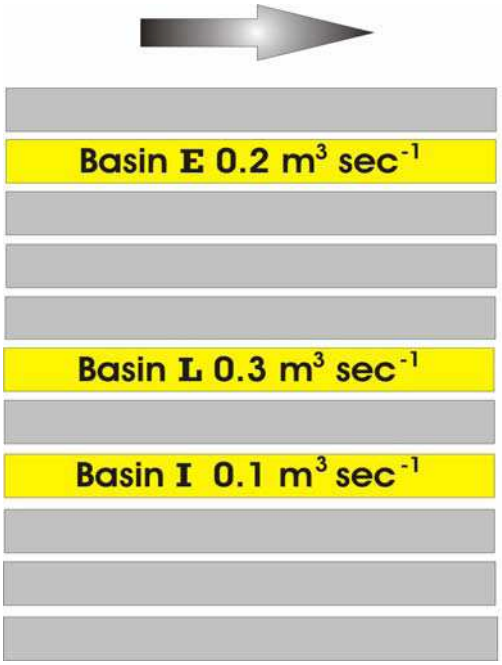


Fig. 8.3.1; Schematic diagram representing the discharge lagoon basins: in yellow are highlight the basins named I, E and L with a water flow set up to 0.1, 0.2, 0.3 m^3 sec^{-1} respectively.

Tab. 8.3.1; Levels of chemical and physical parameters monitored in the inflow and outflow water of three discharge lagoon basins (I, E and L) after water flows alterations (Fig. 7.4). The symbol (* § ‡) indicated the significative difference ($P < 0.05$) after the comparisons (*ANOVA*) with inflow and outflow levels of each basins.

		Basin I 0.3 m³/sec		**Basin E 0.2 m³/sec**		**Basin L 0.1 m³/sec**	
Parameters		Inlet	Outlet	Inlet	Outlet	Inlet	Outlet
Temperature (°C)	mean	26.77	28.02	27.00	27.92	26.76	27.42
	d.s.	0.98	1.58	0.98	1.57	1.07	1.85
Oxygen (mg L^{-1})	mean	8.73	9.90	8.98§	12.96§	8.60	7.44
	d.s.	0.58	2.45	0.85	3.58	0.82	2.72
% Saturation O_2	mean	113.67	131.83	117.60	171.60	112.20	98.40
	d.s.	7.89	35.19	12.07	51.83	11.30	38.23
pH	mean	7.26	7.42	7.28§	7.57§	7.31‡	7.93‡
	d.s.	0.15	0.15	0.11	0.18	0.15	0.35
Salinity (g L^{-1})	mean	35.33	35.33	35.20	35.20	35.20	35.20
	d.s.	0.52	0.52	0.45	0.45	0.45	0.45
TSS (mg L^{-1})	mean	0.24	0.20	0.21§	0.33§	0.26	0.39
	d.s.	0.04	0.07	0.01	0.05	0.04	0.16
SSS (mg L^{-1})	mean	0.22	0.17	0.20§	0.28§	0.23	0.32
	d.s.	0.04	0.09	0.03	0.07	0.04	0.17
USS (mg L^{-1})	mean	0.02	0.04	0.02	0.04	0.02‡	0.05‡
	d.s.	0.01	0.02	0.01	0.03	0.01	0.02
COD (mg L^{-1})	mean	46.60	39.60	49.75	68.25	73.75	46.25
	d.s.	16.46	25.89	20.17	45.51	49.82	14.52
NH_4-N (mg L^{-1})	mean	0.94	1.00	0.94	0.85	1.04	0.76
	d.s.	0.15	0.28	0.20	0.23	0.18	0.28
NO_2-N (mg L^{-1})	mean	0.18	0.17	0.18	0.20	0.18‡	0.08‡
	d.s.	0.02	0.01	0.02	0.02	0.02	0.03
NO_3-N (mg L^{-1})	mean	0.57	0.53	0.70	0.60	0.64	0.52
	d.s.	0.22	0.17	0.24	0.22	0.24	0.22
SRP (mg L^{-1})	mean	0.49*	0.42*	0.50	0.42	0.52	0.47
	d.s.	0.01	0.07	0.03	0.08	0.07	0.15

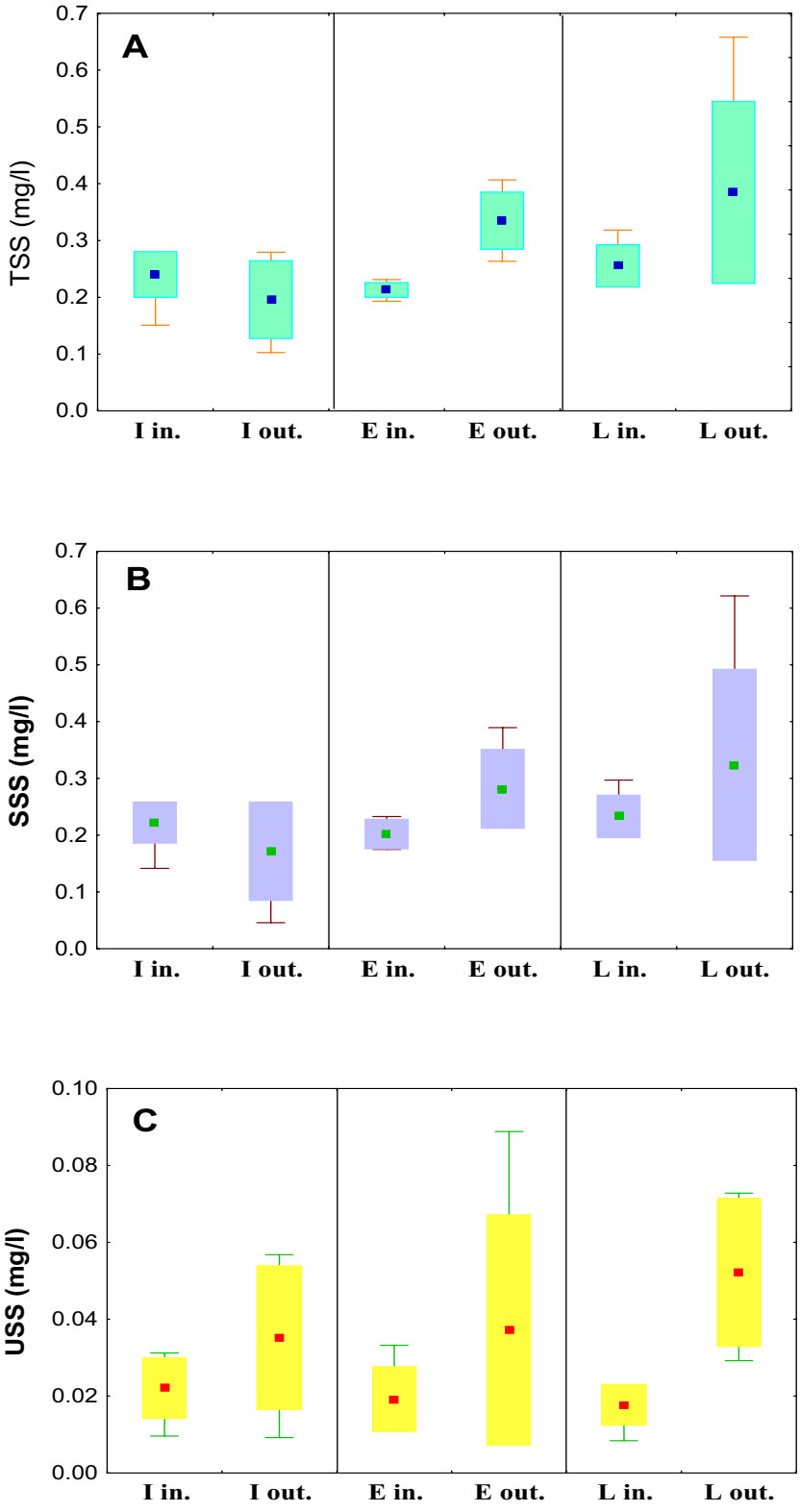


Fig. 8.3.2; Concentrations of TSS (A), SSS (B) and USS (C) monitored in the inflow and outflow water of three discharge lagoon basins (I, E and L) after water flows alterations (Fig. 8.3.1).The square, box and whiskers indicated the mean value, mean ± d.s. and min-max value respectively.

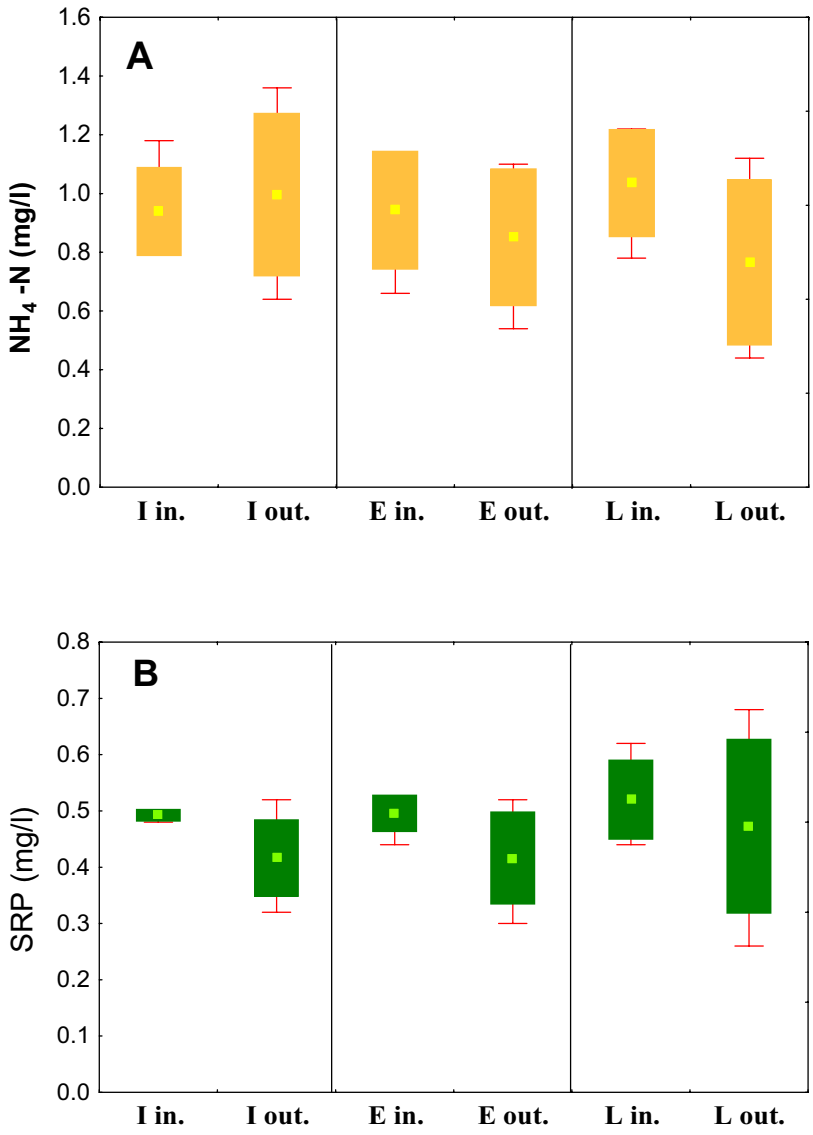


Fig. 8.3.3; Concentrations of NH_4-N (A) and SRP (B) monitored in the inflow and outflow water of three discharge lagoon basins (I, E and L) after water flows alterations (Fig. 8.3.1).The square, box and whiskers indicated the mean value, mean ± d.s. and min-max value respectively.

Tab. 8.3.2; Levels of chlorophyll and transparency monitored in the site D and in the inflow and outflow water of three discharge lagoon basins (I, E and L) after water flows alterations (Fig. 8.3.1).

	D	I out.	E out.	L out.
Chlorophyll (mg/m^3)	25.3 ± 12.01	26.7 ± 1.70	29.3 ± 8.01	49.8 ± 2.16
Transparency (m)	0.6 ± 0.08	0.6 ± 0.02	0.4 ± 0.01	0.3 ± 0.02

8.3.2 Gracilaria verrucosa:

Table 8.3.3 and figure 8.3.4 show the results of the experimental trials to evaluate the NH_4-N removal rate of a commercial macroalga *G. verrucosa.* After about 100 hours to constant artificial lighting (150 Watt), the NH_4-N mean level dropped from 0.73 ± 0.22 mg L^{-1} to 0.21 ± 0.15 mg L^{-1} with a NH_4-N removal rate like to 162 mg/day for kg (d.w.) of macroalga *G. verucosa* . In the dark conditions and in the control experiments the NH_4-N concentrations showed a narrow variability in the experiment time and the mean values were included in the range of 0.78-0.68 and 0.82-0.67 mg L^{-1} respectively with light fluctuations.

Tab. 8.3.3; Results of experimental trials with application of *G. verrucosa*: mean NH_4-N concentrations ± d.s. in light, dark conditions and control.

	mean NH_4-N concentrations ± d.s. (mg L^{-1})		
Time (hours)	Light	Dark	Control
0	0.73 ± 0.22	0.70 ± 0.21	0.77 ± 0.24
16	0.73 ± 0.19	0.72 ± 0.24	0.82 ± 0.12
23	0.51 ± 0.09	0.72 ± 0.36	0.77 ± 0.19
89	0.44 ± 0.27	0.78 ± 0.34	0.67 ± 0.24
95	0.21 ± 0.15	0.68 ± 0.37	0.67 ± 0.27

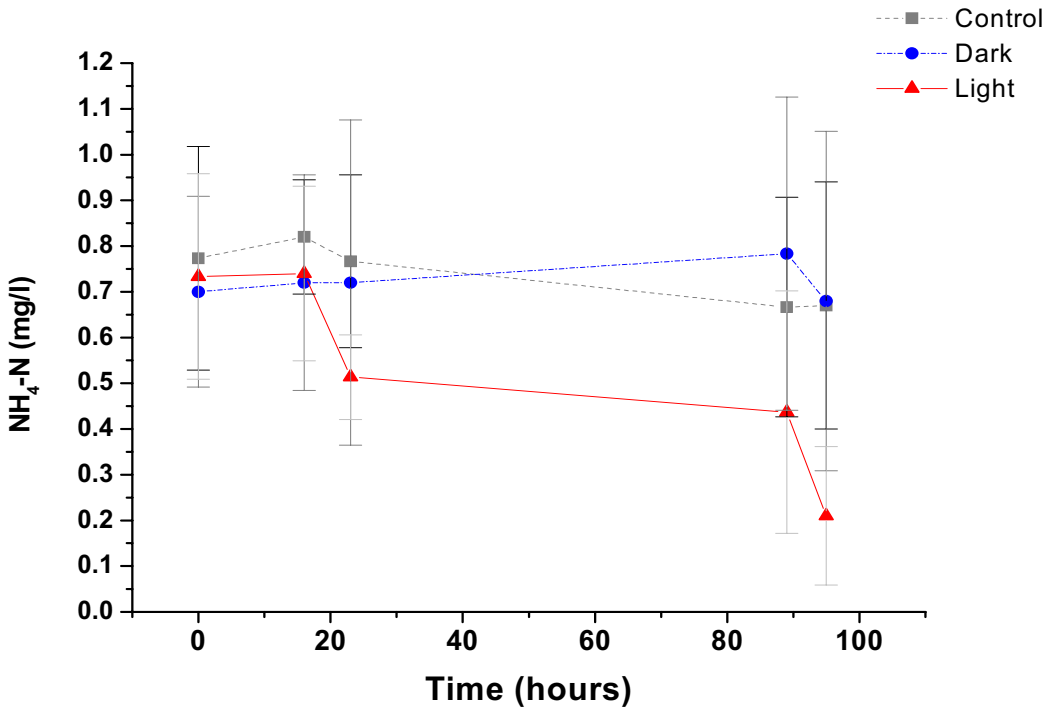


Fig. 8.3.4; Results of experimental trials with application of *G. verrucosa*: the symbol indicate the mean NH_4-N concentrations while the whiskers the relative standard deviation.

8.3.3 Bacterial-enzyme bio-promoter

Aerobic conditions:

Table 8.3.4 and figure 8.3.5 show the results of bacterial-enzyme bio-promoter applications (Aqua-Clear®, Eurovix S.r.l.) in aerobic conditions, and equivalent controls, for study the improvement of nitrification processes through the ability to reduce NH_4-N levels in the water. The means of the oxygen concentrations, temperature and pH monitored in the experiment were 7.0±0.11 mg L^{-1}, 28.6±0.4 °C and 9.0±0.1 unit in the bio-promoter trials and 7.2±0.17 mg L^{-1}, 28.4±0.40 °C and 6.92±0.08 unit in the controls respectively. After NH_4Cl added at the start time, the mean concentrations of NH_4-N increased up to 76.67±3.06 mg L^{-1} in the bio-promoter trials, and 79.67±2.08 mg L^{-1} in the controls; after 72 hours the NH_4-N mean concentrations dropped up to 0.78±0.19 mg L^{-1} and 30.67±3.51 mg L^{-1} in the same systems respectively (Tab. 8.3.4 and Fig. 8.3.5).

Tab. 8.3.4; Concentrations of NH_4-N in aerobic bio-promoter experimental trials and controls.

Time (hours)	NH_4-N (mg L^{-1})	Bio-promoter	Control
0	mean	76.67	79.67
	d.s.	3.06	2.08
24	mean	37.27	53.00
	d.s.	8.54	4.58
48	mean	10.50	40.67
	d.s.	6.07	3.21
72	mean	0.78	30.67
	d.s.	0.19	3.51

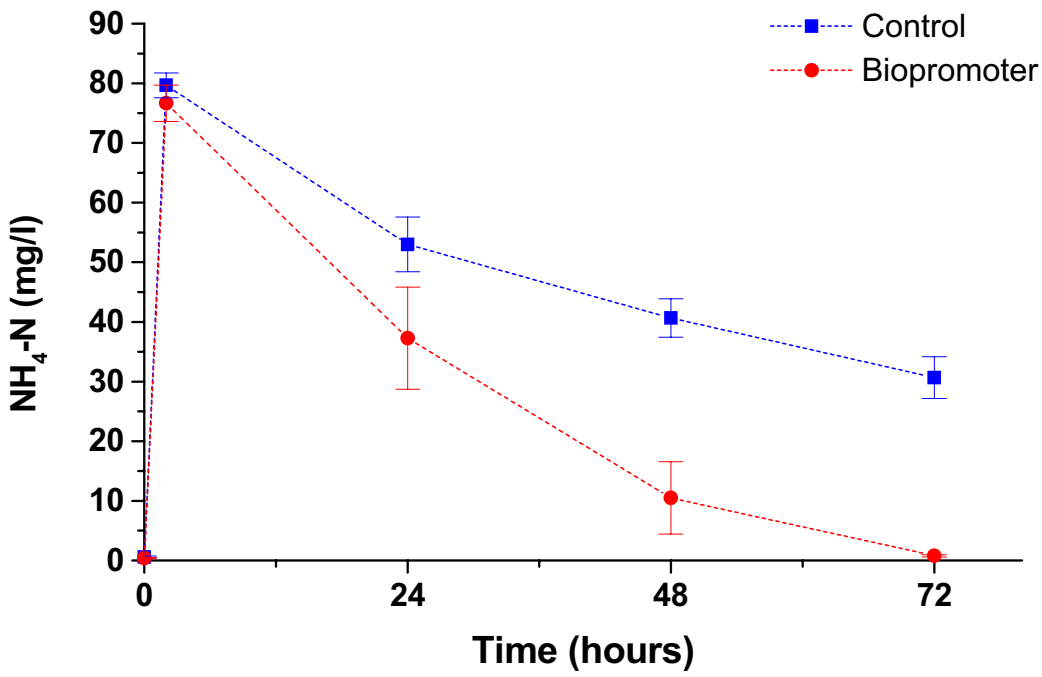


Fig. 8.3.5; Results of experimental trials with bio-promoter application and controls in aerobic conditions: the symbol indicate the mean NH_4-N concentrations while the whiskers the relative standard deviations.

Figure 8.3.6 shows the mean percentages of NH_4-N removal monitored into the experimental bio-promoter applications and equivalent controls. In the first 24 hours the means percentage of NH_4-N removal were 51.62±9.07 % and 33.53±4.30 % in the bio-promoter trials and in the controls respectively. During the experiments, these percentages increased in the bio-promoter applications while decreased in the control systems. The higher percentages of NH_4-N removal were monitored in the bio-promoter trials at the third day of the experiment with values like to 91.6±2.26 % (Fig. 8.3.6). Figure 8.3.7 show the corresponding mean NH_4-N removal rate (mg NH_4-N/h) calculated on the ground of NH_4-N concentrations monitored in the bio-promoter applications and controls. The removal rate decreased during the experiment in the both systems. In the first 48 hours, the NH_4-N removal rate within the bio-promoter applications were higher than those observed in the controls system with higher mean values like to 1.64±0.23 and 1.11±0.12 mg NH_4-N/h respectively. After 72 hours the NH_4-N removal rate were similar in the bio-promoter applications and controls with values like to 0.40±0.24 and 0.41±0.08 mg NH_4-N/h respectively (Fig. 8.3.7).

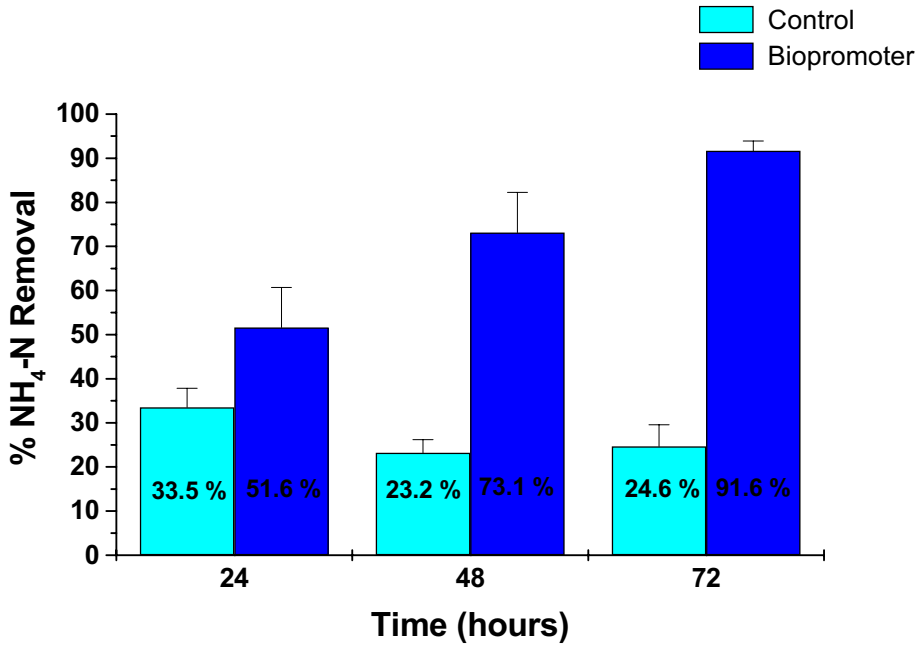


Figure 8.3.6; Mean percentage of NH_4-N removal ± d.s. calculated in the bio-promoter applications and controls under aerobic conditions.

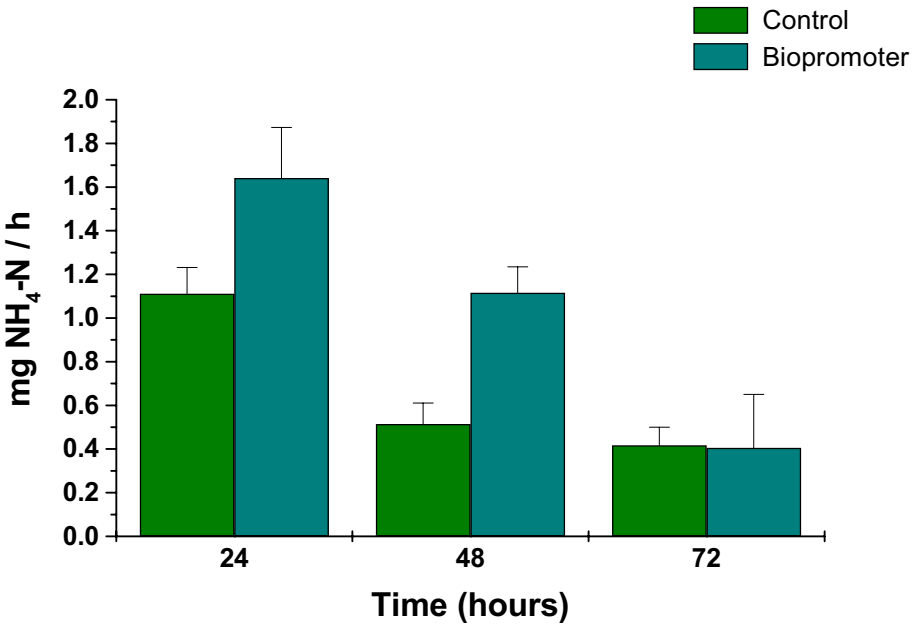


Fig. 8.3.7; Mean NH_4-N removal rates ± d.s. (mg NH_4-N/h) calculated in the bio-promoter applications and controls under aerobic conditions.

In additions to the study of NH_4-N patterns, in these experimental trials were monitored also the ability of bacteria-enzyme bio-promoter to reduce the levels of SRP. Table 8.3.5 and figure 8.3.8 show the SRP concentrations in bio-promoter trials and equivalent controls. After $Na_2H_2PO_4H_2O$ added at the start time, the mean concentrations of SRP increased up to 41.66±5.99 mg L^{-1} in the bio-promoter trials, and 48.67±2.52 mg L^{-1} in the controls. After 72 hours the SRP mean concentrations dropped up to 7.85±0.25 mg L^{-1} and 17.20±2.60 mg L^{-1} in the same systems respectively (Tab. 8.3.5 and Fig. 8.3.8).

Tab. 8.3.5; Concentrations of SRP under aerobic bio-promoter experimental trials and controls.

Time (hours)	SRP (mg L^{-1})	Biopromoter	Control
0	mean	41.66	48.67
	d.s.	5.99	2.52
24	mean	21.00	34.33
	d.s.	6.24	2.89
48	mean	9.75	24.50
	d.s.	0.25	3.04
72	mean	7.85	17.20
	d.s.	0.25	2.60

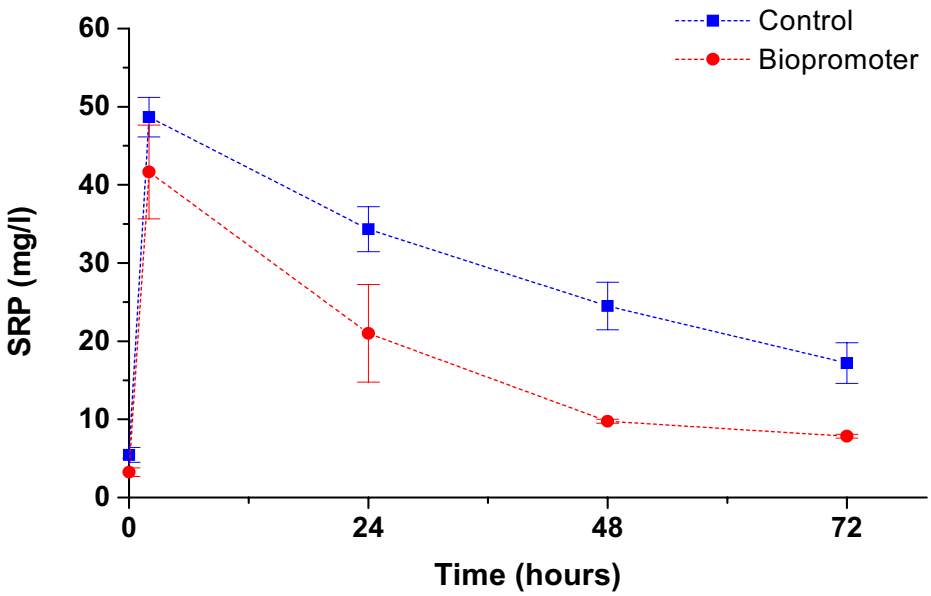


Fig. 8.3.8; Results of experimental trials with bio-promoter application and controls in aerobic conditions: the symbol indicate the mean SRP concentrations while the whiskers the relative standard deviations.

Figure 8.3.9 shows the mean percentages of SRP removal monitored into the experimental bio-promoter applications and equivalent controls. In the first 24 hours the means percentage of SRP removal were 49.84±10.56 % and 29.5±3.04 % in the bio-promoter trials and controls respectively. During the experiments, these percentages resulted constant in the control systems but decreased after 72 hours in the bio-promoter applications with values to 19.49±0.50 (Fig. 8.3.9). Figure 8.3.10 show the corresponding mean SRP removal rate (mg SRP/h) calculated on the ground of SRP concentrations monitored in the bio-promoter applications and controls. The SRP removal rate decreased during the experiment in the both the systems. In the first 48 hours, the SRP removal rate in the bio-promoter applications were higher than those monitored in the controls system with higher mean values like to 0.86±0.19 and 0.59±0.04 mg SRP/h respectively. After 72 hours the SRP removal rate in the bio-promoter applications were lower than to the control systems with mean values like to 0.08±0.001 and 0.30±0.05 mg SRP/h respectively (Fig. 8.3.10).

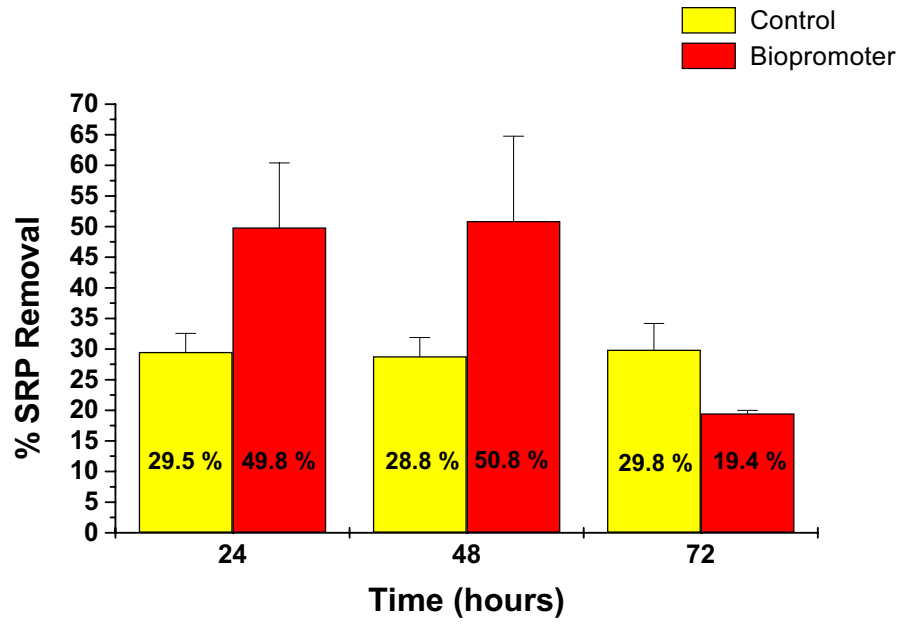


Figure 8.3.9; Mean percentage of SRP removal ± d.s. calculated in the bio-promoter applications and controls under aerobic conditions.

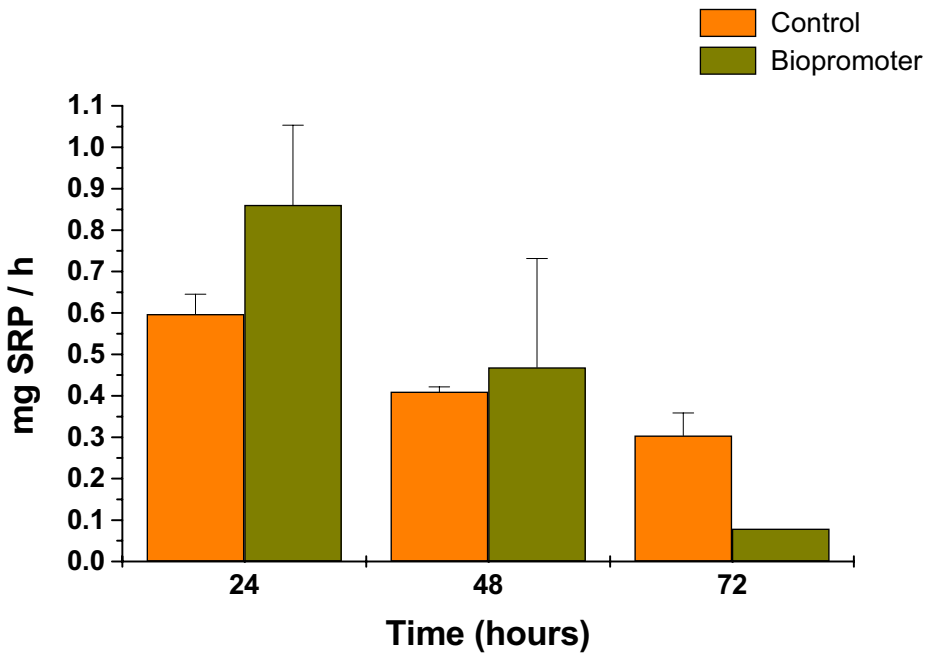


Fig. 8.3.10; Mean SRP removal rates ± d.s. (mg SRP/h) calculated in the bio-promoter applications and controls under aerobic conditions.

Partial anoxic conditions:

Similar experimental trials without added of NH_4Cl and $Na_2H_2PO_4H_2O$, were carried out in partially anoxic conditions obtained without air exchange in the prepared systems. In these conditions, the oxygen levels dropped from 7.10±0.01 to 0.52±0.38 mg L^{-1} and the mean levels of temperature and pH were 24.55±1.07 °C and 7.57±0.56 unit in the bio-promoter trials and 24.3±1.07 °C and 7.53±0.56 unit in the corresponding controls respectively. The table 8.3.6, figures 8.3.11 and 8.3.12 show the NH_4-N and SRP concentrations during these experiments. The mean NH_4-N concentrations increased after 48 hours from 0.38±0.01 to 22.13±2.01 and from 0.38±0.01 to 9.73±0.12 mg L^{-1} in the bio-promoter applications and in the control trials respectively. After this time the mean NH_4-N concentrations dropped up to 6.17±0.23 and 2.97±0.21 mg L^{-1} in both systems. After 72 h the aeration was restored, and at the end of the experiments the mean NH_4-N concentrations monitored were 3.80±0.69 and 1.33±0.12 mg L^{-1} in the bio-promoter trials and in the control systems, respectively (Fig. 8.3.11).

Tab. 8.3.6; Concentrations of NH_4-N and SRP in bio-promoter experimental trials and controls, under partially anoxic conditions.

		NH_4-N (mg L^{-1})		SRP (mg L^{-1})	
Time (hours)		Bio-promoter	Control	Bio-promoter	Control
0	mean	0.38	0.38	11.00	11.00
	d.s.	0.01	0.01	0.01	0.01
24	mean	15.80	5.27	12.60	11.47
	d.s.	1.40	0.50	0.53	0.12
48	mean	22.13	9.73	15.87	11.73
	d.s.	2.01	0.12	1.15	1.17
72	mean	6.17	2.97	22.93	12.00
	d.s.	0.23	0.21	3.51	1.97
98	mean	4.47	2.07	4.47	3.00
	d.s.	0.31	0.12	1.75	0.69
144	mean	3.80	1.33	5.27	4.73
	d.s.	0.69	0.12	0.31	2.12

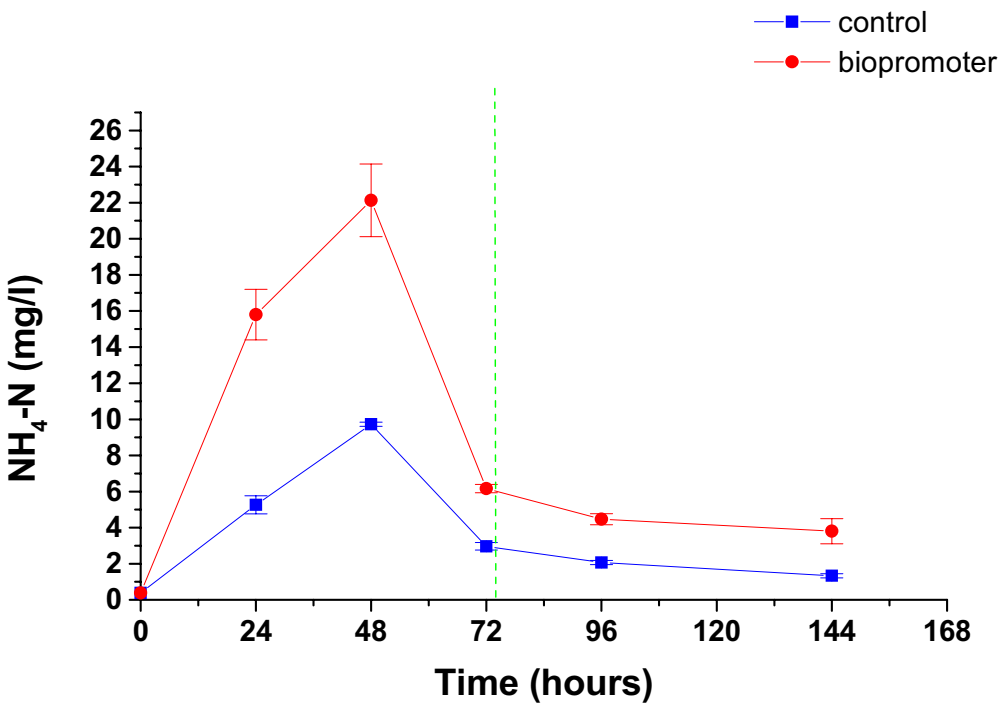


Fig. 8.3.11; Results of experimental trials with bio-promoter application and equivalent controls in partially anoxic conditions: the symbol indicate the mean NH_4-N concentrations, the whiskers the relative standard deviations and the broken line the recovery of aeration.

Figure 8.3.12 shows the SRP levels in bio-promoter trials and controls in partially anoxic conditions. After 72 hours the mean SRP concentrations increased from 11.0±0.01 to 22.93±3.51 mg L^{-1} in the bio-promoter trials while the mean controls levels at this time (12.0±1.97 mg L^{-1}) were similar to the initial values (11.0±0.01 mg L^{-1}). After 72 hours the aeration was restored and the mean oxygen levels increased again up to 6.00±1.07 mg L^{-1} in the bio-promoter trials and 6.48±1.07 mg L^{-1} in the control. In these conditions the mean SRP concentrations decreased in the bio-promoter and in the control systems up to 4.47±1.75 and 3.0±0.69 mg L^{-1} respectively (Fig. 8.3.12).

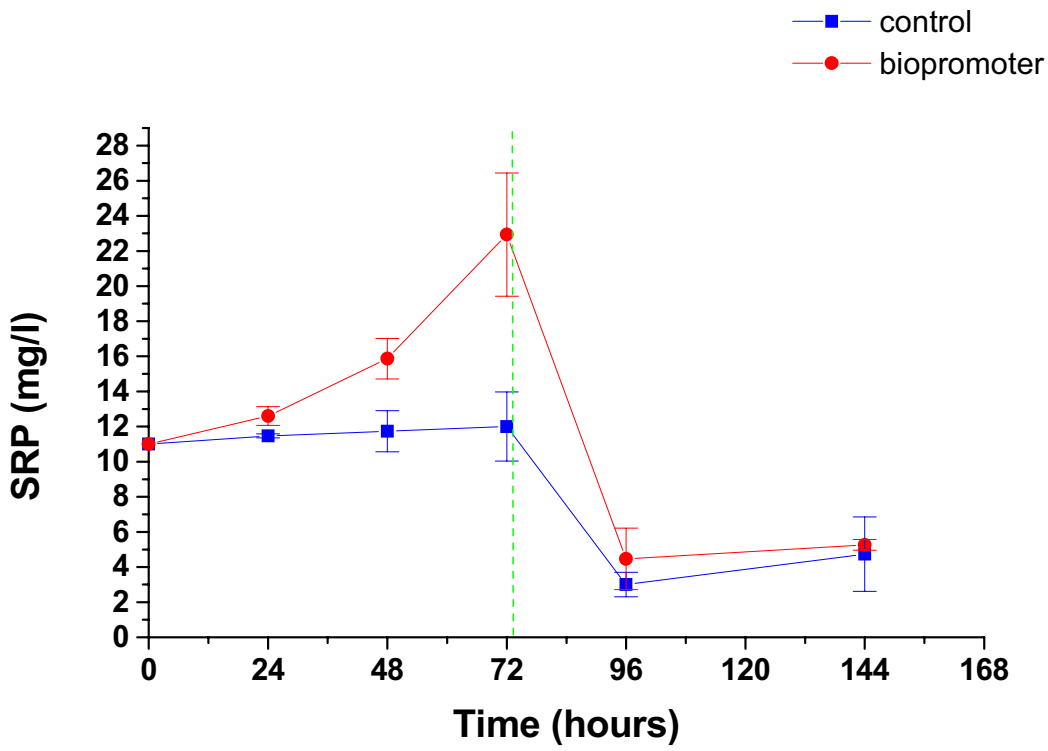


Fig. 8.3.12; Results of experimental trials with bio-promoter application and equivalent controls in partially anoxic conditions: the symbol indicate the mean SRP concentrations, the whiskers the relative standard deviations and the broken line the recovery of aeration.

9. DISCUSSION:

Water temperature is one of the most important variable affecting aquaculture production since the rates of feed intake and of all the biochemical processes are temperature dependent. The relationship between temperature and a typical metabolic process is oxygen consumption. Growth is the result of many metabolic processes, and each aquatic species has a characteristic range of upper and lower lethal temperature limits and a range of temperature considered "optimum" for growth and health. Many species suitable for aquaculture will survive over a wide temperature range, but the temperature range for maximum growth is narrower. For example, a specie might tolerate temperatures of 5-36°C, but the range for maximum growth might be from 25°C to 30°C only (Boyd, 1998). Generally, tropical and subtropical species will not grow well when water temperatures fall below 25°C and water temperature below 10° or 15°C may kill them. Warm-water species which are native at temperate climates, grow best at temperature between 20°C and 28°C, but they can survive at temperature near 0°C. Cold-water species, as salmonids, grow better at temperature below 20°C, and they may die when temperature exceed 25°C. In this study the temperature monitored in spring (16.66±2.01 - 17.15±2.51°C) was lower than in summer (25.81±1.70 - 27.61±2.34°C) period in which the values monitored were similar to the range of temperature considered "optimum" for growth an health for *D. labrax*, about to 20-22°C (Cataudella *et al*., 2001). Water temperature also affects the natural productivity of aquatic ecosystems and directly or indirectly regulates other water quality variables.

The salinity of surface water is influenced primarily by climate, and by the topography and geology of the draining area, and in coastal zones depend on the relative amounts of fresh river and seawater that are mixed together. In areas with pronounced wet and dry seasons, salinity shows a great seasonal variation mainly due to high river discharge rates during the wet season that cause a salinities decline. On a shorter term, salinity at a given point in the coastal zone decreases during ebb tide. During this study the salinity of inflow water in spring period was 25 g L^{-1} lower compared to the value monitored in the summer period. The salinity variation is important for osmoregulation processes. Each species has an optimum salinity range, outside of this range, the fish must expend considerable energy for osmoregulation at the expense of growth processes. If salinity deviates too much from the optimum range, the fish will be die because it cannot maintain homeostasis. In this study, the highest mean values of salinity were monitored during summer period (37.2 ± 1.94 g L^{-}

[1]) and resulted lower than the tolerated salinity values for *D. labrax* in Mediterranean aquaculture, reputed as about 45 g L^{-1} (Saroglia *et al*., 1992).

Although not considered a "nutrient" in the practical sense, the availability of oxygen is commonly the next factor limiting aquaculture production after the animals' food requirements are met. The reason that dissolved oxygen is so important stems for the crucial role played by oxygen in aerobic respiration and relatively low concentrations of dissolved oxygen available in water. It's solubility is controlled by water temperature and salinity. Notice that the solubility of oxygen in water decreases as water temperature and salinity increases. For these reasons, in spring period the dissolved oxygen concentrations monitored in the site of sampling were higher than those monitored in summer period, with a mean difference of 3 mg L^{-1}.

There are various biological, physical and chemical factors that could affected the dissolved oxygen concentration. Concentrations at any one time depend on the relative rates of five major processes: air-water gas transfer, sediment oxygen uptake, animal respiration, plankton respiration and photosynthesis. The plankton metabolism as the major component affecting dissolved oxygen dynamics during periods of warm water temperatures. Within the range of phytoplankton standing crops typically found in aquaculture ponds, phytoplankton photosynthesis is the largest source of oxygen and plankton respiration (predominantly phytoplankton respiration) is the largest sink for oxygen (Boyd, 1998). For these reasons after the passage into head and discharge lagoon system in both the seasons, the mean oxygen concentration in the water monitored in daily sampling increased following the photosynthesis activity of phytoplankton and this process is confirmed to concomitant pH increment. The lower average oxygen concentrations and the higher standard deviation monitored at the end of the discharge lagoon system in the nictemeral cycle confirmed the plankton respiration during the night time.

Oxygen consumed by the fish under culture depends on animal size and species, water temperature, activity, time after feeding, and environment dissolved oxygen concentration. The mean range of oxygen consumption rates is about 205-500 mg O_2/Kg body weight/h (Boyd, 1990). Oxygen is consumed faster by fish that have recently eaten than by fasted fish. The increased oxygen consumption is due to the dynamic action of feed. In the study, after the waters flow through the passage fish on-growing ponds, the oxygen concentration decreased respect to the inflow levels and mechanical aeration is therefore required to maintain values above those stressful or lethal for fish culture. In the time of sampling, the

mean levels of oxygen never dropped below 5 mg L^{-1} at the outflow of fish on-growing ponds.

Coastal water bodies represent heterogeneous resource, characterized by high spatial and temporal variability of the most water quality parameters (Joyce *et al*., 2005). This aspect is confirmed to the higher range of minimum and maximum values and to the high levels of standard deviations obtained after statistical analysis of temperature, pH, TSS, N-NH_4 and SRP data sets monitored in the inflow water. In both the examined seasons the HLS was efficient in the removal of most of the TSS, COD, NH_4-N and SRP contained in the take water. This efficiency was higher in the spring period respect to the summer period (60.37%, 14.89%, 65.38%, 17.6% and 45.10%, 8.06%, 32.43%, 8.00% respectively). This results suggest that high chemical precipitation, sedimentation, nitrification and denitrification processes proceeded concurrently in the HLS wetlands. The processes occurring in the HLS were efficient also to reduce the standard deviation values. These controls of the daily and seasonal variations of the most important water quality parameters is important to maintain an adequate and satisfactory environment for fish grow.

Wastes from aquaculture may contain a variety of constituents which are not removed from the system during harvesting. The constituent include dissolved or particulate organics, total suspended solids, nutrients such as nitrogen and phosphorus and specific organic and inorganic compounds (i.e. therapeutants). These constituent originate primarily from uneaten feed and metabolic wastes from the fish. Respect to the feed N and P contents, nitrogen and phosphorus retentions range between 10 and 49% and 17 and 40% respectively. Similarly, N and P releases in feces range from 3.6% to 35% and 15% to 70% respectively. Lastly, dissolved N and P excretions range from 37% to 72% and 1% to 62%, respectively (Peidrahita, 2003) (Tab. 9.1).

Tab. 9.1; Nutrient excretion and retention rates (as percentages of constituent present in the feed consumed) (Piedrahita, 2003).

Retained		In feces (particulate)		Excreted (dissolved)		Type of fish	Reference
N	P	N	P	N	P		
49	36	14	55	37	9	*A. salmon*	Johnsen et al., 1993; Bergheim and Åsgård, 1996
	17–19		48–54		28–34	*A. salmon*	Holby and Hall, 1991
11	32					Carp	Avnimelech and Lacher, 1979
27	30					Channel catfish	Boyd, 1985
10	40	35	15	55	45	Sea bass	Lemarié et al., 1998
30		10		60		Sea bream	Porter et al., 1987
19–26						Sea bream	Krom et al., 1995
30		13		57		Rainbow trout	Beveridge et al., 1991
25	30	15	70	60	0	Rainbow trout	Håkanson, 1988; Pillay, 1992
21–22	18.8	3.6–5.4	19–22	59–72	60–62	Tilapia hybrid	Siddiqui and Al-Harbi, 1999

The total N, P and TSS discharge and resulting hypothetical effluent concentrations for a number of culture systems and for cold and warm species are shown in table 9.2 (Chen *et al*., 2002). The calculations assume that all the constituents that are nor retained are uniformly distributed in the effluent, and do not differentiate between dissolved and particulate forms.

Tab. 9.2; Hypothetical effluent concentrations for different type of culture systems assuming that no treatment takes place within the systems and the constituent is uniformly distributed in the effluent. The total constituent production is used regardless of whether it is in the solid or dissolved form (Piedrahita, 2003) .

System type	Water use		Calculated effluent concentration[a]		
	kg fish/year/(l/min)[b]	l/kg fish[c]	mg N/l[d]	mg P/l[e]	mg TSS/l[f]
Cold water fish					
Single pass	1.4	375,000	0.2	0.02	1.3
Serial reuse	6	88,000	0.7	0.08	5.7
Partial reuse	50	10,500	5.7	0.67	48
Fully recirculating	160	3,300	18	2.1	152
Warm water fish					
Serial reuse	16	33,000	2.4	0.8	42
Ponds	294	1,800	44	15	780
Recirculating through wetlands	145	3,600	22	7.8	390
Fully recirculating	5,000	105	760	27	13,000

[a] Effluent concentrations calculated as: (Constituent production, (kg constituent)/(kg feed)) × (Feed conversion ratio, (kg feed)/(kg fish))/(Water use, (l/kg fish)) × (10^6 (mg constituent)/(kg constituent)). Feed conversion ratios are 1.0 and 2.0 for cold and warm water fish, respectively.

[b] After Chen et al. (2002).

[c] Calculated assuming a 365-day year.

[d] N production. For cold water fish: 0.06 kg N/kg feed, assuming a 50% protein feed and 30% N retention as fish biomass. For warm water fish: 0.04 kg N/kg feed, assuming a 35% protein feed and 30% N retention as fish biomass.

[e] P production. For cold water fish: 0.007 kg P/kg feed, assuming a 1% P feed and 30% P retention as fish biomass. For warm water fish: 0.014 kg P/kg feed, assuming a 2% P feed and 30% P retention as fish biomass.

[f] TSS production. For cold water fish: 0.5 kg TSS/kg feed (Chen et al., 1997). For warm water fish: 0.7 kg TSS/kg feed (Chen et al., 1997).

Following feed input, 2.8 and 4.5 q/pond/day in spring and summer period respectively, and metabolic activity, the outflow waters from on-growing fish pond contained higher levels of TSS, COD, NH_4-N and SRP than to that monitored in the inflow water. The spring concentrations of these environment compounds were lower than in the summer because the metabolic activity and the feed ration were lower. During the spring month, the increment of TSS, NH_4-N and SRP were about 20.91, 0.32 and 0.11 mg L^{-1} respectively, while the increments monitored in summer period were 13.77, 0.47 and 0.19 mg L^{-1} respectively. Reports from different investigations on nutritional composition of untreated aquaculture effluents resulted in wide ranges: TSS 5-50 mg L^{-1}, SRP 0.06-0.15 mg L^{-1}, and NH_4-N 0.5-1.1 mg L^{-1} (Bergheim *et al*., 1993; Ackefors and Enell, 1994; Kelly *et al*., 1994; Dumas *et al*., 1998; Bergheim and Brinker, 2003). In the present study, the level of quality variables examined in untreated aquaculture effluents were similar to those quoted in literature, except for SRP showing higher values in both seasons (0.25 and 0.42 mg L^{-1} in spring and summer periods respectively). It has been shown that the nutritional

composition of aquaculture effluents depends on various parameters concerning hydraulic management, oxygen and feeding strategy (Summerfelt *et al*., 1999; Cripps and Bergheim, 2000).

Nutrient (N and P) removal from the water flowing out from the fish farms, is essential for protecting the receiving water body by eutrophication and therefore for the potential reuse of the water (Lin *et al*., 2002). Suspended solid, mainly generated from feed residual and fecal releases, included among the pollutant to be removed from aquaculture effluent. These solids can represent an additional source of oxygen demand, ammonia, and phosphorus as they decompose, decreasing the quality of receiving waters. The removal of solids, organic matter, ammonia, nitrite and phosphorus are critical for a aquaculture systems. Solids are usually removed using physical facilities, including sand and mechanical filters. Biological processes such as submerged biofilters, rotating biological contactors and fluidized bed reactor era employed in the oxidation of organic matter, nitrification, or denitrification. These treatment methods present some disadvantage for example they produce sludge, require extra power supply, frequent maintenance and solids processing is required. Wetlands, a natural treatment systems, have been used for aquaculture wastewater treatment. Various biotic and abiotic processes regulate pollutants removal: sedimentation, filtration, adsorption, chemical reaction, vegetations uptake, nitrification, denitrification and other microbial processes (Boyd, 1998). Aquaculture wastewater is a good candidate for solids removal using wetlands because of its low solids levels and because of the organic content in the carried solids (Lin *et al*., 2002).

The nutrient concentrations in the waters flowing out from the DLS monitored in the samples indicate the ability of this system to control aquaculture effluents quality, although a seasonal variability was monitored. In spring period, the process occurred in DLS reduced the levels of TSS, COD, NH_4-N, NO_2-N, and NO_3-N. In summer period, the DLS reduced NO_2-N, NO_3-N levels, although increments of TSS, COD, NH_4-N, were monitored in the outflow water from DLS in comparison to the inflow. Such increments were attributed to the mortality of the vegetation community present in the DLS, dominated by *C. linum*. Taylor *et al*. (2001) showed that vegetative growth processes of eight "green tide" algae including *C. linum*, were significantly affected by temperature (Fig. 9.1), all species growing at temperatures within the range of 10-20 °C, while temperature above 25°C promoted rapid growth over short time periods, but a prolonged exposure caused algae tissue damage. Taylor *et al*. (2001) also demonstrated that growth of all the examined algae were significantly correlated with salinity water from 23.8 up to 27.2 g L^{-1} (70-80%

full seawater) (Fig. 9.2). Moreover all the algae tested showed a wide tolerance to salinity: from 3.4 to 34 g L^{-1} (10 to 95% full seawater) (Fig. 9.2). This result indicate that the most important limiting factor for the alga growth in DLS is temperature, since the values measured in the summer period (27.6°C ± 2.3) were adverse for the growth of *C. linum*, that is the dominant species present in DLS.

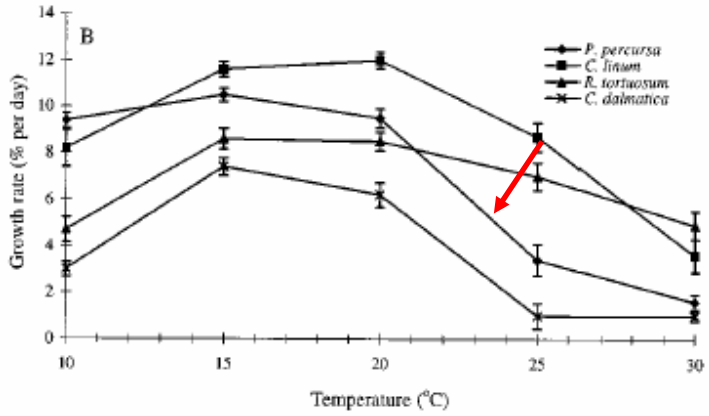


Fig. 9.1; Growth of *C. linum* and other "green tide" algae under a range of temperature conditions (Error bars represent SE, *n=3*); the arrow indicate the *C. linum* growth (Taylor *et al*., 2001).

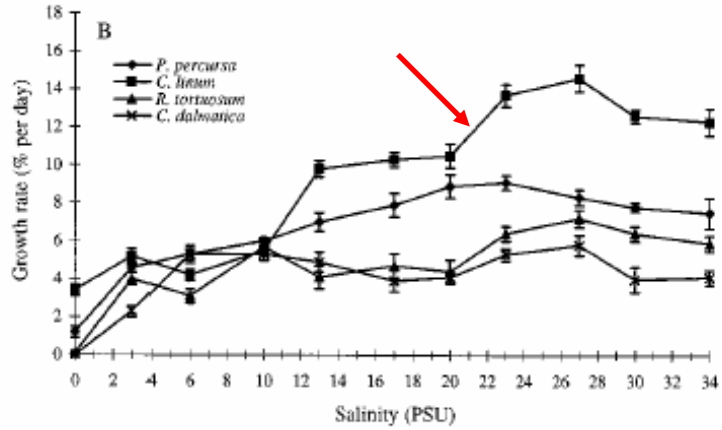


Fig. 9.2; Growth of *C. linum* and other "green tide" algae in a range of salinity levels (Error bars represent SE, *n=3*); the arrow indicate the *C. linum* growth (Taylor *et al*., 2001).

Panella *et al*. (1999) adopted a wetland-pond system, operating at hydraulic retention time between 2.8 and 3.0 day, to process and recycle the outflow waters from intensive aquaculture systems. They reported the purifying performance of the wetland-pond system

with removal percentages of 33% (0.69 g m^{-2} d^{-1}) for BOD_5, 14% (0.46 g m^{-2} d^{-1}) for suspended organic solids, 41% (0.015 g m^{-2} d^{-1}) for NH_4-N, 27% (0.419 g m^{-2} d^{-1}) for NO_3-N, and 58% (0.015 g m^{-2} d^{-1}) for PO_4-P. Additionally, Lin *et al*. (2005) reported the reduction efficiency of pollutant in FWS wetland operating at hydraulic retention time between 2.6 h in spring period and 1.6 h in summer period. They reported a removal percentage of 25.8% for TSS, 24.0% for NH_4-N and negative values for SRP in spring season while reported 20.3% for TSS, 27.7% for NH_4-N and 1.9% for SRP in summer season. At the outflow of the discharge lagoon system, operating at hydraulic retention time of 7.2 h and 6.0 h in spring and summer respectively, were revealed removal rates as 12.72% (16.6 g m^{-2} d^{-1}) for TSS and 39.02% (0.40 g m^{-2} d^{-1}) for NH_4-N in spring period, and 19.05% (0.24 g m^{-2} d^{-1}) for SRP in summer period. The TSS, COD, NH_4-N concentration reduction in summer period were resulted negative.

Also, the comparison of the *K* values defined in this study with those from literature showed, for some cases, similar *K* values (Tab. 7.6). These differences in the performance may be due to the difference in the diverse operating conditions (including hydraulic loading rate and influent concentration) or pollutant loading rates (defined as hydraulic loading rate multiplied by influent concentrations).

The removal efficiency for various environmental quality parameters from aquaculture systems via constructed wetlands is correlated to the hydraulic residence times (HRT) (Schulz *et al.*, 2003). This aspect is partially confirmed by the nutrient concentrations in the wetland outlets monitored in the experimental trials conducted in three discharge lagoon ponds with different water flow (0.3, 0.2 and 0.1 m^3/sec in basin I, E and L respectively). The higher mean removal of TSS was monitored in the basin I (about 16%) with HRT like to 0.35 day while in the other two basins were monitored an increment of TSS concentrations. Similar patterns were monitored for the fraction of settleable suspended solids. All these increments were attributed to the mortality of the vegetation community present in the DLS (0.87±0.08, 0.73±0.06 and 1.81±0.42 kg m^{-2} in the basin I, E and L respectively). The un-settleable fraction of TSS increased in the all wetland outlets respect to the inflow levels, the increment values being significant only in the basin L. This fraction was represented by phytoplankton communities, confirmed by an increment of chlorophyll (1.4-24.5 mg m^{-3}) and reduction of transparency (0-0.23 m), that were marked in the outflow basin with lower HRT.

After sedimentation and filtration of suspended solids, a number of processes occurred in constructed wetland. Kadlec and Knight (1996) identified microbial respiration of carbon

components to CO_2 in the aerobic zones of the wetland to be a major processes of the carbon cycle. In anaerobic zones, nitrate, iron, and sulphate reduction and methanogenesis occurred under evaporation of CO_2 or CH_4^+ (methanogenesis), respectively. In the present study, carried out in the diurnal time, the aerobic conditions were found in the effluents of all wetlands (dissolved oxygen range: 7.44-12.96 mg L^{-1}). Therefore, aerobic microbial decomposition of organic matter was promoted. COD removal was monitored in the basin I and L and because of higher effluent interactions with microbial soil flora, these rate were higher in the basin L (about 36%) (although not significantly). Nevertheless, in the basin E the COD outflow level were higher than to that monitored in the inflow site. Higher internal growth and build-up of biomass in constructed wetlands could decrease COD removal efficiency (Shultz *et al*., 2003).

Nitrogen elimination in wetland begins with microbial ammonification processes. Nitrification or oxidation of ammonia (ammonificated and excreta ammonia) to nitrate, as an oxygen-demanding processes, occur in two steps involving microbial species such as *Nitrosomonas* and *Nitrobacter*. The decrease in quantity of inflowing NH_4-N can be attributed to high microbial ammonification and nitrification within each wetland basin, promoted by dissolved oxygen. Removal of nitrogen in this study was recorder in the E and L basin (although not significantly) and was highest in the basin L (36%) at HRT of 1.06 day, indicating that HRT directly influences removal processes. This was confirmed by significantly NO_2-N removal rate monitored in basin L (55%), and higher percentage of NO_3-N reduction. The reduction mechanisms for phosphorus in wetland with free water surface include sedimentation, precipitation and adsorption reaction through ligand exchange of inorganic dissolved phosphorous in aluminium-, iron-, manganese hydroxides/oxides, calcium and clay minerals, and biochemical nutrient removal by plants. The removal of phosphorus in constructed wetlands generally involves rapid adsorption processes and slower chemical reactions, leading to the formation of a solid phosphate phase. Sorption at iron and aluminium compounds of the soil seems to be the most important processes under presented aerobic conditions (Shultz *et al.*, 2003). In this study, the SRP removal was similar in the I and E wetland basins with values to about 14% (significantive), 16%, while was lower in the 9.6% L basin and these removal processes were promoted by mean oxygen levels of 7.44-12.96 mg L^{-1} .

Among excreted compounds, ammonia is the main product of nitrogen excretion in most teleost fish (Mommsen and Walsh, 1992). Whereas ammonia concentration is often the limiting water quality parameter in intensive aquaculture systems, physiological studies

concerning ammonia excretion have been conducted in the laboratory and need to be validated in aquacultural condition (Thomas and Piedrahita, 1998). Biological processes transforming ammonia also occur and are often system-specific. A conceptual model for nitrogen transformations and removal in intensive system is shown in figure 9.3. Nitrogen added to the system as feed was either transformed into fish nitrogen, or uneaten or not digested, or excreta as dissolved nitrogen (mainly urea and ammonia). Feces and uneaten food were removed by sedimentation and could be remineralized to ammonia or stored in the sediments. Ammonia could be transformed into nitrite and nitrate by nitrification or simply disappeared through a black box (loss). The ammonia loss flux was an aggregation of processes of primary production and gas loss (volatilization or denitrification). Due to shade covering over the fish ponds, ammonia loss processes may have only occurred in the lagoon or the reservoir pond. Lefebvre *et al*. (2001) showed that most of the particulate nitrogen produced by fish was discharged from the fish ponds into the lagoon, in which most of the particulate matter (> 90%) produced in the fish pond settled (Hussenot *et al*., 1998). As a consequence, significant ammonia releases from the sediment occurred in the lagoon system. Finally, the concentrations of various forms of nitrogen depended on the rate of water renewal in each compartment.

A mass balance approach is a useful method to predict fish nitrogen excretion and transformation (Lefebvre, 2001). The Lefebvre's models was applied into the discharge lagoon ponds for evaluated the total ammonia nitrogen mass balance of the lagoon system itself (gN h^{-1}). The values of N-Ammonia forecasted by the model was 0.11 g m^{-3} in spring period and this values was similar to the spring analytical data (0.25 ± 0.06 g m^{-3} in basin E and 0.16 ± 0.10 g m^{-3} in basin F) and was included in the minimum and maximum range monitored in the F basin (0.06-0.43 g m^{-3}). Nevertheless the value of N-Ammonia forecasted by the model in the summer period (0.14 g m^{-3}) resulted underestimate when compared to the summer data (0.82 ± 0.13 g m^{-3} in E basin and 0.67 ± 0.14 g m^{-3} in F basin) and was out off the minimum and maximum range. Because of the conditions monitored in the discharge lagoon systems that limit the growth of the macroalgae present in these basins, it was supposed an improvement of this model represented by algae decomposition rate obtained from experimental trials in partial anoxic condition. The model was so improved with an additional component represented by a new equation with algae biomass as an independent variable. With the max algae density monitored in the farm lagoon ponds (1.81 kg m^{-2} ww), the nitrogen ammonia level obtained after

application of new equation (0.39 g m^{-3}) was a better evaluation of the summer sampling data and it was included in the minimum and maximum range of the F basin's exit. The modified Lefebrve's algorithm allows a good forecast tool for N-ammonia levels at the discharge lagoon ponds but, despite the modify that has been applied, the model needs further specific site calibrations. The mainly NH_4-N sources resulted the inflow ammonia concentrations and nitrification processes while the mainly sinks were the processes of primary production. The ammonia flux from the sediment predicted by the model (11.4 g NH_4-N h^{-1}) was similar to NH_4-N release from the sediment monitored in the experimental trials (9.9 gNH_4-N/h).

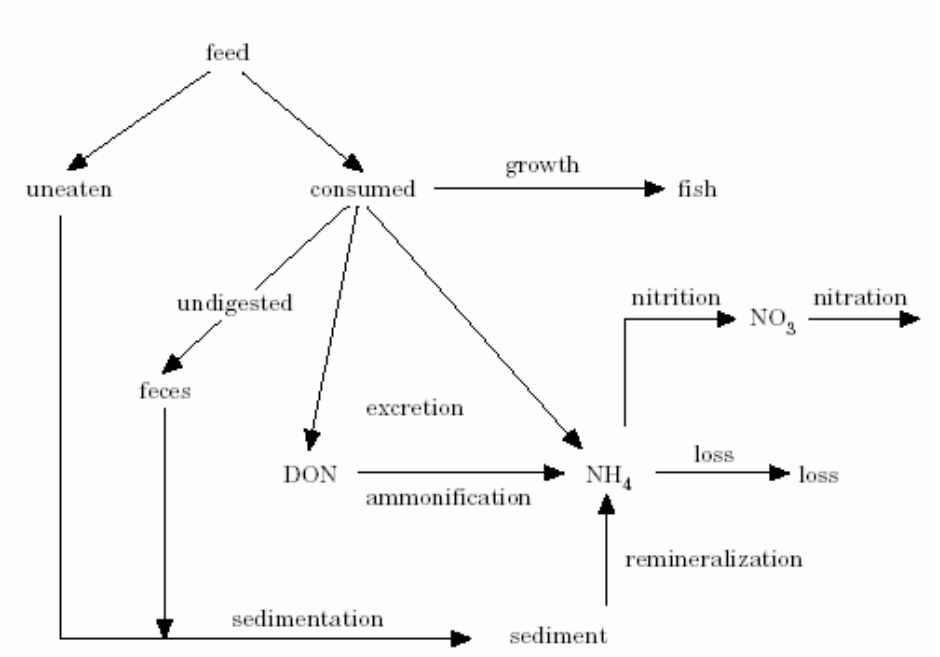


Figure 9.3; Conceptual model of the nitrogen fluxes in a land-based mariculture ecosystem (Lefebvre, 2001).

Most treatment processes (i.e. phyto-depuration in wetland system), don't considered the processes occurred in the sediment or in the column and resulted inadequate for the removal of aquaculture wastes, including ammonia. The utilization of this algorithm is a strong tool for the N-ammonia control in land based aquaculture, as it may be utilized to design focused interventions, either in the water column for improved the ammonia loss by primary production or in the sediments for improved the nitrification processes and ammonification of organic nitrogen forms.

For improving the ammonia loss by primary production some experimental trials were conducted by use of a commercial algae *Gracilaria verrucosa*. A number of agarophytes, including *G. verrucosa*, have been used as marine vegetables or traditional medicinal herbs for over 1000 years (Chen Jia Xin, 1989). Where many seaweed species have traditionally been consumed for many centuries in the Far East, including *G. verrucosa*, are authorized for human feeding in some Europe country, they remain a neglected vegetable, in spite of their high protein content (up to 30% dry weight) (Mebeau and Fleurence, 1993); this value is comparable to that of legumes, such as soya bean. However, these seaweeds have became an industrially important raw material for processing agar some decades ago. *G. verrucosa* is rich in galactans, especially agar, which are mainly used for they technological properties in food agriculture, cosmetic and pharmacy industries (Lahaye and Kaeffer, 1997). The most widespread *G. verrucosa* is the attached form of algae growing on a rocky substrate. However, there is an unattached form of the same species found in lagoons. The unattached form contains much more agar and twice as much lipids as the attached one (Khotimchenko and Levchenko, 1997). *G. verrucosa* is abundant in the lagoon areas which are nutritionally rich and have strong water flows and sandy and muddy bottoms (Ilknur, 2004). At the present Gracilaria is one of the most important raw materials for producing agar and it is also easier to farm. Gracilaria is a group of euryhaline seaweeds; under natural conditions the specific salinity in which the plant can grow out ranges from 5.0-38.0 ‰. Experiments and field surveys have shown that the optimum specific salinity range is 11-30 ‰ (Chen Jia Xin, 1989). Gracilaria is also an eurythermal plant which can grow at 5 to 30°C. Optimum temperature varies with species; for example, the optimum temperature of *G. verrucosa* is 15-25°C (Chen Jia Xin, 1989). The mean temperature (27.61±2.34°C) and salinity (37.18±1.94 g L^{-1}) monitored in the discharge lagoon system in summer period were similar to the optimum salinity and temperature range of this commercial algae. In this study was showed also the capacity of autochthonous *G. verrucosa* to removed NH_4-N with a rate as 162 mgNH_4-N/kg algae (d.w.)/d in light condition and this capacity could be used for improving the ammonia reduction in the discharge lagoon system. For all these reasons, application of Gracilaria could be supposed by controlled culture in each lagoon basin, with a biomass removal for the commercial uses. There are three mainly culture methods of this algae: mud flat, floating culture and pond culture. The floating culture are illustrated in figure 9.4 and probably is the better methods to use for an hypothetical culture because allow to use the total surface of the each discharge lagoon basin. Considering the NH_4-N removal rate by

G. verrucosa (162 mgNH_4-N/kg algae d.w./d), the range of the density culture with floating method (3000-18000 kg algae d.w. per ha) and the surface of each basin (about 1 ha), the hypothetical removal efficiency ranges from 0.48 to 2.90 kg NH_4-N d^{-1}. These removal efficiency applied to the NH_4-N levels in each lagoon basin (about 12.5 kg NH_4-N d^{-1}) could lead an N-ammonia reduction of 4-23%.

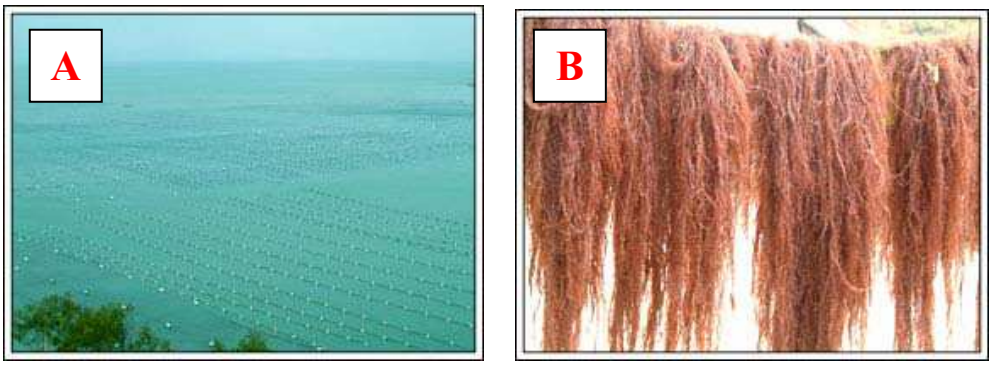


Fig. 9.4; Floating culture of Gracilaria in China (A) and his grow on "seeding rope" (B) (Internet source).

As well as the use of *G. verucosa* for increase the NH_4-N uptake in the water column, the applications of bio-promoters strongly improves the natural nitrification processes both in the water column and in the sediment. These products are recently applied in the treatment of eutrophication lakes or lagoons. In this study were tested in experimental pilots, the abilities of a commercial bacterial-enzyme bio-promoters (Eurovix® Srl) to reduce the NH_4-N and SRP levels in aquaculture wastewater both in aerobic and partially anoxic condition. For these experimental trials were used water coming from a aquaculture closed recirculate system and sediment collected from discharge lagoon basin. In aerobic condition the application of bio-promoter determined higher percentage of NH_4-N removal (max value 91.6±2.26 %) and higher removal rate (max value 1.64±0.23 mg NH_4-N/h) than those monitored in the control systems. The percentage of NH_4-N removal increased while NH_4-N removal rate decreased during the experimental trial because of the N-ammonia concentration reduction. The presence of bacterial (*Nitrosomonas* and *Nitrobacter* spp) and enzyme improved the oxidation processes with the consequence to oxidation of ammonia. These experimental trial have demonstrated the improvement of nitrification process by the application of bio-promoter in aerobic condition respect to the natural efficiency in the NH_4-N removal.

In partially anoxic condition were observed a particular NH_4-N pattern; in fact the NH_4-N concentrations in the first 48 hours increased both in bio-promoter trials (22.13 mg NH_4-N L^{-1}) and control (9.73 mg NH_4-N L^{-1}) due to reduction processes in anoxic condition, but after this time the NH_4-N levels dropped in both system, with higher efficiency in the bio-promoter trials (about 72%). Probably, this reduction was possible thanks to the anaerobic ammonium oxidation (anammox) process.

As well as the ability to NH_4-N remove by bio-promoter mixture, were also evaluated the consequences of this application on soluble reactive phosphorus concentration. In the SRP experimental trials with aerobic conditions, the added of bio-promoter mixture promoted higher percentage of SRP removal rate respect to the control trials. In the bio-promoter trial, was monitored, after 48 hours, the highest percentage of SRP reduction (50.8±15.0 %) while highest SRP removal rate was monitored after 24 hours (0.86±0.19 mg SRP/h). In partially anoxic condition the SRP concentration increased increased both in bio-promoter trials (22.93 mg NH_4-N L^{-1}) and control (12.0 mg NH_4-N L^{-1}) but after added of aeration the SRP levels dropped in both system, with higher efficiency in the bio-promoter trials (about 80%). Traditional methods for phosphorus removal from waste water are based on chemical precipitation of phosphorus with mainly iron and aluminium salts. However, due to the technical and economical constraints of these chemical processes, the use of alternative, biological treatment methods is steadily increasing. Enhanced biological phosphorus removal (EPBR) is the most common biological phosphorus-removal method. The SRP pattern in both anoxic and aerobic experimental condition was proved of the possible presence of these processes. In this way, the application of the bacterial-enzyme bio-promoter in the wetland system is suggested in aerobic condition ($\geq$ 7.0 mg O_2 L^{-1}) for avoid the potential release of phosphorus forms or in a controlled system by aerobic-anaerobic cycles, with sludge removal.

Water colour has, throughout history, been perhaps as much a phycological as a physiological sensation. There is the inescapable tendency for an observer to associate a personal sense of aestheticism with water colour and thereby ascribe perceived water quality criteria to a personal sensation of water colour. Strong reactions to aquatic colour have created both justifiable and unjustifiable emotions in people for centuries. Perceived aquatic colour, in addition to being a function of the individual response characteristics of each observer's eyes, is also a function of the position of the sun, the location and orientation of the observer, the state of the water's surface, and the atmospheric conditions under which the viewing is performed. The perceived colour of the same water body at the

same time could quite easily become moot. It was, therefore, early recognized that means of recording water colour were required that would retain the physiological nature while removing the subjective nature of direct visible observation; chromaticity provides such a means. The observed colour of a natural water body is a direct consequence of the interaction of the incident downwelling solar and sky irradiances with whatever optically responsive organic and inorganic matter comprise the water column at the instance of observation (i.e. Chlorophyll, Suspended matter and dissolved organic carbon). These materials absorb and scatter radiation in a spectrally selective manner. Thus a measure of natural aquatic colour is logically provided by the volume reflectance spectrum within the range of wavelengths to which the human eye is sensitive (Bukata *et al*., 1995) .

From the perspective of the human observer, colour is the result of the interplay between the light spectrum reaching the eye and the spectral response of that eye. Colour perception is most developed in the central region of the human retina. As is incorporated within the Young-Helmholtz theory, this region of the retina (for normal vision) is tri-chromatic (i.e. responsive to the three spectral regions red, green, and blue). Therefore, any perceived colour can be created by appropriately proportioning red, green, and blue components forms the basis of chromaticity analyses. A measured upwelling irradiance spectrum E(λ) may, therefore, be related to a perception of visual colour through chromaticity analyses that integrate the sensitivity of the human eye with the irradiance spectrum impinging upon it. Such an integration results in tristimulus values X', Y', and Z', from which the chromaticity coordinates X (red), Y(green) and Z (blue) may be determined.

Many studies (Bukata *et al*., 1995; Gallie 1993) have demonstrated the possibility to measure levels of chlorophyll, suspended solids and transparency in the water bodies by chromaticity analysis with use of multispectral scanning spectrometer (MSS) mounted aboard the satellites. Nevertheless, the application of this method in aquaculture is limited. Results from this preliminary study highlighted certain aspects regarding the possibility of extracting also for the relatively small basins of land based farms, useful information about water quality from remotes sensors essentially based on spectral and chromaticity analysis. The qualitative images obtained show a spatial variability of the chlorophyll, suspended solids and transparency both in the marsh land and in the fish farm.

The comparison of the levels of total suspended solids registered in different sites of the fish farm, during field measurement with the results of the elaboration of chromaticity analysis show good correlation between the trends observed experimentally with those resulting from the applied algorithms, despite the fact that these elaborations were made

using a single image and dated 03/09/04, about a month after the sampling. More, the spatial TSS variability monitored in the discharge lagoon system was confirmed to the qualitative image obtained after chromaticity analysis. The potential of remote sensing data for providing further information to assess water quality is high and this data are particular useful for understanding important aspect of the functioning and dynamic of water variables among spatial components between and among aquaculture systems and the surrounding basins.

This preliminary investigation using satellite remote sensing to assess water quality in land based aquaculture and surrounding basins demonstrated the possibility to obtain simply qualitative and relatively semi-quantitative data, even in the absence of the reference data. This method can also be seen as a relatively simple system for successive temporal observations on a specific site providing complementary information to the conventional methods and can also identify areas where change are occurring and where more detailed information must be gathered. However, for quantitative analysis purpose, in situ measurements remain indispensable, either for absolute atmospheric corrections or for ecological calibration of the remote sensing data. With further refinement in analytical techniques and calibration of models, the use of optical information data from satellite sensors could be very useful for assessing environmental impact of aquaculture and developing better simulations over a variety of conditions over time and space scale. Satellite remote sensing could be especially appropriate for initial water quality mapping and continued monitoring program of land based aquaculture, also to prevent complex negative phenomenon that may be scattered in the surrounding basins.

10. CONCLUSIONS:

This study allowed to draw some important considerations about the relationships between coastal aquaculture and the surrounding environment. In this contest the dynamics of the inflow and outflow water bio-depurations by lagoon systems were analysed in a coastal fish-farm and a global monitoring system for assess the water quality of coastal aquaculture was proposed. Three principal conclusions were drew:

- The seasonally quality of inflow waters in coastal aquaculture can be controlled by wetland head lagoon system. This system can actually control the most important water quality parameters, such as temperature, salinity, pH, TSS, COD, nitrogen and phosphorus, that may influence aquaculture production. The use of wetland unit requires no mechanical facilities and energy input. As well, this study showed that wetland system reduced most of the deoxygenating and eutrophicating matter contained in the outflow waters. In spite of this positive result it has to be noted that during the summer season high algae mortality can reduce the performance of the discharge lagoon system. This lower efficiency could be improved by controlling the biomass of algae by vegetation harvesting.

- The improvement of effluent treatment by constructed wetland is based on the optimization of abiotic and biotic processes by water flow controls and biological processes increase. Despite the observed alga mortality, the study showed that the removal efficiency of some environmental quality parameters is correlated to the hydraulic retention time (HRT). In fact, lower water inflow and consequently higher HRT, improved the removal efficiency of COD, NH_4-N and NO_2-N. The applications of nitrogen ammonia mass balance (Lefebvre) and its improvement, permitted to program focused intervention both in the water column and in the sediment. The utilization of the new algorithm is a strong tool for the N-ammonia control in aquaculture system studied. The NH_4-N removal efficiency from aquaculture wastewater by commercial algae *G. verrucosa* and the environmental conditions recorded in the discharge lagoon systems, showed the possibility to carry out a controlled culture in each discharge lagoon basin by floating methods with an increment of N-ammonia reduction from wastewater like to 4-23%. A

further improvement of the wetland performance could be achieved by the increasing of nitrification processes with the use of commercial bacterial enzyme-biopromoter. The application of these product strongly improves the natural processes both in the water and in the sediment. The commercial bacteria-enzymel bio-promoter (Eurovix® Srl) utilized, reduced the NH_4-N and SRP concentrations from aquaculture wastewater with higher efficiency (51.6-91.6% for NH_4-N and 19.4-49.8% for SRP) than natural conditions (23.2-33.5% for NH_4-N and 28.8-29.8% for SRP). Some experimental trials conducted in aerobic and partially anaerobic conditions suggested the application of this mixture in aerobic condition to avoid the potential release of ammonia nitrogen and phosphorus forms.

- The relationships between coastal aquaculture and surrounding environment are important for the management of the aquaculture activity and for the protection of the biodiversity in the receiving basin. The potential of remote sensing data providing further information to assess water quality is high and these data are particular useful to understanding important aspect of the functioning and dynamics of water variables. The preliminary investigation by the use of satellite remote sensing to assess water quality in land based aquaculture and surrounding basins demonstrated the possibility to obtain qualitative information even in the absence of reference data. However, when quantitative data are made, an in situ data set remains indispensable for ecological calibration of the remote sensing data. With further refinement in analytical techniques and calibration of models, the use of optical information data from satellite sensors could be very useful for assessing environmental impact of aquaculture and developing better simulations over a variety of conditions over time and space scale. Satellite remote sensing could be especially appropriate for prevent complex negative phenomenon that may be scattered in the surrounding basins.

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