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# THE UTILISATION OF ACID SULPHATE SOILS FOR SHRIMP (Penaeus monodon) CULTURE ON THE WEST COAST OF SRI LANKA

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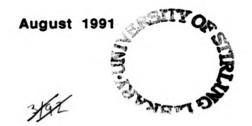
A thesis presented for the degree of Doctor of Philosophy to the University of Stirling

By

J.M.P.K. Jayasinghe, B.Sc., M.Phil.

Institute of Aquaculture University of Stirling Stirling, Scotland.

U.K.



### DECLARATION

I declare that this doctoral thesis has been composed by myself and that it embodies the results of my own research. It has neither been accepted nor is being submitted for any other degree. Where appropriate, all sources of information have been duly acknowledged.

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### DEDICATION

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# THIS THESIS IS MOST AFFECTIONATELY DEDICATED TO MY LOVING PARENTS

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### Abstract

Continued pressure on land resources for shrimp culture has resulted in many shrimp culture developments on acid sulphate soils in South East Asia which are marginal or difficult to manage. The present study included a survey to identify and classify different acid sulphate and potential acid sulphate soils in the areas earmarked for shrimp culture on the West Coast of Sri Lanka. This was supported by on farm investigations into the behavior and kinetics of metals in culture ponds, time series studies on water and soil quality over a culture cycle, plus morphological and histopathological changes in cultured shrimps and monitoring of calcium and magnesium contents in selected tissues.

Although the general environment and water quality criteria in the study areas provided promising conditions for culture of *Penaeus monodon*, survival (35.1%) and production (1240 kg/ha/ crop) were found to be significantly lower on acid sulphate soils than that on neutral soils.

The stability of metals, particularly that of iron, which is governed by the redox potential-pH relationships of the pond environment, appears to play a significant role in the processes that increase the potential stress to the shrimps cultured in an acid sulphate environment.

Under acid sulphate conditions, shrimps showed elevated levels of iron (119.9  $\mu$ g/g dry wt) and manganese (38.4  $\mu$ g/g dry wt) in their muscles and unusually high levels of these heavy metals were recorded in gills (1588 and 93.2  $\mu$ g/g dry wt of iron and manganese respectively) and carapace (778 and 34  $\mu$ g/g dry wt of iron and manganese respectively) during the latter part of the culture period. Calcium levels in the carapace were relatively low (136 to 260 mg/ g dry wt) throughout the culture period and showed a negative correlation with culture time (r = -0.950; p = .001).

Accumulation of hydrated oxides of iron in gills as a result of oxidation of pyrites was confirmed by the Eh-pH relationships monitored in the pond environment and by histochemical, SEM and TEM studies. These insoluble oxides appear to be primarily responsible for gill colour changes and concomitant histological changes in gill, heart and hepatopancreatic tissues. They are clearly detrimental to the normal gill functions of cultured shrimps.

Statistically significant correlations were observed between iron in shrimp gills and muscles with iron in the surface sediments (p = .004 and .010 respectively) and the culture period (p = .013 and .010 respectively). Manganese in gills and carapace of cultured shrimps was correlated to the iron concentration in those tissues (p = .016 and .002 respectively).

Traditional management strategies (drying the pond bottom, liming and artificial aeration) although creating promising conditions for shrimp culture under favourable soil conditions, create adverse conditions by favouring the formation of iron (III) oxides in ponds on acid sulphate soils.

Detailed studies on mapping, classification and identification of profile forms in coastal soils provided vital information necessary for land use planning and development of these sediments in shrimp culture. Development of soil classes; sulphidic sand, unripe sulphidic peat, unripe sulphidic muck, acid sulphate muck, raw acid sulphate muck and raw acid sulphate clay have the most serious implications on cultured shrimps and the environment. Ripe clay with sulphidic subsoil, ripe clay with raw acid sulphate sub-soil, half ripe clay with acid sulphate subsoil appear to be the least harmful soil classes for shrimp culture among the sediment types investigated.

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# CHAPTER 1

### GENERAL INTRODUCTION.

### 1.1. Definition of acid sulphate soils.

Acid sulphate soils develop as a result of the drainage of parent material rich in pyrite (Dent, 1986). These soils are characterised by a pH lower than 4 in water (Bloomfield and Coulter, 1973). Brinkman and Pons (1973) describe acid sulphate soils as those soils that have somewhere within a 50 cm depth a pH less than 3.5 or 4. Actual acid sulphate soils are commonly referred to in the literature as cat clays and have pale yellow mottles and streaks of jarosite (KFe<sub>3</sub>(SO<sub>4</sub>)<sub>2</sub>(OH)<sub>6</sub>). Acid sulphate soils occur in many coastal areas under brackish to saline conditions in all climatic zones of the world (Pons, 1973; Breemen, 1976). The total area under acid sulphate condition has been estimated at about 15 million ha (Pons, 1973).

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Acid sulphate soils are closely associated with oxidising pyritic material and a large number of other sulphate minerals such as coguimbite  $(Fe_2(SO_3)_39H_2O)$ , natrojarosite  $(NaFe_3(SO_4)_2(OH)_6)$ , Alunite  $(KAl_3(SO_4)_2(OH)_6)$ , and also ferric hydroxides and ferric sulphates (Breeman, 1973; Poernomo, 1983). The pH of these soils can fall to a value as low as 2.5, directly or indirectly related to the release of sulphuric acids resulting from the oxidation of pyrites, or rarely from the oxidation of other sulphur compounds (Brinkman and Pons, 1973; Hechanova, 1983; Poernomo, 1983). Potential acid sulphates are soils that are poorly drained and highly pyritic with a nearly neutral or slightly acidic reaction in the field, but which contain higher concentrations of pyrites that will become severely acidic if pyrite is oxidised after draining (Hechanova, 1983; Poernomo, 1983). In recent classification systems the criterion used to classify potential acidity is the fall of pH to a value of less than 4 during three months of moist incubation (Dent, 1986).

### 1.2. Distribution of acid sulphate soils.

About 70% of the world's acid sulphate deposits are found in monsoonal humid tropics where the tidal areas are covered with dense mangrove forests. Such tidal marshes are often sparsely vegetated under temperate climates (Breemen, 1976; Singh, 1985). An abundance of organic matter from mangroves, saturation with water and the ubiquity of sulphates from brackish or sea water leads to anaerobic sulphate reduction. If iron is available in sediments, the resulting sulphides produced are fixed as pyrites (Breemen, 1976; Dent, 1986).

There are also acid sulphate soils documented from temperate regions although the distribution is not very extensive. Reclaimed and drained areas with sulphidic sediments have been recorded from the Netherlands (Pons, 1973), Sweden and Finland (Metson <u>et al.</u>, 1977). Acid sulphate areas also have been reported from the United States of America (Lynn and Whittig, 1966); Canada (Clark <u>et al.</u>, 1961) and from the United Kingdom (Bloomfield

and Coulter, 1973). Murakami (1969) reports acid sulphate soils in reclaimed land along the coastal areas of Japan while Krym (1982) records the occurrence of acid sulphate soils associated with peaty gley soils in the Soviet Union.

One third of the world's acid sulphate soil sediments are distributed in South East Asia and South Asia (Singh, 1985). An estimated area of about 11 million ha of acid sulphate and potential acid sulphate soils are in South East Asia, South Asia, Africa and in Latin America where a tropical climate prevails (Murakami, 1969; Andriess <u>et al.</u>, 1973; Bloomfield and Coulter, 1973).

Neue and Singh (1984); Singh (1985) and FAO/ UNESCO (1974), have listed the distribution of acid sulphate soils in South and South East Asia where the total area covered by acid sulphate soils has been calculated as 5.§ million ha. Indonesia and Vietnam record the largest extent of acid sulphate soils in the South, South East and East Asian regions (Table 1.1). Although there are records of the occurrence of acid sulphate and potential acid sulphate soils, and rough aerial estimates in Sri Lanka (NARA, 1986), so far no attempt has been made to estimate their exact distribution and extent.

1.3. Aquaculture in acid sulphate soil areas.

Brackish water aquaculture is a rapidly expanding form of aquaculture within South and South East Asia, with an annual growth in production of around

Table. 1.1. Distribution of acid sulphate soils in South and South East Asia.

Country	Extent (ha)	Source
Banoladesh	700,000	Neue and Singh, 1984; Singh, 1985
Burma	180,000	FAO / UNESCO, 1974
India	390,000	Singh, 1985
Indonesia	2.000.000	Singh, 1985
Cambodia	200.000	FAO / UNESCO, 1974
Malavsia	160.000	Neue and Singh, 1984; Singh, 1985
Philippines	527,000	Singh, 1985
Sri Lanka	16.000	NARA, 1988
Thailand	778.000	Lin, 1986
Vietnam	1,000,000	Singh, 1985

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70% during years 1975 to 1985, and it is also spreading widely in Latin America, and some parts of Africa. In 1989 a total of 1.09 million ha were estimated to be under shrimp culture with an approximate annual production of 565,000 mt. The main species behind this expansion are shrimps of the genus *Penaeus*. With increasing international markets for shrimps, the perceived high profitability of shrimp farming and growing markets for other brackish water finfish species, more and more coastal areas are likely to be converted for aquaculture use, but without proper soil surveys, land evaluations and environmental impact assessments. A considerable proportion of the coastal swamps in the tropics contain acid sulphate or potentially acid sulphate soils. The location of aquaculture facilities on acid sulphate soils is therefore inevitable.

Penaeid shrimps, milkfish, mullets and *Tilapia* spp. are the most important cultured species in the coastal areas of South and South East Asia. *Penaeus monodon* (tiger shrimp) dominates the world's cultured shrimps by species contributing 46% of production (Fig. 1.1). Other dominant species include western white shrimp (*P.vannamei*) and Chinese white shrimp (*P. chinensis*) which contribute 14% and 21% respectively. All the other species represent only 19 % of the total (World Shrimp Farming, 1990).

The greatest areas under shrimp aquaculture in the Eastern hemisphere are in Indonesia, China, Vietnam and Bangladesh; this is followed by Thailand, the Philippines and India as indicated in World Shrimp Farming (1990). However

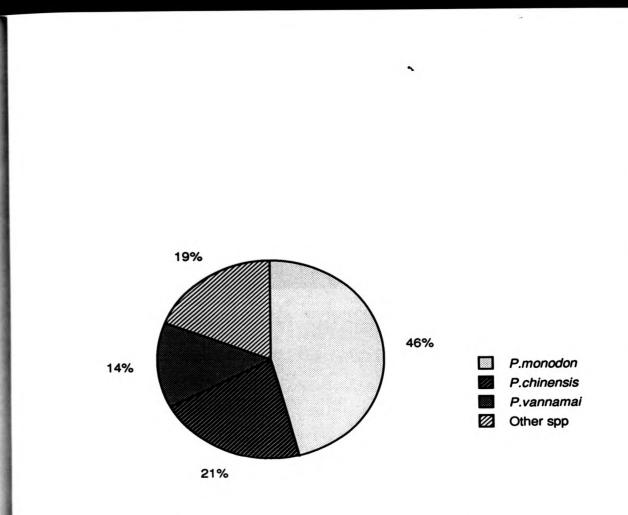


Fig. 1.1. World's cultured shrimp production by species (Source :World Shrimp Farming, 1990) rate of production (kg of shrimp produced per ha) varies very widely among different countries, from less than 200 kg to several thousand kg/ha (Table 1.2).

Global production of cultured shrimps has grown very rapidly during recent years from around 90,000 mt in 1980 to an estimated production of 565,000 mt in 1989 representing 26% of the global supply of marine shrimps (Phillips et al., 1990). In 1990 the world's shrimp farmers produced a record crop of 663,000 mt, up by 17% from the recorded harvest in 1989 (World Shrimp Farming, 1990). Shrimp culture production during the period 1985 to 1989 showed a 146 % increase while fin fish and mollusc production has increased by only 41% and 38% respectively during the same period (FAO, 1989). The recent predictions for farmed shrimp production in Asia by the year 2000 will be between 800,000 and 900,000 mt contributing around 50% to the global shrimp production (Phillips, et al., 1990). On the other hand, with the increased profits generated in the shrimp culture industry and the availability of more complete technology for production of shrimps, more and more farms are switching from brackish water fish culture to shrimp culture, or at least are incorporating shrimps in fish ponds in poly culture. About 98% of the recent expansion in brackish water aquaculture (850 to 1100 ha) in Sri Lanka is for shrimps (NARA, 1988).

Table. 1.2. Summary of the shrimp farming industry in the eastern hemisphere (World Shrimp Farming, 1990).

Country	% Production	Total production (mt)	ha in production	kg/ ha	No. of farms
China	28	150,000	150,000	1,000	1,000
ndonesia	22.5	120.000	300,000	400	3,000
Thailand	20.5	110.000	60,000	1,800	3,000
ndia	9	32.000	60,000	533	2,000
Philinnines	22	30.000	50.000	600	3,000
Vietnem	55	30.000	160.000	188	1,000
Taiwan	5	30.000	8.000	3,750	2,000
Ranladach	45	25,000	100.000	250	1,000
lanan -	- F	3.500	500	2,000	165
Other	-	5,000	5,000	1,000	400

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### 1.4. Formation of coastal acid sulphate soils.

Acid sulphate soils have been defined as soils with one or more horizons containing acid sulphate material. They are rich in various iron, aluminium and manganese compounds (Brinkman and Pons, 1973). Acid sulphate soils are developed from sediments that have a high content of sulphides, which have been fixed and accumulated by the reduction of sea water (Kevie, 1973). These sediments occur in narrow coastal zones on swampy land which is regularly flooded by brackish water; they can have serious adverse affects on aquaculture projects in such areas.

1.4.1. Formation of pyrites and potential acid sulphate soils.

Dent (1986) summarizes the essential chemical processes of acid sulphate soil formation as firstly the formation of pyrite in a water logged environment and the subsequent oxidation of this pyrite following natural or artificial drainage. According to Beers (1962); Pons (1973); Bloomfield and Coulter (1973) common factors associated with pyrite formation are: a source of sulphates, a source of iron, metabolized organic mater, sulphur reducing bacteria, an anaerobic environment and time.

The processes leading to pyrite formation involves bacterial reduction of sulphates to sulphides, partial oxidation of sulphide to elemental sulphur and interaction between ferrous or ferric ions with sulphides and elemental sulphur (Rickard, 1973; Breeman, 1976). Sulphur reducing bacteria such as Desulfovibrio desulfuricans and Desulfoto maculatus are mainly involved in

the process of pyrite formation (Metson <u>et al.</u>, 1977). These bacteria reduce the sulphate ion to sulphides utilizing the decomposable organic matter such as lactates. Oxidation of organic matter provides the energy requirements of sulphur-reducing bacteria. The sulphate ion serves as the electron sink for their respiration and is thereby reduced to sulphide (Dugan, 1972; Rickard, 1973).

The generalized reaction between organic matter [CH<sub>2</sub>O] and sulphate is represented by the equation :

 $SO_4^{2}$  +2 CH<sub>2</sub>O ---> H<sub>2</sub>S +2 HCO3<sup>-</sup> (Breemen 1976).

Taking a known metabolizable substrate such as lactate, the reaction can be written as:

2 CH<sub>3</sub>CHOH.COOH + SO<sub>4</sub><sup>2-</sup> --> 2CH<sub>3</sub> COOH + HCO3<sup>+</sup> + CO<sub>2</sub> + HS<sup>-</sup> + H<sub>2</sub>O The hydrogen sulphide can then react with the ferrous iron present in the reduced sediments (Singh, 1985):

 $Fe (OH)_2 + H_2S \quad ----> FeS + H_2O$ 

This results in the formation of iron monosulphide (FeS). The iron originates mostly as iron (III) oxides in the sediments but is reduced to iron (II) by bacteria (Dent, 1986). The formation of pyrite by combination of iron monosulphide with elemental sulphur (Rickard 1973) can be written as follows:

FeS + S -----> FeS<sub>2</sub> (Pyrite).

Dent (1986) summarizes the formation of pyrites with iron (III) oxides as a source of iron using the following overall equation.

 $\begin{aligned} & Fe_2O_{3(n)} + 4SO4^2_{(m)} + 8CH2O + 1/2 O_{2(m)} - \cdots > 2FeS_{2(n)} + 8HCO_3_{(m)} + 4H_2O \\ & Iron (III) sulphate organic dissolved \\ & oxide^1 & ions^2 & matter oxygen \end{aligned}$ 

1 from sediments

2 from sea water

Little is known at present about the rate of pyrite formation in the natural environment but direct participation from dissolved  $Fe^{2+}$  and polysulphide may under favourable conditions yield pyrite within a day (Howarth, 1979). Potential acidity can only develop if at least part of the alkalinity formed during sulphate reduction (represented as HCO<sub>3</sub><sup>-</sup>) is removed from the system by diffusion or flushing by tidal action (Dent, 1986).

#### 1.5. Pyrite oxidation in acid sulphate soils.

The development of sediments with pyrites into shrimp ponds expose these sediments to the air initiating the oxidation of pyrites and the generation of acidity. The bunds (pond embarkments) constructed out of sediments with pyrites, are exposed to air throughout and the bottom of ponds are exposed only when they are drained. Oxidation of pyrites consists of several steps and involves both chemical and microbiological processes (Bloomfield and Coulter 1973; Dent, 1986).

According to Dent (1986) oxidation of pyrites in acid sulphate soils takes

place in several stages. Initially, dissolved oxygen reacts slowly with pyrite vielding iron (II) and sulphates or elemental sulphur:

$$2FeS_2 + 7O_2 + 2 H_2O -----> 2FeSO_4 + 2H_2SO_4$$
  
or  
 $FeS_2 + 1/2 O_2 + 2H^+ -----> Fe^{2+} + 2S + H_2O$ 

At very low pH values  $Fe^{2+}$  is further oxidized to  $Fe^{3+}$  by iron oxidizing bacteria of the species *Thiobacillus ferroxidans* (Robert <u>et al.</u>, 1969): 4 FeSO<sub>4</sub> + 2H<sub>2</sub>SO<sub>4</sub> + O<sub>2</sub> -----> 2 Fe <sub>2</sub>(SO<sub>4</sub>)<sub>3</sub> + 2H<sub>2</sub>O

Iron (Fe<sup>3+</sup>) can remain in the solution in appreciable amounts only at pH values less than 4 and is a more effective oxidant for pyrite and elemental sulphur than is oxygen:

 $\text{FeS}_2$  + 14 Fe<sup>3+</sup> + 8 H<sub>2</sub>O -----> 15 Fe<sup>2+</sup> + 2SO<sup>2</sup><sub>4</sub> + 16 H<sup>+</sup>

Precipitation of iron (III) due to hydrolysation occurs at higher pH values:  $Fe^{3+} + 3 H_2O \longrightarrow Fe(OH)_3 + 3H^*$ 

The *Thiobacillus thiooxidans* bacteria can oxidize elemental sulphur to sulphuric acid:

2S + 3 O<sub>2</sub> + 2 H<sub>2</sub>O ----> 2 H<sub>2</sub>SO<sub>4</sub>

In the absence of acid, basic ferric sulphate is also formed during pyrite

oxidation with the hydrolysis of ferric sulphate and oxidation of ferrous sulphates:

$$Fe_2SO_4 + 2H_2O ----> 2 Fe(OH)SO_4 + H_2SO_4$$
  
 $4FeSO_4 + 2 H_2O + O_2 ----> 4 Fe(OH) SO_4$ 

They appear as yellow mottles and streaks (basic ferric sulphate) and red deposits (ferric hydroxides) in acid sulphate soils. These deposits are frequently observed in shrimp culture ponds on the West Coast of Sri Lanka.

Acid sulphate soils conditions can develop only if the potential acidity represented by the pyrite is greater than the acid neutralizing capacity of the soil. The acid neutralizing capacity of a soil depends on several properties such as the amounts of carbonates, exchangeable bases or easily wetherable silicates present.

Breeman (1976) has provided a model of pyrite oxidation under climates with pronounced wet and dry weather periods. Oxidation of pyrite may continue after flooding, using the oxidative capacity that was stored as iron (III) oxide during the dry season. Even so the supply of oxygen appears to be the rate limiting factor in the oxidation of pyrites under acid conditions in the field (Dent, 1986). Excavated pyritic material during the construction of ponds oxidize faster than that of the sediment <u>in situ</u>. 1.5.1. Other oxidation products of pyrites in acid sulphate sediments. Colloidal iron (III) oxides are common in drainage water where active oxidation of pyrite is taking place; goethites appear to be the most commonly found iron oxide. Sometimes goethite may be transformed to haematite (Dent, 1986).

2 FeO.OH ----> Fe<sub>2</sub>O<sub>3</sub> + H<sub>2</sub>O

goethite haematite

Jarosite, KFe<sub>3</sub> (SO<sub>4</sub>)<sub>2</sub> (OH)<sub>6</sub> is identified in acid sulphate sediments under strongly oxidizing, severely acid conditions. Dent (1986) has summarized the formation of Jarosites from pyrites as:  $FeS_2 + 15/4 O_2 + 5/2 H_2O + 1/3 K^* ---->$ 

 $1/3 \text{ KFe}_{3}(SO_{4})_{2} (OH)_{6} + 4/3 SO^{2}_{4} + 3H^{+}$ 

According to Breeman (1973), at lower pH (<4) under strongly oxidised conditions, jarosite occurs in a stable iron phase.

1.6. Problems for aquaculture in acid sulphate and potential acid sulphate areas.

The development of aquaculture in acid sulphate and potentially acid sulphate soil areas has led to a large number of problems associated with acid sulphate soil conditions. Poor growth (Singh, 1980 a, 1980 b, 1980 c, 1981, 1982 a, 1982 b; Neue and Singh, 1984 ; Lin, 1986); development of severe acidity (Webber and Webber, 1978; Brinkman and Singh, 1982; Singh, 1985; Singh and Poernomo, 1984); poor fertilizer response and low natural productivity (Hechanova, 1983; Ladja, 1983; Simpson <u>et al.</u>, 1983; Cook <u>et al.</u>, 1984; Matla, 1984; Sing, 1985; Simpson and Pedini, 1985; Dent, 1986; Lin, 1986); mass fish kills (Dunn, 1965; Singh, 1982a & 1982 b; 1985); and accumulation of iron hydroxide in gills of shrimps (Nash <u>et al.</u>, 1988; Nash, 1990) are the most commonly documented problems associated with aquaculture in actual or potential acid sulphate soil areas.

Although precise estimates are not available, a large proportion of the aquaculture developments which have taken place in the coastal mangrove areas of the tropics are located in acid sulphate or potential acid sulphate soil areas. In the Philippines, out of a total of 170,000 ha of coastal fish and shrimp farms approximately 100,000 ha (60%) are affected by acid sulphate soil conditions (Cook et al., 1984). Most of the shrimp culture sites in Sri Lanka (850 to 1000 ha) are located in acid sulphate/ potential acid sulphate soil areas (NARA, 1988).

In these and other countries most of the detrimental effects so far identified have been attributed to the production of excessive amounts of sulphuric acid in the pyrite oxidation processes (Poernomo, 1983; Dent, 1986).

1.6.1. Soil and water acidity.

Due to the production of sulphuric acid by oxidation of pyrites, acid sulphate soils have a low pH, usually below 4, after the process of sun-drying during pond preparation. The release of sulphuric acid can continue during the grow out period, when acid can leach from the pond dikes into the ponds. This effect is more pronounced during rainy periods after prolonged dry spells. Sudden fish kills (Dunn, 1965; Singh, 1985) and shrimp kills (Simpson <u>et al.</u> 1983) are reported under these circumstances. Seepage through dikes and the entry of acidic dike pore water are other ways in which the pH of pond water may be reduced. Lethal levels of acidity (pH levels around 3) can be reached very rapidly under circumstances of heavy rains after prolonged dry spells (Dunn, 1965).

Alternatively, chronic sublethal levels of pH can occur under seepage through dikes and through dike pore water. The effects of these sub lethal levels on pond biota in general are perhaps most detrimental on a long term basis (Cook et al., 1984).

The effects of acidity in aquaculture related to fish have been widely studied (Swingle, 1961; Fromm, 1980; Wickins, 1981, 1984 a, 1984 b; Boyd, 1982, 1989; Chiu, 1988 a, 1988 b). Although the cause of acidity is different in acid sulphate soil environments the effects of low pH can be considered similar to those observed under other circumstances. The physiological effects include increased mucous production in gills to protect the epithelium

(Beamish, 1972; Daye and Garside, 1975), uptake of H<sup>\*</sup> ions through gills lowered blood pH (McDonald, 1983; Muniz and Livestad, 1980) and changes in osmoregulatory capacity due to changes in ionic composition (Dunson <u>et</u> <u>al.</u>, 1977). These recorded implications of low pH can be expected in fish cultured under poorly managed acid sulphate conditions.

The acid death point for shrimps has been established at 4 (Boyd, 1989). pH values between 4 and 6 result in poor growth (Boyd, 1989; Chiu, 1988 a, 1988 b). Such low pH values commonly occur in South East Asian shrimp culture ponds constructed in acid sulphate soil areas, unless special management strategies are followed.

#### 1.6.2. Poor growth and production.

Slow growth and poor production are two other adverse conditions frequently observed in aquaculture under acid sulphate conditions (Singh, 1982 a; Hechanova, 1983, 1984; Cook et al., 1984). The growth rate and also the general condition of the culture organisms are impaired by the culture environment generated due to acid sulphate conditions. Although the problem may gradually recede in about a decade after pond construction, the production remains low, in the order of 0.6 mt/ ha / year in milkfish culture (Singh, 1985) compared to an average of 1.5 mt/ ha / year in ponds fertilised and managed in a similar manner in non acid sulphate soil areas (Singh, 1982 b). Similarly Poernomo (1983) has recorded significantly lower yields of milk fish (167 kg /ha /crop) under experimental conditions in untreated ponds when

compared to the yield of 369 to 400 kg/ ha /crop in reclaimed ponds using repeated tilling, drying and flushing.

In experiments with Oreochromis niloticus and Puntius gonionotus, Lin (1986) recorded a yield of only 426 kg/ ha after a 5 month culture period in ponds constructed in acid sulphate soil areas when compared to ponds which received frequent water exchange, liming and higher dozes of inorganic and organic fertilizers as reclamation measures (1528/ kg /ha /5 month culture period) under similar acid sulphate conditions.

In Sri Lanka the average production recorded for intensive shrimp farms is 4,000 to 5,000 kg/ ha/ year. But in ponds affected by problems related to acid sulphate soil conditions the average production is only 500 to 1,500 kg/ ha /year (NARA, 1988).

According to Singh (1982), Macrobrachium rosenbergii are more seriously affected by acid sulphate soil conditions than fish species such as milkfish and Tilapia spp. Simpson et al., (1983) reported that the yields of Penaeus merguiensis and Penaeus monodon cultured under low stocking densities in acid sulphate areas are lower than those of other non-acidic areas. Production of P. monodon in excavated tidal ponds built on acid sulphate soils in the coastal areas of Malacca has yielded only 270 kg/ ha over a period of 90 to 100 days of culture (Cook et al., 1984).

1.6.3. Poor natural productivity.

The factors relating to acid sulphate soils which adversely affect the cultured stock have a similar effect on the other microbial, planktonic and benthic organisms of the pond environment. In addition, by adversely affecting the nutrient dynamics such as phosphate fixation and release, acid sulphate conditions also reduce the primary productivity and this effect can be conveyed through the food chain.

Webber and Webber (1978) noted that microorganisms grow slowly in pond environments under acid sulphate conditions, while Singh (1985) has indicated that insufficient growth of algae for aquaculture needs is a common problem in these environments. In an experiment to evaluate different reclamation measures, poor phytoplankton production in acid sulphate soil ponds has also been recorded by Simpson and Pedini (1985). The same authors have observed encrustations of benthic algae in the pond environment with iron hydroxides destroying the algal mat on the pond bottom. Poernomo (1983) observed relatively low lab-lab production (230 g/ m<sup>2</sup> ash free dry weight) in untreated ponds when compared to 883 g/ m<sup>2</sup> ash free dry weight in reclaimed ponds.

1.6.4. Poor fertilizer response.

Phosphorus and nitrogen are critical nutrients in acid sulphate soil areas (Attanandana and Vacharotayan, 1989). Very low efficiency of phosphate fertilization is another problem encountered in fish ponds constructed on acid

sulphate soils (Hechanova, 1983; Poernomo, 1983 ;Singh, 1985). It is a commonly accepted fact that the soluble phosphorous added to acid sulphate soils is largely changed into iron and aluminium phosphates (Chang and Chu, 1961). In aquaculture ponds which receive frequent treatment of lime, formation of calcium phosphates can remove soluble phosphorous from the pond environment.

The dynamics of phosphorous are extremely complicated in acid sulphate soils particularly under flooded conditions (Alva and Larsen, 1982). According to Ladja (1983), phosphorous retention by the mineral fraction of an acid sulphate soil is believed to be the result of the reaction of orthophosphate ions with iron and exchangeable aluminium in the soil, these are capable of precipitating phosphorous as phosphate compounds at low pH and in oxidised conditions. In addition, leaching aluminium and iron from pond dikes after heavy rains also can form complex insoluble phosphates utilising dissolved phosphates in pond water (Poernomo, 1983). Willet (1989) stated that during submergence of acid sulphate soils after flooding soil reduction processes can lead to an increase in the concentration of soluble phosphorous, but when iron (III) oxides are present they will resorb any phosphorous which is released.

### 1.6.5. Toxic effects of iron, aluminium and manganese.

Soluble iron, aluminium and manganese occur in high concentrations in water of poorly managed ponds constructed in acid sulphate / potential acid

sulphate soil areas (Cook <u>et al.</u>, 1984; Singh, 1985; Simpson and Pedini, 1985; Lightner, 1988), particularly under low pH, and higher Eh conditions. A sudden influx of these heavy metals into the pond environment has been observed after heavy rains following prolonged droughts (Poernomo, 1983; Singh, 1985). Ionic imbalance brought about by these influxes can result in large mortalities at lethal concentrations, or can weaken the cultured stock at sub-lethal concentrations.

The toxic effects of aluminium and manganese on fish have been well documented for different fish species (Muniz and Livestad, 1980; Honda <u>et</u> <u>al.</u>, 1983; Dallinger <u>et al.</u>, 1987; Young and Harvey, 1986). The histopathological condition of the gills is the best indicator of the impact of heavy metals (Freeman and Everhart, 1971; Livestad and Muniz, 1976; Driscoll <u>et</u> <u>al.</u>, 1980; Odonnell <u>et al.</u>, 1984). Prolonged exposure to sub-lethal concentrations may result in fusion of primary gill lamellae; reduction of the space between secondary gill lamellae; hypertrophy in the gill epithelium and increase in the number of mucous cells. Higher concentrations may result in clubbing at the proximal end of primary lamellae; hypertrophy of lamellar epithelium; fusion of secondary lamellar tips; increase in the number of mucous cells or increase in the size of chloride cells and sloughing of the epithelial lining (Livestad and Muniz, 1976; Henry and Atchison, 1979; Baker and Schofield, 1981; Lee and Harvey, 1986). The recorded effects of iron on cultured shrimps under acid sulphate conditions (Wickins, 1984 b; Nash <u>et al.</u>, 1988; Lightner, 1988; Nash, 1990) concern mainly accumulation of ferric hydroxides in the gill lamellae as well as on the other cuticular surfaces of the body carapace.

# 1.6.7. Effects on shrimp / fish health.

Various processes which take place in the acid sulphate pond environment can create serious problems for the general health of cultured organisms. Mass fish deaths or shrimp kills are frequently recorded in ponds constructed on acid sulphate soil areas (Dunn, 1965; Singh, 1985). These mass mortalities are closely associated with the heavy rains after prolonged drought conditions (Dunn, 1965).

Drainage water flushing over exposed acid sulphate soils on the dikes of ponds, and in their near vicinity, may wash in large amounts of acids, (thereby lowering pH) and aluminum, iron and manganese ions beyond the tolerance limits of culture organisms, resulting in sudden fish / shrimp kills. Sub-lethal effects of low pH, toxic metals and related processes can create chronic stress resulting in slow growth and higher susceptibility to diseases and parasitic invasions (Poernomo, 1983; Singh, 1985). Lightner (1988) describes poor survival, poor growth rates and decreased moulting frequency as the gross signs of such adverse conditions for shrimp in the acid sulphate soil areas.

Non-specific signs of illness such as lethargy, weakness, anorexia and the gathering of shrimp in shallow waters especially near the dikes, are often observed in shrimp culture ponds constructed in acid sulphate soil areas prior to mass shrimp kills (Nash et al., 1988; NARA, 1988; Nash, 1990).

Discolouration of gills, lamellar ferric hydroxide accumulation and encrustation of the lamellar epicuticle with iron are very common conditions in cultured shrimps affected by acid sulphate conditions (Simpson <u>et al.</u>, 1983; Lightner, 1988; Nash <u>et al.</u>, 1988; Nash, 1990) which Cook <u>et al.</u>, (1984) have described as the red gill disease. Extensive ferric hydroxide accumulations are detrimental to the normal gill functions. Clogging of gills will lead to hypoxia resulting in necrosis of the myocardial tissue (Nash <u>et</u> <u>al.</u>, 1988; Nash, 1990).

Chronic soft shell syndrome which was recorded under poor soil and water conditions (Baticados et al., 1986) has also been recorded in shrimps cultured under acid sulphate conditions (Simpson et al., 1983; Simpson and Pedini, 1985; Nash et al., 1988). The shrimps suffering from soft shell syndrome show poor calcification (Simpson et al., 1983), they are also more susceptible to cannibalism, are relatively weaker and experience high mortality rates (Baticados et al., 1988). The market value of these shrimps is much lower than that of the normal (hard shelled) shrimps.

The various stages in the process leading to melanisation of shrimp gill epithelium has been observed in shrimps by Nash (1988), together with epibiont fouling. *Epistylis*-like ciliates and *Zoothamnium* sp. have been identified as the most commonly occurring fouling organisms. Nash (1988) has also observed filamentous and non filamentous bacteria in tissues of sick shrimps that shows gill discolouration, but he considered them as secondary colonisers.

# 1.7. Reclamation measures and management strategies.

Various reclamation methods and management strategies have been discussed and reviewed to help overcome these acid sulphate problems; they are summarised in Table 1.3. These are primarily aimed towards neutralising acidity or removing the source of acidity. Removal of heavy metals or prevention of influx of these metals into the culture environment, and measures to increase primary productivity, or increase the efficiency of fertilizers are some other important considerations. In general, the conditions for fish production in acid sulphate soil areas are heavily dependent on the degree of reclamation.

# 1.8. Survey mapping and classification of acid sulphate soils.

Sediment quality is one of the crucial parameters in planning and managing ponds for aquaculture (Apud <u>et al.</u>, 1989; Chiu, 1988 a; Poernomo, 1990). In most conventional assessments, soil is evaluated in terms of its compactibility, Table. 1.3. Reclamation measures and management strategies suggested for aquaculture on acid sulphate soils.

Management strategy/ reclamation measure.

Liming to increase pH (1;4;6;9;10;14;15;16) Repeated drying and flushing pond bottom.(12;3;4;14;) Repeated tilling, drying and flushing pond bottom.(10;11;13;15) Leaching of pond dikes.<sup>(2,6;10;15)</sup> Construction of dikes with non-mangrove soil.(13;16) Reduction of the size of dikes, increase pond size and water depth<sup>(3;12;13)</sup> Removal of iron by drying and flushing.<sup>(3;13)</sup> Addition of impervious layer of rice hull, marfon, fertilix etc.<sup>(5,5;13)</sup> Inoculation of ponds with algae.<sup>(6;13)</sup> Stocking large size, hardy fish species.<sup>(6;10;13;14)</sup> Continuous water exchange.<sup>(3;13;15;16)</sup> Addition of higher dozes of phosphate fertilizer in dissolved form and more frequently.<sup>(2;4;7;8;10;13;14;15)</sup> Regular monitoring of water quality data related to acidity impacts. (2.4.12,13) Addition of organic fertilizer.<sup>(5;13)</sup> Growing of vegetational cover on dikes.<sup>(3;13)</sup>

Source in parenthesis; 1- Boyd(1989); 2-Brinkman and Singh(1982); 3-Cook<u>et.al.</u>,(1984); 4- Dent(1986); 5- Hechanova(1983); 6- Hechanova(1984); 7-Ladja(1983); 8-Lin(1986); 9- Matla(1984); 10- Neue and Singh(1984); 11-Poernomo(1983); 12-Simpson <u>et. al.</u>,(1983); 13-Simpson and Pedini(1985); 14-Singh and Poernomo(1984); Singh(1985); 15-Singh(1985); 16- Webber and Webber(1978) water retention capacity and acidity under field conditions (Aqua Business Associates, 1987; Tseng, 1987; Apud <u>et al.</u>, 1989). In these evaluations, soils with higher percentages of clay, high compactibility and near neutral or slightly basic pH levels in the field are considered best for aquaculture developments.

In aquaculture, soils are mainly classified according to their texture, using particle size distribution as a measurement of texture. The previous classification systems for acid sulphate soils (FAO / UNESCO, 1968; FAO / UNESCO, 1970; USDA, 1970; FAO /UNESCO, 1974; Soil Survey Staff, 1975) do not provide the information necessary for detailed land use planning nor that required for stipulating detailed management strategies for pond culture.

In this present study a soil classification system has been established, with the help of the ILRI classification system suggested by Dent (1986), to classify the acid sulphate coastal swamps of Sri Lanka in terms of their suitability for shrimp culture. In this system, soil acidity, potential acidity, composition and texture, ripeness and the detailed profile form of the sediments are considered in combination in classifying sediments. This classification system provides much more of the information needed for land use planning, management and reclamation of acid sulphate soil resources in brackish water aquaculture.

# 1.9. Behaviour of heavy metals.

Acid sulphate soils contain relatively high proportions of iron, manganese and aluminium compounds. Pyrites (FeS2) amounts upto 10% under severe acid sulphate conditions (Dent, 1986). However no attempt has yet been made to study the behavior of these heavy metals in aquaculture ponds on acid sulphate soils. The behaviour of iron and manganese in aquaculture ponds is important in explaining the physical, chemical and biological problems associated with culturing shrimps in acid sulphate soil areas. The behaviour of iron and manganese in natural systems can be explained in terms of redox potential and pH (James, 1954; Jeffrey, 1960; Ponnamperuma et al., 1967; Ponnamperuma, 1972; Collins and Buol, 1970 a & b). Composite Eh-pH diagrams are available for various iron or manganese systems (Breemen, 1976; Collins and Buol 1970 a & b). In agriculture various models have been constructed to explain the processes pertaining to pyrite oxidation (Breeman, 1976; Dent, 1986). Pyrite being the main compound responsible for acid sulphate conditions, models of this nature are of prime significance in stipulating management strategies. No attempt has yet been made to explain the behaviour of iron in terms of the Eh-pH relationship in the environment, or to model the process of pyrite oxidation in aquaculture ponds.

# 1.10. Specific aims and the objectives of the study.

The present study was undertaken with the view of investigating the processes behind the physical, chemical and biological problems related to shrimp culture under acid sulphate soil conditions in coastal swamps of the West Coast of Sri Lanka.

The experimental design included a field survey on Eh-pH relationships in ponds under different shrimp culture practices to understand the behaviour of iron and manganese compounds in the shrimp culture environment, a time series study of physical and chemical environmental conditions in ponds on acid sulphate soils during the culture of *Penaeus monodon*, studies on the kinetics of iron, manganese and aluminium in shrimp ponds, studies on calcium and magnesium accumulation in selected tissues of cultured shrimpsgod monitoring of black /brown gill syndrome and studies on the histopathology of shrimps cultured under acid sulphate conditions. Finally, a detailed survey to identify and classify the different acid sulphate and potential acid sulphate sediments, was also carried out in the areas earmarked for future shrimp culture development on the West Coast of Sri Lanka.

# MATERIAL AND METHODS

## 2.1. Study area.

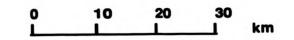
Sri Lanka is a tropical island located between 6 and 10° North. The present study was carried out on the West Coast of the island (between 7 and 9° North), where 95 % of all shrimp farming activities are taking place at present and more than 80 % of the areas earmarked for shrimp culture are located. The Landsat image (Plate 2.1) shows the Northern part of the study area. This extended along approximately 260 km of coast line. The major lagoon systems of the area includes Puttlam lagoon, Chilaw-Mundal lagoon complex, Negombo lagoon, Bolgoda lagoon and Bentota estuary. Over 70 % of the potential areas for shrimp culture have been identified in the coastal areas of the Chilaw and Mundal lagoon system (NARA, 1988).

#### 2.2. General environmental conditions

Data on monthly rainfall, wind direction, monthly average air temperature and the monthly diurnal range of air temperature were obtained from two principle meterological stations of the Department of Meteorology of Sri Lanka. These stations are situated at Puttlam, in the arid zone and Katunayaka in the wet zone. Tidal data were obtained from charts of predicted tides for the West Coast of Sri Lanka (Department of Meteorology, 1988).

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# Plate. 2.1. Landsat image showing the study area. (source: Department of Survey, Sri Lanka)



2.3. Survey of Eh-pH relationships in different shrimp culture systems. A general survey was carried out at shrimp culture sites developed on acid sulphate soils in the western coastal areas of Sri Lanka. The redox potential (Eh) and pH of the water (Bibby model smp 1 Eh-pH meter) and sediments (Bibby model smp1 with Russel type cept 11/ rod / 280 /1.5 m electrode) were measured between 1300 and 1500 hours at or around 4 weeks after stocking, in 51 shrimp culture ponds. Redox potential and pH of pond water were measured at three sites in each pond at 0.5m depth in the water column. Identical measurements were made at 1 to 2 cm depth in sediments from three sites in each pond by directly inserting the Eh probe.

Ponds were selected to cover the following major types of systems and management practices employed in the area :

a) Culture systems, with relatively high stocking densities and intensive aeration.

b) Semi-intensive systems with moderate stocking densities and no artificial aeration systems.

c) Extensive culture systems with low to moderate stocking densities (usually small scale operations).

#### 2.4. Culture trials

Shrimp ponds at a private shrimp farm located in Marambatiya, the West Coast of Sri Lanka (7° 9" North) bordering the Chilaw lagoon, were used for culture trials which included studies on (1) growth, survival and production; 2.3. Survey of Eh-pH relationships in different shrimp culture systems. A general survey was carried out at shrimp culture sites developed on acid sulphate soils in the western coastal areas of Sri Lanka. The redox potential (Eh) and pH of the water (Bibby model smp 1 Eh-pH meter) and sediments (Bibby model smp1 with Russel type cept 11/ rod / 280 /1.5 m electrode) were measured between 1300 and 1500 hours at or around 4 weeks after stocking, in 51 shrimp culture ponds. Redox potential and pH of pond water were measured at three sites in each pond at 0.5m depth in the water column. Identical measurements were made at 1 to 2 cm depth in sediments from three sites in each pond by directly inserting the Eh probe.

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#### 2.4. Culture trials

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(2) time series recordings of the environmental conditions in the culture ponds; (3) kinetics of iron, aluminium and manganese in shrimp grow-out ponds; (4) histo-pathology of shrimp exposed to acid sulphate soil conditions; and (5) studies on the tissue calcium and magnesium levels in relation to environmental conditions.

The farm was first established in 1986 for commercial production on a intensive/ semi-intensive scale of black tiger shrimps for the export market. During feasibility studies an estimated target production of 20 mt/ ha/ year had been proposed. The site used for construction of this farm was a mangrove swamp dominated by *Avicennia* and *Rhizophora* species. The total extent of the farm is 50 ha. Thirty ponds of 0.5 ha each had already been constructed and brought into operation at the time of this study.

The ponds are 1.5 m deep (pond bottom approximately 20 cm above MSL) with earthen bunds. Each pond has one sluice gate which connects to a single main supply and drainage canal.

The main water supply for the farm is Chilaw lagoon. The chemical, physical and biological water quality parameters of this water source have been monitored and were recommended as favourable for brackish water shrimp farming (NARA, 1986).

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# 2.4.1. Pond preparation.

Normal pond preparatory and management procedures practised by semiintensive and intensive farms in Sri Lanka (NARA, 1986) were followed in (Annex ?) the present study. The pond preparatory procedures were in accordance with the general guide lines given by Apud <u>et al.</u> (1989), Kongkeo (1990).

The ponds were drained and dried for 12 days. Lime was broadcasted evenly over the pond bottoms and dikes at the rate of 1.5 mt/ ha. After filling the pond to a depth of about 20 cm tea seed cake was applied (150 kg/ ha). Fertilisation was carried out at the rate of TSP, 20 kg /ha, and urea 50 kg/ ha. The water level was then raised to a depth of about 80 cm and post larvae were introduced after 5 days. Hatchery reared post larvae (PL 15) were stocked at a density of 20 post larvae/  $m^2$  in June 1989.

Three ponds (P3, P11 and P21) out of those prepared and stocked as described above were randomly selected. The records for these ponds kept by the farm are summarised in Table 2.1.

#### 2.4.2. Pond management

Table 2.2. summarises the information regarding feeding, water exchange, and water depth maintenance during the culture period. The feed used was President feed (President feed Company, Taiwan). Feeding was provided according to guidelines provided by the manufacturers.

al monitoring.	Average production (kg / pond / cycle)
Table. 2.1. Farm record for the ponds used for the shrimp culture and environmental monitoring.	No. of culture cycles completed
Farm record for the ponds used	Date of first stocking
Table. 2.1. F	Pond no.

B	1986. 11. 22	07	950
P11	1986. 11. 16	07	1150
P21	1987. 02.01	06	1200

•

According to information provided by the feed company, the proximate composition of feed includes 36-42 % crude protein, 2.8 to 3.0 % crude fat and 3 % crude fibre. Feed was broadcasted evenly in the ponds twice daily. In addition two feeding trays of approximately 1  $m^2$  were also used to monitor food consumption by the shrimps.

Water exchange was started at the end of week 2 at a rate of 2 % daily; this was progressively increased to a rate of 10 to 15 % daily (Table 2.2). Paddle wheel aeration was started during the 4th week of culture. Aeration was done normally during 0400 to 1000 hour daily, using two paddlewheel aerators (TEAM, Model TA 333, 1 HP) per pond.

#### 2.4.3. Sampling

Weekly sampling for shrimps was carried out using a cast net from the 2nd to 8th weeks and in the 10th week. Cast netting was done at 10 to 12 different locations per pond. 60 to 100 shrimps were removed in total and were immediately chilled. These shrimps served as samples for monitoring growth; and for the various studies on kinetics of aluminium, iron and manganese; tissue calcium and magnesium levels; black gill/ brown gill condition and for histo-pathology.

# 2.4.4. Determination of water characteristics.

Determination of salinity, pH, Eh, dissolved oxygen, temperature and turbidity was carried out *in-situ* while all other parameters were determined in the

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Table. 2.2. Info	rmation on feed,	water exchange rate, w	Table. 2.2. Information on feed, water exchange rate, water depth and aeration during the culture period.	uring the cutture period.
Culture time (weeks)	Feed (type)	Water exchange rate (% / day)	water depth (m)	Artificial aeration
2-3	starter	2	0.70	
4-5	starter	5 - 10	1.0	+
6-7	grower	10 - 12	1.2	٠

•

+

1.2

grower 10 - 15

8 - 10

laboratory. For the parameters to be analyzed at the laboratory, three samples were collected from each pond near the sluice gate, middle and at a point close to the blind end of the pond. For *in-situ* measurements, three readings were recorded at each sampling point. Sampling was carried out weekly, during the 2nd to 6th week and thereafter bi-weekly until the end of the culture period. Samples were collected between 0800 and 1000 hours.

Except for sulphides and total suspended solids, samples were collected from surface layers and near the bottom by means of a Ruttner sampling bottle. Samples were collected in 500 ml polythene bottles except for sulphides. General precautions suggested by APHA (1985) and Stirling (1985) were followed in sample pre-treatments, sample preservation, transportation and storage.

All spectrophotometric analyses were performed in either a Hach DR/3000 microprocessor controlled spectrophotometer, or a Uvicon 810 spectrophotometer.

#### 2.4.4.1. Salinity.

Salinity was measured using a refractometer (American Opticals) in water samples collected from bottom layers as well as from surface layers.

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# 2.4.4.2. pH

Water pH was measured using a Hachone (model HH 43800-00) portable pH meter, which has a relative accuracy of 0.01 % of the reading.

#### 2.4.4.3. Eh

Redox potential was measured using an Eh electrode (Russel smp 2, 1.5 m) attached to a Bibby smp 1 Eh-pH meter or by means of a Hanna ORP Redox tester with a range of  $\pm$  999 mV. Measurements were made by immersing the electrode firstly 5 cm below the surface, then lowering the electrode further nearer to the bottom. Triplicate readings were recorded for each measurement

#### 2.4.4.4. Dissolved oxygen.

Dissolved oxygen was measured using a portable oxygen meter (Jenway Portlab 9070) with a range of 0 to 19.9 mg /l. This has a resolution of 0.1 mg/l. Direct measurements were taken in the field as in the case of redox potential.

#### 2.4.4.5. Dissolved phosphorous.

Samples preserved by adding 20 mg of HgCl<sub>2</sub>, were filtered through GF/C filter paper and used for the analysis of dissolved phosphorus.

The principle of the method involved the reaction of phosphates with molybdate to form molybdo-phosphoric acid in the acidified solution; this was then reduced to the molybdenum blue complex as described in APHA (1985) and Stirling (1985). Absorbance of standards and samples were measured against reagent blank spectrophotometricaly at 882 nm. This method has a sensitivity range of 0.005 to 2.00 mg /l.

#### 2.4.4.6. Nitrite.

Samples preserved and filtered in a similar manner to those for analysis of dissolved phosphorus were used for the determination of nitrites.

Reaction of nitrite with sulphanilamide to form a diazonium compound which reacts with N-(1-naphthyl)-ethylene-diamine dihydrochloride (NED) to form a strongly coloured azo compound as described in APHA (1985) and Stirling (1985) was the principle behind the method followed. Absorbence of standards and samples were measured spectrophotometrically against a reagent blank at 540 nm. The sensitivity range for this method is 0.001 to 0.50 mg/l.

# 2.4.4.7. Nitrate

A cadmium/ copper column was used to reduce nitrates to nitrites (Strickland and Parsons, 1972; APHA, 1985; Stirling 1985). The column was prepared by washing 20 g of cadmium filings with 100 ml of 2M HCl and rinsing with distilled water. 40 ml CuSO<sub>4</sub> solution was added and the contents swirled until the blue colour disappeared.

Water samples were allowed to run through the columns and then were analyzed for nitrites as described earlier. In the case of higher concentrations the initial samples were diluted.

# 2.4.4.8. Sulphides.

The samples were collected in 250 ml glass stoppered sample bottles and were treated immediately with 5 ml of 1 M zinc acetate solution to precipitate the sulphides. Sulphides can react with N.N-diethyl-pphenylenediamine sulphate in the presence of iron to yield a blue dye which can be measured spectrophotometrically. The detailed methodology suggested by Strickland and Parsons (1972) and Stirling (1985) was followed.

Absorbence of standards and samples was measured against a reagent blank spectrophotometrically at 670 nm. The limit of detection is about 2  $\mu$ g S<sup>2</sup>/l (Stirling, 1985).

#### 2.4.4.9. Chlorophyll a.

Chlorophyll a concentration, with correction for phaeo-pigments, of the water sample was determined according to the methods described by Strickland and Parsons (1972), APHA (1985) and Stirling (1985). 100 ml of water sample was filtered through millepore HA fibre 0.45  $\mu$ m filter paper in the field with the help of milliphore hand filter unit. In the laboratory pigments were extracted with 25 ml of 90 % acetone. Extracts were centrifuged and absorption was measured spectrophotometrically in cells of 4cm at 665 nm and 750 nm. Calculation was based on the following formulae (APHA, 1985; Stirling, 1985): Chl-a ( $\mu$ g/l) = A x K (A<sup>o</sup><sub>ess</sub> - A<sup>o</sup><sub>ess</sub>) x v2 / v1 x L

Where,  $A^{\circ}_{665}$  and  $A^{\circ}_{665}$  are the corrected absorbances at 665 nm before and after acidification respectively, v1 is the volume of water sample filtered in litres, v2 is the volume of acetone extract in ml and L is the path length of the spectrometer cuvette in cm. A is the absorbance coefficient for chlorophyll and K is the ratio expressing correction for acidification.

# 2.4.4.10. Temperature

Water temperature was measured to the nearest 0.5 °C near the pond surface (10 cm below the water level) and near the bottom using a mercury thermometer.

#### 2.4.4.11. Turbidity

Turbidity of the water samples collected was measured in the field using a portable turbidimeter (Hach Portlab model 1680). This has a resolution of 0.1 NTU in the range 0 to 10 NTU.

#### 2.4.4.12. Total suspended solids

Total suspended solids were determined by filtering 1 fof pond water through a pre-washed, pre-dried, and pre -weighed 47 mm Whatmann GF/C filter paper using a suction pump as described by Stirling (1985). The filter papers were then placed on an aluminium foil, dried at 105°C for 12 hours, cooled and re-weighed. The suspended solid content was then calculated from the difference of weight. 2.4.5. Determination of sediment characteristics.

# 2.4.5.1. Sampling for sediment characteristics.

Sediment samples were collected weekly during weeks 2 to 6 and in week 8 and week 10 of the culture trials (as in the case of water quality). Sediment samples were collected in triplicate at each of 3 points as described for water quality determination, using a stainless steel auger with cylindrical attachments. These cylinders are 30 cm in length and are interchangeable. The auger was inserted into the surface layers of sediments during the sample collection. The cylindrical portion was removed and the sediment sample was pushed from the cylinder by means of a screw device on to a polythene sheet. The surface sediment (first 3 cm) was removed for analytical work.

#### 2.4.5.2. Soil pH .

Soil pH was measured using a Hach pH meter [Hachone model HH 43800-000]. The procedure involved is described in section 2.7.

### 2.4.5.3. Soil Eh.

Soil Eh measurements were carried out *in-situ* by immersing the Eh electrode (Russel type cept 11 /rod/ 280 1.5 m) attached to Bibby model smp 1 Eh /pH meter into the surface sediments as described earlier.

# 2.4.5.4. Soil organic matter content

Organic matter content of the soil was determined by ignition of 8 to 10 g of soil samples in triplicate in a muffle furnace at 350 °C for 16 hours as described in section 2.7.

# 2.4.5.5. Soil particle size.

Analysis of soil particle size was done prior to the pond preparation. The same procedure described in section 2.7 was used for soil particle size analysis.

2.5. Kinetics of iron, manganese and aluminium.

Water samples, sediment samples and shrimp samples collected during the regular sampling programme for time series studies as described earlier were used to study the kinetics of iron, aluminium and manganese in shrimp culture systems.

In addition, for comparison, samples of wild shrimps were collected (from Chilaw lagoon) and from a pond at the same farm where they had been exposed to acid sulphate soil conditions for an 18 week period.

The water, sediment and tissue samples were processed for analysis by atomic absorption spectrometry. Either a Perkinelmer 2280 or a Pye Unicam SP 9 atomic absorption spectro photometer was used for analysis. Iron concentrations in processed samples were measured by directly aspirating samples and standards into an air-acetylene flame. A wave length of 252.3 nm and slit width of 0.2 nm were selected as instructed by the manufacturers (Analytical methods for atomic absorption spectrophotometry: instruction manual for Perkinelmer 2280).

The aluminium concentrations were measured by directly aspirating samples and standards into a nitrous oxide-acetylene flame. A wave length of 309.3 nm and a slit width of 0.7 nm was used.

The samples to be analyzed for manganese were directly aspired into an airacetylene flame. The wave length selected was 279.5 nm and the slit width was 0.2 nm

2.5.1. Iron, aluminium and manganese in water.

Water samples collected from the bottom layer during the regular sampling programme for water quality were also used for heavy metal analysis. Samples were filtered through a 0.45  $\mu$ m membrane filter, then were transferred to 500 ml-polythene bottles. The samples were acidified with 2 ml of extra pure nitric acid and stored in a refrigerator at -4 °C.

Sub-samples in triplicate were used for analysis of iron, aluminium and manganese by atomic absorption spectrophotometry.

2.5.2. Iron, aluminium and manganese in sediments.

Aliquots of the surface sediment samples were used for the analysis of iron, aluminium and manganese. The material was acidified with a few drops of nitric acid and then stored in polythene containers in a refrigerator at -4 °C.

Sediment samples were oven dried to a constant weight at 60 °C and sieved through a 1 mm mesh. For sediment samples to be analyzed for total iron and manganese, aliquots of 0.5 g to 1.0 g in triplicate from each sample were suspended in extra pure concentrated nitric acid in tall digestion tubes following the method described by Dallinger and Kautzky (1985). The sample tubes were gradually heated on a heater block and were left standing for 72 hours at 90°C, shaking them several times per day. The supernatant was filtered through pre-washed Whatmann GF/C papers and diluted to a known volume. The concentrations of heavy metals were measured in an atomic absorption spectrophotometer.

The sediment samples to be analyzed for the available aluminium soil extracts were prepared according to FAO (1973). The extracting solution used was a mixture of 0.5 n ammonium acetate and 0.02 m EDTA at pH 4.65. Twenty g of oven dried soil was extracted with 100 ml extracting solution in a mechanical shaker for 30 minutes, the filtrate of the resulting solution was collected in polythene bottles.

# 2.5.3. Iron, aluminium and manganese in tissues.

Randomly selected sub-samples of shrimps (25 to 30 per sample) collected during regular sampling was used for the analysis of aluminium, iron and manganese concentrations in the gill, muscle and carapace. They were transported to the laboratory in iced regiform containers. In the laboratory shrimps were rinsed with double distilled water, and gills, carapace and abdominal muscles were carefully dissected. The samples were stored at  $-21^{\circ}$ C.

The dry ashing method proposed by Dallinger and Kautzky (1985) was followed in processing tissue samples for heavy metal analysis. Tissue samples were wrapped in tissue papers and oven dried at 60 °C. Sub samples of 0.3 to 0.6 g in triplicate were digested in 15 ml concentrated nitric acid / concentrated sulphuric acid 1:1 mixture for 48 hours in tall digestion tubes at 70 °C on a heating block. The remaining solution was filtered through pre washed 0.45  $\mu$ m GF/C paper and diluted to 25 ml. Metal concentrations were measured in an atomic absorption spectro-photometer.

#### 2.6. Pathology of cultured shrimps

During the regular sampling programme of cultured shrimps observations were made to differentiate colour and other changes in their gills and to group them into following categories:

Category 1: Normal gills - gills with normal transparent colour, with no

visual sign of any accumulations.

Category 2: Brown gills - light brown coloured gills with a somewhat opaque appearance when compared to normal gills.

Category 3: Black gills - shrimps with dark brown or black gills, opaque and with very clear visual signs of foreign particles on the gills.

A randomly selected sample of 10 to 15 brown gilled/ black gilled shrimps/normal gill shrimps were sacrificed by injecting Davidson's fluid (Bell and Lightner, 1988) into the soft tissue of the live animals. In addition shrimps of the same weight classes were collected from the immediate natural environment for comparison. The gills, hepatopancreas and hearts of these shrimps were dissected and removed for morphological and pathological studies.

For light microscopic studies (LM) specimens were fixed in Davidson's fluid (Bell and Lightner, 1988) and processed for wax embedding. Five micron sections and whole mounts of gills were stained either with haematoxylin and eosine (H&E) for general examination or with Perl's Prussian blue for the demonstration of iron (Clark, 1981).

Gills were preserved in phosphate-buffered gluteraldehyde for electron microscopic (EM) studies. These gills were later treated with phosphatebuffered 1 % osmium tetroxide, and were dehydrated in 70% acetone and processed separately for scanning and transmission electron microscopy. Gills to be processed for scanning electron microscopy (SEM) were further dehydrated in 100% acetone and were critical-point dried using liquid carbon dioxide as a transitional fluid in a Polaron-E 1000 critical point drier. The gills were then mounted on aluminium stubs using colloidal graphite paste, coated with gold in an Edwards S-150 B Sputter coater and were examined under an ISI-60A or Phillips PSEM 500 scanning electron microscope.

For transmission electron microscopy (TEM), gills were dehydrated in ethanol and propylene oxide and were embedded in Epon 812 medium hard resin (TAAB). Thin sections cut on an LKB-III ultratome in the silver/ gold colour region were double stained with uranyl acetate and lead citrate and were examined under a Phillips 301 electron microscope.

Calcium and magnesium concentrations of the carapace and abdominal muscles were determined in the samples collected during the regular sampling programme. The tissue digestion prepared for atomic absorption spectrometric analysis (section 2.5.3.) were also used for the analysis of calcium and magnesium concentrations. The samples and standards were directly aspired into an air-acetylene flame. A wave length of 422.7 nm and slit width of 0.7 nm was used for calcium while for magnesium wave length of 285.2 nm and slit width of 0.7 nm was used.

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2.7. Classification and mapping of acid sulphate / potential acid sulphate coastal swamps in the West Coast of Sri Lanka.

#### 2.7.1 Soil survey:

Different methods of soil survey are discussed for acid sulphate and potential acid sulphate soils by Dent and Young (1981); Bos and Mensvoort (1984) and Dent (1986). In the present survey, the methods suggested by Dent (1986) were followed.

Land use maps (1: 10000) for Puttlam, Gampaha, Panadura and Aluthgama (Department of Survey, 1983), aerial photographs (1:6000 in black and white year, 1984) covering the coastal swamps of Negombo and Chilaw were collected from the Remote Sensing Centre of the Department of Survey Colombo. Reconnaissance survey has been carried out by NARA (1986) in a preliminary investigation to identify potential areas for shrimp culture. This has indicated the location of acid sulphate / potential acid sulphate soils on the West Coast.

Areas earmarked for shrimp culture development in the coastal swamps on the West Coast of Sri Lanka (NARA, 1986) were identified in the land use maps. The areas were divided into 2 ha plots and sampling was carried out one per each plot. This intensity of sampling is regarded as satisfactory for soil surveys for management problems (Dent and Young, 1981; Dent, 1986) and for aquaculture purposes (FAO, 1985). At each sampling point soil samples were taken at 3 depths (0-10 cm; 40-60 cm; 80-100 cm) using a stainless steel Dachnowsky, screw or cylindrical auger depending on the sediment type. Samples were placed in thin walled polythene bags. The air inside the bag was squeezed as much as possible. The sample bags were enclosed in an outer bag which was sealed and transported to the laboratory.

# 2.7.2. Soil analysis.

In the laboratory investigations were carried out for the following: existing acidity or potential acidity, soil ripeness, soil particle size, organic matter content.

# 2.7.2.1. Existing or potential acidity.

Existing acidity (pH) was measured by mixing 10 g of air-dried soil with 50 ml distilled water. The suspension was stirred and pH was measured using a Bibby model smp 1 pH meter after 18 hours of equilibrium as described by FAO (1973).

Soil samples indicating a pH over 4 were used for the determination of potential acidity. About 250 g of moist samples were incubated in open thinwalled polythene bags to stimulate the oxidation process as described by Dent (1986). Soil pH was measured after three months of moist incubation following the same procedure as that for existing acidity.

# 2.7.2.2. Soil particle size

Samples of 15 g oven dried soil at 105 °C were first ground to free separate particles, treated with 100 to 150 ml hydrogen peroxide (30 volume) to remove the organic matter and then 2ml of 2N sodium hydroxide was added to disperse the particles. The silt and clay fraction was separated using the wet pipette method as described by Meanns and Parcher (1965) and Smith and Atkinson (1975). The sediments settled were washed through No. 300 mesh sieve (0.05 mm aperture diameter) to separate the sand fraction (Means and Parcher, 1965).

# 2.7.2.3. Organic matter content.

Organic matter content was estimated by ignition of finely ground soil samples of 5g in triplicate in a muffle furnace for 16 hours at 375 °C. Dent (1986) has indicated that wet oxidation with dichromate reagent is unsuitable for acid sulphate soils because the chromate reacts with pyrites. At a temperature of 375 °C, carbonates, pyrites, and most clay minerals are stable, so the weight loss is attributable to the oxidation of organic matter (Dent, 1986).

#### 2.7.2.4. Soil ripeness.

Soil ripeness was determined at the initial stage of the survey by the method described by Pons and Zonneveld (1965) using water content, organic matter content, and particle size distribution, The quantity of water in grams absorbed by one gram of clay in the soil, is derived from the equation:

n = A - 0.2R / L + bH

Where :

A = the percentage of water in the soil under field conditions, calculated on dry soil basis;

R = the non-colloidal part of the soil (% sand + silt);

L = percentage clay;

H = percentage organic matter;

b = the ratio of the water absorption capacity of organic matter to clay (3 for well humified organic matter, 4 for partly decomposed organic matter)

Later in the survey, due to the large number of samples involved, the degree of ripening was assessed in the field following the simple method described by Pons and Zonneveld (1965) and by Dent (1986) which involves squeezing the soil by hand (Table 2.3).

2.7.3. Determination of soil classes and soil profile forms.

The ILRI classification (Dent, 1986), with slight modification, was used to assign soils to various classes. The soil properties considered were acidity or potential acidity at various horizons from 0 to 100 cm depth and texture, composition and ripeness of the soil profile at various depths down to 100 cm. Table. 2.3. Field method for determination of soil ripeness (n-value) and soil ripeness class. (From Dent, 1986).

Ripeness class.	Description	n-value
Totally unripe	fluid, and flows between the fingers when	0 = > 2 0
Practically unripe	squeezed. mud is very soft, can be squeezed through the finders by very dentle pressure.	n = 1.0-1.4
Half ripe	mud is fairly soft, sticky, and can be squeezed through the fingers.	n = 0.7 - 1.0
Nearly ripe	material is fairly firm, it can be kneaded but not soueezed through the fingers.	n =<0.7

2.7.3.1. Acidity and potential acidity.

A pH value of less than 4 is considered as the primary criterion for acid sulphate soils, while the criterion for potential acidity is a fall in pH but to a level below 4 during three months of moist incubation.

2.7.3.2. Soil composition and soil texture.

The terms clay, peat, and sandy soils were used to classify soil types according to the following criteria:

<u>Clav soils</u>: soils where more than fifty percent of the material is silt + clay. <u>Peat soils</u>: soils have more than twenty percent organic matter by mass (if the mineral component has no clay) to more than thirty percent organic matter by mass (if the mineral component is fifty percent or more clay).

Sandy soils: soils where more than fifty percent of the material is sand (more than 0.05 mm diameter).

2.7.3.3. Degree of ripening (n- value).

The ripening values described by Pons and Zonneveld (1965) Brinkmann and Pons (1973) are used in the ILRI classification (Table 2.3) and were used in describing soils in the present study.

# 2.7.3.4. Limiting values for ILRI classification.

The limiting values considered for individual characteristics of acid sulphate soils and related soils are given in Table 2.4. Several potential acid sulphate and acid sulphate soil categories have been identified and are grouped under Table. 2.4. Limiting values considered for the individual characteristics of acid sulphate soils and potential acid sulphate soils for different profile forms (Adapted from Dent, 1986).

	cid subhate soil conditions (pH less than 4)			
Within 0 to 20 cm				
Within 40 to 60 cm	a2			
Within 80 to 100 cm	a3			
otential acidity :				
otential acid sulphate soil co	butential acid surphate soil conditions (pH falls below 4 after three months moist incubation).	ree months moist incubation	Ĵ.	
Vitin 0 to 20 cm	E			
Mithin 40 to 60 cm	<b>p</b> 2			
Within 80 to 100 cm	5d			
fexture and composition :				
Clay	_	han 40 cm thick. Where pe	aty	
	surface horizon ispresnt, this is less than 20 cm thick.	is less than 20 cm thick.		o
	Peat laver more than 40 cm thick.	hick.		•
	Sand laver more than 40 cm thick. Where peaty layer is present	thick. Where peaty layer is	present	
	this is less than 20 cm thick			•0
	Integrated peat and mineral soil not fulfilling the above thickness	oil not fulfilling the above	thickness	
	criteria			
	Cineria tes esite			o/c
	Mineral top soils			c/o, s/o
Ripeness :	•			
In clay, peat or muck:				
	n-value	An to 60 cm denth	80 to 100 cm depth	sm death
	0 10 50 CM 06bm	to to out in the time of the		1
Inripe	> 0.7	×1.4		EM
Half rive	> 0.7	0.7-1.4		W2
Disa with under a th ani	202	> 0.7	> 1.0	Ĩ
The with the sub out	202	< 0.7	> 1.0	2

organic soils, sandy soils and clayey soils. Table 2.5 presents the major categories of acid sulphate soils and potential acid sulphate soils under the ILRI classification.

# 2.7.4. Mapping of different soil classes and soil profile forms.

The various soil classes identified were included in maps (1: 10,000 scale) traced from land use maps. Soil profile forms were interpreted from aerial photographs (1: 6,000 scale, black and white) obtained from the Department of Survey of Sri Lanka using methods described by Coche (1985) and Loubersac (1985). Photographs in sequence were arranged under a stereoscope to obtain the necessary overlap for stereoscopic interpretation. Soil boundaries were then identified on a transparent overlay on the photographs.

# 2.8. Land use pattern and vegetation.

Relavent land use information used was sourced from land use maps (1: 10,000) and 1" scale maps of the Department of Survey, Sri Lanka. The statistical information presented is based on data collected at the Puttalam and Gampaha Government Agent's office on livestock, population and proposed / operational farm sites. Other information presented was collected through interviews and historic records. Land use categories for farms, both operational and proposed, are based on the land use maps together with personal observation on operational farms.

Table. 2.5. Major categories of potential acid sulphate and acid sulphate soils. (Adapted from Dent, 1986)

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	Organic soils		Sandy soils	G	Clayey soils		
Undrained not potentially acid	untipe peat and muck			untipe clay			
potential acid sulphate soils	unnipe sulphidic peat and muck		suphidic sand	unripe sulphidic clay			
acid sulphate soils	raw acid sulphate	ripe acid sulphate	raw acid sulphate sand	raw acid sulphate	ripe acid sulphate clay	ripe acid sulphate clay	
	pear and muck	pcet and muck	acid sulphate sand	clay	with raw sub-		
associated non acid sulphate	pest and muck ripe pest and with unripe muck	ripe peat and muck	pues		ripe clay with unripe sub-soil	ripe clay	ripe acid aluminium clay
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# 2.9. Statistical analysis.

Cluster analysis (Kendall, 1975; Howarth and Larsen, 1983) was carried out by the fast cluster method using the statistical package SAS (see Annex 8). Goodness of clusters were checked using the procedure described by Howarth and Larsen (1983). The dispersion or scatter of all values about their respective group (W), the dispersion of all values about the grand mean (T) and the dispersion between the groups (B) was calculated using the following formulae, considering only one parameter Eh or pH.

$$W_{m} = \Sigma^{n1} (x_1 - \bar{x}_1)^2 + \Sigma^{n2} (x_2 - \bar{x}_2)^2$$

$$W_{nH} = \Sigma^{n1} (y_1 - \bar{y}_1)^2 + \Sigma^{n2} (y_2 - \bar{y}_2)^2$$

 $T_{m} = \Sigma (x - \hat{x})^{2}$ 

 $T_{pH} = \Sigma (y - \bar{y})^2$ 

 $B_{Bh} = n_1 (\bar{x}_1 - \bar{x}_1)^2 + n_2 (\bar{x}_2 - \bar{x}_1)^2$ 

$$B_{arr} = n_1 (\dot{y}_1 - \dot{y}_2)^2 + n_2 (\dot{y}_2 - \dot{y}_2)^2$$

Where  $x_1, y_1, x_2, y_2$  are Eh and pH values of respective clusters,  $\dot{x}_1, \dot{y}_1, \dot{x}_2, \dot{y}_2$ are mean values of respective Eh and pH clusters  $\dot{x}$ ,  $\ddot{y}$  are grand means of all Eh and pH values.

This approach can be extended to express the relationships between different Eh-pH clusters as follows:

$$W_{Ba\,eH} = \Sigma^{t} \left( X_{Ba} - \ddot{X}_{Ba} \right) \left( X_{pH} - \dot{X}_{pH} \right)$$

$$T_{BapH} = \Sigma^{\star} \left( X_{Ba} - \bar{X}_{Ba} \right) \left( X_{pH} - \bar{X}_{pH} \right)$$

$$B_{Bh oH} = n_k \left( \bar{\mathbf{x}}_{Bh} - \bar{\mathbf{x}}_{Bh} \right) \left( \bar{\mathbf{x}}_{pH} - \bar{\mathbf{x}}_{pH} \right)$$

Where: k is the over all number of clusters for Eh and pH and  $n_k$  is the number of observations for each cluster.

The test statistic described by Wilks (Wilks, 1931) known as Wilks' lambda (^) was determined using the ratio |W| / |T| as described by Marriott (1971) and Howarth and Larson (1983) to confirm the success of clustering, where |T| and |W| are determinants. Determinants |W| and |T| were calculated using following:

 $|W| = |(W_{\rm m} \times W_{\rm pH}) - (W_{\rm m}_{\rm pH})^2|$  $|T| = |(T_{\rm m} \times T_{\rm pH}) - (T_{\rm m}_{\rm pH})^2|$ 

Regression analysis.- Linear regression analysis was carried out to determine the correlations between dependent and independent variables (sediment Eh, sediment pH, sediment organic matter with culture period; metals in tissues with those metals in sediments and with culture period).

Partial correlation analysis. Pearson product-moment correlation analysis was carried out to determine correlations between tissue metal concentrations, soil metal concentrations, metal concentrations in water and culture time from the time series studies on culture of shrimps.

A stepwise linear regression method which allows in determining correlations among a number of variables was used for the analysis of manganese in gills with iron in gills, iron in sediments and culture period; calcium in carapace with iron in gills, iron in sediments and culture period.

Cluster analysis was performed using Statistical Analysis System (SAS, 1982) at the Department of Statistics and Computer sciences, University of Colombo, while all other analysis was carried out using Statgraphics version 3 at the Institute of Aquaculture.

All the graphs were plotted using an Apple II Macintosh computer with Cricket graph software.

# CHAPTER 3

GENERAL ENVIRONMENTAL ASPECTS OF SHRIMP FARMING IN SRI LANKA

# 3.1. Background Information.

Climatic conditions have been identified as one of the most important factors to be considered in site selection for shrimp farming (Chiu, 1988 a; Poernomo, 1990). The crucial climatic factors for site selection, planning, constructing or reclaiming ponds on acid sulphate soils, and for stipulating management strategies for shrimp farming include, climatic zones, seasons, total rainfall and its seasonal distribution, air temperature and wind and tidal characteristics of the area (Poernomo, 1990).

The climate has a substantial influence on soil profile depth and texture, organic matter content and nitrogen content. The processes of clay mineral synthesis in soil involve interactions between climate, parent material and drainage conditions. Herath (1973) has related the different types of clay sediments found in the coastal areas of Sri Lanka to the prevailing climatic zones. Similarly differences in the properties of different acid sulphate soils have been explained in accordance with the prevailing climate (Kevie, 1973; Brinkman and Pons, 1973; Bloomfield and Coulter, 1973). The humid and monsoonal climate of the tropics and moist temperate climates favour the formation of acid sulphate soils (Dent, 1986).

# 3.2. Climatic zones.

Sri Lanka lies in the monsoonal region of South East Asia and has a humid tropical climate (Domros, 1974). The west coast of Sri Lanka, where there are substantial activities in shrimp farming, occupies all three major climatic zones: arid zone, dry zone, and wet zone. The divisions into these different climatic zones are primarily based on the distribution and amount of rainfall. Various other climatic factors important to successful planning and management of shrimp culture, such as air temperature and humidity, also vary between these various climatic zones.

The study area, which includes the coastal areas around Puttalam lagoon and the northern part of the Mundal lagoon lie within the arid zone, while those of southern Mundal lagoon are located in the island's dry zone. The dry zone also includes the coastal areas of Chilaw lagoon. Coastal areas of Negombo lagoon, Bolgoda lagoon and Bentota estuary occupy the wet zone.

#### 3.3. Monsoonal seasons.

There are four seasons based on the predominant wind directions, namely south-west monsoon, north-east monsoon, first inter-monsoon and second inter-monsoon seasons. The duration of each season is given in Table 3.1.

## 3.4. Rainfall.

The total annual rainfall and the distribution of rainfall throughout the year is important in the formation of acid sulphate soils and their subsequent

Table. 3.1. The main climatic seasons in Sri Lanka.

Season	Period of the year	Duration (% of the year)
First inter-monsoon	March to mid May	21
South-west monsoon	mid May to September	36
Second inter-monsoon	October to November	16
North-east monsoon	December to March	25

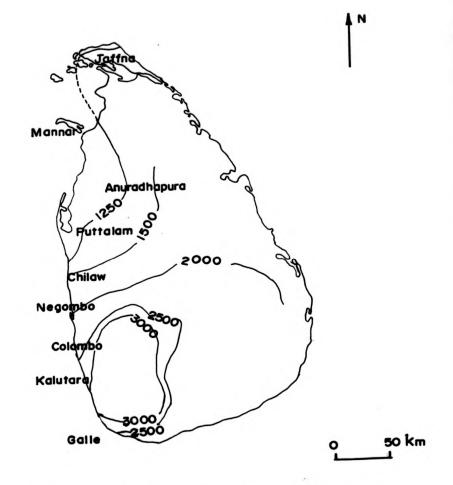
properties (Dent, 1986). Rainfall is also related to land drainage and river run off which are contributory processes in acid sulphate soil formation.

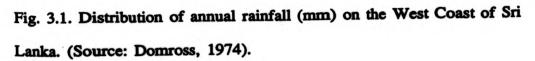
Bloomfield and Coulter (1973) indicate that the behaviour of sulphidic mud is affected by rainfall distribution. In areas where there is rain throughout the year, sulphidic deposits remain in a water logged and reduced condition, while in areas where there is a pronounced dry season strongly acid sulphate conditions can develop (Dent, 1986). Higher rainfall also enhances the organic matter content of soils.

Rainfall governs the salinity distribution in the coastal waters of Sri Lanka (Aruldpragasam and Jayasinghe, 1984). The dilution of sea water with rain water affects the sulphate content of coastal waters and therefore the sulphate reduction processes during potential acid sulphate soil formation.

3.4.1. Annual distribution of rainfall.

The distribution and pattern of annual rainfall in Sri Lanka has been described by Bamford (1929); Jamesion (1936); Ekanayaka (1945); Thambayahpillay (1954, 1958, 1959, 1960); Jayamaha (1959) and by Domross (1974). Fig. 3.1 shows the distribution of annual rainfall in the western coastal areas of Sri Lanka. Considering the annual totals for a thirty year standard meterological period, the coastal area around Puttalam lagoon and the northern part of Mundal lagoon receives the least amount of rain (less than 1250 mm annually). Coastal areas around the southern part of the Mundal lagoon lie in





between the 1250 mm and 1500 mm isohyets. The coastal areas around Chilaw lagoon receive an annual rainfall of 1500 mm to 2000 mm. All the other coastal swamps on the west coast of Sri Lanka are in the wettest zone, between the 2000 mm and 3000 mm isohyets.

# 3.4.2. Seasonal distribution of rainfall.

The seasonal distribution of rainfall in the West coast (Fig. 3.2) clearly shows differing and greatly diverging rainfall conditions among the seasons. The rainy seasons coincide with the two intermonsoonal periods while North East and South West monsoonal seasons remain relatively dry.

# 3.4.3. Monthly distribution of rainfall.

The role of rainfall distribution in the formation of acid sulphate soils and in determining their various properties have been described by Kevie (1973), Bloomfield and Coulter (1973) and Dent (1986). The monthly rainfall values give a more detailed description of rainfall throughout the year. These are shown for two selected meterological stations in the wet zone and arid zone of the west coast in Fig. 3.2. On this coast, there is a change from a relatively wet period to a comparatively dry period twice each year. During the observation period the observed rainfall pattern followed this typical rainfall pattern with double maxima. Some rainfall is evident every month in the wet zone.

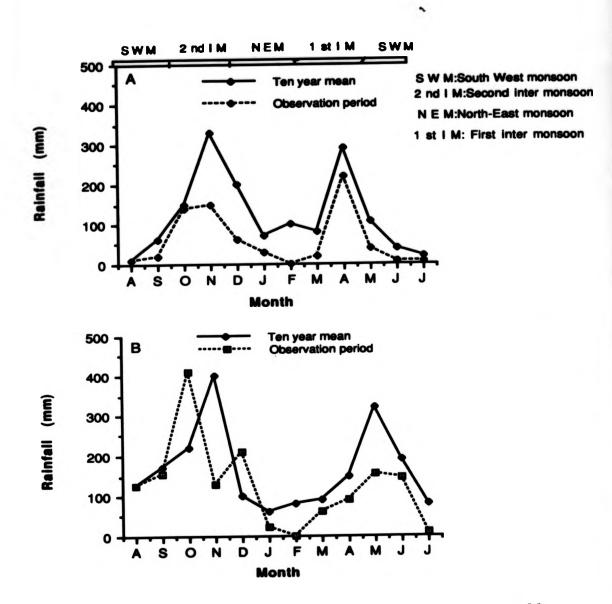


Fig. 3.2. Monthly mean rainfall (10 year average) and the monthly rainfall totals for the observation period, August 1988 to June 1989 at two principal meteorological stations in arid zone (A) and (B) wet zone.

3.4.4. Rainfall and shrimp culture in acid sulphate soil areas.

In acid sulphate soil areas sudden fish kills have been recorded during intermonsoonal months when there was heavy rainfall after prolonged drought periods (NARA, 1986). A similar incidence has been recorded by Dunn (1965) in acid sulphate soil areas of Malaysia.

Reclamation of ponds on acid sulphate soils through repeated drying and flushing is more effective and economical in dry weather periods (Hecanova, 1984; Singh and Poernomo, 1984; Singh, 1985) and in Sri Lanka monsoonal seasons appears to be the most suitable periods for such reclamation measures.

Other important considerations related to monsoonal seasons and rainfall include the lowering of salinity in water sources creating problems for water exchange in ponds, high turbidity and the threat of physical damage to ponds by floods and the danger of agro-chemicals being washed into ponds from surrounding agriculture areas.

## 3.5. Air temperature.

Air temperature has been identified as an important factor that affect the organic matter content and associated properties of soils (Young, 1976). It also influences the composition of clay minerals (Hearth, 1973) which are important in the formation of potential and actual acid sulphate soils (Horn and Chapmann, 1968; Breemen, 1973).

Conditions in Sri Lanka are described as thermic diurnal, i.e. the differences in air temperatures are predominantly diurnal and the seasonal variations are comparatively low. Fig. 3.3 presents the monthly means of daily temperature and monthly mean diurnal range in air temperatures from typical arid zone and wet zone meterological stations situated in the west coast of Sri Lanka for the period December 1988 to November 1989. The highest daily fluctuations in temperature were observed during the months of December, January, and February.

The water temperature of culture ponds tends to follow the same pattern of variation as the air temperature (NARA, 1988). Thus shrimps cultured in arid and dry zones are likely to experience relatively higher diurnal fluctuations in temperatures than those cultured in the wet zone.

# 3.6. Wind characteristics.

Wind is a force that can influence estuarine circulation and affect the salinity distribution (Perkins, 1974). Pritchard (1967) has noted that wind stress can cause reversed flow in brackish water bodies, while Bowden (1967) has stated that wind effects can sometimes dominate the tidal effect. Winds can enhance the tidal effect and influence the penetration of sea water if they flow in the same direction as the tidal current.

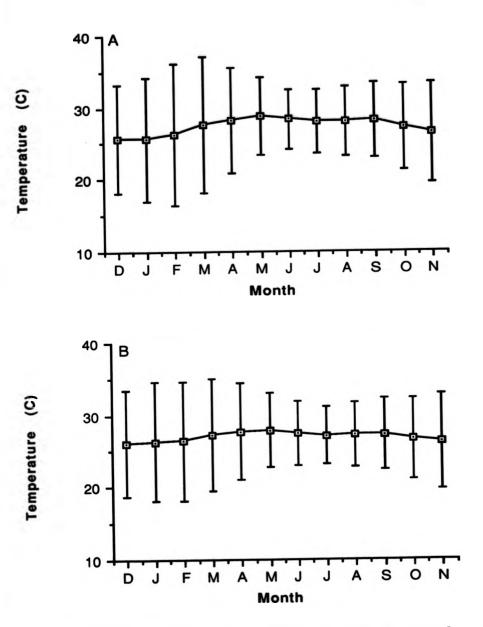


Fig. 3.3. Monthly average air temperature and monthly diurnal range (I) of temperature for the observation period, December 1988 to November 1989 at two principal meteorological stations in arid zone (A) and wet zone (B).

Wickramasuriya (1969) has explained the influence of cyclonic wind activity on the formation of sand bars at the sea out-falls of the estuaries and lagoons along the west coast of Sri Lanka. It is evident that winds can influence sea water mixing and the inundation of tidal flats from sea water and both factors are important in processes contributing to the formation of acid sulphate sediments. Winds also influence the rainfall distribution pattern. Several studies by Jayamaha (1959), Thambyahpillay (1954, 1958, 1960) and Domross (1974) have described the relationship between monsoonal winds and rainfall over the coastal areas of Sri lanka.

Wind characteristics have been identified as an important factor in evaluating sites for shrimp farming (Poernomo, 1990). Orientating ponds in the same direction as the dominant winds appears to enhance the natural processes of oxygenation.

### 3.6.1. Surface wind conditions.

The winds of the western coastal areas of Sri Lanka consist of two main components, identified as monsoonal winds and land and sea-breezes. Monsoonal winds are linked with the monsoonal seasons, while land and seabreezes are developed throughout the year. Monsoonal winds have two components; the winds blowing with a predominantly westerly component are referred to as south-west monsoonal winds, while the winds with a predominantly easterly component are referred to as north-east monsoonal winds (Domross, 1974). The land and sea-breezes are strongest in the

# intermonsoonal months.

3.6.2. Annual and monthly distribution of wind directions.

Annex. 3.1 presents the annual distribution of wind direction over the arid zone and wet zone of Sri Lanka's western region, while annex 3.2 and 3.3 shows the monthly distribution of wind direction in those climatic zones. The dominant winds are from the west, south west and north east.

### 3.7. Tidal characteristics.

Tides have an important influence on various estuarine and lagoon processes. In addition to influencing salinity, the inundation of coastal swamps with tidal water is an essential phenomenon in the formation of acid sulphate soils in coastal areas (Kevi, 1973; Dent, 1986). The pattern of succession of brackish water vegetation such as mangroves has been related to the degree of tidal inundation by Andriesse <u>et al.</u>, (1973) and Bloomfield and Coulter (1973).

The tides in the coastal areas of Sri Lanka are semidiurnal in nature, with two high tides and two low tides each day. Fig. 3.4 shows a typical predicted monthly tidal curve from published tide tables for the west coast of Sri Lanka (Department of Meteorology, 1988). The prediction takes into account the effects of seasonal meterological changes, but does not include the effect of temporary and unpredictable meteorological conditions, or of localized effects due to topographical features such as shelter or sand bars.

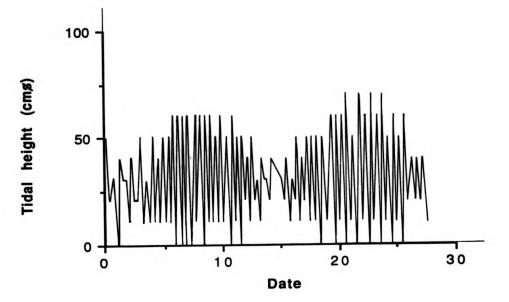


Fig. 3.4. Predicted monthly tidal curve for West Coast of Sri Lanka for the month of September, 1989. (Source: Department of Meteorology, 1989)

The following tidal characteristics have been recorded for the west coast of Sri Lanka (Department of Meteorology, 1988: heights above mean sea level):

Mean lower low water of spring tides+0.02 m.Mean low water of spring tides+0.06 m.Mean low water of neap tides+0.30 m.Mean high water of neap tides+0.42 m.Mean high water of spring tides+0.72 m.

Although there are usually some variations between predicted tides and the actual water levels recorded in lagoons and estuaries, the results of observations in various lagoons adjoining coastal swampy areas of Negombo, Chilaw lagoons and in the north Mundal and south mundal coastal areas, indicate that water level fluctuations follow the predicted pattern at most times (NARA, 1988).

Tidal characteristics are important considerations in site selection for shrimp farms as they affect the water supply and drainage during pond operation. According to Poernomo (1990), for better management and economical pond operations, semi-intensive and extensive ponds should be between slightly above mean low water level and slightly below mean high water level to enhance the tidal exchange.

# CHAPTER 4

TIME SERIES STUDY OF ENVIRONMENTAL CONDITIONS IN PONDS ON ACID SULPHATE SOILS DURING THE CULTURE OF Penaeus monodon.

This chapter is introduced with a review of the limited amount of information published on this important but often neglected environmental aspects of shrimp farming.

# 4.1. Introduction

4.1.1. Redox potential-pH relationships and the behaviour of iron compounds.

The problems of acid sulphate soils in aquaculture are presently related primarily to potential acidity and secondarily to associated high levels of iron, aluminium and other metals. Slow growth, low survival, sudden mortalities, poor natural production, poor fertilizer response and severe economic losses due to disease outbreaks in acid sulphate areas in many regions of Asia, have been attributed to severe acidity due to oxidation of pyrites. (Neue and Singh, 1984; Webber and Webber, 1978; Cook <u>et al.</u>, 1984; Simpson and Pedini, 1985; Singh, 1985; Lin, 1986). Several authors (Simpson <u>et al.</u>, 1983; Cook <u>et al.</u>, 1984; Singh, 1985; Nash <u>et al.</u>, 1988) have also mentioned the probable adverse effects on cultured organisms of metals associated with acid sulphate soils.

Pyrite  $(FeS_2)$  is the main chemical compound responsible for acid sulphate conditions but surprisingly little information is available on the behaviour of iron compounds in aquaculture ponds, particularly the effects of iron on the physical, chemical and biological problems associated with culturing shrimps in acid sulphate soil ponds.

The processes responsible for the formation of acid sulphate soils are well identified and documented (Beers, 1962; Pons, 1973; Bloomfield and Coulter, 1973) and have been described in detail in Chapter 1. Dent (1986) summarised the formation of pyrites with iron (III) oxides as a source of iron using the following overall equation:

 $Fe_{2}O_{1}$  (s) +4  $SO_{4}^{2}$  (aq) +  $8C_{2}H_{2}O$  + 1/2  $O_{2}$  (aq)-----> 2FeS. (s)

$$+ 8HCO_3^{-}$$
 (aq)  $+ 4 H_2O_{-}$ 

Iron encrustation on the cuticle of shrimps has been reported by Simpson  $\underline{et}$ <u>al.</u> (1983), Wickins (1984 a) and Lightner (1988) in acidic culture conditions. Brown discolouration of gills is the most common symptom revealed. A detailed study by Nash <u>et al.</u> (1988) has demonstrated lamellar ferric hydroxide accumulation in *Penaeus monodon* cultured in acid sulphate soil areas. These authors also describe the resultant hypoxic changes in other tissues of shrimps due to clogging of respiratory surfaces by the iron oxides. Bioaccumulation of iron (Patric and Loutit, 1978) in the culture organisms as well as the biotransference and biomagnification of iron in aquatic ecosystems (Dallinger et al., 1985, 1987) are some other important considerations for the development of aquaculture in acid sulphate soils.

The chemical behaviour of iron compounds in the culture environment will play an important role in determining the influence of iron on cultured organisms. This behaviour is governed by the redox potential (Eh) and pH of the pond environment. There have been several studies of the behaviour of iron compounds in relation to Eh-pH conditions in agriculture (James, 1954; Jeffrey, 1960; Ponnamperuma <u>et al.</u>, 1967; Collins and Buol, 1970 a; Collins and Buol, 1970 b; Ponnamperuma, 1972), but no studies relating to aquaculture have been conducted. Hence, one objective of the present study was to investigate the stability relations of iron compounds as a function of Eh and pH in shrimp culture ponds developed in an acid sulphate soil area.

4.1.2. Redox potential-pH relationships and the behaviour of manganese compounds.

Manganese is one of the main elements in acid sulphate soils which may also be involved in bioaccumulation, biotransference and biomagnification in aquatic environments (Patric and Loutit, 1978). Manganese also has been identified as an ameliorating agent for some forms of heavy metal toxicity due to its ability to incorporate other metals by adsorption, ion exchange or co-precipitation (Stauber and Florence, 1985). The behaviour of the manganese compounds in natural systems has been often explained in terms of redox potential (Eh) and hydrogen ion activity (pH) (Collins and Buol, 1970 a, 1970 b).

Manganese exists in four main forms in acid sulphate environments: (1) water soluble, (2) exchangeable, (3) reducible and (4) in residual forms in soil (Collins and Buol, 1970a). Oxidation of manganese in acid sulphate soils may occur during pond construction, as well as during pond drying when sediments are exposed to air. Reduction may follow once the aquaculture ponds are filled with water. Determination of the oxidation/ reduction potential can give a qualitative measure of the intensity of these changes, but this is not normally monitored in the context of shrimp farming.

Reduction of the soil proceeds in the sequence predicted by thermodynamics and produces an array of redox systems ranging from the  $O_2$ -H<sub>2</sub>O system in highly oxidised conditions, to the H<sup>+</sup>-H<sub>2</sub> system in strongly reduced states. MnO<sub>2</sub> (pyrolusite), Mn<sub>2</sub>O<sub>3</sub> (bixbyite), Mn<sub>3</sub>O<sub>4</sub> (hausmannite) and Mn(OH)<sub>2</sub> (pyrochroite) are the most common oxidised compounds of manganese in the acid sulphate soil environment (Ponnamperuma, 1972). The stability relationships of different manganese compounds as a function of Eh and pH are illustrated by the Eh-pH diagram given in Fig. 4.5. The acidic pH conditions and reduced Eh conditions favour the formation of water soluble manganese, while relatively higher pH values favour the formation of its insoluble oxide and hydroxide forms. When the shrimp culture ponds are filled with water after drying, the exchange of gases between soil and atmosphere is virtually stopped. Anaerobic conditions developed in the pond bottom stimulate the anaerobic bacteria to reduce Mn (III) and Mn (IV) compounds to Mn (II) compounds. Equilibrium reactions (Lindsay, 1979) of the different oxidised and reduced compounds of the most abundant manganese forms in the acid sulphate pond environment are given below.

 $Mn(OH)_2 + 2H^*$  ----->  $Mn^{2*} + 2H_2O$  (log K°=15.19)

 $MnO_2 + 4H^+ + 2e$  .....>  $Mn^{2+} + 2H_2O$  (log K° = 41.89)

 $Mn_2O_3 + 8H^+ + 2e$  -----> 2  $Mn^{2+} + 3H_2O$  (log K° = 51.46)

 $Mn_3O_4 + 8H^+ + 2e$  -----> 3  $Mn^{2+} + 4H_2O$  (log K° =63.03)

These Mn (II) species can get into the overlying water. Acid sulphate soils high in manganese and organic matter have been reported to accumulate soluble  $Mn^{2+}$  to as high as 90 ppm within two weeks of submergence (Collins and Buol, 1969).

Intensive systems have high stocking densities and are aerated artificially. They maintain fairly high oxidised Eh values while the Eh values of semiintensive and extensive prawn culture systems show relatively low Eh values.

Manganese precipitates can accumulate in the pond bottom which is the ecological niche occupied by the cultured shrimps. *Penaeus monodon* is sensitive to heavy metal precipitates (Apud <u>et al.</u>, 1989) and these precipitates have a tendency to accumulate in between the gill lamellae, causing obstructions to respiratory currents (Nash <u>et al.</u>, 1988). Deposits on the exoskeleton may also provide an environment suitable for fouling organisms. On the other hand dissolved manganese can enter into the culture organisms via permeable surfaces and can accumulate on the carapace (Bryan and Ward, 1965) and in the internal organs.

# 4.1.3. Water quality.

The information available on water quality management in aquaculture concentrates mainly on fresh water systems (Alabaster and Lloyd, 1982; Boyd, 1982 and 1989). Comparatively little information deals with aspects of water quality management in brackish water culture, despite the importance of coastal shrimp farming.

Water quality parameters can have a direct influence on growth, survival and production of farmed shrimps and also on the occurrence of diseases. Water quality management becomes increasingly important as culture practices progress from extensive towards semi-intensive and intensive systems.

The effects of water depth, circulation and artificial aeration on the water quality dynamics of extensive, semi-intensive and intensive culture shrimp

ponds have been described by Carpenter et al. (1986), Sanares et al. (1986), and Boyd (1989). There are generally accepted levels for nutrients, some heavy metals, ammonia, sulphide and nitrite in shrimp ponds, and the most favourable temperature, turbidity, salinity and dissolved oxygen levels and pH ranges to promote optimum growth, survival and production of *P. monodon* and have been well documented (Wickins, 1981; Sanares et al., 1986; Chiu, 1988 a and 1988 b; Apud et al., 1989; Boyd, 1989; Poernomo, 1990). An oxygen budget, which shares the proportions of the total oxygen consumed by the different respiratory components of a shrimp pond has been recently worked out by Madenjian (1990).

# 4.1.3.1. Salinity.

Shrimps are very sensitive to changes in salinity in the culture environment and the preferred salinity range is highly dependent on the species concerned. *Penaeus monodon* survive and grow well in low salinities (Boyd, 1989). Its generally accepted optimum range of salinity for growth and production is 10 to 25 % (Chiu, 1988 a and b). Chiang and Kuo (1988) and Apud et al. (1989) have suggested that slightly narrower ranges of salinity (15 to 25% and 15 to 20% respectively) are most favourable.

#### 4.1.3.2. pH.

Comparatively little information is available on the effects of pH on shrimps when compared to that recorded for fish (Swingle, 1961; Boyd, 1979). A pH range of 6 to 9 has been identified as optimal for growth. The ranges 4 to

6 and 9 to 11 result in slow growth, while pH levels of 4 and 11 have been identified as the acid and alkaline death points respectively. Boyd (1989) suggested that it is safe to assume that the shrimp may respond to pH in much the same way as fish.

Apud <u>et al.</u> (1989) indicated the pH range 6.0 to 8.5 as suitable for culturing *P. monodon* while Chiu (1988 a and 1988 b) has indicated a narrower range of pH (6.8 to 8.7) for favourable growth and production. According to Chiang and Kuo (1988) the optimal range of pH for tiger shrimp is 7.5 to 8.8.

# 4.1.3.3. Redox potential.

Redox potential together with pH determines the stability of different heavy metal compounds (Collins and Buol 1970 a, 1970 b; Ponnamperuma, 1972; Breemen, 1976) in the aquatic environment. Despite the growing awareness of redox potential in aquaculture, little attention has been given so far to monitoring Eh values in shrimp culture ponds. Rajyalakshmi <u>et al.</u> (1988) has recorded Eh ranging from -97 to 760 mV in shrimp culture ponds in India.

# 4.1.3.4. Dissolved oxygen.

Dissolved oxygen can be considered as the most critical water quality variable in aquaculture (Boyd, 1989). Dissolved oxygen levels above 3.7 mg/l are considered favourable for shrimp culture by Apud <u>et al.</u> (1989) while Chiu (1988 b) states a slightly lower level (> 3.5 mg/l). According to Boyd (1989) dissolved oxygen levels around 5 mg /l provides the best conditions for shrimp growth. Recent information (World Shrimp Farming, 1991) suggests the maintenance of dissolved oxygen levels around 3 mg/l is sufficient in shrimp ponds even up to stocking densites of 40 Pl (post-larvae 20)/ $m^2$ .

## 4.1.3.5. Phosphorus.

Phosphorus is considered to be one of the important primary nutrients in the pond environment. Phosphates have been identified as one of the chief limiting factors in the production of fish in ponds excavated in acid sulphate areas (Watts, 1968). The chemical equilibrium between phosphate in sediments and water dominates the phosphorus cycle in the pond and this cycle is extremely important in regulating primary productivity.

According to Boyd (1982) the average concentration of total phosphorus in unfertilised ponds is around 0.026 mg /l, while those receiving periodical applications of fertilizer show phosphate levels of around 0.175 mg/l. Experiments in ponds in partially reclaimed acid sulphate soils in the Philippines with higher doses of phosphate fertilisers applied showed phosphate levels ranging from 0.19 mg /l to 0.55 mg /l (Singh, 1985).

#### 4.1.3.6. Nitrates.

Nitrate is a primary nutrient in the shrimp pond environment. In brackish water ponds with moderate to high salinity, diatoms are the dominant planktonic organisms. They require fairly large amounts of nitrogen and in these ponds nitrogen is a more important limiting nutrient than phosphorus (Boyd, 1989).

According to Boyd (1982), nitrate-N in ponds which receive fertilizer is around 0.016 mg /l and in unfertilized ponds the recorded nitrate-N values are around 0.004 mg /l. According to Boyd (1973), nitrate -N levels as high as 25-100 mg /l are not toxic to fish. For good growth of salmonids, nitrate-N levels in recirculating systems should be in the range 5.6 to 7.9 mg NO<sub>3</sub> N/l (Boyd, 1979). Wickins (1981) has stated that the NO<sub>3</sub>-N levels of 175 mg NO<sub>3</sub>-N can affect the growth of the fresh water prawn *Macrobrachium* whereas growth of *P.monodon* is not affected by levels even up to 200 mg NO<sub>3</sub> -N / l.

#### 4.1.3.7. Nitrites.

Nitrite in the pond environment can result from the reduction of nitrates by bacteria (Hollerman and Boyd, 1980). Imbalances in the reactions involved in nitrification can lead to an accumulation of nitrite in aquaculture ponds. Most of the recorded values for nitrite concentrations in ponds vary between 0.5 and 5 mg /l of  $NO_2$  -N (Boyd, 1982).

Relatively low concentrations of  $NO_2$ -N (0.5 mg /l) have been reported to be toxic to cold water fish (Crawford and Allen, 1977). For channel catfishes 96 hr, LC 50 values recorded for  $NO_2$  -N are 4.6 mg /l (Konikoff, 1975) and 13.0 mg /l (Russo and Thurston, 1977).

For *P. monodon*, total NO<sub>2</sub> -N levels less than 1.0 ppm support maximum growth in grow out ponds (Chiu, 1988 b). In hatcheries the accepted levels are below 0.02 mg /l (Apud <u>et al.</u>, 1989). Crustaceans show decreased resistance to nitrite during their moulting periods. According to Wickins (1981) the levels of 6.4 mg NO<sub>2</sub> -N / l reduces the growth of the shrimp *P. indicus* by about 50 % and survival is affected significantly at levels around 60 mg NO<sub>2</sub> -N /l. Chen and Lei (1990) have suggested a "safe level" of 3.8 mg /l nitrite-N for *P. monodon* juveniles. A relationship between nitrite levels and gill damage/ gill colour changes have been indicated by Lightner (1988). Sub-acute or chronic exposure to nitrite levels as low as 2 to 3 ppm can damage the gills, causing changes to their normal gill colour (Lightner, 1988).

## 4.1.3.8. Sulphides.

Certain heterotrophic bacteria under aerobic conditions use sulphates and other oxidised sulphur compounds for metabolism, producing sulphide as the end product. Boyd (1982) has summarised the process as:

 $SO_4^{2} + 8H^{+} - S^{2} + 4H_2O$ 

Although the ions resulting from the dissociation of hydrogen sulphide are not appreciably toxic, unionized hydrogen sulphide is toxic to fish (Boyd, 1982). In this form it can affect egg survival and the growth of fry (Adelman and Smith, 1970) and retard the growth of adult fish (Bonn and Follis, 1967). According to Smith <u>et al.</u> (1976), any detectable level of hydrogen sulphide may have adverse effects on fish production. Apud <u>et al.</u> (1989) suggested that the maximum acceptable level of sulphide in shrimp culture ponds as 3 ppb. According to Kongkeo (1990) a hydrogen sulphide concentration of 1.3 mg/l can cause shock, paralysis and eventual death to shrimps.

The degree of ionization of sulphide is highly dependent on the pH of the environment. Chiu (1988 b) has described the relationship between hydrogen sulphide levels safe for shrimp and pH in ponds under semi-intensive operation. These values are < 0.004 mg/l (pH 6-7); <0.007 mg/l (pH 7-8); <0.04 mg/l (pH 8-9).

# 4.1.3.9. Chlorophyll a.

Although chlorophyll a is not a direct measurement of phyto-plankton concentration, it is a reliable indicator of phytoplankton abundance. Chlorophyll a concentration has been used by several workers to assess fish pond productivity (Wade, 1990).

The recorded ranges for chlorophyll a in fish ponds are from 8.8  $\mu$ g/l to 115.5  $\mu$ g/l (unfertilised) and from 103.4  $\mu$ g/l to 212.3 (fertilised) freshwater ponds in Israel (Hepher, 1962). Boyd (1973) has reported a range of 5  $\mu$ g/l to 30  $\mu$ g/l for chlorophyll a in unfertilised ponds and values ranging from 20  $\mu$ g/l to 130  $\mu$ g/l in ponds fertilised with nitrogen and phosphorus fertilizer. In shrimp culture ponds with continuous aeration, measurements revealed higher chlorophyll levels (around 100  $\mu$ g/l) when compared to ponds without aeration (around 53  $\mu$ g/l).

4.1.3.10. Temperature.

Temperature influences the distribution, abundance and development of aquatic organisms. They are tolerant certain temperature ranges outside of which they are unable to function (Hyns, 1970; Tatarko, 1970; Fast, 1985; Opuszynski et al., 1989). Temperature also influences other parameters important in aquaculture, including dissolved oxygen level, nutritional requirements, feeding, growth and digestion rate (Hilton and Slinger, 1981; Fast, 1985).

Recorded temperature ranges for successful growth and survival of *P.monodon* are 26 to 32°C (Chiu, 1988 a and b), 28 to 33°C (Apud <u>et al.</u>, 1989), 26 to 33 °C (Poernomo, 1990). However, a narrower range of temperature 29 to 30 °C supports optimal growth (Poernomo, 1990).

#### 4.1.3.11. Turbidity.

Turbidity in the pond environment can be a result of either inorganic or organic matter. Organic sources include plankton and high concentrations of humic substances in aquaculture ponds (Boyd, 1979). Inorganic sources of turbidity are mainly negatively charged colloidal clay particles which remain dispersed and in suspension (Fast, 1985).

Turbidity from suspended particles of clay is generally undesirable for aquaculture (Boyd, 1982) as the resulting reduction in light penetration reduces photosynthetic efficiency. Besides, it can interfere with pond fertilisation as the particles may adsorp or absorb phosphorous (Lennen et al.. 1985). According to Poernomo (1990) turbidity related to soil particles upto 150 FTU can be acceptable, while the optimum range for shrimp growth is 20 to 30 FTU.

## 4.1.3.12. Total suspended solids.

Water sources often used in shrimp culture have heavy loads of suspended solids. These particles may either originate by erosion in the drainage basin of the water source, or as effluent from shrimp farms themselves in culture ponds. Suspended solids consist mainly of soil particles and particles of organic matter (Boyd, 1989). Suspended clay particles are considered undesirable in shrimp ponds (Chiu, 1988 b) but according to Wickins (1981) shrimp growth is not likely to be limited up to total suspended solid levels of 15 mg/ 1.

# 4.1.4. Soil quality.

Soil type and quality are an extremely important consideration in pond culture. Soils absorb and release nutrients in the pond environment. These nutrients determine pond primary productivity and also the type and quantity of natural food available for the cultured organisms. Soil is the main and the most economical source of material for dike building.

The adverse influences of unfavourable soil properties on the pond environment and its cultured organisms are seen very clearly in the case of acid sulphate soil conditions (Simpson <u>et al.</u>, 1983; Poernomo, 1983; Cook

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et al., 1984; Singh, 1985; Simpson and Pedini, 1985; Nash <u>et al.</u>, 1988; Nash, 1990). Because shrimps are benthic organisms, pond soil conditions are even more critical in shrimp culture than in the culture of other aquaculture species in ponds (Boyd, 1989).

4.1.5. Growth, survival and production.

Slow growth, low survival and poor production have been described as common problems in shrimp culture on acid sulphate soils (Simpson <u>et al.</u>, 1983; Cook <u>et al.</u>, 1984; Simpson and Pedini, 1985; Nash <u>et al.</u>, 1988; Nash, 1990).

Table. 4.1 presents a summary of survival rates, sizes at harvest, normal culture periods and production data in semi-intensive and intensive culture systems under favourable soil conditions. These data form the basis for comparision of the effects of acid sulphate conditions on shrimp culture as monitered in the present study.

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Table. 4.1. Stocking densities, survival rates, size at harvest and production data from semi- intensive / intensive culture systems for *Penaeus monodon*. (Adapted from Kungvankij and Kongkeo. 1988; Kongkeo, 1990)

Stocking density (PL/m2)	Stocking size	Culture period (weeks)	Survival rate (%)	Size at harvest (g)	Equivalant produc- tion (mt/ha/year)
5 -10	PL 25	16 -20	80	33 -40	2.5 -5.0
10 -50	PL 15	16 -20	20	30 -33	5.0 -20.0
50 -100	PL 15	16	65	25 -30	20.0 -40.0
PLE Past Porvac					

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## 4.2. Results.

# 4.2.1. Eh-pH relationships and the behaviour of iron

The Eh-pH relationship observed in water and bottom sediments of 51 shrimp ponds surveyed is given in Fig. 4.1. The values of Eh and pH in this figure were also used for cluster analysis, the results of which are given in Table 4.2. Two clusters could be recognized for both sediments and water. Cluster 1 represents the Eh-pH relationship in extensive and semi-intensive shrimp culture systems (low or moderate stocking density, non-aerated). The Eh-pH relationship of the intensive systems (high stocking density, well-aerated) falls into cluster 2. The same two clusters were observed in both water and in sediments. More reduced redox conditions were observed in sediments in comparison to water, and in water and sediments in non-aerated systems with moderate to low stocking density, in comparison to well-aerated systems with high stocking densities. The pH values in both clusters were slightly basic with slightly higher pH values in cluster 2.

Howarth and Larsen (1983) have used dispersion or scatter (W); dispersion of all values about the grand mean (T); and dispersion between the groups (B) in describing the goodness of clusters obtained. The values obtained for (W), (T) and (B) for the Eh-pH data are given in Table. 4.3. For any given set of data (T) is fixed, so a good grouping criterion is one which minimises (W), or maximises B (Horwarth and Larsen 1983).

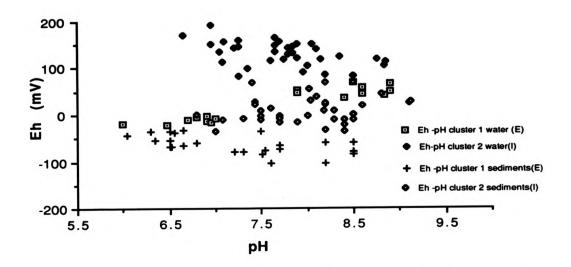


Fig. 4.1. Eh-pH relationships observed in water and sediments of 51 shrimp culture ponds on acid sulphate sediments, West Coast of Sri Lanka. (E) extensive and semi-intensive ponds, (I) intensive ponds.

Table. 4.2. The cluster means, standard deviations and centroid distances for different clusters identified for Eh-pH values in water and sediments of the surveyed shrimp culture ponds (N=51).

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	Cluster	Eh	PH	Centroid
		(mV)		distance.
later	1	21.29 ± 33.79	7.66 ± 0.98	
				109.0
	2	130.29 ± 26.50	7.73 ± 0.50	
Sediments	1	-64.85 ± 19.17	7.16 ± 0.81	
				118.50
	2	22.35 ± 25.24	7.93 ± 0.59	

Table. 4.3. Within group dispersion (W), between group dispersion (B) and total dispersion (T) matrices for Eh-pH data for the water and bottom sediments in shrimp culture ponds developed in acid sulphate soil areas (N=51).

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ter:			
Within group			
dispersion (W)	:	Eh	PH
	Eh	41463.0	730.98
	PH	730.98	25.63
Between group			
dispersion (B)	) :	Eh	PH
	Eh	134651.0	86.16
	PH	86.16	0.06
Total dispers	ion		
- (T)	:	Eh	PH
	Eh	176103.0	340.17
	pH	340.17	25.69
ttom sediments:	-		
Within group			
dispersion (W	) :	Eh	PH
	Eh	14411.43	-48.07
	PH	-48.07	15.71
Between group			
dispersion (B		Eh	PH
-	Eh	63386.05	572.93
	PH	572.93	4.67
Total dispers	ion		
- (T)	:	Eh	PH
	Eh	77809.0	522.33
	PH	522.33	20.83

Results of the determination of Wilks' lambda (^), which is a test statistic originally derived by Wilks (1932) as described by Howarth and Larsen (1983), are given in Table 4.4. Wilks' lambda decreases inversely with the number of clusters (k). Criterion  $^{k^2}$  plotted against k will decrease from a value of 1.0 for k=1 to about 0.5 at k=10 and successful grouping will be partitioned such that the corresponding  $^{k^2}$  value is below this general trend. The values calculated for  $^{k^2}$  (Table 4.4) are 0.88 and 0.66 for water and sediments. Since those values fall below the general trend (Howarth and Larsen, 1983), the grouping can be considered statisticaly valid and meaningful in practice.

Iron can exist in acid sulphate soils in various forms. The mobility of iron and the physico-chemical behaviour of iron in natural systems is controlled to a large extent by redox potential and pH (James, 1954; Ponnamperuma et al., 1967; Ponnamperuma, 1972; Collins and Buol, 1970 a, 1970 b; Breemen, 1976). Reduction of the soil proceeds in the sequence predicted by thermodynamics and produces an array of redox systems ranging from the  $O_2$ -H<sub>2</sub>O system to the H<sup>+</sup>-H<sub>2</sub> system.

Iron and sulphur, moreover, combine in two minerals characteristic of acid sulphate soils: pyrite and jarosite. The stability relations of these compounds as a function of Eh and pH are shown by the Eh-pH diagram for pyrite, jarosite, and ferric oxide (Fig. 4.2). Pyrite is stable over a wide pH range but only under reduced conditions. Jarosites occur under strongly oxidised and

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Table. 4.4. Determination of Wilk's lambda ( ^ ) for Eh-pH data collected from (a) water and (b) sediments of the shrimp culture ponds developed in acid sulphate soil areas (N = 51).

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Water:

 $|W| = | (41463 \times 25.63) - (730.98)^{2} |$   $|T| = | (176103 \times 25.69) - (340.17)^{2} |$   $^{=} |W| / |T|$ = 0.22

For two clusters ^  $k^2 = 0.88$ 

Bottom sediments:

|W| = |(14411.43 x 15.71 ) - (-48.07) \* |
|T| = |(77809 x 20.83 ) - (522.03 ) \* |
^ = |W| / |T|
=0.16
For two clusters ^k \* =0.66

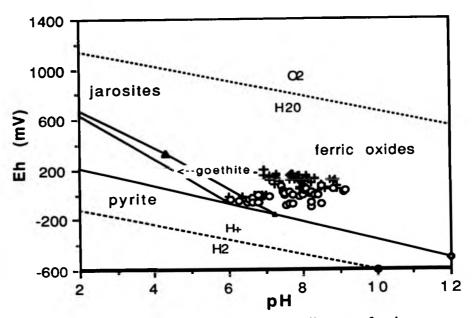


Fig. 4.2. Composite Eh-pH stability diagram for iron systems at  $25^{\circ}$ C and the observed Eh-pH relationships for water and sediments of shrimp culture ponds in Sri Lanka (N =51).(+) Eh-pH relationships for water, (o) Eh-pH relationships for sediments.

acid conditions. Ferric oxides, the oxidation products of pyrite, occur over a wide Eh range and at pH values above 4.

Iron (III) oxides are present in soils as  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> (haematite),  $\tau$ -Fe<sub>2</sub>O<sub>3</sub> τ-FeOOH (lepidocrocite) and (goethite), α-FeOOH (maghemite), Fe(OH)<sub>3</sub>.nH<sub>2</sub>O (hydrated ferric oxide). Of these, Fe(OH)<sub>3</sub>.nH<sub>2</sub>O is the least stable in nature, But in soils that undergo reversible oxidation-reduction over short periods of time " ferric hydroxide" may be the dominant form of iron (III) oxide (Ponnamperuma et al., 1967). Also, because it is the most reactive of the oxides, it may be regarded as the principal solid species involved in redox equilibria in flooded soils. Although ferric hydroxide is not a definite chemical compound, its apparent standard free energy of formation has been calculated from its solubility product and given as -166.0 kcal per mole at 25°C (Latimer, in Ponnamperuma et al., 1967). Solutions of strongly acid soils may also contain  $[Fe(H_2O)_5(OH)]^{2+}$  and  $[Fe(H_2O)_4(OH)_2]^+$  as the products of the hydrolysis of the hexaquo ion  $[Fe(H_2O)_6]^{3+}$ , but will tend to condense to colloidal gels, forming a reddish brown gelatinous precipitate, as pH increases.

Some other reduction products of iron (III) likely to be present in ponds constructed in acid soil areas are the hexaquo iron (II) ion,  $[Fe(H_2O)_6]^{2*}$  and the solids, ferrous carbonate (FeCO<sub>3</sub>), ferrous phosphate (Fe<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>.8H <sub>2</sub>O), ferrous sulphide (FeS), ferrosoferric hydroxide (Fe<sub>3</sub>(OH) <sub>4</sub>), a hydrated variant of (Fe<sub>3</sub>O<sub>4</sub>) ferrosoferric oxide, and perhaps Fe <sub>4</sub>(OH)<sub>10</sub>, a black compound of

Fe<sub>3</sub>(OH)<sub>8</sub> and Fe<sup>2+</sup> (Ponnamperuma <u>et al.</u>, 1967; Collins and Buol, 1970 a, 1970 b; Ponnamperuma, 1972).

In most acid sulphate soils, part of the ferric iron released by pyrite oxidation is tied up in jarosites but the bulk is present in free oxide form (Breemen, 1976). From Fig. 4.2, the majority of Eh-pH values observed during this study are located in the region where ferric oxides are the most stable form of iron. However, the Eh-pH relationships observed for the low /moderate stocking density, non-aerated, systems were located closer to the pyrite zone than the those of high stocking density, aerated, systems. This finding indicates that Eh-pH conditions are more favourable for the formation of iron oxides / hydroxides in intensive systems and thus cultured organisms in such intensive systems are more likely to be exposed to iron hydroxides.

4.2.1.1. Models of pyrite oxidation in shrimp culture ponds.

Pyrite is only stable under slightly oxidised conditions (Fig. 4.2). The most important stages in pyrite oxidation has been summarised by Bloomfield and Coulter (1973), Breemen (1976) and by Dent (1986). According to Dent (1986), pyrite oxidation can be described by the following formula:

 $FeS_2 + 15/4 O_2 + 7/2 H_2O -----> Fe(OH)$ ,  $+2 SO_4^2 + 4H^*$ 

Several models have been developed to describe pyrite oxidation in agriculture (Breemen, 1976; Dent, 1986), but none yet describe oxidation in shrimp aquaculture ponds. In Sri Lanka and other countries in Asia, ponds are commonly dried before stocking. Following 2 to 3 weeks of drying, they are then filled with brackish water. An illustration of the oxidation and reduction processes which take place in the sediment environment during drying and filling the pond is given in Fig. 4.3. During drying, oxygen diffuses into the sediments and Fe (II) ions are oxidised to Fe (III) ions or oxides. At low pH, some Fe (III) can remain in wet layers and can diffuse to the pyrite surface where it is reduced to Fe (II), liberating acidity. Fe (II) may then diffuse back towards the oxidation front where it is oxidised. This may result in the formation of ferric oxides (Plate 4.1). After filling, redox potential decreases and Fe (II) ions can migrate out of the sediments into the overlying waters (Dent, 1986).

Similar processes can explain the various oxidation and reduction processes in shrimp ponds with and without aeration (Fig. 4.4). After filling, the redox potential of the soil will tend to decrease, resulting in an increase in pH. This process will promote the release of Fe<sup>2+</sup> into the water, particularly in unmanaged ponds where there is no adequate neutralising agent, such as lime, to neutralise acidity. The reactions can be described as follows:

Fe(OH)<sub>3</sub> + 3H<sup>\*</sup> + e -----> Fe<sup>2\*</sup> + 3H<sub>2</sub>O. 3 Fe(OH)<sub>3</sub> +H<sup>\*</sup> + e ----> Fe<sub>3</sub>(OH)<sub>8</sub> +3Fe<sup>2\*</sup> +8H<sub>2</sub>O. Fe<sub>3</sub>(OH)<sub>8</sub> + 8H<sup>\*</sup> + 2e ----> 3Fe<sup>2\*</sup> + 8H<sub>2</sub>O

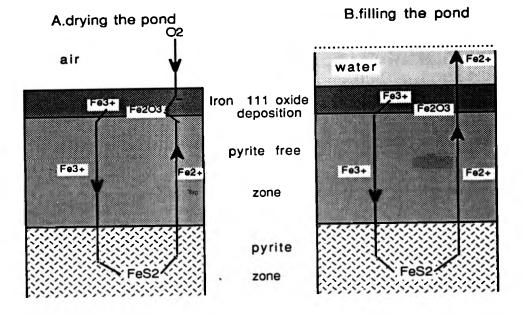


Fig. 4.3. Model of pyrite oxidation in shrimp culture ponds constructed from acid sulphate soils. (A) during pond drying, (B) after filling ponds with water.

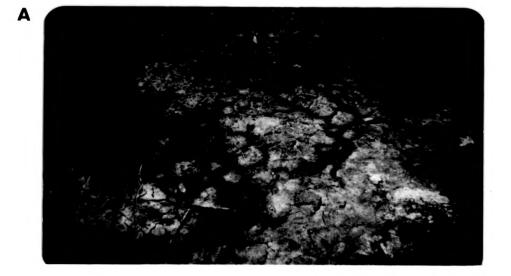


Plate. 4.1. Iron hydroxide accumulation on sedment of a shrimp pond developed from acid sulphate soils during drying the pond (A). Close up view of iron oxide formation on pyrite bearing soil (B).



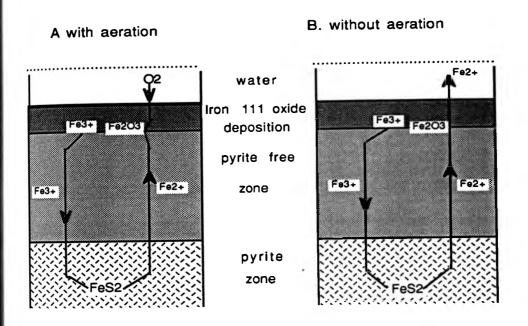


Fig. 4.4. Model of pyrite oxidation in shrimp culture ponds constructed from acid sulphate soils. (A) with aeration, (B) without aeration.

During pond aeration, these reactions will take place in the opposite direction and redox conditions will favour the formation of ferric hydroxides. The basic pH values maintained by the application of lime during culture practices will also enhance these processes.

Iron hydroxides are colloidal in nature and tend to precipitate on to the sediment-water interface, the niche occupied by cultured penaeid shrimps and their natural benthic food organisms. Most cultured shrimps are nocturnal in behaviour and live half buried in sediments during the day time (Apud <u>et al</u>, 1989). The fineness of the grain size of the iron hydroxide precipitate increases as repeated oxidation followed by reduction takes place, as during continued drying and flooding cycles (Breeemen, 1976). Thus, there may be a tendency for the fineness of iron hydroxide precipitates to increase over several culture cycles, increasing the possibility of accumulation of iron hydroxides in shrimp gill filaments.

The iron concentrations in gills, abdominal muscles and in the abdominal carapace of the cultured shrimps in relation to sediment iron concentration, culture time in a time series study will be discussed in detail in Chapter 5. Results of a parallel histochemical study and SEM and TEM studies on the gill structure and deposits are presented in Chapter 6.

4.2.1.2. Redox potential-pH relationships in water and sediments of culture ponds during a 10 week culture cycle.

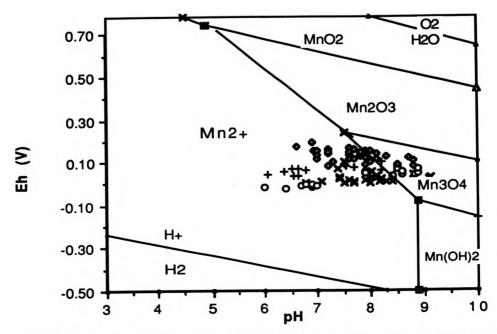
The redox potential- pH relations observed in pond water and in bottom sediments during a 10 week cycle of *P. monodon* culture in the experimental ponds are discussed in sections under water quality in this chapter. The EhpH values in water and sediments fall into the goethite (FeO.OH) and ferric oxide areas of the Eh-pH diagram (Fig. 4.2).

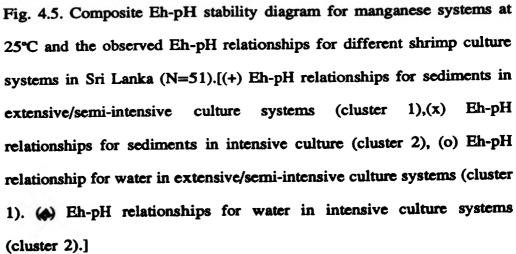
4.2.2. Eh-pH relationships and behaviour of manganese.

The observed Eh-pH relationships for extensive and semi-intensive culture systems fall into cluster 1 in both water as well as in sediments, while cluster 2 represents the Eh-pH relationships of the intensive culture systems in Fig. 4.5. It is apparent that  $Mn^{2*}$  is the most stable form of manganese in most of the ponds. However, the Eh-pH relationships of the intensive culture systems partially lie in the  $Mn_3O_4$  field indicating the tendency to form precipitates of manganese hydroxides in such ponds.

The concentration of total manganese in gills, abdominal muscles and in the abdominal carapace of P. monodon sampled in intensive ponds on acid sulphate soil and the correlation of these concentrations with sediment manganese, sediment iron concentrations and culture time are discussed in Chapter 5.

A parallel histological study using specific stains and SEM techniques showed





that deposits in between gill lamellae of these cultured shrimps may contribute to the relatively high concentrations of manganese in gills (Chapter 6). Moreover the shrimps with high concentration of manganese in their gills also exhibited brown gill and black gill syndrome which has been identified as a common pathological sign in the shrimp grown in acid sulphate soil areas (Nash <u>et al.</u>, 1988).

#### 4.2.3. Water quality.

The salinities observed in the surface and bottom waters of the studied culture ponds are shown in Fig. 4.6. The salinities at the surface fluctuated between 15 and 21 %, while the those near the bottom varied from 16 to 21 %. During most of the sampling periods the pond water remained homogeneous (without vertical stratification) with respect to salinity.

Fluctuations in pH values during the culture period are shown in Fig. 4.7 A. The pH values in the surface waters were slightly higher than those closer to the bottom throughout the sampling period. The pH varied from 7.7 to 8.4 in the surface waters and between 7.4 and 8.3 in the bottom layer.

Fig. 4.7 B. presents the variations in Eh values observed during the study period in the surface and bottom layers of the ponds. Although the Eh fluctuations of the surface and bottom waters followed the same pattern, the surface waters remained relatively more oxidised than the bottom waters throughout the sampling period. The variations in Eh values were between 58

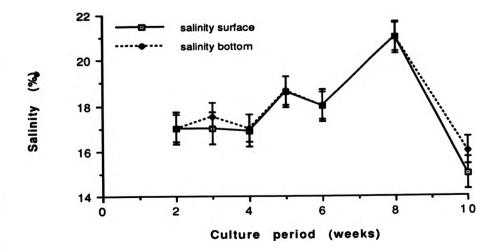


Fig. 4.6. Variation in average salinity  $(\pm sd)$  in surface and bottom water during the culture cycle in shrimp culture ponds constructed on acid sulphate soils.

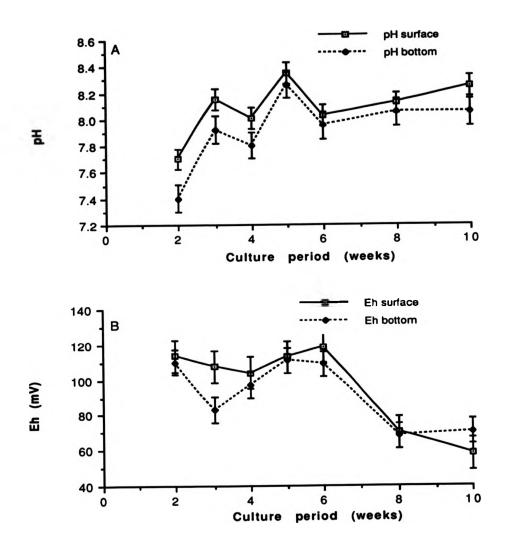


Fig. 4.7. Variation in average (a) pH and (b)  $Eh_{,(\pm sd)}$  in surface and bottom water during the culture cycle in shrimp culture ponds constructed on acid sulphate soils.

mV and 118 mV of the surface and 68 mV to 110 mV at the bottom. However, at both levels Eh became lower during the latter part of the culture cycle, indicating more reduced conditions with time.

In this present study the dissolved oxygen concentration in the surface waters fluctuated between 6.8 and 7.8 mg/l, while fluctuations of the bottom waters were between 6.3 and 7.4, ppm (Fig. 4.8 A). The dissolved oxygen levels were relatively high during the initial period of culture and declined gradually towards the fourth week, thereafter they rose to reach a maximum value. Dissolved oxygen levels near the pond bottom followed the same trend as the surface waters, but the values decreased with time through the culture period.

The total phosphorus concentration in the surface and the bottom waters of the shrimp culture ponds studied are shown in Fig. 4.8 B. The recorded phosphorus concentrations in the surface waters of the ponds varied between 0.01 ppm and 0.06 ppm. The highest concentrations were recorded in the first two weeks. The phosphorus concentrations of the bottom waters were slightly lower than those at the surface, but followed a similar pattern of changes with time except for the fourth, sixth and tenth weeks. The variations were between 0.01 ppm and 0.06 ppm.

The nitrate concentration of the surface water layers in the studied ponds varied between 0.56 ppm to 2.53 ppm while in the bottom water it fluctuated

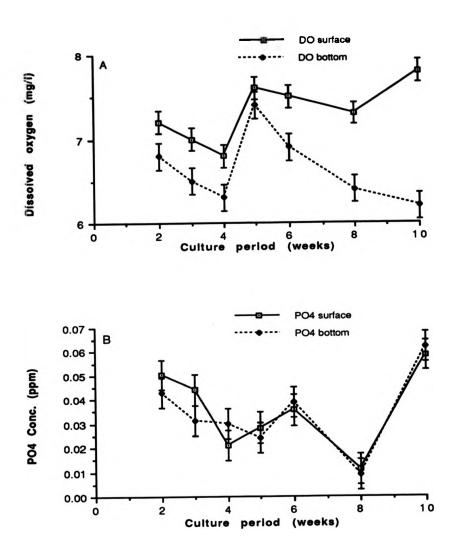


Fig. 4.8. Variation in average (A) dissolved oxygen and (B) phosphate concentration,  $(\pm sd)$  in surface and bottom water during the culture cycle in shrimp culture ponds constructed on acid sulphate soils.

between 0.26 ppm and 2.32 ppm (Fig. 4.9 A).

In the present study, the nitrite concentration varied between 0.47 ppm and 0.01 ppm in the surface waters of the pond. In the deeper layers of water it fluctuated between 0.46 and 0.02 mg/l. The highest nitrite concentration was recorded in the fifth week of the culture cycle for both surface and bottom waters (Fig. 4.9 B).

The sulphide concentration of pond water fluctuated between 0.016 and 0.040 ppm (the pH range 7.4 to 8.3) and is shown in Fig. 4.10 A. The highest sulphide concentrations recorded occurred in weeks 6 to 10.

Fig. 4.10 B illustrates the change in chlorophyll a concentration in the culture ponds during the culture period; values ranged from a minimum of 1.65  $\mu g/l$  l in week 2 to a maximum of 13.3  $\mu g/l$  in week 5.

Fig. 4.11 A presents the water temperatures observed in the surface and bottom layers of the ponds during the culture cycle. The water temperature of the surface waters fluctuated between 25.5°C and 29.0°C, while the bottom temperature ranged between 25.0°C and 29.5°C.

The turbidity values recorded in the bottom waters were consistently higher than those of the surface waters. Fig. 4.11 B shows the variation in turbidity observed at the surface and bottom of the ponds during the culture period.

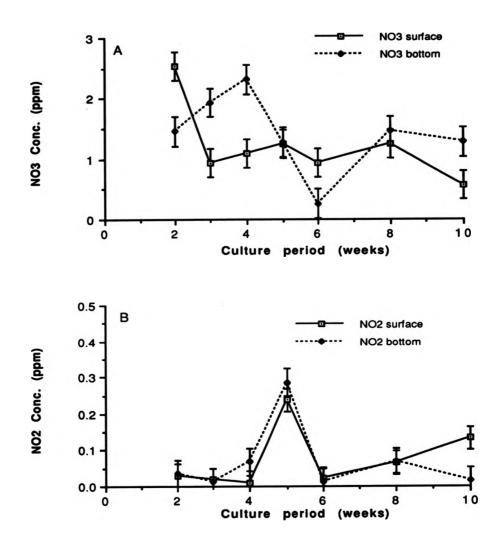


Fig. 4.9. Variation in average (A) nitrate concentration and (B) nitrite concentration,  $(\pm sd)$  in surface and bottom water during the culture cycle in shrimp culture ponds constructed on acid sulphate soils.

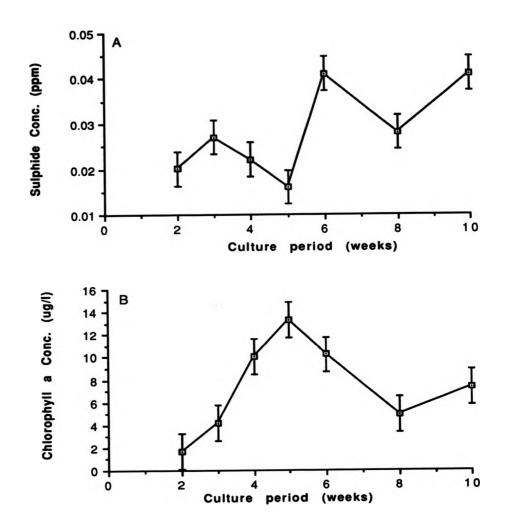


Fig. 4.10. Variation in average (A) sulphide concentration and (B) chlorophyll a concentration,  $(\pm sd)$  in water during the culture cycle in shrimp culture ponds constructed on acid sulphate soils.

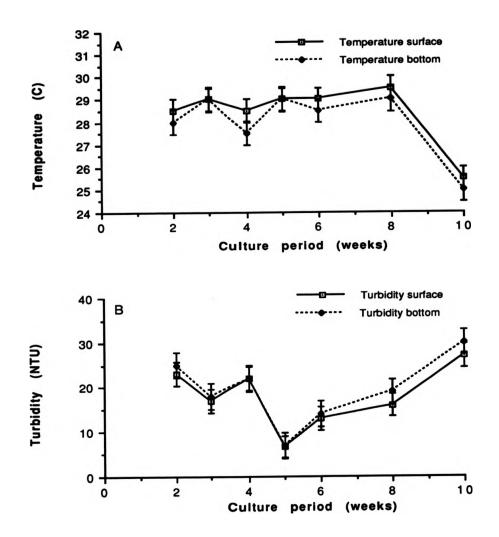


Fig. 4.11. Variation in average (A) temperature and (B) turbidity  $(\pm sd)$  in surface and bottom water during the culture cycle in shrimp culture ponds constructed on acid sulphate soils.

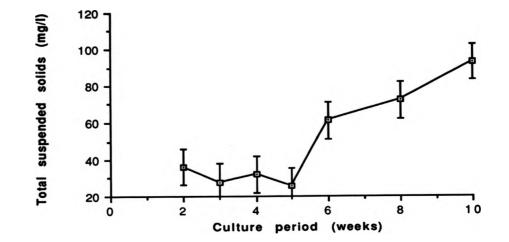
Turbidity fluctuated from a minimum of 6.5 NTU to a maximum of 27 NTU at the surface and from 7.0 NTU and 30 NTU in the bottom water layer. The lowest value occurred in week 5.

The observed fluctuations in total suspended solids are given in Fig. 4.12. Total suspended solids fluctuated between 26 and 93 mg/l during the culture period, with the highest levels occurring during the latter part of the study from about week 6.

### 4.2.4. Soil quality

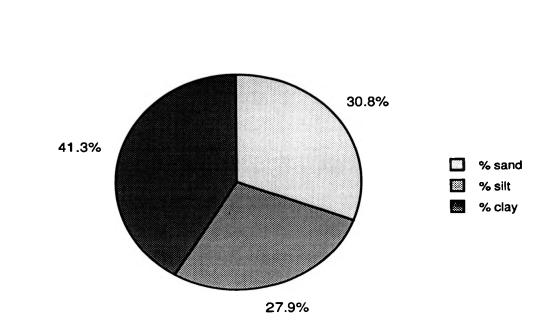
Fig. 4.13 shows the average distribution of the sand, silt and clay particles in the pond sediments. The sediments contained a relatively high percentage of clay (41.3  $\pm$  7.15), followed by silt (27.9  $\pm$  9.2) and sand (30.8  $\pm$  6.24). According to conventional classification systems based on triangulation of the basic soil textural classes sediments can be classified as clayey sediments (FAO, 1985).

The variations in sediment redox potential are shown in Fig. 4.14 A. These fluctuated between +19 and -118 mV during the experimental period. Sediments remained in more oxidised states during the initial period of culture but progressively became more reduced during the culture period. Consequently, a strong negative correlation was observed between sediment Eh and the culture period (r= -0.939; p = .0008).



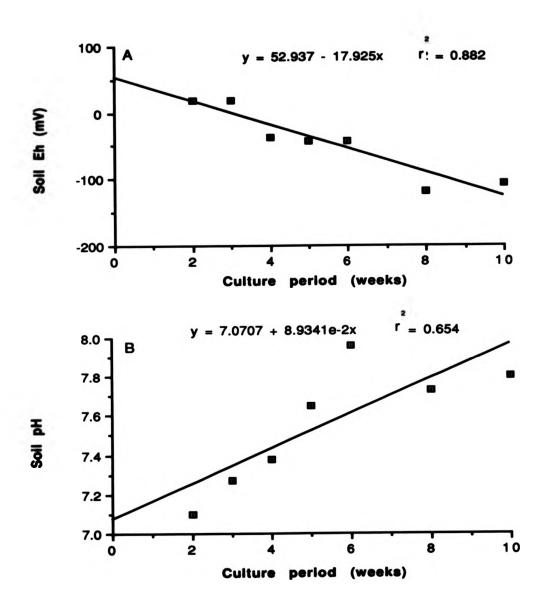
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Fig. 4.12. Variation in total suspended solids  $(\pm sd)$  in pond water during the culture cycle in shrimp culture ponds constructed on acid sulphate soils.



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Fig. 4.13. Average particle size distribution (% by weight) in the soils of shrimp culture ponds studied. (clay < 0.002 mm; silt 0.002 - 0.05 mm; sand 0.05 - 2.0 mm).



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Fig. 4. 14. Variations in average sediment (A) Eh and (B) pH with culture period in studied shrimp culture ponds.

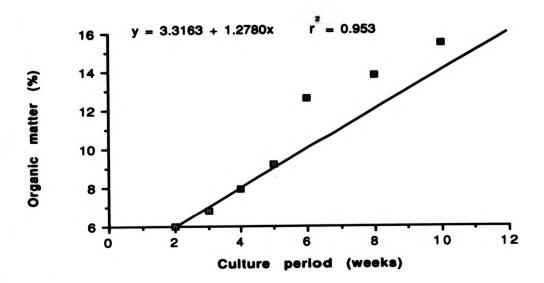
Variations in sediment pH during the culture cycle are shown in Fig. 4.14 B. The sediment remained slightly basic throughout the culture period, and fluctuated in pH over a narrow range from 7.1 to 7.96. The sediment pH was not highly significantly correlated to the culture period (r = 0.801; p= .027).

The variation in organic matter content in sediments of the studied ponds is shown in Fig. 4.15. The percentage of organic matter content in pond sediments varied between 6.01 and 14.19 and showed a tendency to increase during the culture period. The correlation between organic content and time was significant (r = 0.976; p = <.001).

The fluctuations in total iron, total manganese and available aluminium in pond sediments recorded during the culture period are discussed in detail in Chapter 5 in relation to the kinetics of these heavy metals in culture ponds.

4.2.5. Growth, survival and production of shrimps in ponds sited on acid sulphate soils.

In the ponds, shrimps recorded an average weight of 23.6 g after 10 weeks. Fig. 4.16 shows the size frequency of shrimps each week during the experimental period while Fig. 4.17 shows the variations in their mean weight. The survival rate of the cultured shrimps varied between 26.8 and 44.9% with a mean of 35.1 %. The variation in total production was from 975 kg /ha /crop to 1326 kg / ha /crop with a mean of 1242 kg /ha /crop.



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Fig. 4. 15. Variation in average, organic matter content (%) with culture period in sediments of studied shrimp culture ponds.

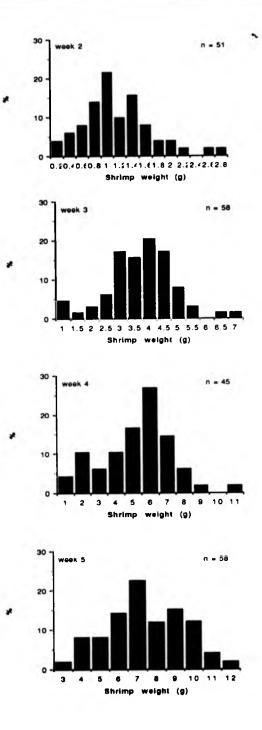


Fig. 4.16. Size frequency distribution of cultured shrimp in weeks 2 to 10 of the production cycle. Data have been grouped into the following weight classes: week 2-0.2 g; week 3-0.5 g; week 4 to 6-1.0 g; week 7-2.0 g; week 8 to 10-1.0 g.

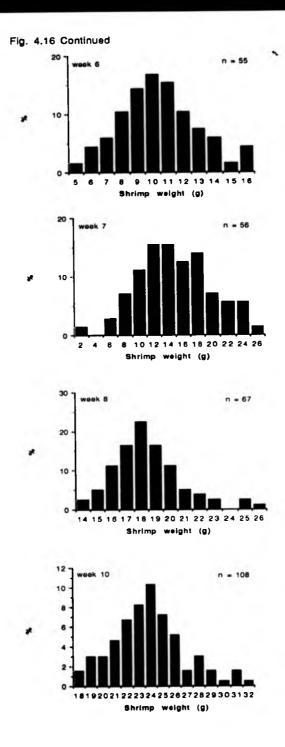


Fig. 4.16. Size frequency distribution of cultured shrimp in weeks 2 to 10 of the production cycle. Data have been grouped into the following weight classes: week 2-0.2 g; week 3-0.5 g; week 4 to 6-1.0 g; week 7-2.0 g; week 8 to 10-1.0 g.

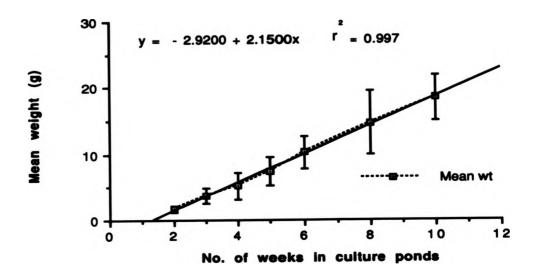


Fig. 4.17. Mean weight  $(\pm sd)$  of shrimp recorded each week during the culture period. Means calculated from weights of 51 to 150 shrimp. Regression (solid line) fitted by method of least squares.

## 4.3. Discussion

## 4.3.1. Behaviour of iron and manganese.

The results of this study show for the first time something of the significance of redox potential-pH relations in the water and sediments of shrimp culture ponds developed on acid sulphate soils.

The accumulation of iron has been shown by several studies to be an important problem causing significant mortalities and sub-lethal stress in cultured shrimps (Simpson <u>et al.</u>, 1983; Wickins, 1984 a; Lightner, 1988; Nash <u>et al.</u>, 1988; Nash, 1990). The present study has quantified the conditions under which this accumulation may occur. It also indicates that aeration and liming of culture ponds on acid-sulphate soils may actually promote conditions which favour the formation of harmful ferric hydroxides, contrary to normal management practices which advocate use of aerators and lime application (Chiu, 1988 b; Apud <u>et al.</u>, 1989; Boyd, 1989; Poernomo, 1990).

Rainbow (1988) did not report on manganese but has suggested that deposition of other metals such as iron, on various tissues may contribute to very high concentrations. The present study suggests that determining iron and manganese concentrations in sediments together with selected tissues of cultured shrimps may be useful as a general indicator of stress generated by acid sulphate soil conditions.

The study also suggests that more emphasis should be placed on manipulating the Eh-pH of pond environments to ensure that the pond conditions do not favour the formation of potentially harmful precipitates. However, the liming and aeration practices normally employed are likely to result in formation of these precipitates on farms with acid sulphate soils.

In summary, the results indicate that more attention should be given to monitoring and manipulation of redox-pH in water and sediments of shrimp culture ponds to minimise risks associated with ferric hydroxide precipitation and manganese hydroxide. Under conditions where iron accumulation cannot be prevented, care should be taken to regulate the use of artificial aeration and liming of ponds as far as possible.

## 4.3.2. Water quality

The salinities recorded during this study remained within the favourable ranges for *P. monodon* (Chiu, 1988 b; Apud <u>et al.</u>, 1989; Boyd, 1989). It may be noted that, in spite of the acid sulphate nature of the pond sediments, the pH values of the surface waters as well as the bottom waters remained basic throughout the sampling period. However, in the present study the higher pH values would have increased the possibility of promoting the formation of insoluble iron and manganese compounds as indicated by the Eh-pH stability diagrams (Breemen, 1976; Collins and Buol, 1970 a, 1970 b) for these heavy metals. On acid sulphate soils, low pH has been recorded as the primary

problem by Neue and Singh (1984); Simpson <u>et al.</u>(1983); Singh (1985) and Lin (1986) in partially reclaimed or unreclaimed ponds, but the ponds used in this investigation had received lime before and during the present study and recorded basic pH values.

The Eh-pH relationships observed in this study fall within the range where hydrated oxides of the iron and  $Mn^{2+}$  ions of manganese are stable in their respective Eh-pH diagrams as indicated earlier.

Respiration amounted to 22.5% of the total output of oxygen in an oxygen budget calculated for fish ponds in Alabama (Boyd, 1989). Recent studies in intensively managed shrimp ponds (Madenjian, 1990; World Shrimp Farming, 1991) indicate that the percentage of total oxygen consumed at night by shrimp is only a very small part of the total pond respiration, which includes respiration in the water column and sediments. It amounts to only 3 % of the total in the early stages of culture, increasing to 6.3 % during the latter part of the culture period. This suggests that controlling the other main respiratory components, in the water column and sediments could be an important aspect of shrimp culture pond management.

From what has been revealed about the Eh-pH relationships and the behaviour of heavy metals such as iron and manganese in the pond environment it is concluded that highly oxygenated conditions have an adverse impact on shrimps cultured in acid sulphate soil areas through their stabilising effect on the insoluble hydrated oxides of iron and manganese.

Considering the near saturation levels of dissolved oxygen recorded in the shrimp culture ponds in this study, and the "safe level" of dissolved oxygen (around 3 mg/l) for intensive shrimp ponds (World Shrimp Farming, 1991) there is scope to reduce present aeration levels to minimise the formation of harmful hydrated oxides of heavy metals in acid sulphate soil ponds.

The land use pattern of an area may influence the phosphorus concentration in adjacent ponds. Activities such as animal husbandry and agriculture activities have been shown to increase the phosphorus concentration of ponds constructed in such areas (Boyd, 1982).

The major land use categories of the study area include considerable amounts of agricultural land. These ponds have been fertilized with phosphate fertilizers (T.S.P) at a rate of 20 kg per ha during the pond preparatory stages.

The poor response to phosphate fertilizer and low phosphate concentrations due to phosphate fixing properties of acid sulphate soils are well documented (Chang and Chu, 1961; Watts, 1968). In the acid sulphate soil environment oxidation processes promote the precipitation of phosphates together with iron (III) hydrated oxides decreasing the phosphate concentration of the overlying water. This tendency has been clearly demonstrated by Attanandana and Vacharotayan (1989) and by Willett (1989). The relatively low phosphate levels recorded in this study also indicate the possibility of phosphate fixation in the acid sulphate soil environment.

In acid sulphate soils under oxidised conditions the formation of  $SO_4^2$  is more favoured (Ponnamperuma, 1972), thus under the oxidised conditions observed in the present study, high levels of sulphide would not be expected. Moreover the pH levels observed in the present study were in the range of 7.0 to 8.5.

## 4.3.3. Soil quality.

The sediment of the area where the studied culture ponds are located falls into the potential acid sulphate soil class of ripe clay with sulphidic subsoil, and the detailed soil profile form of p3cw1 according to the ILRI classification system suggested for acid sulphate soils (Dent, 1986). The sediments contained a relatively high proportion of clay (34.4 to 48.7 %). In general the soil texture of the ponds falls within the acceptable ranges for aquaculture recommended by FAO (1985) and Pillay (1990).

These ponds had received considerable amounts of lime during their preparatory stages and showed slightly basic pH values. The process of drying ponds prior to stocking exposes the sediments to air so that they are in an oxidised state at the beginning of the culture period. Filling the ponds with water before stocking, prevents the diffusion of oxygen from air into the sediments and lowers the soil redox potential.

Organic matter can play an important role in ponds constructed in acid sulphate soil areas. Under aerobic conditions organic matter generates electrons and the principal electron sink is oxygen. But in culture ponds where an anaerobic environment exists, the decomposition of organic matter continues to activate reduction processes. According to Jenne (1968); Ponnamperuma et al. (1967) and Ponnamperuma, (1972) the sequence of reduction in acid sulphate sediments is firstly NO<sub>3</sub>, followed by manganese oxide, iron (III) oxide and sulphates.

This reduction is accompanied by increase in  $Fe^{2+}$  ions as follows: Fe(OH)<sub>3</sub> +2H<sup>+</sup> +1/4 CH<sub>2</sub>O -----> Fe<sup>2+</sup> +11/4 H<sub>2</sub>O +1/4 CO<sub>2</sub>

The amount of iron hydroxide will decrease in the sediments thereby increasing the  $Fe^{2+}$  ion content.

Jenne (1968) lists various organic substances, especially those containing hydroxy and /or carboxilic functional groups (phenols, polyphenols, gallic acid, tannic acid etc), that can reduce both ferric and manganic oxides reasonably fast. Organic matter can bring about these changes in the following three main ways:

(a). Direct reduction of ferric and manganic oxides by organic matter.

(b). Indirect reduction of organic matter via lowering of pH and Eh of the

system because of the other organic matter reactions.

(c).Direct biological reduction of oxides.

4.3.4. Growth, survival and production of shrimps in ponds sited on acid sulphate soils.

In the areas of Sri Lanka where problems related to acid sulphate soil conditions exist, shrimps are harvested before 10 weeks to prevent economic losses due to mass mortalities (NARA, 1988). The shrimps harvested in the present study after a ten week period were smaller than the size expected under favourable soil conditions with similar management strategies. In intensive culture practices the expected weight of harvested shrimps is around 35 g (Kungvankij and Kongkeo, 1988) and production is in the range of 8 to 10 mt/ha /year based on 25/ m<sup>2</sup> stocking density; 16 to 20 week culture period; 2 to 2.5 crops per year (Kungvankij and Kongkeo, 1988). The total production in the present study (0.975 to 1.3 mt/ ha/ crop) was well below these targeted production rates and the average production rate of 4.5 mt /ha/ year in Sri Lanka (World Shrimp Farming, 1990).

The survival rates of cultured shrimps under the present experimental conditions (26.8 % to 44.9 %) were low in comparison to the normal range of 60 to 70 % survival recorded using similar management practices under more favourable soil conditions (Kungvankij and Kongkeo, 1988; Apud <u>et al.</u>, 1989).

# CHAPTER 5

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THE KINETICS OF IRON, MANGANESE AND ALUMINIUM IN SHRIMP CULTURE PONDS DEVELOPED ON ACID SULPHATE SOIL AREAS.

# 5.1. Introduction.

Acid sulphate soils include among their characteristics the presence of relatively high concentrations of harmful metals. These heavy metals can be influenced by various processes in the environment to accumulate in culture organisms, be released or undergo other chemical changes which may be detrimental to plant and animal life (Ponnamperuma, 1972; Lindsay and Schwab, 1982; Singh, 1985).

Pyrites (FeS<sub>2</sub>), the main chemical compound responsible for acid sulphate soil conditions can be present in levels as high as 10 % by weight (Breeman and Morman, 1978). Other important materials suspected of being harmful to cultured organisms include compounds of manganese and aluminium.

The utilisation of acid sulphate soil in pond culture results in processes that lead to various chemical changes related to the stability of the metal compounds. Usually potential acid sulphate soils are located at relatively deeper layers of the soil profile. During the construction of ponds these deeper layers are exposed to air, stimulating the oxidation of pyrites. The resulting acidification caused by the production of sulphuric acid can induce the release of toxic concentrations of Al<sup>3+</sup>, Mn<sup>2+</sup>, and often Fe<sup>2+</sup>(Pons, 1973; Singh, 1985; Dent, 1986). Filling these ponds with water prior to stocking then induces soil reduction processes as a result of the limited supply of oxygen. This also influences the stability relations of iron, manganese and aluminium compounds in the environment. Other activities such as liming, artificial aeration and water exchange affect the redox potential-pH relationship in both the sediment and water. It has been well established that the stability relations of various iron, manganese and aluminium compounds in the environment are controlled by the Eh-pH relationships in the immediate vicinity (Ponnamperuma <u>et al.</u> 1967; Collins and Buol, 1970 a, 1970 b; Breeman, 1976). These stability relationships are extremely important in understanding the processes which lead to stress and pathological changes in cultured animals and the bioaccumulation of these metals.

Accumulation of metals and their distribution in the various tissues of organisms has been extensively investigated. Metal concentrations in aquatic organisms have been attributed to various causes: to industrial activity and urban effluent (Barker and Schofield, 1981; Leed and Belanger, 1981; Badsha and Goldspink, 1982; Khalaf et al., 1985; Correa, 1987); to metals in feed and contamination of feed (Dabrowski, et al., 1981; Dallinger and Kautzky, 1985; Dallinger, et al., 1987); and to acidification of waters due to acid rains (Lee and Harvy, 1986; Haines et al., 1987; Segner et al., 1988). Several studies have shown that metal levels in fish increase in association with metal levels in the water, sediments or in their food (Mathis and Cummings, 1973; Patrick and Loutit, 1978).

Manganese is one of the dominant inorganic elements in acid sulphate soil areas. In the shrimp culture environment manganese can exist either in reduced or oxidised forms. Pyrolusite  $(MnO_2)$ , bixbyite  $(Mn_2O_3)$ , hausmannite  $(Mn_3O_4)$  and pyrochroite  $(Mn(OH)_2)$  are the oxidised forms of manganese compounds most commonly found in acid sulphate soil environments (Collins and Buol, 1970a, 1970 b). The soluble  $Mn^{2+}$  is the reduced form of manganese. The Eh- pH relationships and the stability of different manganese compounds are discussed in detail in chapter 4. Although soluble  $Mn^{2+}$  and  $Mn_3O_4$  appears to be the most stable form of manganese in the shrimp culture systems developed in acid sulphate soil areas, the possibility of  $Mn_2O_3$  or  $Mn(OH)_2$  occurring under relatively higher lower Eh and high pH conditions cannot be ruled out.

Aluminium is one of the dominant elements in soil and one of the most important inorganic components in the acid sulphate soil areas. So far very little effort has been made to understand the bioavailability, bioaccumulation and possible pathological effects of aluminium in organisms cultured in acid sulphate soil areas. The information available deals mainly with the effect of various types of organic matter on the exchangeable aluminium and dissolved aluminium levels of fish ponds constructed on acid sulphate soils (Hechenova, 1983; Poernomo, 1983; Singh, 1985). On the other hand extensive studies have been carried out on pathology, toxic effects and bioaccumulation of aluminium in fish and other aquatic organisms in relation to acid rains and the acidification of waters by other forms of pollution (Muniz and Livestad, 1980; Karlsson <u>et al.</u>, 1986).

Gibbsites and Kaolinite are the most abundant forms of aluminium in the sediments and the solubility of these compounds controls the concentration of dissolved aluminium in the pond water. According to Karlsson <u>et al.</u> (1986) bioavailability of aluminium with respect to its accumulation in fish tissues depends mainly on the pH. Between pH values of 4.0 and 5.2 aluminium can be acutely toxic to fish at concentrations as low as 0.1 mg/l (Driscoll <u>et al.</u>, 1980). At pH level 4 and below the toxic effects of the elevated hydrogen ion concentrations are more important than the presence of low concentrations of aluminium (Schofield and Trojnar, 1980). The ambient aluminium concentration in the environment is also important to aluminium accumulation in tissues (Exly and Phillips, 1988). pH values lower than 5.5 has been shown necessary for significant aluminium bioaccumulation in fish tissues (Odonnell et al., 1984).

The work described in this section was conducted in parallel to the time series studies on the pond environment during the culture of shrimps. The stability relationships of different heavy metal compounds in the acid sulphate soil environment have not been fully investigated in the context of heavy metal effects on cultivated aquatic organisms.

## 5.2. Results.

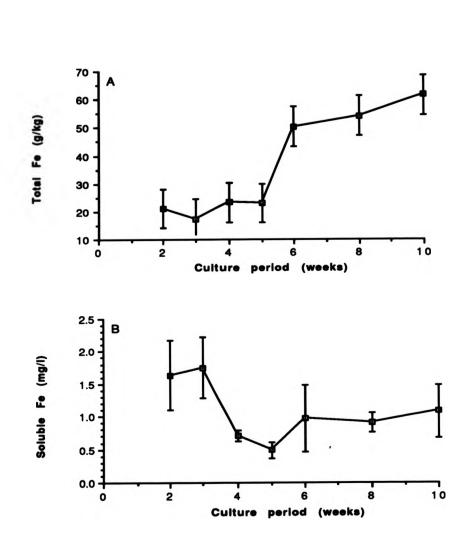
# 5.2.1. Iron in sediments.

The variation in the total iron concentrations in sediments during the culture cycle is given in Fig. 5.1 A. The fluctuation in total iron concentration was between 17.40 g /kg and 61.48 g /kg. Relatively low concentrations were recorded up to the 5th week of culture, then a very sharp increase in total iron was observed from a level of 22.9 g/kg in week 5 to a level of 50.2 g /kg in the 6th week.

It may be noted that artificial aeration was initiated during the 4th week of culture using paddle wheel aerators. By increasing the oxidation processes, aeration would have been the major factor contributing to elevated iron values in the sediments due to accumulation of precipitated hydroxides.

## 5.2.2. Iron in water.

Dissolved iron concentrations measured in the pond water over the culture cycle are given in Fig. 5.1 B. These varied from 0.49 mg/l to 1.75 mg/l. Values were higher in the initial period of culture indicating that there was an influx of ferrous ions from reduced sediments after the pond was filled with water. With the initiation of aeration during the fourth week, the total iron concentration dropped and remained low throughout the rest of the culture cycle.



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Fig. 5.1. Iron concentrations in (A) surface sediments (total), and (B) in water (soluble) in relation to the culture period.

5.2.3. Iron in gills of cultured shrimps.

The gills of shrimps showed the highest concentration of iron of the tissues studied. Fig. 5.2 A shows total iron concentration in shrimp gills during the culture cycle. The values range from a minimum of 738  $\mu$ g /g dry wt (2nd week) to 1588  $\mu$ g /g dry wt (6th week).

The total iron concentration in gills showed a highly significant correlation (r=0.911, p=0.0043) with the total iron concentration in sediments and a significant correlation (r=0.867, p=0.0135) with the culture period indicating that iron probably accumulates steadily during the culture period. There was no clear correlation between the iron content of the gills and the concentration of dissolved iron in the culture medium.

5.2.4. Iron in carapace cuticle of cultured shrimp.

The concentrations of iron in the carapace cuticle of shrimp were lower than those in the gills, but higher than those of the abdominal muscles (Fig. 5.2 B). Carapace iron values were between 359  $\mu$ g / g and 778  $\mu$ g /g dry wt and were significantly correlated (r=0.871, p=0.0105) with the length of the culture period; this again indicates that iron accumulates in the shrimp during the culture period. The concentrations of total iron in sediments did not produce any significant correlation (r =0.6959, p=0.082) with the concentrations of iron in the cuticle as in the case of gills, neither were they correlated with the iron concentration in water.

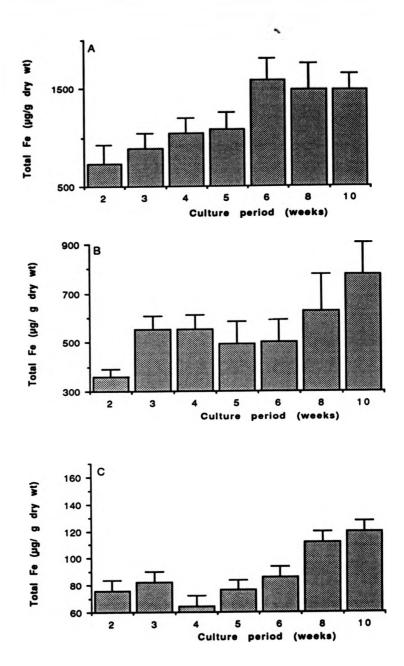


Fig. 5.2. Total iron  $(\pm sd)$  in (A) gills, (B) carapace cuticle and (C) abdominal muscle of shrimp in relation to the culture period.

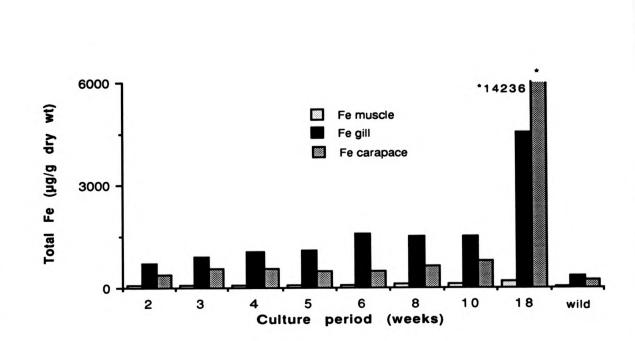
5.2.5. Iron in muscles of cultured shrimps.

The variation in the total concentration of iron in the abdominal muscles of shrimps during the culture period is given in Fig. 5.2 C. Iron concentration in muscles varied from 76  $\mu$ g /g dry wt (2 nd week) to 119.9  $\mu$ g /g dry wt (10 th week).

The total iron concentration in muscle showed a significant correlation with the culture period (r =0.8716, p=0.0105), and with the total iron concentration in sediments (r=0.8649, p=0.01195).

5.2.6. Iron in wild shrimps and shrimps exposed longer period for acid sulphate environment.

Fig. 5.3 compares the concentrations of iron in gills, abdominal carapace and in the muscles of cultured shrimps, shrimps exposed to acid sulphate soils for 18 weeks and wild shrimps of similar weight. The iron concentrations of all the tissues in wild shrimp showed relatively low levels in contrast to the tissues of shrimps exposed for a period of 18 weeks. There was a 13-fold increase in iron in gills and a 1.8-fold increase in muscles of the cultured shrimps when compared to those of wild ones. The cuticle of shrimps exposed for acid sulphate soil conditions for 18 weeks had unusually high concentrations of iron. It may also be noted the shrimps exposed to the acid sulphate conditions for a longer period showed the familiar black gill syndrome and had heavy deposits of iron in their gills and carapace.



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Fig. 5.3. Comparison of total iron concentrations in muscles, gills and carapace cuticle of shrimps cultured in acid sulphate soil conditions and shrimp collected from the wild.

## 5.2.7. Manganese in sediments.

Fig. 5.4 A presents the variation in total manganese concentration in the sediments of the shrimp ponds during the studied culture cycle. The total manganese concentration fluctuated from a minimum of 74 mg / kg to a maximum of 227 mg / kg. During the initial period of culture, manganese concentrations were relatively higher than those during the latter part of the study. There was a sharp drop in the manganese concentration from a value of 227 mg /kg (4th week) to a value of 95 mg /kg (5th week); the levels remained low for the rest of the culture period.

#### 5.2.8. Manganese in water.

The soluble manganese concentration in the pond water fluctuated between 0.15 mg / 1 and 0.27 mg /1 (Fig. 5.4 B). No definite trend was evident in the way that manganese fluctuated in concentration during the culture period.

# 5.2.9. Manganese in gills of cultured shrimps.

The variations in manganese concentration in gills during the culture period are given in Fig. 5.5 A. Total manganese concentration in gills fluctuated from a minimum of 26.04  $\mu$ g/g dry wt (2nd week) to a maximum of 93.22  $\mu$ g / g dry wt (10 th week). A progressive increase in manganese concentration was observed during the culture period. The manganese concentration in gills indicated a highly significant positive correlation (r =0.986, p =<0.001) with the culture period, but no significant correlation with the manganese concentration in the sediments or water.

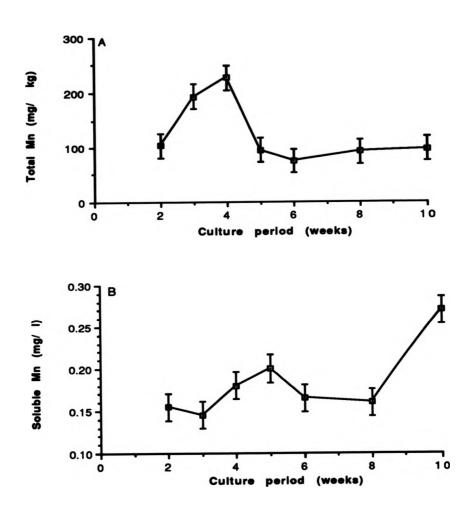


Fig. 5.4. Manganese concentrations  $(\pm sd)$  in shrimp culture ponds (A) in surface sediments (total), (B) in water (soluble) in relation to the culture period.

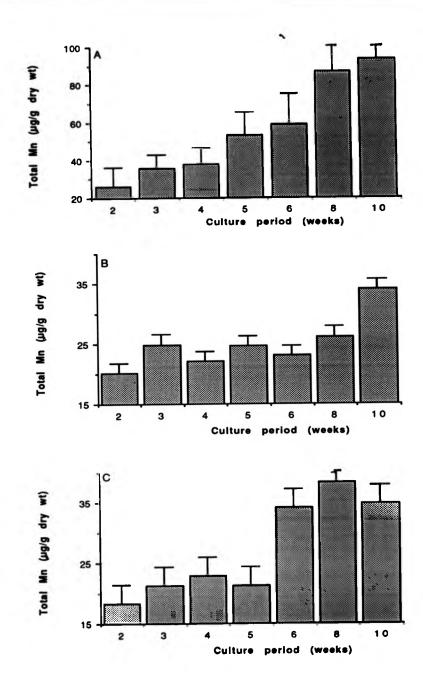


Fig. 5.5. Total manganese  $(\pm sd)$  in (A) gills, (B) carapace cuticle and (C) abdominal muscle of shrimp in relation to the culture period.

5.2.10. Manganese in carapace cuticle of cultured shrimps.

Fig. 5.5 B describes the variation in manganese concentration in the carapace cuticle during the culture period. This fluctuated from a minimum of 20  $\mu$ g/g dry wt (2nd week) to a maximum of 34  $\mu$ g /g dry wt (10 th week). In the latter part of the culture cycle the shrimp showed a significantly higher concentration of manganese (r=0.885, p=0.0140), but the correlations relating dissolved manganese in water and in sediments to the carapace concentration of manganese in the shrimp were not significant.

## 5.2.11. Manganese in muscle of cultured shrimps.

The change in the total manganese concentration in shrimp abdominal muscle during the culture period is shown in Fig. 5.5 C. It varied from a minimum of 18.37  $\mu$ g / g dry wt (2nd week) to a maximum of 38.42  $\mu$ g /g dry wt (8th week). The relative manganese concentration in muscle was low during the 2nd to 5th weeks of culture when compared to that in weeks 6 to 10. The concentration of manganese in shrimp muscle was highly correlated to the culture period (r=0.888, p=.009) and did not show any significant correlation with the manganese concentration in water and sediments .

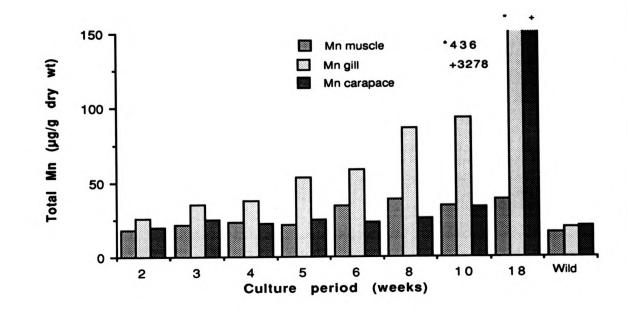
5.2.12. Manganese in wild shrimps and shrimps exposed for 18 weeks in acid sulphate conditions.

The manganese concentration in gills, carapace and muscles of wild shrimps sampled from the immediate surrounding natural environment, and of the same weight class as the 18 week pond cultured shrimps, were very low (Fig. 5.6). In comparision, the abdominal muscular tissue of the cultured shrimp showed an approximately 2 fold increase, the carapace a 1.6 fold increase and a 4.6 fold increase in gills of the shrimps cultured for an 18 week period when compared to the wild ones.

5.2.13. Inter-relationships of iron and manganese in cultured shrimps. Fig. 5.7 shows the stability relations of different iron and manganese compounds in the acid sulphate soil environment. Soluble  $Fe^{2+}$  and  $Mn^{2+}$  are the stable forms of iron and manganese in relatively low pH and Eh conditions. Iron tends to precipitate in advance to manganese as Eh and pH increases. The Eh-pH relationships observed for different shrimp culture practices (Chapter 4) are included in this figure. It is apparent according to Eh-pH relationships that iron oxides are the most stable form of iron, while soluble  $Mn^{2+}$  is the most stable form of manganese in shrimp culture ponds.

Pearsons product movement correlation matrix on iron and manganese in different tissues of shrimps, in sediments, and in water is given in Table 5.1. Statistically significant correlations observed for the iron concentration in gills and manganese concentration in gills (r=0.844; p =0.016), manganese concentration in carapace with the iron concentration in carapace (r= 0.9248; p=0.002) indicate the close association of manganese with the iron in shrimps cultured on acid sulphate soil.

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Fig. 5.6. Comparison of total manganese concentrations in muscles, gills and carapace cuticle of shrimps cultured in acid sulphate soil conditions and shrimp collected from the wild.

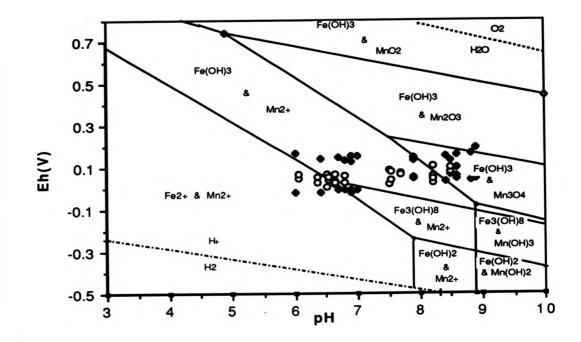


Fig. 5.7. Composite Eh-pH stability diagram for iron and manganese systems at 25°C (Eh-pH relationships observed for o- extensive and semi-intensive,  $\blacklozenge$  intensive shrimp farms ).

Table. 5.1. Pearson product movement correlation matrix for manganese and iron in gills, cuticle carapace and in muscles

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	1	2	3	4	5	6
1	1.0000	. 8274	. 8949	. 8558	. 8288	. 9092
	.0000	.0216	.0065	.0141	.0212	.0045
2	. 8274	1.0000	. 5849	.5636	. 9249	.8254
	.0216	.0000	.1677	.1877	.0029	.0222
3	. 8949	. 5849	1.0000	.9469	. 6770	.8525
	.0065	.1677	.0000	.0012	. 0948	.0237
4	. 8558	. 5636	. 9469	1.0000	. 6397	. 6856
	.0140	.1877	.0012	.0000	. 1232	.0891
5	. 8288	. 9249	. 6770	. 6379	1.0000	.7644
	.0212	.0029	. 0948	.1232	.0000	.0454
6	. 9092	. 8254	. 8205	. 6856	.7644	1.0000
	.0045	.0222	.0237	.0891	.0454	.0000

1-Mn in gill, 2-Mn in carapace cuticle, 3-Mn in muscles, 4-Fe in gills, 5-Fe in carapace cuticle, 6-Fe in muscles (coefficient level and below significance level)

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The possibility of co-precipitation of manganese together with iron as suggested by Collin and Buol (1970. b) and Ponnamperuma (1972), is reflected in these correlations. In the oxygenated environments  $Fe^{2+}$  can become more easily oxidised than  $Mn^{2+}$ . Jenne (1968) has indicated that in the presence of freshly precipitated ferric hydroxide,  $Mn^{2+}$  can oxidise and precipitate at relatively lower Eh-pH combinations than values indicated in normal Eh-pH diagrams. The precipitated iron hydroxides have a very high capacity to absorp  $Mn^{2+}$  or to co-precipitate (Collin and Buol, 1970. b) as indicated below:

 $Fe(OH)_3(colloidal) + Mn^{2*} + 2H^* - MnO_2(colloidal) + 4H_2O + 2Fe^{2*}$ 

Stepwise linear regression analysis (Table 5.2) between manganese in gills and a combination of variables, (iron in gills, iron in sediments and culture period) produced a highly significant positive correlation ( $r^2 = .933$ , p = .0073). The manganese in the gills of shrimps appears to be closely correlated with the iron in the gills of shrimps and iron in the sediments of the ponds. The resultant regression equation is given in Table 5.2.

## 5.2.14. Aluminium in sediments.

The variation in available aluminium in the sediments of the shrimp culture ponds used in this study is illustrated in Fig. 5.8 A. The recorded aluminium levels fluctuated between 265 mg / kg and 338 mg/kg, without showing any definite trend in relation to the culture period. In comparison, the recorded values for available aluminium are around 190 mg /kg in fish ponds

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Table. 5.2. Stepwise linear regression equations for Mn in gills, Fe in gills, Fe in sediments and culture period.

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Step. 1 :
Mn in gills = -22.4356 + 0.06578 Fe in gill.
(r<sup>2</sup> =.7324 , p =0.01400)
Step. 2 :
Mn in gills = 6.61533 +0.00663 Fe in gill + 0.00115 Fe in
sediments.
(r<sup>2</sup> = 0.8390, p =.0295)
Step. 3 :
Mn in gills = 8.995 -0.0035 Fe in gill +0.00008 Fe in sediments
+ 8.8604 culture period.
(r<sup>2</sup> = .9733, p = .0073)
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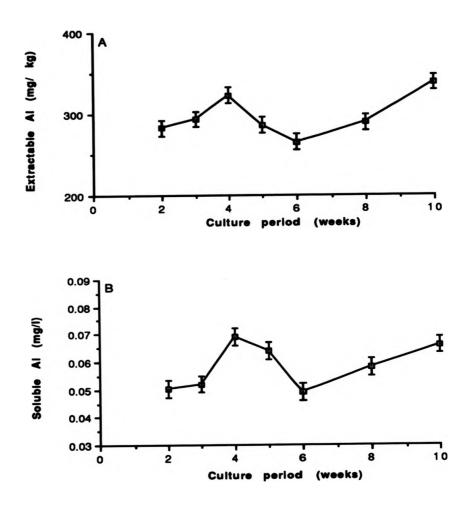


Fig. 5.8. Aluminium concentrations  $(\pm sd)$  in shrimp culture ponds (A) in surface sediments (extractable), (B) in water (soluble) in relation to the culture period.

developed in acid sulphate soil areas (Singh, 1985).

## 5.2.15. Aluminium in water.

The dissolved aluminium concentration in the pond water is shown in Fig 5.8 B. Relatively low concentrations of soluble aluminium were observed in the culture ponds. The variation was between 0.049 mg /l and 0.069 mg /l. Although there was no definite trend with time, the pattern of variation was similar to those for the aluminium concentrations of water and sediments.

In polluted and acidified areas the aluminium levels recorded in fish ponds were around 100 to 700  $\mu$ g/1 (Dickson, 1983; Karlssen <u>et al.</u>, 1986). The values recorded in the present study are actually lower than those recorded in fish farms in polluted and acidified areas by the same authors.

5.2.16. Aluminium in gills of cultured shrimps.

The variation in total aluminium concentration in gills during the culture cycle is shown in Fig. 5.9 A. The concentrations recorded were highest at week 3 then showed a gradual decline with time through the culture period. The range in aluminium concentration was 6.93  $\mu$ g /g dry wt and 11.370  $\mu$ g /g dry wt. These values are relatively low when compared with the values recorded for farmed trout by Karlsson <u>et al.</u>, (1986) in acidified areas (20 to 90  $\mu$ g / g wet wt). In un-polluted areas the concentrations of aluminium in gills of farmed trout are around  $2\mu$ g /g wet weight and agree with the values recorded in this study. At this level of aluminium concentration the fish do

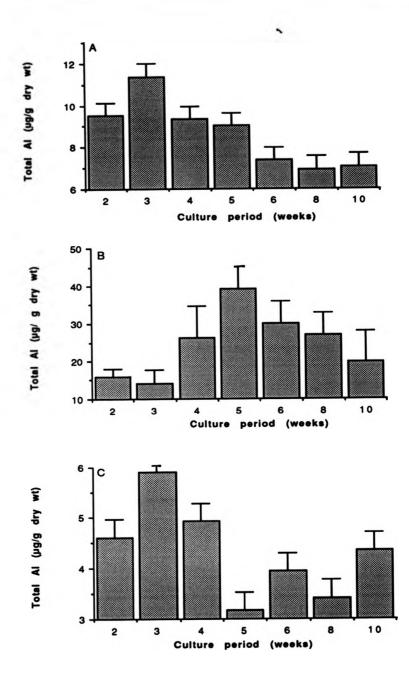


Fig. 5.9. Total aluminium  $(\pm sd)$  in (A) gills, (B) carapace cuticle and (C) abdominal muscle of shrimp in relation to the culture period.

not show any sign of stress or pathological conditions (Karlsson <u>et al.</u>, 1986). Given this evidence, the pathological changes observed in the gills of shrimps in this study cannot be related to the aluminium content of the gills.

# 5.2.17. Aluminium in carapace of cultured shrimps.

Total aluminium levels recorded in the carapace were higher than those recorded for shrimp gills and muscles (Fig. 5.9 B). The fluctuation was from a minimum of 14.03  $\mu$ g/g dry wt to a maximum of 39.2  $\mu$ g /g dry wt. Although the values recorded indicate relatively high concentrations at the mid-culture stage, no definite trend was observed with time. The higher concentration perhaps may be due to the aluminium particles in the sediments deposited on the carapace, as in the case of iron and manganese.

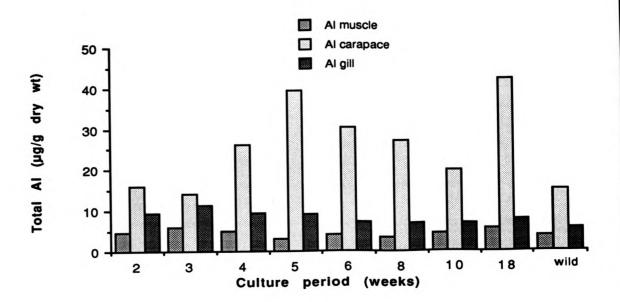
# 5.2.18. Aluminium in muscle of cultured shrimps.

Fig. 5.9 C shows the fluctuations in aluminium levels in the abdominal muscles of shrimp during the culture period; the range was from 3.17 to 5.90  $\mu$ g/g dry wt. Relatively higher concentrations were observed in weeks 2 to 4 when compared to the rest of the culture period. Under acidified environmental conditions the reported values by Karlsson et al. (1986) (1.8 to 2.6  $\mu$ g /g wet wt) are higher than the values of this study. The values recorded by them in the same study for farmed trout in unpolluted areas agree well with the concentrations of the aluminium in the muscles of shrimps in this study. No significant correlations were observed between aluminium concentration in muscles and the culture period as in the case of

iron and manganese. Also, the aluminium in water or sediments are not significantly correlated with the muscle aluminium concentration.

5.2.19. Aluminium in wild shrimps and shrimps exposed for 18 weeks in acid sulphate conditions.

Fig. 5.10 compares the aluminium concentrations in muscles, gills and carapace cuticle of the cultured shrimps in the present study, cultured shrimps exposed to acid sulphate soils for 18 week period and wild shrimps of equivalent size of 18 week shrimps. As in the case of other heavy metals measured, except for accumulation in the carapace, there were no significant increases in other tissues of cultured shrimps when compared to wild shrimps.



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Fig. 5.10. Comparison of total aluminium concentrations in muscles, gills and carapace cuticle of shrimps cultured in acid sulphate soil conditions and shrimp collected from the wild.

### 5.3. Discussion

Iron concentrations in fish tissues has been studied under various environmental conditions. The recorded total iron concentrations in muscles varies between 2.85 and 11.8  $\mu$ g / g wet wt (Honda <u>et al.</u>, 1983; Leed and Belanger, 1981) in wet tissues and in dry tissue samples recorded values varied between 26.5 and 58.5  $\mu$ g /g dry wt (White and Rainbow, 1987). Comparatively little information is available on the concentrations of iron in crustaceans. The lowest iron concentrations are normally found in deep sea crustaceans while relatively higher values occur in coastal crustaceans (Rainbow, 1987).

White and Rainbow (1987) have also indicated the enzymatic iron requirement for decapods as 27.4  $\mu$ g /g dry wt. The muscles of cultured shrimps in this study (76  $\mu$ g /g dry wt to 119  $\mu$ g /g dry wt) had higher levels than those but the values. However, the muscles of wild shrimps had iron concentrations closer to the values calculated by White and Rainbow (1987). Very high concentrations recorded for crustaceans have usually been related to sediment particles in gills or elsewhere on the body (Rainbow, 1988). Parallel histological investigations in the present study have revealed the presence of deposits containing iron particles in between gill lamellae and on the carapace cuticle (chapter 6).

The extremely high concentrations of iron recorded in the gills and carapace cuticle of cultured shrimp in the present study are consistent with the work carried out by Nash <u>et al.</u> (1988), in acid sulphate soil areas and also the investigations by Wickins <u>et al.</u> (1984 b) and Lightner (1988) which all suggest the possibility of accumulation or deposition of iron particles on various tissues of shrimps. The type of iron compounds that are deposited could be predicted using the Eh -pH relationships of the pond environment. These details and the results of Eh-pH relationships and pathological investigations are discussed in Chapters 4 and 6 respectively.

The available literature pertaining to the presence of manganese in muscle and gills of aquatic organisms mainly concerns fish. The manganese values recorded in this study in cultured shrimp are relatively higher than the values recorded for fish species by Honda <u>et al.</u>, (1983) (1.42 to 5.59  $\mu$ g / g wet wt), Khalif <u>et al.</u> (1985) (3.42  $\mu$ g /g wet wt) and by Dalinger and Kautzky (1985) (4.4  $\mu$ g /g dry wt). Values recorded for the manganese concentrations in gills are relatively higher than those of muscles and vary between 16.5 and 38.5  $\mu$ g /g dry wt (Dalinger and Kautzky, 1985). The shrimp gills in the present study showed values similar to those above during the initial period of culture and thereafter increased to considerably higher values. According to Rainbow (1987) the body manganese concentration of coastal decapods usually lies in the range of 5 to 50  $\mu$ g/g dry wt.

There is a possibility of these values being elevated due to the presence of sediments containing manganese in gut and also due to the conditions where the ambient physicochemical conditions promotes the deposition of manganese

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from solutions on the carapace (Bryn and Ward, 1965). The enzymatic requirement of manganese in decapods is around 3.9  $\mu$ g /g dry wt. The values recorded for oceanic crustaceans are lower than those recorded for coastal decapods (White and Rainbow, 1987). According to Bryn and Wade (1965), manganese is found in close association with exoskeleton or in other calcified parts such as the ossicles, teeth and gastric mill in decapods. In crustaceans the lowest manganese concentrations (around 0.8  $\mu$ g /g wet wt) are found in abdominal muscles. The values recorded for shrimps cultured in acid sulphate soils are much higher than the above recorded values. The high concentrations recorded in gills in this study is a result of deposition of particles containing manganese in between filaments as suggested by Bryn and Wade (1965).

Metal uptake by crustaceans can take place via contaminated food or water. Dellinger and Kautzky (1985) have demonstrated a positive relationship between heavy metal concentrations in sediments and the accumulation of heavy metals via food chains in the aquatic environment, finally resulting in highly elevated concentrations in food fishes such as rainbow trout.

Heavy metal uptake from water when the metals are in soluble form is through permeable surfaces, especially across the gills in the case of adult crustaceans (Bryn, 1964; Jennings and Rainbow, 1979; White and Rainbow, 1982, 1984 a, 1984 b) but perhaps more generally over the body surface in larvae or in smaller forms with relatively little tanning or calcification in the cuticle. The dominant role of gills in the process of metal uptake in shrimps

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has been well documented (Patric and Loutit, 1978; Aoyama et al., 1978).

Heavy metals enter animal body tissue passively. Aquatic organisms are very sensitive even to very low concentrations of heavy metals in the external media. A possible scenario for heavy metal uptake would involve metal out side the cell binding with membrane-associated ligands for transport across the hydrophobic cell membrane into the cell interior where further ligands of higher affinity for the metal are available to bind the transport metal (Rainbow, 1987). In the case of iron, it is transported into the cell by an extracelluar iron-rich protein transferrin. When this metal is absorbed, it can be incorporated into an another iron-rich protein, ferritin inside the cell (Williams, 1981 a, 1981 b; Rainbow, 1988). Transferrins have been isolated from decapod crustaceans (Guray and Negrel, 1980).

The concentrations of heavy metals measured in this study as dissolved compounds in pond water are relatively very low. This is predictable under acid sulphate conditions as the redox potential-pH relationships operating promote the precipitation of iron as described earlier (Chapter 4). Although the soluble Mn<sup>2+</sup> ion is more stable according to stability diagrams, the possibility of co-precipitation and adsorption of manganese with iron compounds can remove a considerable proportion of the dissolved form from the aqueous medium.

Taking the stability relations of the different heavy metal compounds into

account, the absorption of heavy metals via food is of more importance in acid sulphate pond environments than through the water. Insoluble hydrated iron oxides are the most expected form of heavy metal according to the stability relationships. Shrimps, being benthic feeders, are likely to ingest metal-rich sediments together with their food, as suggested by Rainbow (1987). Benthic food organisms themselves may also contain relatively high concentrations of iron and manganese in such an environment.

Iron also can be get readily dissolved in the acid gastric contents, even when present as forms sparingly soluble in the external media. Some workers have identified an iron binding factor referred to as gastroferrin and this can absorb both ferrous ions as well as ferric ions (Ford <u>et al.</u>, 1984).

Absorption of manganese can take place via the stomach and hepatopancreas of shrimp. From the stomach, absorption into the blood can occur very rapidly. Bryan and Wade (1965) have demonstrated a build up of manganese in blood when manganese is directly introduced to the stomach. They have also suggested the possibility of very high manganese levels in muscles and gills as a result of contamination of these tissues with manganese-enriched blood.

Physico-chemical conditions in the shrimp culture systems developed in acid sulphate soil areas cannot much influence on the bioavailability of aluminium which is important in accumulation processes. This is reflected in the

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relatively low levels of aluminium recorded in muscles and gills in the present study. As in the cases of iron and manganese, no significant correlations were observed with culture period, or aluminium concentrations in water or sediments with aluminium levels in muscles, gills or carapace. Among the three elements studied, aluminium appears to be the least influential on bioaccumulation and the other processes which can contribute towards stress and early pathological signs in cultured shrimps reared in an acid sulphate soil environment.

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## CHAPTER 6

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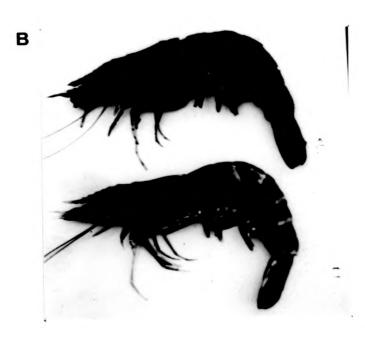
# PATHOLOGY OF THE SHRIMPS CULTURED ON ACID SULPHATE SOILS.

#### **6.1.** Introduction.

Impaired growth, poor production and heavy mortalities of shrimps in ponds developed on acid sulphate and potential acid sulphate soils (Simpson et al., 1983; Cook et al., 1984; Simpson and Pedini, 1985) are often associated with several common clinical signs and histopathological changes (Nash et al., 1988; Sindermann, 1989; Nash, 1990). The clogging of gills by iron hydroxide (Wickins, 1984; Lightner, 1988; Nash et al., 1988); changes in normal gill colour, hypoxic changes in gill and heart tissue (Nash et al., 1988); soft shelled condition (Baticados et al., 1986) and secondary invasions of pathogens through injuries in gills and other tissues due to acidity and deposits (Nash et al., 1988; Nash, 1990) are some of the commonly recorded pathological conditions in shrimps cultured on acid sulphate soils. Plate 6.1 shows the brown and black-gilled shrimps from culture ponds on acid sulphate soils in contrast to normal (disease free) shrimps from ponds with no acid sulphate soil conditions.

Normal (disease free) Penaeus monodon from culture ponds in Plate. 6.1. Thailand, situated in a coastal area with no acid sulphate soils (A) in contrast to brown and black- gilled Penaeus monodon from ponds developed on acid sulphate soils in Sri Lanka.





6.1.1. Gills, hepatopancreas and heart tissues.

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Gills are the primary site of respiration and osmoregulation in shrimps (Robertson, 1960). Physiological, histological and ultrastructural studies on fish gills and crustacean gills (Quinn and Lane, 1966; Baker, 1969; Jones, 1975; Papathanassiou and King, 1983; Stern <u>et al.</u>, 1984; Papathanassiou, 1985) have shown that heavy metal ions can interfere with respiration and osmoregulation by disrupting the structure of the gill cells in fish and crustaceans. Gills are also highly sensitive to other stressors and are susceptible to diseases and parasitic infestations which pose serious problems in shrimp culture systems (Foster and House, 1978).

The appearance of brown-gilled shrimps (Plate 6.2 A) is the first sign prior to large mortalities, disease outbreaks and other associated problems such as poor production and poor quality of cultured shrimps in acid sulphate soil areas (NARA, 1988). The brown gill condition is usually followed by the black gill condition (Plate 6.2 B) in culture ponds. During this period large number of dead shrimps are observed in ponds (NARA, 1988).

The hepatopancreas is a vital and major organ involved in diverse metabolic activities. It is primarily responsible for the synthesis and secretion of digestive enzymes and subsequent uptake of nutrient materials. Other major functions of the hepatopancreas include excretion, storage of organic reserves, and lipid and carbohydrate metabolism (Gibson and Barker, 1979; Stroch and Lehnert-Moritz, 1980). Infestations by virus, bacteria and by ricketsial Plate. 6.2. Penaeus monodon reared on acid sulphate soil ponds indicating brown- gill condition (A) and black- gill condition (B). organisms are reflected in this organ. It is of vital importance to diagnose different infections in shrimps (Couch, 1978, Lightner, 1988) and to assess the nutritional condition of shrimps (Vogt <u>et al.</u>, 1985 & 1986).

The heart tissues are also vulnerable to infestations by pathogens (Couch, 1978, Lightner, 1988). In addition, hypoxia induced changes can be identified in heart tissues (Nash <u>et al.</u>, 1988; Nash, 1990).

Changes in the chemical environment can greatly influence the behaviour and stability of the different chemical compounds responsible for acid sulphate soil conditions (Nuhg and Ponnamperuma, 1966; Ponnamperuma <u>et al.</u>, 1967; Collins and Buol, 1970 a), which in turn, might influence clinical signs and pathological changes in the culture organisms.

The stability of various iron and manganese compounds in relation to redox potential-pH in the acid sulphate soil pond environment has been discussed in detail in Chapter 4. Insoluble hydrated ferric oxides are the most stable form of iron, while the co-precipitated form of manganese with iron is the most probable form of manganese under the prevailing chemical and environmental conditions (Ponnamperuma et al., 1967, Collins and Buol, 1970 a, 1970 b) of shrimp culture ponds located on acid sulphate soils.

The management strategies employed in shrimp culture systems can obviously influence the stability relations of different iron and manganese compounds in the culture environment through their redox potential-pH relationships. Because shrimps live in close association with the pond sediments they are more likely to be affected by changes in the sediment environment. Several authors (Lightner, 1988; Nash <u>et al.</u>, 1988; Nash, 1990) have discussed the pathological changes in shrimps cultured on acid sulphate soils, but no attempt has been made to describe histopathological conditions observed in shrimps in relation to the stability relationships of different iron and manganese compounds in the aquaculture environment.

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6.1.2. Magnesium and calcium concentrations in shrimp tissues.

Calcium and magnesium are among the most commonly deposited minerals during the biochemical mineralisation processes of crustaceans (Travis, 1960 and 1963; Brannon and Rao, 1979). Previous workers have described changes in levels of calcium in the exoskeleton and muscles in relation to moulting and exposure to barite (Brannon and Rao, 1979), during various phases of the moulting cycle (Numnoi, 1934; Skinner, 1962; Dall, 1965 a and 1965 b; Mills and Lake, 1976; Fiber and Luiz, 1984), and with water hardness (Brown et al., 1991).

The effect of reduced pH on carapace calcium and magnesium levels in P. monodon and the effects of hypercapnic sea water on growth and mineralization by penaeid shrimps have been reported by Wickins (1984 a and 1984 b). Baticados <u>et al.</u> (1986) studied the soft shell syndrome in cultured shrimps inculuding investigations on variations in levels of carapace calcium in relation to water quality, soil quality, organic matter content and pesticides in the culture environment.

Factors known to influence mineralisation in crustaceans includes temperature, physiology, external levels of calcium, pH and bicarbonates (Gibbs and Bryan, 1972; Greenaway, 1974; Wickins, 1984 a and 1984 b). Hains (1981) has described a related problem, the effects of pH changes in the aquatic environment, resulting from acid rains, on the mineralisation of crustaceans.

Soft shell syndrome in cultured shrimps in acid sulphate soil areas has been widely reported (Webber and Webber, 1978; Singh, 1985; Simpson et al., 1983; Simpson and Pedini, 1985; Nash et al., 1988; Nash, 1990). In Sri Lanka up to 48 % soft shelled shrimps have been observed in harvests from acid sulphate soil areas (NARA, 1988). Although regular monitoring was not carried out for soft-shell shrimps during the present study, 37 % of the shrimps harvested after a 10 week period exhibited soft shelled condition. Thus, this condition represents a considerable technical and economic problem. Shrimps suffering from soft shelled syndrome are generally weaker, are more susceptible to predators and pathogens and have a low market value. However, no attempt has been made to investigate the soft shell syndrome in relation to the various physico-chemical processes occurring in the acid sulphate pond environment.

#### 6.2. Results.

The data collected on water quality (Chapter 4) is summarized in Table 6.1.

#### 6.2.1. Gill colour.

Fluctuations in the percentage of brown gill / black gilled shrimps in the culture ponds are given in Fig. 6.1. Brown gill syndrome was first observed during the 5 th week of culture. The percentage of brown-gill shrimps gradually increased up to seven weeks, from where there was a rapid increase to a peak of 40% during the 8 th week. A considerable number of dead shrimps were observed at this stage, as the shrimps became lethargic and migrated towards the peripheral areas of the ponds.

Dark brown or black gilled shrimps were observed after the 7 th week and increased progressively in occurence towards the 10th week when 9% of the total population exhibited the black-gilled condition.

6.2.2. Stability relations of iron in acid sulphate soils and brown gill syndrome.

A stability diagram for different iron compounds (pyrite, jarosite, ferric oxides, goethite) in the acid sulphate soil environment in relation to Eh- pH conditions is given in Fig. 6.2. The observed redox potential and pH values for the water and sediments of the culture ponds throughout the culture period (Chapter 4) are also included in this stability diagram.

Parameter	Surface layers	ers		Bottom layers	rs	
	range	mean	sd	range	mean	sd
Salinity (ppt)	15-21	17.4	± 1.90	16-21	17.8	± 1.57
płł	7.7-8.4	8.08	± 0.20	7.4-8.2	7.9	± 0.25
Eh (mV)	58-118	97.7	± 23.7	68-111	92.6	± 18.7
(mod) Od	6.8-7.8	7.31	± 0.35	6.2-7.4	6.64	± 0.42
Phosphate (ppm)	0.01-0.06	0.04	± 0.01	0.01-0.06	0.03	± 0.02
Witrates (ppm)	0.56-2.52	1.23	±0.61	0.26-2.34	1.42	± 0.69
Nitrites (ppm)	0.01-0.24	0.07	± 0.08	0.01-0.28	0.07	± 0.09
Sulphides (ppm)	•	1		0.02-0.04	0.03	± 0.01
Chlorophyll a (µg/l)	1.70-13.3	7.73	± 4.07	-	-	•
<b>Temperature</b> °C	25.5-29.5	28.4	± 1.3	25.0-29.5	27.9	± 1.48
Turbidity (NTU)	6.5-27.0	17.8	± 6.68	7.0-30.0	19.3	± 7.5
Total suspended solids (mg/l)	26.0-93.2	49.7	± 25.93	•	T	-
Dissolved aluminium (ppm)	3	P		0.05-0.07	0.06	± 0.003
Dissolved iron (ppm)	1			0.49-1.75	1.08	± 0.45
Dissolved manganese (ppm)	1	1	Đ	0.15-0.27	0.18	± 0.04

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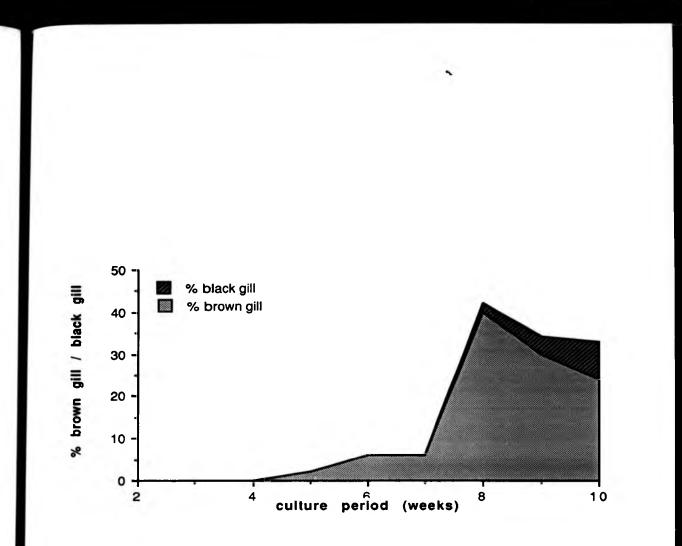


Fig. 6.1. Fluctuations in the percentage of brown and black gilled shrimps in culture ponds during a 10 week culture cycle.

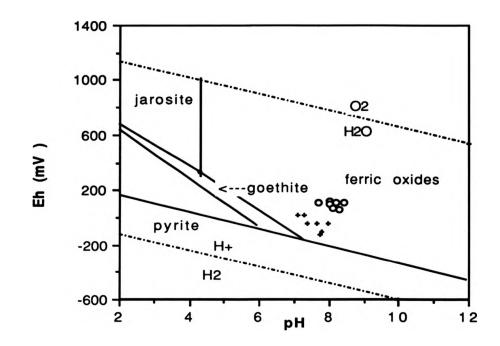
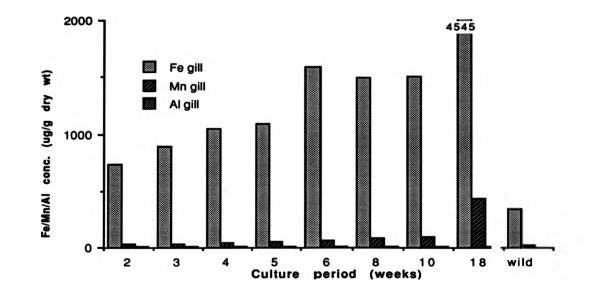


Fig. 6.2. The Eh-pH stability diagram for different iron compounds in the shrimp culture ponds on acid sulphate soils. (Eh-ph relationships observed for water (o), Eh-pH relationships observed for sediments (+) during culture period)

Data collected on variations in total iron, manganese and aluminium contents of the gills during the culture period (Chapter. 5) are presented in summarised form in Fig. 6.3. This figure also includes the concentrations of those heavy metals present in wild shrimps  $(23.5 \pm 2.9 \text{ g} \text{ in weight})$  collected from the lagoon adjoining the culture site, and in shrimps exposed to acid sulphate soil conditions for an 18 week period  $(32.1 \pm 6.5 \text{ g} \text{ in weight})$  in another pond on the same farm.

Relatively high concentrations of total iron were observed in the gills of the cultured shrimps. The iron concentration fluctuated from a minimum of 738  $\mu g / g dry$  wt to a maximum value of 1588  $\mu g / g dry$  wt (6 th week). There was a sharp increase in iron in gills from the 5 th to 6 th weeks. The iron concentrations remained high from week 6 until the end of the culture period. The wild shrimps had a very low concentration of iron (347  $\mu g / g dry$  wt) while the shrimps exposed for the 18 week period had very high concentrations (4545  $\mu g / g dry$  wt). The manganese concentration in gills fluctuated between 26.0 and 93.2  $\mu g/g dry$  wt while the fluctuation in aluminium concentration was between 6.9 and 11.3  $\mu g / g dry$  wt.

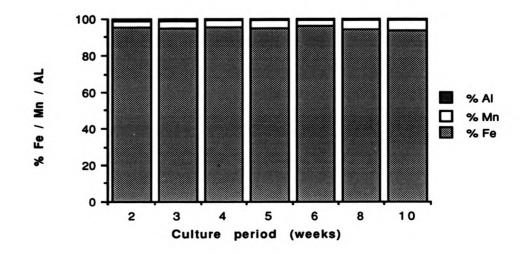
Fig. 6.4 presents the variations in percentage composition of iron, manganese and aluminium in gills during the culture period. Iron predominated in this group, contributing 91 to 96 %. The contribution of aluminium was very low at between 0.1 and 1.2 %, while manganese contributed 3.5 to 5.9 %.



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Fig. 6.3. Variations in total iron, manganese and aluminium concentrations in gills of cultured shrimps and shrimps collected from wild.

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Fig. 6.4. Variations in percentage composition of iron, manganese and aluminium in gills of cultured shrimps.

6.2.3. Histological studies on gills.

Scanning micrographs of the normal and brown gills are given in Plate 6.3. Penaeid gills are dendrobranchiate, consisting of an axis that bears a series of paired branches at right angles along its length. Each branch, in turn, gives rise to numerous perpendicularly oriented gill lamellae, either single or bifurcate (Foster and Howse, 1978).

Lamellae of the brown gills were extensively covered with a heavy layer of coarsely granular material. Deposits were very heavy in-between the gill lamellae of shrimps with black gills when compared to those with brown gills. Detritus was also found trapped in these deposits and the lamellae were swollen.

Histochemical studies on brown gills using Prussian blue stain demonstrated the presence of iron (Clark, 1981). In whole mounts of gills (Plate 6.4 A) the deposits were found concentrated among the gill lamellae. The density of such iron- containing material increased towards the central axis of the gill. The iron deposits became more and more dense with the length of exposure to acid sulphate soil conditions.

In LM histological sections (Plate 6.4 B) the deposits containing iron were found on the cuticular surface. Concurrent TEM sections very clearly indicate the electron dense material presumed to consist of iron and manganese hydroxides on the surface of the cuticle of brown gill-shrimps (Plate 6.5). Plate. 6.3. Scanning electron micrographs of (A) normal gill, (B) late brown gill with heavy deposits (d) on gill lamellae of cultured shrimps in acid sulphate soils. (A x 170; B x 300)



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Iron containing deposits (d) stained with Perl's prussian blue Plate.6.4. in brown gills of the cultured shrimps. (A) whole mount x 250, (B) L.S. of gill x 1700.



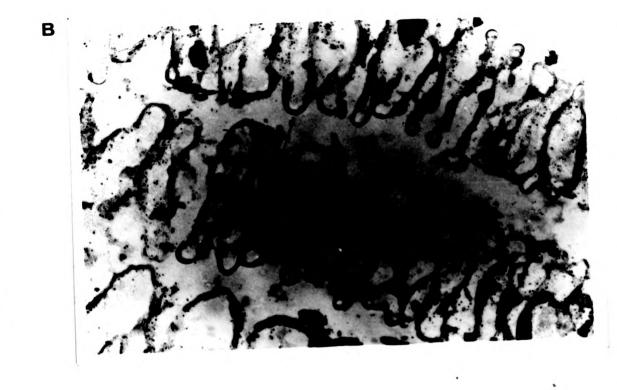
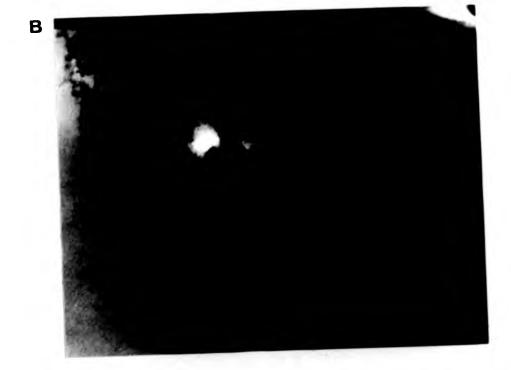




Plate. 6.5. Transmission electron micrographs of (A) normal lamellar cuticle and (B) lamellar cuticle of brown-gilled shrimp showing electron dense deposits; epicuticle (e), endocuticle (n), exocuticle (c) membranous layer (m), deposits (d). (A x 14,700; B x 95,750)



Both brown and black gill-shrimp were frequently found infested with ectocommensal protozoans in LM studies. These ciliated protozoans were found attached the tips of the gill filaments, mainly towards the anterior end, the areas relatively less affected from iron-containing deposits.

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The SEM studies of the parallel gill samples showed the detailed structure of the colony and the mode of attachment of the ectocommensals to the host gill epithelium (Plate 6.6 and 6.7). Zooids or the individuals in the colony were connected to one another by dichotomously branching stalk. The base of the each stalk terminates in a circular disc that is attached to the shrimps gill cuticle. No mechanical damage could be observed to the epicuticle as a result of these infestations. The fine structure of the ectocommensals closely reassembled the peritrichous ectocommensal of the genus *Zoothamnium* as described by Johnson et al. (1973); Overstreet, (1973); Johnson (1976) and Foster et al. (1978).

LM studies of the H&E stained histological sections of gills also revealed structural changes in the gill lamellae of the brown/ black gilled shrimps (Plate 6.8). Histological changes were relatively less severe in brown gills when compared to black gills (Plate 6.9). Lamellae were swollen (Plate 6.10) and the epicuticular layer was found separated in brown-gill shrimps with increased vaculations and dilated blood sinuses in some animals.

Lamellar pillar cells which hold the opposite epithelial layers apart were found ruptured in black gills. Lamellae also indicated hypertrophic and Plate. 6.6. Scanning electron micrographs of the gills showing (A) a colony of *Zoothamnium sp.* in brown-gilled shrimps cultured on acid sulphate soils, (B) enlarged zooid. zooid (z) stalk (s), (Ax 1100; Bx 4500)



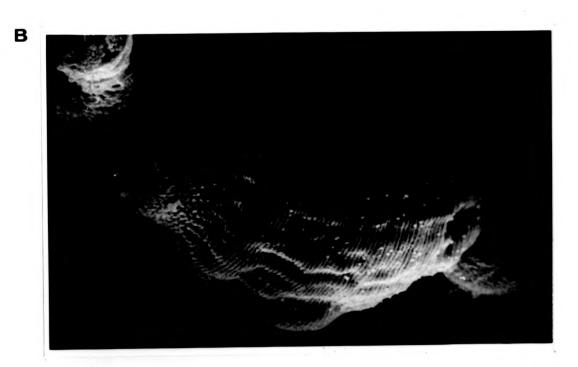


Plate. 6.7. Scanning electron micrographs showing the mode of attachment of *Zoothamnium sp.* to the gill filament of the shrimp; stalk (s), basal disc (b), zooid (z), gill lamellae (f). (A x 900; B x 3,500)





Plate. 6.8. Sections of (A) normal gill and (B) brown-gill showing cuticular separation (c), and dilated blood sinuses (v) in shrimps cultured on acid sulphate soils. pillar cell process (p) (H & E; A x 1,700; B x 1,2000).

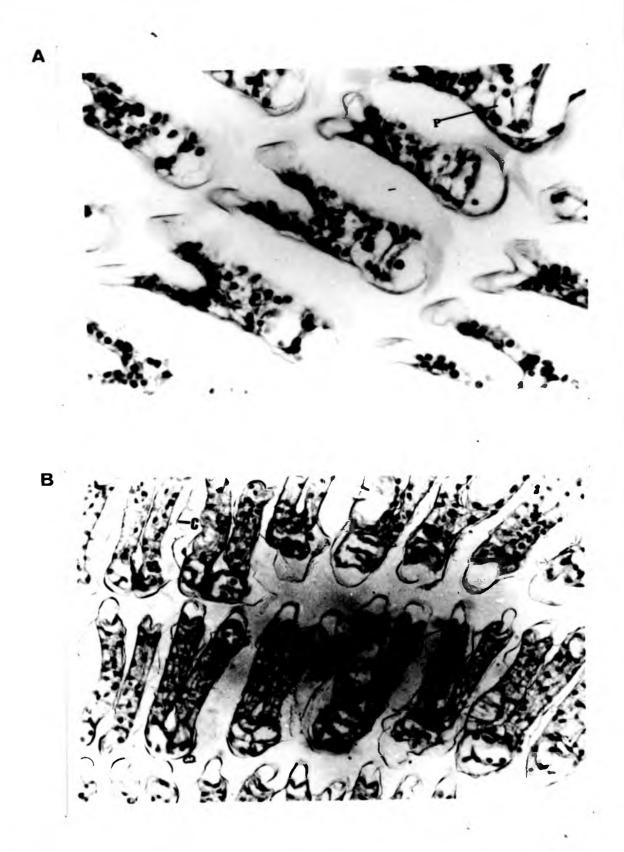


Plate. 6.9. Sections of (A) early black -gill, (B) late black-gill showing haemocytic infiltrations (h), dilated blood sinuses (s) and severe lamellar destruction. (H & E; A x 1,700, B x 800)

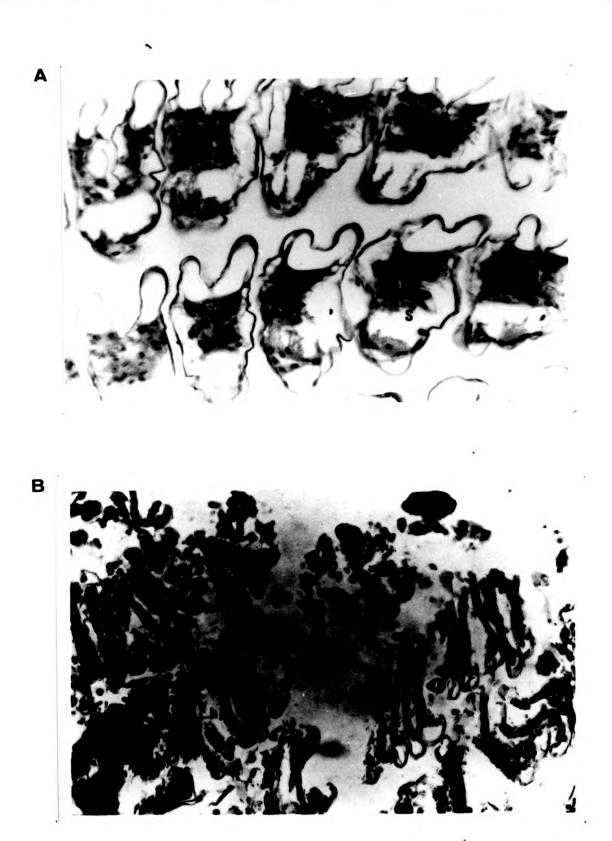
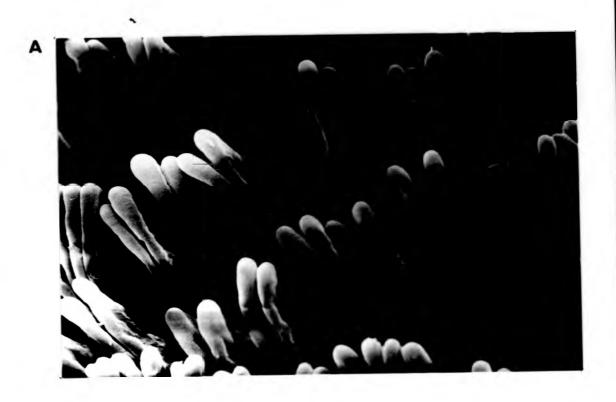


Plate. 6.10. Scanning electron micrographs of enlarged gill lamellae of (A) normal gill and (B) brown gill of shrimps showing lamellar swelling. (A x1300; B x 1250)





hyperplastic changes. Haemocytic infiltration and encapsulations were very frequent in black gills.

The separation of the cuticle and epicuticle of the gill was clear in brown gilled shrimps in transmission electron microscopic observations. Damage to the gill lamellar tissue beneath the cuticle was also very clear in TEM studies of brown gilled shrimps. The gill lamellae of brown-gill shrimps were with mitochondria exhibiting deteriorating cristae, less endoplasmic reticulum, less complex golgi apparatus with more vesicles when compared to normal gills (Plate 6.11). The nuclei of the lamellar cells were in the processes of degeneration in gill lamellae of brown-gilled shrimps.

### 6.2.4. Histological studies on hepatopancreas.

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All the cell types described by Staniar <u>et al.</u> (1968); Gibson and Baker (1979); Dall and Moriarty (1983) were well represented in the tubules of the hepatopancreas of the shrimps with normal gill colour (Plate 6.12 A and 6.13 A). Considerable areas of the tubules were occupied by very clear, well vacuolated R-cells (Restzellan). Darkly stained F-cells (Fibrillenzellan) and Bcells (Blasenzellan) were observed in normal shrimps (Bell and Lightner, 1988). The lumens of the tubules were filled with secretions.

The hepatopancriatic tubules of the brown gilled shrimps were dominated by B-cells (Plate 6.12 B and 6.13 B). R-cells were very poorly represented. The lumens of the tubules were without secretions. The F-cells were not observed Plate. 6.11. Transmission electron micrographs showing the structure of the gill lammelar tissue of (A) normal gill, (B) disturbed lamellar tissue of brown-gilled shrimps; cuticle (c), epicuticle (e), endocuticle (n), membranous layer (m), nuclious (n), mitochondria (a) (A x 12,100, B x 5,500)

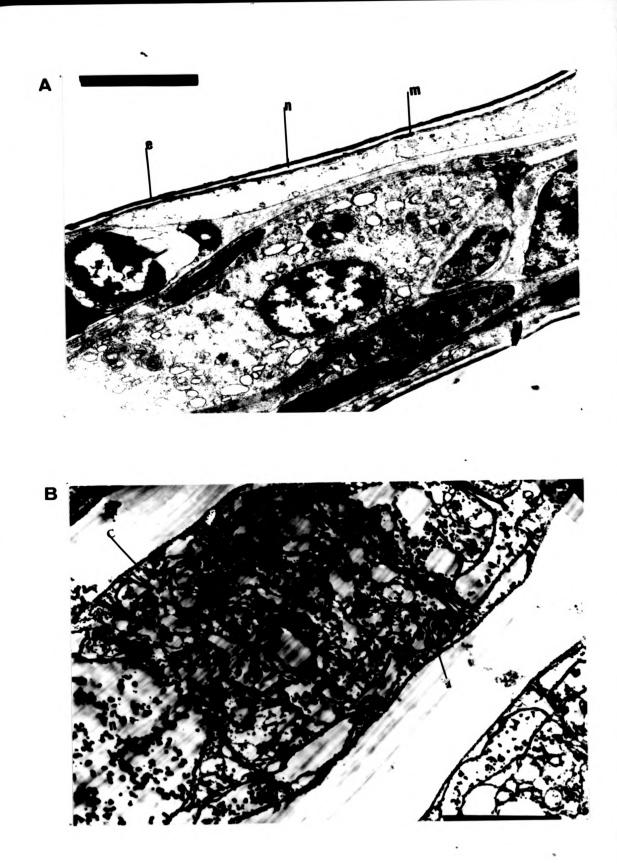


Plate. 6.12. Longitudinal sections of hepatopancreatic tubules of (A) normal-gilled, (B) brown-gilled shrimps cultured on acid sulphate soils; B-cells (b), lumen (I), r-cells (r). (H & E; A x 450; B x 450)



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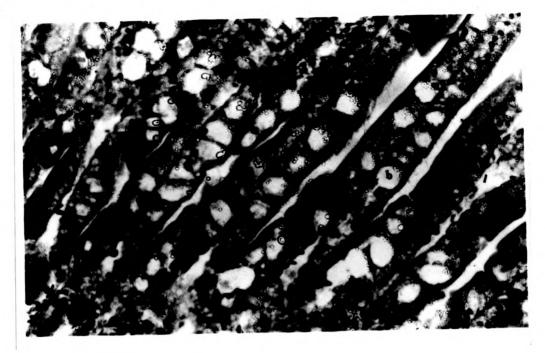
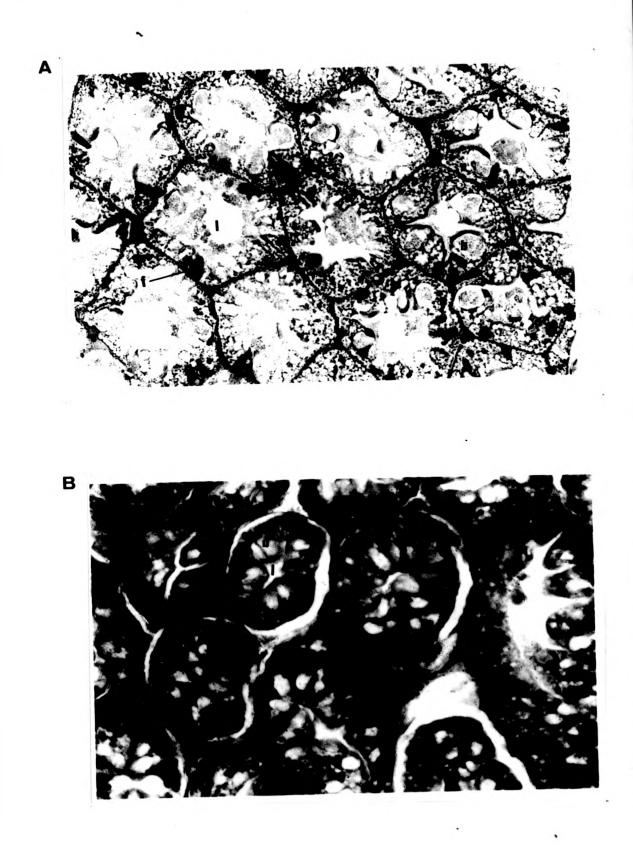
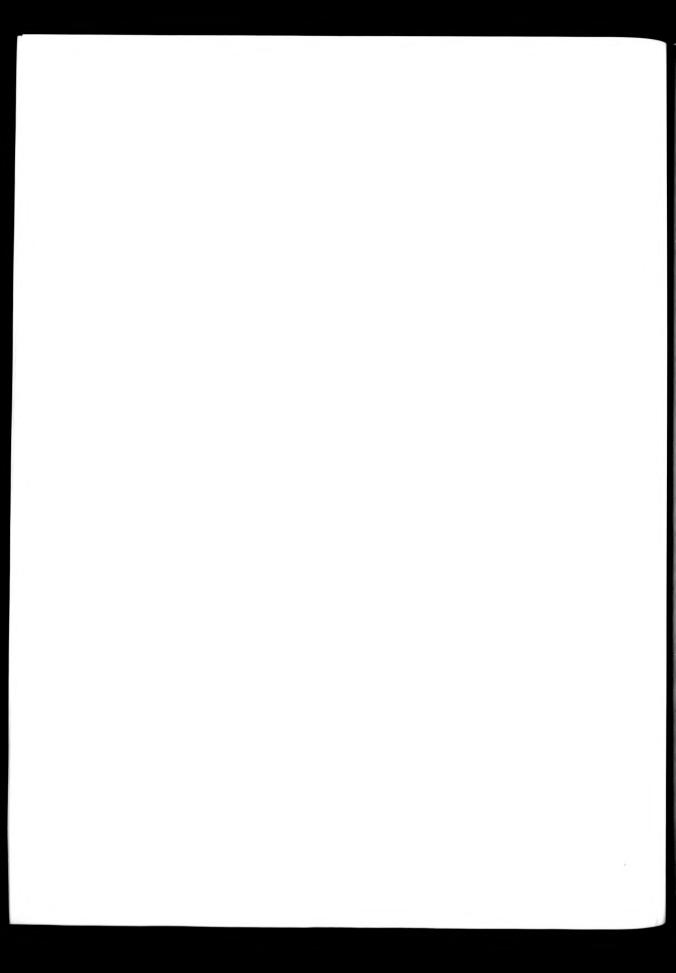


Plate. 6.13. Transverse sections of hepatopancreatic tubules of (A) normal-gilled, (B) brown-gilled shrimps cultured on acid sulphate soils; B-cells (b), f-cells (f), lumen (I). (H & E; A x 450; B x 450)





frequently. It may be also noted that signs of parasitic infestations were observed in hepatopancreatic tissues of the brown-gilled shrimps. Haemocyte infiltrations and hemocytic encapsulations were observed in haemosynuses of tubules. In black-gilled shrimps the hamocytic infiltrations and encapsulations were more common. Chromatin marginated, hypertrophid nuclei were also observed at this stage.

6.2.5. Histological studies on heart.

Plate 6.14 shows the myocardium of the normal shrimps. The nuclei of the myocardial cells and the nuclei of associated satellite cells were very clear as in the normal heart tissue (Bell and Lightner, 1988). Even at the brown-gilled stage there were no visual changes in the normal structure of the myocardial tissue (Plate 6.15).

Necrotic areas in the myocardium were observed in shrimps at the blackgill stage (Plate 6.16). Myocardial fibres appeared split and fragmented into multiple rounded, shrunken masses of myltivacuolated tissue. The nuclei of the fragmented areas showed evidence of disintegration. Haemocytic infiltrations and encapsulations and even nodule formations were noted near necrotic areas indicating signs of severe infestations.

Plate. 6.14. Transverse section of the heart of normal-gilled shrimp cultured on acid sulphate soils; nucleii (n), muscle bands (m). (H & E; x 250)

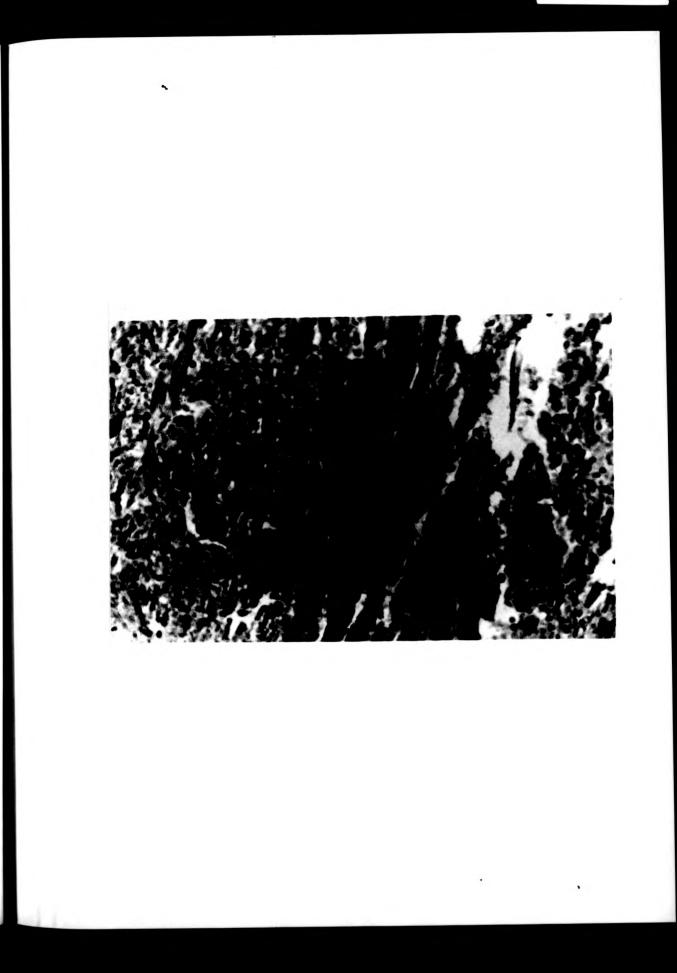
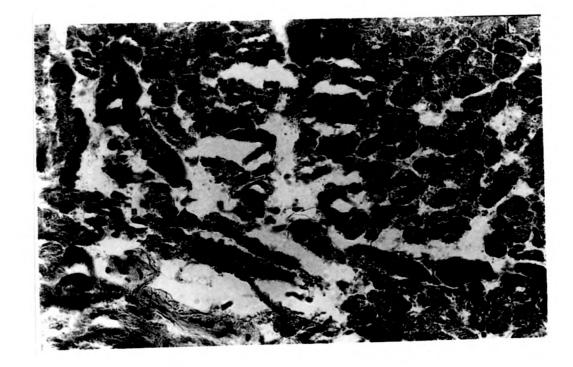


Plate. 6.15. Transverse section of the heart of brown -gilled shrimp cultured on acid sulphate soils; nucleii (n), muscle bands (b) haemocytic infiltrations (h). (H & E: x450 )



Plate. 6.16. Transverse section of the heart of black-gilled shrimp cultured on acid sulphate soils showing myocardial necrosis (n) and myocardial fragments (f). (H & E; x 650)



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6.2.6. Calcium and magnesium concentrations in carapace and muscle. Calcium and magnesium concentrations recorded in the carapace (cuticle) and muscle of shrimps cultured over a period of 10 weeks are shown in Fig. 6.5 and 6.6. The calcium concentration of the carapace varied between 136.1 mg / g dry wt and 260 mg / g dry wt. The calcium concentration decreased during the culture period, especially between week 5 and 6. The carapace magnesium concentration was between 0.83 mg / g dry wt and 1.19 mg /g dry wt and showed a less consistent trend of change with time compared to calcium.

The calcium concentration in muscle varied between 1.54 mg/g dry wt and 1.94 mg/g dry wt while magnesium ranged from a maximum of 0.0730 mg /g dry wt to a minimum of 0.0212 mg /g dry wt. Neither the magnesium in the carapace cuticle nor in the muscles showed any correlation with the culture period.

There was a strong tendency for the calcium concentration in muscles of cultured shrimps to decrease with time (r = -0.945, p = .0011). Highly significant negative correlations were obtained between the calcium concentration in the carapace cuticle and (a) the total iron concentration in the gills (r = -0.966, p = < 0.001); and (b) iron in the pond sediments (r = -0.9728, p = .00023).

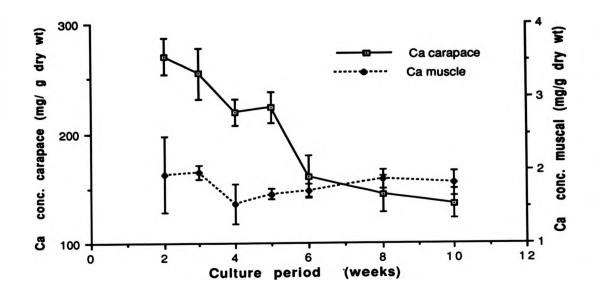


Fig. 6.5. Variations in calcium concentration  $(\pm sd)$  in carapace and muscles of cultured shrimps during the culture cycle in ponds on acid sulphate soils.

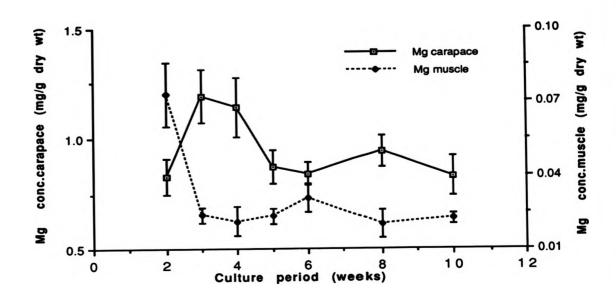


Fig. 6.6. Variations in magnesium concentration  $(\pm sd)$  in carapace and muscles of cultured shrimps during the culture cycle in ponds on acid sulphate soils.

The results of a stepwise linear regression between carapace calcium concentration, gill iron concentration, the iron concentrations in pond sediments and the culture period are given in Table 6.2. There is a strong correlation between calcium concentration in the cuticle, and a combination of these three variables.

#### 6.3. Discussion.

Stressors generated through poor water quality, poor soil quality and deteriorating environmental conditions are most likely to provoke histological changes and disease outbreaks resulting in poor growth and low survival in shrimp culture systems (Lightner, 1988; Sindermann, 1989; Liao, 1989; Nash, 1990). The variations in the basic water quality parameters such as pH, salinity and dissolved oxygen concentrations recorded in the present study were within the favourable ranges specified by Apud <u>et. al.</u> (1989), Chiu (1988 b), Boyd (1989) and Poernomo (1990) for shrimp culture systems. Results also revealed that the ranges of nitrites, sulphides and suspended solids were within the recognised safe limits (Wickins, 1981; Chiu, 1988 a&b; Boyd, 1989). The concentrations of iron, manganese and aluminium levls in water during the culture period did not reach the harmful levels recorded in fish ponds on acid sulphate soils by Poernomo (1983) and Singh (1985).

Brown/ black gill syndrome has been widely reported in cultured as well as in wild crustaceans (Rinaldo and Yevich, 1974; Lightner and Redman, 1977; Greig et al., 1982; Lightner, 1988; Sindermann, 1989 and Nash et al., 1988). Table. 6.2. Stepwise linear regression of Ca in carapace cuticle with iron in gill, iron in pond sediments and culture period.

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Step. 1 : Ca(cc) = 377.916 - 0.14958 Fe(g). $r^2 = 0.9340, p = .00039$ 

Step. 2 : Ca(cc) = 339.717 - 0.07207 Fe(g) - 0.00151Fe(s). $r^2 = 0.97926, p = .0004$ 

Step. 3 : Ca(cc) = 338.38 - 0.06636Fe(g) -0.000917Fe(s) -4.97727 cul.p  $r^2 = 0.9896$ , p = .0018

Ca(cc)- calcium in carapace cuticle, Fe(g)- Fe in gills, Fe(s)- Fe in sediments, cul.p- culture period.

Environmental degradation (Sawyer, 1982; Sawyer et al., 1983; Estrella, 1984; Sindermann, 1989); infestations with epiphytic and epizoic organisms (Couch, 1978; Lightner, 1988; Sindermann, 1989); exposure to heavy metals (Couch, 1978; Greig, 1982) and accumulation of iron-containing deposits (Nash et al., 1988; Nash, 1990) have been related to the appearance of brown/ black gills. According to available literature, blacking is attributed to the deposition of melanin as a defensive mechanism, at the sites of tissue necrosis and haemocytic infiltration due to conditions mentioned above (Sawyer et al., 1983; Lightner, 1988, Sindermann, 1989). Melanin is usually found in melanophores which are black or brown pigment bearing cells, stellate in form with a central soma and a number of radiating process (Rao, 1985). In the present study melanophores were not observed in gills of the affected shrimps at the brown gill stage, suggesting that melanisation is only associated with the subsequent black-gill stage.

Wickins (1983); Lightner (1988); Nash <u>et al.</u> (1988); and Nash (1990) have indicated the presence of iron deposits among gill lamellae or encrustations on gill lamellae of shrimps cultured on acid sulphate soils or acidic culture systems rich in iron. The very high concentrations of iron, manganese recorded in gills apears to be due to deposits in-between gill lamellae which were clearly demonstrated by the LM ,SEM, TEM studies.

The Eh-pH relationships observed for pond water and sediments of the shrimp culture ponds are discussed in detail (Chapter 4). They suggest the predominance of iron (III) hydroxides in culture ponds. According to Ponnamperuma et al. (1967) and Breeman (1976) the most common forms of iron (III) oxide in the acid sulphate environment includes  $\alpha$ -Fe<sub>2</sub>O<sub>3</sub> (haematite),  $\tau$ -Fe<sub>2</sub>O<sub>3</sub> (maghemite),  $\alpha$ -FeOOH (goethite),  $\tau$ -FeOOH (lepidocrosite) and Fe(OH) <sub>3</sub>.nH<sub>2</sub>O (hydrated ferric oxid<sup>5</sup>).

As in the case of the shrimp culture environment where reversible oxidation and reduction can take place (Nugh and Ponnamperuma, 1966; Ponnamperuma et al., 1967), hydrated iron oxide (Fe(OH)<sub>3</sub>.nH<sub>2</sub>O) is expected to be the most dominant form of iron. This compound is a colloidal gel and forms a reddish brown precipitate. This precipitate can settle on the bottom of the pond which is the immediate environment of the cultured shrimps. Shrimps also show diurnal behavioural changes and live half buried in the bottom sediments during the day time (Fig. 6.7). This suggests the possibility of iron hydroxide precipitate entering the gills together with the respiratory current.

Soluble  $Mn^{2+}$  is the expected most stable form of manganese in the acid sulphate soil environment according to the Eh-pH relationships observed in the present study (Chapter 4, but in the presence of iron hydroxide precipitates, manganese has a very strong tendency to co-precipitate with iron (Jeen, 1968; Collins and Buol 1970 b) according to the following equation: Fe(OH), (colloidal) + Mn<sup>2+</sup> + 2H<sup>+</sup> -----> MnO<sub>2</sub> (colloidal) +H<sub>2</sub>O +2Fe<sup>2+</sup> Fig. 6.8 presents the relationships between total iron and total manganese concentrations in gills observed in this study, which indicate highly



Fig. 6.7. Adult *Penaeus monodon* burrowing partially in mud. (Source: Apud et al., 1989)

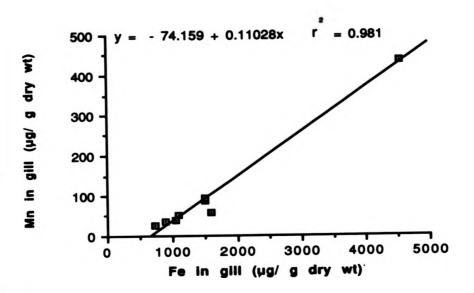


Fig. 6.8. Relationship between iron in gills and manganese in gills of cultured shrimps during 2 to 10 week period and shrimps exposed for 18 week period to the acid sulphate soil conditions.

statistically significant correlation ( $r^2 = 0.981$ , p = 0.001) between total iron and manganese in gills.

According to Foster <u>et al.</u> (1978); Lightner and Redman (1977); and Couch (1978), *Zoothamnium* sp. have a peritrichus mode of attachment. Here the ectocommensals cause no mechanical damage to the underlying tissue and elicit little response by the shrimp's haemocytes.

The histology and the function of various cell types of the hepatopancreatic tubules are well documented (Stainer <u>et al.</u>, 1968); Hopkin and Nott, 1980; Dall and Moriarty, 1983). B-cells are characterised by a large single vacuole containing digestive enzymes. The F-cells synthesize digestive enzymes which accumulate in vacuoles that enlarge by pinocytocis of nutrients and fluids from the tubular lumen. Fatty acids, neutral lipids and phosphor lipids (Momin and Rangneker, 1975) and lipid droplets have been identified in R-cells. According to Loizzi and Peterson (1971) and Momin and Rangneker (1975), R-cells are the major site of lipid absorption, digestion and lipid metabolism. These cells also metabolize and store glycogen.

Hopkin and Nott (1980) and Dall and Moriarty (1983) indicate that B-cells can presumably develop from the F-cells in the fasting animals, when no luminal nutrients are present for absorption and development. R-cell activity is an indication of active lipid absorption, digestion and lipid metabolism in shrimps (Dall and Moriarty, 1983). The predominance of B-cells and the lack of R-cell activity in the tubules of brown-gilled shrimps indicate that they are in a poor nutritional state in spite of receiving the normal recommended ration of supplementary feed.

Although evidence of light infections was observed, the histology of the hepatopancreas of the brown-gilled shrimps was within normal limits. In the hepatopancreas of the black-gilled shrimps more severe infections were evident. As indicated by Lightner (1988), chromatin marginated hypertrophoid nucleii observed in the present study could be considered as an early sign of viral infection.

Histological changes related to depleted oxygen supply to myocardial tissue and evidence of severe infestations were observed in black gilled shrimps. The iron containing deposits observed among gill lamellae can obstruct the normal respiratory current affecting the normal gas exchange via the respiratory surface.

In summary, the brown-gill stage in shrimps cultured in ponds on acid sulphates soil appears to be related to the colour of the iron (III) hydroxide which predominates the iron containing deposits among gill lamellae. These deposits can inflict damage to respiratory and osmoregulatory tissues of gills at this stage. The histology of hepatopancreas and heart tissue were within normal histological limits, even when there were early signs of mild infestation. Shrimps were in a very poor nutritonal state at this brown-gill stage.

At the black-gill stage, considerable damages to the tissues of the gills and heart could be observed, together with evidence of severe infestations. The stressors generated through the factors related to stability of iron compounds in acid sulphate soil conditions at the brown-gill stage, appears to affect the normal balance between pathogen, environment and host, provoking disease outbreaks at a later stage of the culture cycle.

Wickins (1984 b) has stated that the gills of crustaceans take part in ionic exchanges and the mobilization of a proportion of exoskeletal mineral carbonates. Calcium uptake from the environment is via gills in fish (Greenaway, 1985) and calcium transportation sites have been identified in gills of crustaceans. Adegboye (1987) has indicated that the gills of crayfish are responsible for the uptake of calcium from the environment. Dall (1964 a, 1964 b) has stated that in metapenaeids gills acounts for 90 % of the total calcium uptake to the body.

Calcium and magnesium require an active transport mechanism for their uptake into cells. The occurrence of Na-K ATP-ase which is identified as an enzymatic basis for active calcium transport (Stern <u>et al.</u>, 1984) has been reported in the gills of decapod crustaceans.

Dall (1965 a) has indicated that the intermolt carapace contains a calcium concentration around 190 mg/ g dry wt, while that of exuviae is around 247 mg/ g dry wt in metapenaeid shrimps. The magnisium levels recorded for the carapace of these shrimps vary from 1.35 mg/ g dry wt (intermolt carapace) to 2.2 mg/ g dry wt (in exuviae). Brown et al. (1991) have recorded calcium concentrations of 128 to 224 mg/g dry wt and magnesium concentrations of 2.64 to 5.19 in the intermolt carapace of *M. rosenbergii* exposed to water with different hardnesses. Baticdos et al. (1986) observed relatively low levels of calcium (23.2 mg/ g wet wt) in soft shelled shrimps when compared to normal (hard shelled) animals (27.2 mg/ g wet wt). Wickins (1984 b) has shown that there is an increase in calcium levels in shrimps with increased exposure to hypercapnic sea water. This trend was not observed for magnesium levels in cuticle.

The high incidence of soft shelling in the shrimps grown in acid sulphate soil areas has been related to various conditions. According to Simpson <u>et al.</u> (1983) the depletions in carbonate alkalinity can affect the mineralization processes in shrimp cuticle. The low phosphorus concentrations in water can increase the chances of soft shelling (Baticados <u>et al.</u> 1986). Phosphorus can directly or indirectly act as a limiting factor in soft shell formation and hardening. Phosphorus deficiency in acid sulphate aquaculture ponds has been well demonstrated (Poernomo, 1983; Singh, 1985; Hanchanova, 1983).

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In the present study the concentrations of phosphates recorded were also very low, (from 0.01 to 0.05 ppm) in the culture ponds. Relatively high pH values in the culture environment also have been correlated with the soft shell condition by Baticados <u>et al.</u> (1986). Precipitation of calcium compounds at higher pH values may make them less available to culture organisms (Boyd, 1982).

The correlations obtained between the calcium concentration in the carapace, the iron concentrations in the gills of cultured shrimps, iron concentrations in the sediments of culture ponds (Fig. 6.9), and the culture period suggests that iron has a strongly negative effect on the carapace calcium levels with time (Table 6.3). Based on the observed Eh-pH relationships and other available information (Ponnmperuma <u>et al.</u> 1969; Breeeman, 1976; Collins and Buol, 1970 a; Nash <u>et al.</u>, 1986), the iron in the gills of shrimps as well as in the sediments of these ponds are presumed to be in the form of insoluble colloidal iron hydroxides.

The iron hydroxides that accumulate among the gill lamellae are detrimental to the normal gill functions of cultured shrimps (Nash <u>et al.</u>, 1986 and Nash, 1990). The calcium absorption function of the gills may also be affected by these precipitates. This perhaps may be the main factor contributing to the decrease in calcium concentrations in the carapace cuticle of the shrimps grown on the acid sulphate soil ponds, and hence to the soft shelled condition which subsequently develops.

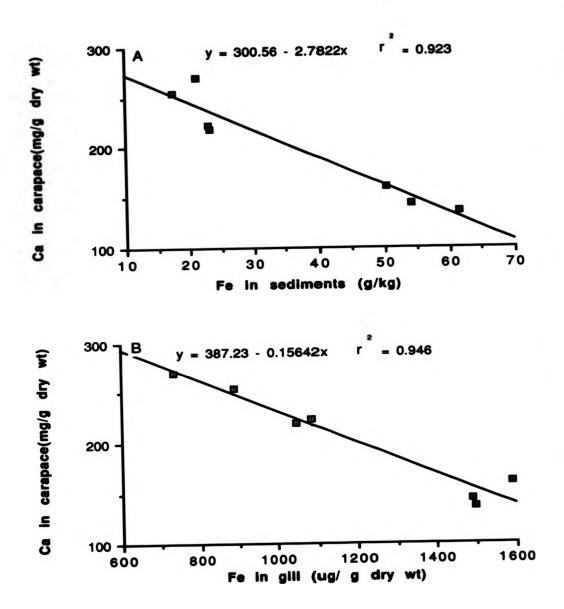


Fig. 6.9. Correlations between calcium concentration in carapace and iron concentration in (a) sediments of culture ponds, (b) in gills of cultured shrimps, in ponds constructed on acid sulphate soils.

Table. 6.3. Pearson product-moment correlation matrix for Ca in carapace, Fe in gills, Fe in sediments and culture period. Culture period 0.8719 0.9245 .0029 0.0000 .0012 0000 -0.9476 .0105 Fe sediment 0.9245 0.9206 0000 1.0000 .0033 .0029 .0002 -0.9727 0.8719 1.0000 0.9206 .0105 0000 .0033 -0.9664 .0004 Fe gill Ca carapace 1.0000 -0.9475 -0.9728 .0004 0000 -0.9664 .0002 .0012 Culture period Ca carapace Fe sediment Fe gill

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Coefficient and below, significance level.

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# CHAPTER 7

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## LAND USE PATTERN AND VEGETATION TYPES IN RELATION TO SHRIMP FARMING IN COASTAL AREAS OF SRI LANKA.

### 7.1. Introduction.

The expansion of the shrimp culture industry has contributed to the socioeconomic welfare of the coastal areas of Sri Lanka. However, this new activity has also resulted in changes in the land use patterns of these areas creating new problems in its wake. Shrimp culture on the west coast commenced in the mid 1980's. The first recorded figure for production from shrimp farming was for 1984 and amounted to 10 mt. Within four years the production of cultured shrimp has risen to 669 mt (NARA, 1988) and shows every sign of increasing further. The revenue from cultured shrimp in 1988 was approximately Rs 174 million and the industry has created 400 to 500 new jobs. The total area of land utilized by shrimp farming is estimated to be in the range of 800 to 1050 ha and could rise to as much as 2000 ha in the next few years. At present the majority of farms that have come into operation are on leased State land. Present Government policy in Sri Lanka is based on the promotion of small scale business and encourages small enterprises.

Shrimp farming practices can be classified into three major categories, intensive, semi-intensive and extensive culture (Apud <u>et al.</u>, 1989). These three types differ in the manner in which they operate, in their land requirements, stocking densities, feed requirements, complexity and investment needs. Table 7.1 illustrates the salient points of the three culture systems. In Sri Lanka all three types are evident but most farms practice either semi-intensive or intensive systems involving *Penaeus monodon*. These two types of culture have a distinct advantage over extensive culture with respect to land requirements, needing less land to produce each ton of shrimps (Table

Table. 7.1. Comparision of three different levels of shrimp culture practiced in Sri Lanka in relation to land requirements.

	Extensive	Semi-intensive	Intensive
Stocking density (No. of PL /ha)	4,000 -5,000	30,000	150,000
Average production (kg/ ha/ year)	300 -5000	1,500	10,800
Extent of land (ha) required to produce 1 mt/ year	5.0	0.67	0.09

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7.1).

Mangroves are ecologically and economically valuable resources both in terms of their direct uses and their contribution to coastal and estuarine fisheries in the study area (Amarasinghe, 1988 a). Most land use conflicts and damage to ecological balance appears to arise in mangrove areas.

Almost all land earmarked or developed for shrimp farming on the West Coast lies in two administrative districts namely, Puttlam and Gampaha. Puttlam administrative districts are subdivided into six administrative subdivisions, while Gampaha is divided into four sub-divisions. The governing bodies of these two districts act independently in taking decisions over land use policies.

#### 7.2. Results

7.2.1. Land use pattern prior to shrimp farming activities.

Fig. 7.1 shows the distribution of different land use categories in the administrative sub divisions of Puttlam District while Fig. 7.2 shows the different land use categories in those of Gampaha District based on the land use maps of the Department of Survey.

In Puttlam District, homesteads, coconut land, paddy land and sparsely used cropland dominates the land use. Dense forest, open forest, forest plantations and scrubland also occupy a considerable land area in this district. Homesteads, coconut plantations, paddy growing areas and marshland dominate the land use categories of Gampaha District.

Scrubland, grassland, sparsely used cropland, mangroves, barren land, coconut land, homesteads, and paddy land have the most potential for shrimp culture development. Annex. 7.1 and 7.2 shows the proportional distribution of potential land for shrimp culture development, water area, mangrove area,

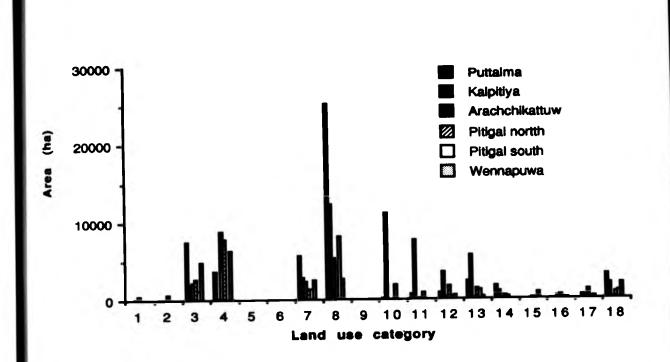


Fig. 7.1. Distribution of different land use categories in the administrative sub-divisions of Puttlam District.(1. built up land; 2. associated non-agricultural land; 3. homesteads; 4. coconut land; 5. cashew plantations; 6. mixed tree; 7. paddy land; 8. sparsely used cropland; 9. other cropland; 10. dense forest; 11 open forest; 12 forest plantations; 13. scrubland; 14. grassland; 15. mangroves; 16. marshland; 17. barrenland; 18. water area)

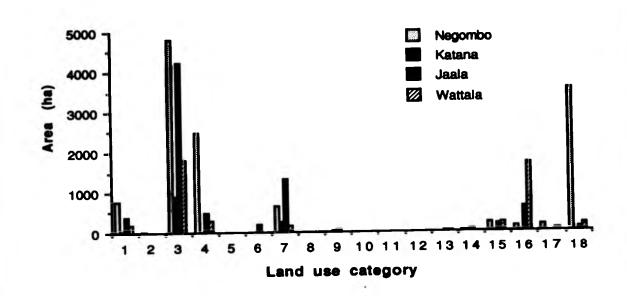


Fig. 7.2. Distribution of different land use categories in the administrative sub-divisions of Gampaha District. (1. built up land; 2. associated non-agricultural land; 3. homesteads; 4. coconut land; 5. cashew plantations; 6. mixed tree; 7. paddy land; 8. sparsely used cropland; 9. other cropland; 10. dense forest; 11 open forest; 12 forest plantations; 13. scrubland; 14. grassland; 15. mangroves; 16. marshland; 17. barrenland; 18. water area )

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paddy growing area and all other land use categories in each administrative sub-division of Puttlam and Gampaha Districts. Potential and existing shrimp culture ventures are situated in the near vicinity of coastal lagoons and saline waterways where there is a close source of saline water.

### 7.2.2. The effects of shrimp culture on land use pattern.

In 1990, the Ministry of Aquatic Resources and Fisheries had approved 896 ha for shrimp farming. However, the actual area will be higher, at around 1000 ha, when small farms constructed on private land are taken into account. These have been constructed without being placed for approval. Applications have been received for a further 2170 ha of Crown land for shrimp culture development (NARA, 1988).

Table 7.2 shows the distribution of proposed/operational farms in the study area by land use category in the year, 1989. The major areas in which farms have been constructed are in main land use types namely, wetland (34.7%), barrenland (29.3%) and agricultural (18.9%) areas. Marshlands, mangroves, coconut land, sparsely used cropland and paddy growing areas are the main subdivisions in which the wetland and agricultural categories fall.

### 7.2.3. Natural plant and animal communities and land use

### 7.2.3.1. Mangrove community.

Sixteen true mangrove species, eleven mangrove-associated species and two salt marsh species have been recorded in the mangals of the West Coast (Aruchelvam, 1969; Kanageratne <u>et al.</u>, 1983; Amerasinghe, 1988 b). Table 7.3 lists the true mangrove species while Table 7.4 lists the mangrove-associated species recorded in the study area.

Avicennia marina, Rhizophora apiculata, Exoecaria agallocha, Aegiceras corniculatus, and Lumnitzera racemosa have been identified as the most predominant species of true mangroves in these areas. Anona glabra, Table. 7.2. Distribution of shrimp farms on the West Coast of Sri Lanka in 1989, categorised by land use.

Type of land	Area of sl ha	Area of shrimp farms ha %	No. of sh No.	No. of shrimp farms No. %
Barren land	294.5	29.3	13	29.5
Agricultural land :	1.05	90	67	6.9
Homesteads	07.0	2.6	5	11.4
Coconut	53.8	5.3	4	9.1
Cropiario	197.5	18.9	16	36.4
Wetland : (Marshlands, Mangroves etc)	340	34.7	Ø	20.5
Unclassified :	159.2	16.4	9	13.6

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Table. 7.3. List of true mangrove species recorded in the study area.

Mangrove species

Source

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Aegiceras corniculatus (L.) Blanco1,3Avicennia officinalis L.1,2,3,4,5Avicennia marina (Forsk) Vierth.1,2,3,4,5Bruguiera gymnorhiza (L.) Lam.1,3,5Bruguiera sexangula (Lour.) Poiret.3Ceriops tagal (Perrottet).1,3,5Excoecaria agallocha L.1,3,4,5Lumnitzera racemosa Willd.1,3,5Nypa fruticans van Wurmp1,2,3,4,5Rhizophora apiculata Blume1,2,3,4,5Sonneratia alba J. Smith1,2,3,5Sonneratia apetala Buch-Ham.1,5,3Sonneratia caeseolaris (L) Engl.1,3,4,5Xylocarpus granatum Koening1,5	Acanthus ilicifolius L.	1,2,3,4,5,
Avicennia officination:Avicennia marina (Forsk) Vierth.1,2,3,4,5Bruguiera gymnorhiza (L.) Lam.1,3,5Bruguiera sexangula (Lour.) Poiret.3Ceriops tagal (Perrottet).1,3,5Excoecaria agallocha L.1,3,4,5Lumnitzera racemosa Willd.1,3,5Nypa fruticans van Wurmp1,2,3,4,5Rhizophora apiculata Blume1,2,3,4,5Rhisophora mucronata Lam1,2,3,5Sonneratia alba J. Smith1,2,3Sonneratia caeseolaris (L) Engl.1,3,4,5	Aegiceras corniculatus (L.) Blanco	1,3
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Sonneratia apetala Buch-Ham. 1,5,3 Sonneratia caeseolaris (L) Engl. 1,3,4,5	Rhizophora mucronata Lam	1,2,3,5
Sonneratia caeseolaris (L) Engl. 1,3,4,5	Sonneratia alba J. Smith	1,2,3
Sonneratia Causeolalio (=, ===,==	Sonneratia apetala Buch-Ham.	1,5,3
Xylocarpus granatum Koening 1,5	Sonneratia caeseolaris (L) Engl.	1,3,4,5
	Xylocarpus granatum Koening	1,5

1. Aruchelvam (1969); 2. Kanegaratne <u>et al.</u> (1983); 3. Marga institute (1985); 4. Wignaraja (unpublished); 5. Amarasinghe (1988 b)

Species	Source
Acrostichum aureum	1,3,4,5
Anona glabra	4,5
Callophyllum inophyllum	5
Cassia oriculata	3
Cerbera manghas	1,4,5
Cissus quadrangularis	3
Clerodendron inerme	1,5
Dalbergia spp.	4
Heritiera littoralis	3,4,5
Hibiscus tiliaceus	2,5
Phoenix zelanica	3

Table. 7.4. Mangrove-associated species recorded in the study area

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1. Aruchelvam (1969); 2. Kanegaratne <u>et al.</u> (1983); 3. Marga institute (1985); 4. Wigneraja (unpublished); 5. Amarasinghe (1988 b) Clerodendron inerme and Acrostichum aureum are the most consistently occurring mangrove-associated species observed. The salt marsh species, Arthrocnemum indicus and Salicornia brachiatsa, were also observed in coastal swamps around Puttlam and north Mundal lagoons. Line transect analysis by Jayasooriya (1988) shows a prominent fringe of Rhizophora apiculata in most of the mangrove areas studied.

### 7.2.3.2. Natural fauna and wild life.

The coastal swamps and brackishwater areas of the West Coast have a very rich natural fauna: 8 species of polychaetes, 17 species of molluscs, 22 species of decapods, 3 species of holothurians, 75 species of fish, 7 species of frogs and toads, 14 species of reptiles, 50 species of birds and 15 species of mammals are known to occur in these areas (De Silva and De Silva, 1982). Coastal areas are also important habitats for birds, both resident species and migrants; these areas act as a vital staging and feeding area for the latter.

The advent of shrimp farming, which has been primarily carried out in these areas or areas bordering them, is believed to have reduced the land available to birds for nesting. Coastal swamps around Mundal are of special importance to ducks, waders and water birds while swamps around Puttlam also offer shelter to seabirds in addition to those found in Mundal. Waders and water birds have been identified as important members of the swamp community around Negombo and Chilaw lagoons. According to the mid-winter fowl census in 1988 (Hoffman, 1988), the coastal swamps of the West Coast support approximately 70,000 birds (Annex. 7.3).

7.2.4. Traditional farming practices and shrimp culture.

Traditional paddy growing occurs in low-lying areas adjoining aquaculture developments. Paddy fields are also found in the upper reaches of the small waterways which feed into the lagoons and estuaries. The total land cultivated with paddy approximates to 20,500 ha. With the advent of shrimp farms the water table has become more saline as a result of the large quantities of water discharged. The effects of this discharge on nearby paddy fields is apparent in terms of the number of complaints received from farmers. A survey in Puttlam District indicated that 11 of 14 small-scale paddy farmers interviewed, noted that one of the major problems they faced was salt water intrusion from the nearby shrimp farms.

The communities living in the coastal areas have also been traditionally involved in animal husbandry. Cattle, buffaloes and goats are the most common forms of livestock reared (Annex. 7.4). Most of the open grazing land and scrubland is used for maintaining these stocks and are also potential areas for shrimp culture.

### 7.2.5. Village expansion and land for shrimp culture.

The competition for land, between village expansion and shrimp culture, is one of the most serious sources of potential conflict and to an extent the problem has already begun.

The population of these areas is increasing at a rate of approximately 3% per year (NARA, 1988). With this increase, the demand for residential land will increase rapidly. The most common source would be from land types that would be most likely to be used for shrimp culture, such as bare land, sparsely used cropland and grassland. The extent of conflicts in interest would be difficult to predict, but a yearly population increase of 10,000 persons (NARA, 1988) for the area has been estimated. This alone will have a significant effect on land demand.

#### 7.3. Discussion.

The development of shrimp culture on the West Coast is still in its infancy, but nevertheless is rapidly expanding. The total land area utilized by shrimp farms approximates to 1,000 ha and a further 2100 ha of state land are under consideration for release to prospective developers.

Estimates of the actual suitable land for shrimp culture on the West Coast ranges between 1800 and 2400 ha (NARA, 1988). At present the use of all suitable land for culture has not been exhausted. However, based on the applications received for more land, and the increasing number of small scale private farms in the area, it is expected that the maximum level will be reached very shortly.

The majority of land already in operation was owned by the government or its agencies; however, the use of private lands for culture has begun and it is expected to significantly contribute to production in the near future.

Based on present information it can be seen that the two land classes most heavily in demand at present for culture are barrenland (inclusive of scrubland) and wetlands. The implications of this on the current land use patterns are twofold. The demand on wetland\$, mostly State owned, is significant, based on present requests. The wetland under request approximates to 89 % of the total wetland surrounding the water fringes. The potential effects of this on wildlife, particularly bird life would be catastrophic.

The loss of mangrove areas due to shrimp farming will have a severe impact on the coastal fishery and coastal communities. At least maintenance of buffer zones (Macintosh, 1982) and demarcation of conserved areas needs immediate consideration and action.

The use of barren and scrubland classes for shrimp culture has important social consequences, especially with respect to village expansion and the raising of livestock. The availability of land for expansion by the coastal communities bordering these areas is minimal, and as such most future expansion will compete with areas where shrimp culture could take place, even though some of these areas might not be suitable for human habitation.

Agricultural land is the least-demand category for shrimp farming. However, it is the effect on paddy land that has the potential to raise the greatest conflict in the future. At present the paddy growing areas that have been converted or are to be converted to shrimp farming are small. However, it is envisaged that more paddy lands will be converted to shrimp in the near future as the higher revenue from shrimp farming will encourage this. Furthermore, paddy cultivation is seasonal and depends on freshwater availability, which is a problem in these areas.

There is a further impact on paddy growing areas in the vicinity and those areas situated along the upper reaches of the waterways; that of salinity intrusion. The full effect of salinity intrusion has not yet been felt as the number of operational farms in these areas is still small. However, complaints on this matter have been recorded and are likely to increase as more farms are constructed on private land.

The present land use pattern has created several important changes in the acid sulphate and potential acid sulphate sediment of these coastal areas of Sri Lanka. Acid sulphate coastal swamps reclaimed for paddy land in these areas have been subjected to repeated drying and flushing for seven to eight decades. During these processes most of the pyrites have been washed away and are in a favourable state for shrimp culture. Conversion of these lands into shrimp ponds will minimize the risks related to acid sulphate soil conditions.

On the other hand, most of the land classified under present land use categories of barren land, marshes and mangroves which are earmarked for shrimp culture development exhibit acid sulphate or potential acid sulphate conditions. Therefore, it is important to consider soil type and distribution in addition to those aspects of land use. Those soil characteristics are dealt with in the following chapter.

# CHAPTER 8

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# CLASSIFICATION AND MAPPING OF ACID SULPHATE SOILS AND POTENTIAL ACID SULPHATE SOILS IN AREAS EARMARKED FOR SHRIMP CULTURE DEVELOPMENT IN SRI LANKA.

## 8.1. Introduction.

# 8.1.1. Geology of the West Coast of Sri Lanka.

Knowledge of geology is essential in understanding the distribution and properties of acid sulphate soils in Sri lanka. The general succession of geological formations and the main mineral deposits in Sri Lanka are given in Table 8.1 while Fig. 8.1 shows the distribution of the main geological formations on the island. Deposits of the Precambrian, Jurassic, Miocene, Pleistocene and Holocene periods are evident in the coastal areas of Sri Lanka. The most recent deposits (Holocene) include both residual and alluvial formations (Wadia, 1941; Herath, 1973). Residual deposits include the deep weathered zones or soils to be found in the central hill country and on the intermediate slopes. Alluvial deposits are concentrated in the flood plains of the river systems draining into the south west coastal areas. Pleistocene deposits are red earth and gravel deposits confined mainly to the North West. Lateritic deposits are well developed in the South West of the island. They are highly sandy and contain more than seventy five percent quartz. The majority of the alluvial clay deposits contain varying amounts of lateritic material (Herath, 1963). Jurassic deposits show a limited distribution and Table.8. 1. General succession of geological formations and main mineral deposits in Sri Lanka. (Adapted from Herath, 1973)

Main geological divisions Era	visions Period	Geological formations	Main mineral deposits
ANTHROPOZOIC	HOLOCENE	recent residual and alluvial deposits, blown sand, coastal sand stone, coral and shell formations, beach mineral sands	kaolin, alluvial clay, silica sand ilmenite, rutile, monasite, zircon garnet, thorianite, clay ochets
Cichord	PLEISTOCENE	laterites, gravels, red earths limestone	laternes, immous non ore limestones
CENOZOIC	MICCEINE		
MESOZOIC	JURASSIC	shales, carbonaceous shales, arkosic sand stones	shales
PALAEOZOIC		absent	•
ARCHAEOZOIC	PRE-CAMBRION	chanockites, meta-sedimentary belt, gnesses, migmatites, intrusives granites dolomites	lime stone,dolamite, magnetite quatz, alabanite, feldspar, graphite, magnetite, mica cordierite, apatite, chert, wollastonite, sillimanite

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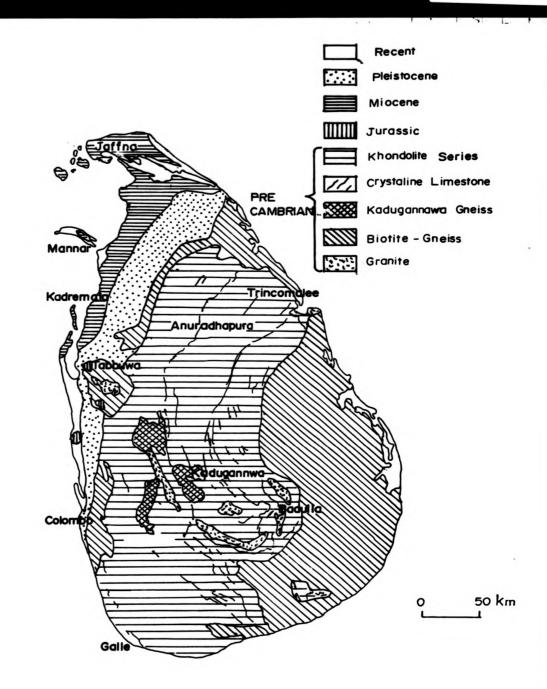


Fig. 8.1. Distribution of the main geological formations in Sri Lanka. (From Herath, 1973)

consist mainly of shales (Herath, 1969).

There are several river systems draining into the coastal areas of Sri Lanka (Fig. 8.2). These rivers have radial distribution and the upper reaches are confined to the central hills of the country.

During the wet season large stretches of the low lying areas are subjected to floods. The alluvial deposits which are detrital clay, silt and sand brought down by streams are deposited in the flood plains. Extensive stretches of alluvium are evident in the Western coastal areas of Sri Lanka.

Lagoons, estuaries and associated swamps are common along the coastal belt and represent potential areas for coastal aquaculture development. The swamps have been subjected to several inundations by sea water during the geological history. The presence of molluscan shells of living species at certain levels in the deposits provide evidence of such inundations.

The deposits of some of these swamps includes the debris of several generations of forests and vegetation that have been swept into the marshes with the remains of brackish water vegetation which grew in the lagoon itself.

8.1.2. Clay deposits and shrimp culture development.

The swamps and marshes associated with brackish water bodies along the

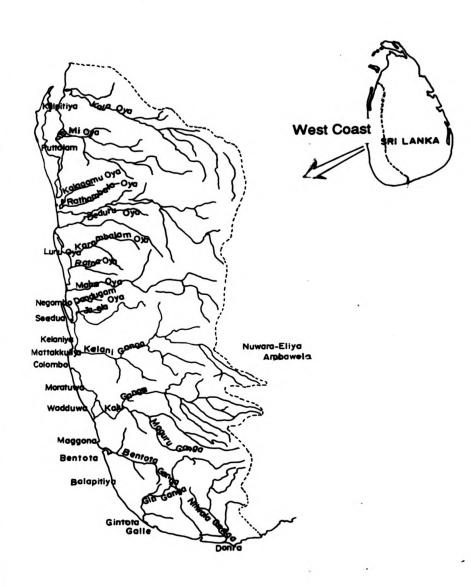


Fig. 8.2. Distribution of main river systems draining into West Coast of Sri Lanka. (From Herath, 1973)

Western coastal belt have been identified as the areas of greatest potential for coastal aquaculture development in Sri Lanka. Clayey deposits are the most predominant sediment type in these areas and such sediments are generally accepted as suitable material for shrimp pond construction and dike building (Chiu, 1988 a; Apud et al., 1989; Poemomo, 1990).

It has been recognised that the behaviour and properties of clay are directly related to their mineralogical composition and texture (Herath, 1973; Young, 1976). In general clays are complex and diverse in composition. They frequently contain many mineral species intimately admixed, all of which have an influence on the sediment's properties as a whole. The predominant minerals are limited in numbers. Geologically, clays are composed of various minerals of primary and secondary origin.

Herath (1973) has classified clayey sediments broadly into three groups, wet zone clays, dry zone clays and intermediate zone clays. This classification indicates that climatic conditions have played an important role in clay mineral development in Sri Lankan soils. Table 8.2 shows the most abundant minerals and other minerals present in these different clay sediments.

Wet zone clays include kaolinite, gibbsite and goethite as the most abundant minerals. These formations are favoured by the wet zone weather conditions. Gibbsite and goethites are not predominant in dry zone clay sediments. Intermediate clayey sediments indicate mineral development features Table. 8.2. The most abundant minerals and other minerals present in different clayey sediments of Sri Lanka. ( Adapted from Herath, 1973)

Group of clay	Most abundant minerals	Other minerals
Wet zone	kaolinite, gibbsite, goethite quartz, ilmenite	montmorillonite, balloysite, mica, biotite, vermiculite, bohemite, * other ferric oxides, other primary resistant minerals
Intermediate zone	kaolinite, quartz, ilmenite	mica, gibbsite, goethite, *other ferric oxides, montmonilonite, other primary resistant minerals.
Dry zone	kaolinite, montmorillonite feldspar, quartz, ilmenite	goethite, *other ferric oxides, mica, calcareous material other primary resistant minerals

Other femic oxides include; magnetite, hematite, amorphous ferric oxides, lepidocrocite, limonite, hydrated amorphous oxides. intermediate between the wet zone and dry zone clays.

# 8.1.3. Classification and mapping of acid sulphate soils.

In Sri Lanka, no attempt has yet been made to identify and classify the acid sulphate soils in the coastal areas although part of these areas have been earmarked for shrimp culture development. In the present study an attempt has been made to identify and map these areas in a manner useful in land use planning and development for shrimp culture.

In early classifications acid sulphate soils have been considered as soils that contain cat clay which are strongly acidic and are with yellow jarosite mottles. Some others considered non-acidic mud clays with a high sulphide content as these sediments may acidify considerably upon drying and oxidation of the sulphides to acid sulphates (Moorman, 1963; Kevie, 1973). In the soil map of the world (FAO /UNESCO, 1968) the name thionic gleysols was used to identify acid sulphate soils; this was later changed to thionic fluvisols (FAO /UNESCO 1970).

# 8.1.3.1. USDA classification system.

In the classification proposed by USDA (1970), acid sulphate soils have been classified in a separate great soil group. This American classification system has subsequently introduced the great soil group sulfaquents.

This group comprises strongly acid soils with a sulphuric horizon with yellow

jarosite mottles and a pH (1:1 in water) less than 3.5 in some part of the top 50 cm of the profile.

# 8.1.3.2 International classification system.

In international classification system (Soil survey staff, 1975) potential acid sulphate soil are recognised by the presence of sulphidic materials, while acid sulphate soils are recognised by the presence of a sulphuric horizon (Dent, 1986).

In potential acid sulphate soils sulphidic material are identified as water logged mineral or organic materials that contain 0.75% or more sulphur (dry weight) mostly in the form of sulphides. They also should have a very low carbonate concentration when compared to that of sulphur. The sulphuric horizon of acid sulphate soil is described as mineral or organic material that has both a pH less than 3.5 (1:1 in water) and jarosite mottles. The main categories of potential acid sulphates and acid sulphate soils identified under above classification and the main properties of those soils are given in annex 8.1.

# 8.1.3.3. ILRI Classification system.

According to Dent (1986), if a classification is to be useful for management and land use planning the classification should be based on soil properties that are important to land use, especially acidity or potential acidity, composition and texture, ripeness, profile form and thickness of the limiting horizons.

The established early classification systems have not considered such properties in combination and therefore are insufficient to provide basic data for land reclamation, management or land use planning. For this reason a new classification has been proposed by the International Institute for the Land Reclamation and Improvement (ILRI) which does consider some of these soil characteristics (Dent, 1986). The criteria and limiting values that determine different soil classes are discussed in chapter 2 under materials and methods.

## 8.2. Results.

8.2.1. Soil classes and soil profile forms in the study area.

The various soil classes identified in the areas earmarked for shrimp culture on the West Coast of Sri Lanka are shown in Figs. 8.4 to 8.6. Different soil profile forms identified in coastal areas of Chilaw and Negombo lagoons are interpreted in aerial photographs (Department of Survey, 1985) in Plates 8.1 and 8.2. Table 8.3 describes the characteristics of the various soil classes identified in the study area.

The coastal swamps around Negombo and Chilaw lagoons consist of acid sulphate soils and potential acid sulphate soils while those of Bolgoda lagoon

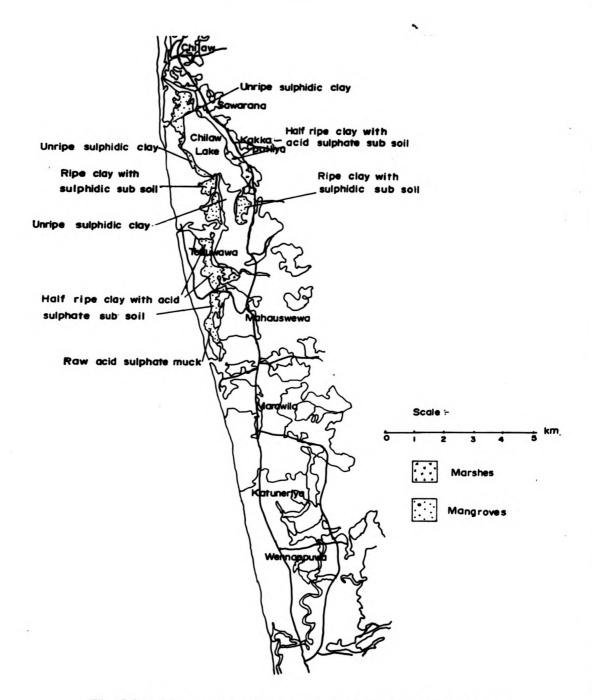


Fig. 8.3. Different acid sulphate and potential acid sulphate soil classes identified in coastal areas around Chilaw lagoon.

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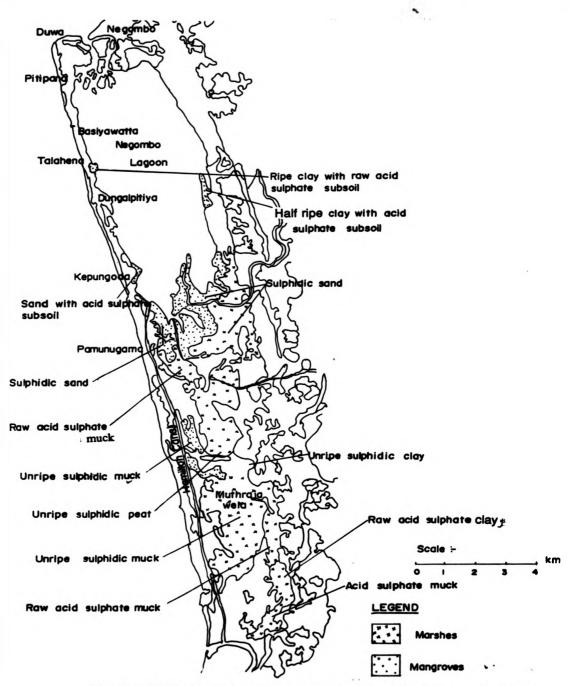


Fig. 8.4. Different acid sulphate and potential acid sulphate soil classes

identified in coastal areas around Negombo lagoon.

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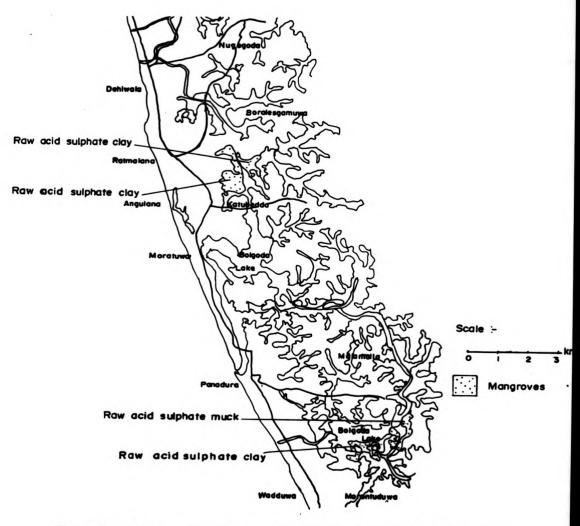


Fig. 8.5. Different acid sulphate and potential acid sulphate soil classes identified in coastal areas around Bolgoda lagoon.

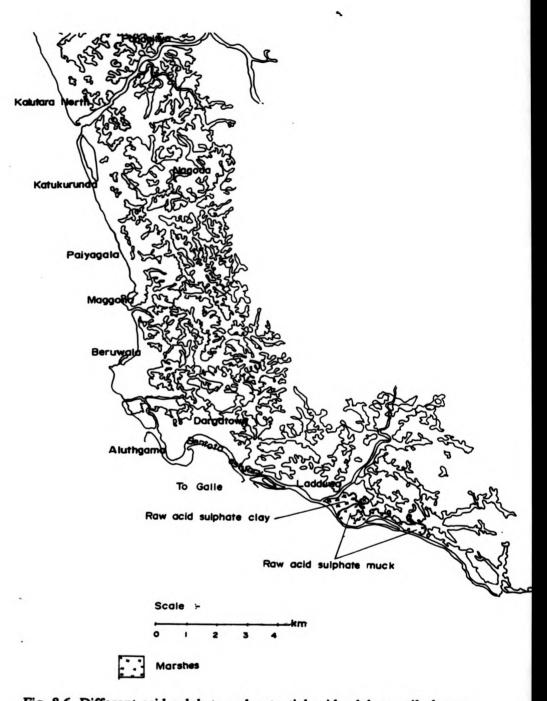


Fig. 8.6. Different acid sulphate and potential acid sulphate soil classes

identified in coastal areas around Bentota estuary.

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Plate. 8.1. Different soil profile forms identified in the coastal areas around Chilaw lagoon interpreted on an aerial photograph (Original photograph : scale 1 : 6000; source Department of Survey, Sri Lanka)



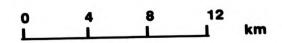
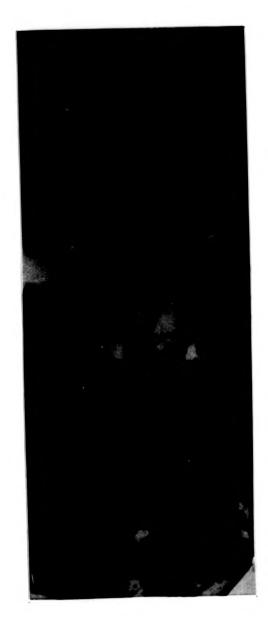


Plate. 8.2. Different soil profile forms identified in the coastal areas around Negombo lagoon interpreted on an aerial photograph. (Original photograph: scale 1: 6000; source Department of Survey, Sri Lanka.



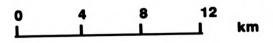


Table. 8.3. Different soil classes identified in the study area and their characteristics.

# a Potential acid sulphate soils.

1. Sulphidic sand :

In these soils sand content is more than fifty percent. pH is greater than 4 but potentially acidic within 100 cm of the depth profile.

2. Unripe sulphidic peat / muck:

Unripe sulphidic soils that are at present water logged but which will become severely acidic when drained. The soils are unripe and n-values are more than 0.7 within 60 cm of the surface and more than 1 within 100 cm. Organic soils and the organic matter content is more than twenty percent of dry mass.

**3.Unripe sulphidic clay:** 

The clay and silt content of these soils are more than fifty percent of the mineral fraction. These soils are water logged ,but contain pyrites that will oxidise after drying and produce acidity in excess of the soil neutralising capacity. pH of the 100 cm region is greater than 4 but after incubation it falls below 4. Unripe and the n-value is greater than 0.7 in the upper 20 cm region of the depth profile. Table. 8.3. continued.

4. Ripe clay with sulphidic sub soil :

These soils have high silt and clay content (more than fifty percent). Potential acidity in the 80 to 100 cm region of the soil profile. Ripe soils n-value in the 0 to 20 cm region is less than 0.7.

## Acid sulphate soils:

1. Acid sulphate muck:

Organic soils with organic matter content greater than twenty percent dry mass. Acid sulphate conditions with pH less than four, the soil is ripe with n-value less than 0.7 to a depth of 60 cm and less than 1 between 60 cm and 100 cm.

2. Raw acid sulphate muck:

Organic soils as above. acid sulphate conditions with a pH less than four. Soils are unripe and n-values are greater than 0.7 within 20 but is between 0.7 and 1.4 in 40 to 60 cm depth region.

3. Raw acid sulphate clay:

Clayey soils with more than fifty percent silt and clay content. pH values are less than four in the most part of the 0 to 100 cm region of the soil profile. They are unripe with n-values greater than 0.7 within 20 cm region.

# Table. 8.3. continued

4. Half ripe clay with acid sulphate sub soil:

Clay content of the soil is as above. pH values less than four in 80 to 100 cm region. n-value is more than 0.7 in the 0 to 20 cm region while at the 40 to 60 cm region the n-value is between 0.7 and 1.4.

5. Ripe clay with acid sulphate sub soil :

Silt and clay content as above. Acid sulphate conditions, pH less than four in the 80 to 100 cm region of the depth profile. n- value below 0.7 in the 40 to 100 cm region.

6. Sand with acid sulphate sub soil:

Sandy soils. Sandy layer more than 60 cms. Soils are acidic (pH less than four) in the 80 to 100 cm region.

n-value: soil ripeness.

rable. 8.4. Comparison of various soil classes and soil profile forms identified at different lagoon / estuarine areas during the Survey.

ILRI soil class	Soil profile form	3	Lagoon / estuarine area	area	
- Andrew States		o	z	8	BE
roteriual add suprate solls.					
Sulphidic sand	p1p2p3s		+		•
-	p1p2s		+		•
Unripe sulphidic peat	p1p2p3ow2		+		•
Unripe sulphidic muck	p1p2p3o/s	•	+		•
Unripe sulphidic clay	p1p2cw2	+	+		•
•	p2p3cw2	+	•		•
Ripe clay with sulphidic sub soil	p3cw1	+	•		•
Acid sulphate soils:					
Acid sulphate muck	a1a2a3os		+		•
Raw acid sulphate muck	a1a2a3o/cw2		+	+	•
•	a1a2a3o/cw3	+			+
Raw acid sulphate clay	ala2a3cw2		•	+	•
	a1a2a3cw3		•	•	+
	ala2cw2		+		•
Half ripe clay with acid sulphate sub soil	a3cw2	+	+		•
Ripe clay with raw acid sulphate sub soil	a3cw1		+	•	•
Sand with acid sulphate sub soil	a3s		+		•

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(C-Chilaw lagoon, N- Negombo lagoon, BO- Bolgoda lagoon, BE-Bentota estuary)

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and Bentota estuary consist only of acid sulphate soils (Table 8.4). The number of soil profile forms found among different lagoon and estuarine systems varied widely. The coastal swamps of the Negombo lagoon showed the highest diversity in profile forms (11), followed by Chilaw (5), Bolgoda(2) and Bentota (2).

#### 8.2.2 Coastal swamps of Chilaw lagoon.

The potential acid sulphate soils found in these areas are unripe sulphidic clays and ripe clays with sulphidic sub-soil. They fall into the profile forms p1p2cw2, p2p3cw2 and p3cw1 (Table 8.4). The acid sulphate soil classes identified were raw acid sulphate muck and half ripe clays with acid sulphate sub-soil and the profile forms were a1a2a3ocw3 and a3cw2. Most of the potential acid sulphates were concentrated more towards the northern region of the lagoon while acid sulphate soils were concentrated at the southern region of the lagoon and the associated water ways. The predominant potential acid sulphate class was unripe sulphidic clay while half ripe clay with acid sulphate sub-soil dominated the acid sulphate soil classes.

#### 8.2.3. Coastal swamps of Negombo lagoon.

The largest coastal swamp of Sri Lanka is distributed in the low lying areas around Negombo lagoon and its associated waterways. There the marshes, mangroves and grass and bare lands amount to 3220 ha in these areas.

Four different potential acid sulphate soil classes (sulphidic sand, unripe

sulphidic peat, unripe sulphidic muck, unripe sulphidic clay) are present, these fall into soil profile forms p1p2p3s, p1p2s, p1p2p3ow2, p1p2p3o/s and p1p2cw2. There are six different acid sulphate soil classes (acid sulphate muck, raw acid sulphate muck, raw acid sulphate clay, half ripe clay with acid sulphate sub-soil, ripe clay with raw acid sulphate sub-soil, with acid sulphate sub-soil) falling in to the soil profile forms of a1a2a3o/s, a1a2a3o/cw2, a1a2cw2, a3cw2, a3cw1 and a3s. Unripe sulphidic muck and sulphidic sand dominated the potential acid sulphate soil classes while acid sulphate muck, raw acid sulphate clay dominated the acid sulphate soil classes.

Most of the acid sulphate and potential acid sulphate are concentrated at the southern end of the Negombo lagoon bordering the water ways associated with this lagoon.

8.2.4. Coastal swamps of Bolgoda lagoon.

Reclamation activities and urbanization has greatly affected the extent of swampy areas around this lagoon and only about 340 ha of swamps remain. Only acid sulphate sediments were identified consisting of raw acid sulphate clay and raw acid sulphate muck; these can be categorized under soil profile forms a1a2a3o/cw2 and a1a2a3cw2.

8.2.5 Coastal swamps of Bentota estuary.

Marshy areas and mangroves are confined to the upper reaches of the Bentota

estuary. Only acid sulphate sediments are evident belonging to the classes of raw acid sulphate clay and acid raw acid sulphate muck. The soil profile forms identified were a1a2a3cw3 and a1a2a3o/cw3.

### 8.3. Discussion.

The coastal areas of Chilaw, Negombo, Bolgoda and Bentota lagoons / estuaries have the conditions required for the accumulation of sulphides as described by Breeman (1976); Dent (1986). The sediments are kaolinite rich (Herath, 1973), and are below mean high water level with dense mangrove vegetation. These marshes are amply flushed tidally by brackish water with a sedimentation rate which has allowed mangroves to persist well below mean high water level for several decades.

Low lying areas associated with the Negombo lagoon and its water ways contain the most extensive coastal swamp areas in Sri Lanka. Most of the swampy areas around Bolgoda lagoon and the Bentota estuary have been subjected to reclamation activities and the remaining marshy areas are confined to a few localized patches. As expected, more diversity in soil profile forms as well as in soil taxonomic classes were observed in the coastal swamps of the Negombo lagoon area.

Relative abundance of various soil classes in different lagoon / estuarine areas are given in Table 8.5. Unripe sulphidic clay is the most dominant soil class in coastal swamps around Chilaw lagoon while sulphidic sand and unripe

ILRI soil class	Lagoo	on/ estu	arine a	rea
	С	N	BO	BE
Potential acid sulphate soils:				
Sulphidic sand	-	+++	-	-
Unripe sulphidic peat	-	+	-	-
Unripe sulphidic muck	-	+++	-	-
Unripe sulphidic clay	+++	+	-	-
Ripe clay with sulphidic sub-soil	++	-	-	-
Acid sulphate soils:				
Acid sulphate muck	-	++	-	-
Raw acid sulphate muck	+	++	+	+++
Raw acid sulphate clay	-	-	+++	++
Half ripe clay with acid sulphate				
sub-soil	+++	+	-	-
Ripe clay with raw acid sulphate				
sub-soil	-	+	-	-
Sand with acid sulphate sub-soil	-	+	-	-

Table. 8.5. Relative abundance of various soil classes in different lagoon / estuarine areas.

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Key- C: Chilaw lagoon, N: Negombo lagoon, BO: Bolgoda lagoon, BE: Bentota estuary, (+++) most abundant, (++) abundant, (+) present, (-) absent. sulphidic muck dominates the soil classes in coastal swamps around Negombo lagoon.

8.3.1. Variations in soil profile forms and soil taxonomic classes.

## 8.3.1.1. Climate.

The coastal swamp systems studied fall into different climatic zones. Those of the Chilaw lagoon area belong to the intermediate and dry zone while the coastal swamps of Negombo and Bolgoda lagoons and the Bentota estuary are in the wet zone.

The wet zone annual rain fall (> 2000 mm) is considerably higher than that of the dry and intermediate zones (< 1250mm and 1500mm respectively). The later have a relatively more prolonged dry season than the wet zone. In most of the catchment areas of the wet zone in Sri Lanka, some rain fall is observed during every month of the year (Domross, 1974) while more dry months (2 to 3 months) without rainfall are typical of the dry and intermediate zones.

The mean annual air temperature of the dry zone (27.8°C)are higher than that of wet zone (26.2°C). In dryer climates ripening and profile development processors are faster and deeper as the dry season becomes more pronounced (Dent, 1986). According to Pons (1973) the rate of development of the soil profile is determined by the degree to which external conditions favour drainage and leaching.

## 8.3.1.2. Land form and sand bar formation.

Land form also has been identified as a factor affecting the occurrence of different soil patterns (Dent, 1986). Sulphidic sediments develop more extensively where clayey sediments accrete slowly in brackish water and simultaneously, copious organic matter is supplied by swamp vegetation. The longer the duration of brackish conditions, the greater is the accumulation of pyrites (Dent, 1986).

The formation of sand bars across the outfalls of lagoons and estuaries of Sri Lanka has been described by Wickramasooriya (1969), and Jayasinghe (1979). These sand bars affect the duration of brackish water swamp conditions and consequently will affect the rate of sulphidic sedimentation.

Negombo lagoon and Bentota estuary are connected to the sea throughout the year while one sea outfall from the Chilaw lagoon is cut off from the sea during the dry season due to sand bar formation. The sea outfall of the Bolgoda lagoon has a record of periodical cutoffs due to previous formation of sand bars, although at present it is kept open continuously by means of artificial rock barrier (Jayasinghe, 1979).

8.3.1.3. Tidal influence and the water level fluctuation.

The tidal range can also influence sediment accumulation and the soil pattern

(Thomas et al., 1979; Thomas and Varly, 1982). Although the predicted tides for the different coastal areas studied are similar, the actual tides and water level fluctuations due to tides were observed to be different (NARA, 1988). The tidal effect and the fluctuations in water level in the environment of the coastal swamps of the Chilaw and Negombo lagoons are more pronounced than those of the coastal swamps of Bolgoda lagoon (Jayasinghe, 1979).

#### 8.3.1.4. Vegetation

Soil differences in mangrove swamps supporting different species of mangroves have been identified by Hesse (1961) and Marius (1982). *Rhizophora* bearing soils are more sulphidic than those colonised by *Avicennia* species. Vegetation fuels the process of pyrite formation by supplying readily decomposable organic matter (Dent, 1986). Relatively more true mangrove species are distributed in the coastal areas of Chilaw lagoon, while the swampy areas of Negombo, Bolgoda and Bentota consist of relatively more mangrove- associated species.

## 8.3.1.5. Previous reclamation activities.

The swamps of the Chilaw lagoon area are relatively less disturbed than those of the Negombo, Bolgoda and Bentota lagoon systems. A larger proportion of the coastal swamps in the Negombo lagoon area was reclaimed for paddy cultivation about 70 years ago but are now abandoned. These early reclamation activities would have included drainage of tidal flats bringing about aeration into the upper soil layers. Reclamation activities would also have affected soil leaching and ripening, processes which contribute to the observed differences in soil patterns.

#### 8.3.1.6. Underlying geology.

Geological differences were also observed in the studied coastal systems. The clayey sediments of the wet zone, dry zone and the intermediate zone differ in their mineral composition (Herath, 1973) which would in turn affect the soil pattern and the distribution of different soil classes in each swamp-land.

As described by Dent (1986), differences in climate, land form, vegetation, tidal influence and differences in underlying geology and reclamation activities, are mainly responsible for the differences in soil profile forms in different areas. The present study is a valid conclusion in the case of the coastal swamps of the West Coast of Sri Lanka.

8.3.2. Acid sulphate/ potential acid sulphate soil classes, soil profile forms and shrimp culture development in West Coast of Sri Lanka. Acid sulphate soil classes exhibit acidic conditions from the initial stages of the pond construction. Potential acid sulphates develop acidity after oxidation of pyrites. Generally, potential acid sulphate soil classes may take relatively longer period for reclamation than those of acid sulphate soil classes. Unripe soil classes have easily deformed structure. The material has little frictional and cohesive strength (Dent, 1986) and cannot be recommended as dike building material. Excavation is harmful in areas where potential acid sulphate sediments exhibits potential acidity in their deeper layers of soil profile. If the acidic layers are deep in the profile form of a particular acid sulphate soil class the ponds can be constructed with out disturbing these layers. Unripe soil classes when developed for ponds can induce several environmental problems such as soil erosion, sedimentation and increased levels of total suspended solids in water sources.

Soil classes sulphidic sand, unripe sulphidic peat, unripe sulphidic muck and unripe sulphidic clay have potential acidic soil layers at 0 to 60 cm depth in their soil profiles while acid sulphate muck, raw acid sulphate muck and raw acid sulphate clay have acidic soil layers at the same depths in their soil profiles. These soil classes will create problems in shrimp culture related to the acid sulphate / potential acid sulphate soil conditions.

The soil class ripe clay with sulphidic sub-soil appears to be the relatively less harmful soil class for the development of shrimp culture where the potential acidity is in 80 to 100 cm layer of the soil profile, soils are firm and provides strong material for dike building. Other soil classes that can be considered in land evaluations in shrimp culture development in their descending order of relative acceptability are; ripe clay with raw acid sulphate sub-soil, half ripe clay with acid sulphate sub-soil, and sand with acid sulphate sub-soil (Table 8.6). These soil types were located only in the coastal swamps around Negombo and Chilaw lagoons in the West Coast of Sri Lanka.

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Table. 8.6. Different soil classes and profile forms least harmful for shrimp culture development.

Soil class	Soil profile form Lagoon area	Lagoon area	Remarks
Ripe clay with sulphidic sub-solil	p3cw1	Chilaw	Potential acidity at 80 to 100 cm in depth profile. Avoid excavation below 50
Ripe clay with raw acid sulphate sub-soil	a3cw1	Negombo	cm to avoid exposure. Acidic pH values at 80 to 100 cm depth Avoid excavation below 50 cm to prev-
Half ripe clay with acid sulphate sub soil	a3cw2	Chilaw Negombo	ent leaching of acidity. Acidity in 80 to 100 cm region. Ripe soil in 40 to 60 cm region which is nood as dike huilding material Avoid
Sand with acid sulphate sub-soil	a3s	Negombo	excavation below this layer. Acidity in 80 to 100 cm layer. Sand la- yer at surface. Does not provide strong material for dike construction.

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# CHAPTER 9

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## SUMMARY, CONCLUSIONS AND RECOMMENDATIONS.

The present study was conducted on the West Coast of Sri Lanka in shrimp ponds developed on acid sulphate soils, with a view to understanding the processes which contribute towards problems in shrimp culture related to the presence of these sediments. The main components of the study included investigations on the behaviour and kinetics of the important metals associated with acid sulphate /potential acid sulphate sediments in the pond environment, and a time series study over a normal culture cycle (10 weeks) of the water quality, soil quality and growth of shrimp in ponds on acid sulphate sediments. Disease related conditions, particularly brown gill syndrome, calcium and magnesium in selected tissues and the general histopathology of cultured shrimps were also monitored. A detailed field survey augmented by land use maps, aerial photographs and other data provided information necessary for mapping and classification of the different acid sulphate/ potential acid sulphate sediments along the West Coast. From this survey soil profile forms in the coastal swamps around the main lagoons and estuarine systems earmarked for shrimp culture development were identified and classified.

Pyrite, the main constituent responsible for acid sulphate soil conditions, has considerable influence on the physical, chemical and biological problems affecting shrimp culture on acid sulphate soils. Stresses generated through various processes involving the behaviour of major elements such as iron and manganese systems in acid sulphate soil increase the risks of disease outbreaks in the culture systems.

The traditional management practices such as drying of ponds, maintenance of basic pH values and intensive aeration, procedures which have a positive effcts on shrimp culture under favourable soil conditions, appears to have serious implications for shrimp culture on acid sulphate sediments. The present study shows that careful monitoring of the pond environment, and use of alternative management strategies to minimize the risk of formation of hydrated oxides, will minimize the potential stress on shrimps, which lead to most of the problems in shrimp culture on acid sulphate soils.

#### 9.1. Main conclusions.

1. The general environment of the West Coast of Sri Lanka offers favourable conditions for shrimp culture development. Salinity (15 to 21 %), pH (7.4 to 8.4), dissolved oxygen concentration (6.2 to 7.8 ppm), and temperature measurements (25.0 to 29.5 °C) of the pond environment recorded over a culture cycle at a commercial shrimp farm at North West Coast were within the favourable ranges for *Penaeus monodon* (Chiu, 1988 b; Apud et al., 1989; Boyd, 1989). Levels of sulphides (0.02 to 0.04 ppm), nitrites (0.01 to 0.28), turbidity (6.5 to 30.0 NTU) and total suspended solids (26.0 to 93.2 mg /l) were within the "safe limits" for shrimps (Chiu, 1988 b; Boyd, 1989; Kongkeo, 1990; Poernomo, 1990). The concentrations of dissolved iron (0.49 to 1.75

ppm), manganese (0.15 to 0.27 ppm) and aluminium (0.05 to 0.07 ppm) in pond water did not reach the elevated levels commonly recorded in poorly managed ponds on acid sulphate sediments (Poernomo, 1983; Singh, 1985). However the important nutrients, phosphate (0.01 to 0.06 ppm) and nitrate (0.56 to 2.34 ppm) showed relatively low concentrations.

2. The soil texture, pH, Eh, and organic matter content fulfilled the general requirements for shrimp culture (FAO, 1985; Pillay, 1990). Pond sediments contained very high concentrations of iron (17.4 to 61.48 g/kg) and manganese (74 to 227 mg/kg).

3. Different shrimp culture systems that employ different management strategies showed clear differences in the Eh-pH relationships of the pond sediments as well as in pond water. These relationships confirmed that iron exists as insoluble iron (III) oxides. Soluble manganese (Mn<sup>2\*</sup>) is the most stable form of manganese under prevailing Eh-pH conditions, although the presence of iron oxides can influence manganese to co-precipitate with iron. The formation of iron hydroxides is more favoured in intensive shrimp culture systems with aeration when compared to semi-intensive /extensive systems without artificial aeration systems.

4. Appreciable influxes of soluble iron, manganese and aluminium ions to pond water were not observed during the culture cycle, but significant increases in total iron (6.0 g/kg/week) and manganese (19.1 mg/kg/week) concentrations were observed in surface sediments of the ponds with culture time.

5. Iron (5.4  $\mu$ g/g dry wt/week) and manganese (2.5  $\mu$ g/g dry wt/week) were found to accumulate in muscles of cultured shrimps during the culture cycle. Unusually high concentrations of iron and manganese in gills (iron up to 1588  $\mu$ g/g dry wt; manganese up to 93.2  $\mu$ g/g dry wt) and in carapace (iron up to 778  $\mu$ g/g dry wt; manganese up to 34  $\mu$ g/g dry wt) were the result of accumulation of oxides in-between the gill lamellae, or of precipitation of oxides on the carapace. Longer term exposure of shrimps to an acid sulphate soil environment (18 weeks) resulted in significantly higher concentrations of these metals (4.53 and 14.24 mg/g dry wt of iron; 436.5 and 327  $\mu$ g/g dry wt of manganese in gills and carapace respectively) when compared to wild shrimps (347 and 219  $\mu$ g/g dry wt of iron; 19.8 and 20.9  $\mu$ g/g dry wt of manganese in gills and carapace respectively).

6. The manganese concentrations in gills were closely correlated to concentrations of iron ( $\mathbf{r} = 0.870$ ,  $\mathbf{p} = .014$ ). The Eh-pH relationships appears to be primarily responsible for these high concentrations and to the processes related to accumulation of these metals in cultured shrimps.

7. Brown-gill syndrome is the main clinical sign associated with iron accumulation in shrimp culture ponds developed on acid sulphate soils. This is followed by a black-gill condition at a later stage. The presence of ironcontaining deposits in gills of cultured shrimps was demonstrated in histochemical studies. Gill infestations with Zoothamnium spp. were also common in brown/ black gilled shrimps, but the peritrichous mode of attachment of these ectocommencels appears to inflict no mechanical damage to gill tissue. Histological changes observed in brown gills included pillar cell rupture, dilations in blood sinuses and haemocytic infiltrations. TEM studies indicated damage to osmoregulatory and respiratory tissues beneath the cuticle at the brown-gill stage. At the black-gill stage severe damage to gill tissue was evident.

8. Morphological differences related to poor nutritional conditions (reduction in R-cells and F-cells) were observed in the hepatopancreas of brown-gilled shrimps. Haemocyte infiltrations and damage to myocardial tissue together with signs of parasitic infestations were observed in the heart of the shrimps with black gills.

9. The calcium concentration in the carapace of cultured shrimps showed a significantly negative correlation with culture time (r = -0.945, p = 0.001). Carapace calcium concentration was also negatively correlated to the iron concentration in gills (r = -0.967, p = .001) and iron concentration in surface sediments. Iron accumulation in the gills appears to interfere with normal calcium uptake through the gills in shrimps cultured on acid sulphate soils.

10. Acid sulphate as well as potential acid sulphate soil classes were identified in coastal areas around Chilaw and Negombo lagoons while only acid sulphate

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sediments were identified in Bolgoda and Bentota lagoon/ estuarine areas. Coastal swamps around Negombo lagoon recorded the highest diversity in acid sulphate and potential acid sulphate soil classes as well as in their soil profile forms.

11. Soil classes with acidic or potentially acidic soil layers in surface layers in the upper 60 cm of their profiles (sulphidic sand, unripe sulphidic peat, unripe sulphidic muck, acid sulphate muck, raw acid sulphate muck and raw acid sulphate clay) appear to have more serious environmental implications, as well as detrimental effects on cultured shrimps. The soil classes that have acidic or potentially acidic sediments in deeper layers in their profile (ripe clay with sulphidic sub soil, ripe clay with raw acid sulphate sub-soil, half ripe clay with acid sulphate sub-soil and sand with acid sulphate sub-soil) appears to be the least harmful soil classes for shrimp culture among these sediment types investigated.

12. Differences in total rainfall and distribution, air temperature, sand bar formations at the sea outfall, water level fluctuations in the estuaries / lagoons, vegetation distribution pattern, reclamation activities and underlying geology, all appear to have influenced the diversity of soil classes and profile forms in the coastal swamps studied.

9.2. Main recommendations for shrimp culture in acid sulphate /potentially acid sulphate soil areas.

Based on the findings of the present study the following are guidelines/ recommendations for alternative management strategies to overcome or minimize the adverse impacts on aquaculture related to acid sulphate soil conditions.

# a. Site survey.

Detailed soil surveys and land evaluations are recommended as part of site selection for shrimp farming, especially in acid sulphate soil areas. Surveys should include investigations on acidity, potential acidity, soil ripeness and soil texture in the 0 to 100 cm soil profile in order to identify the detailed soil profile forms.

## b. Pond design and construction.

Soil profile form should be taken into account during pond construction. Avoid exposure of potential acid sulphate soil layers in the profile. If possible the pond bottom should be constructed slightly below the mean low water level inorder to prevent complete exposure of the pond bottom to air.

#### c. Pond preparation by drying.

Avoid drying the pond bottom to prevent oxidation of pyrites and use alternative strategies to eradicate pathogens and predators. Exposure of the pond bottom to air can also increase the oxidative capacity of the soil, which can continue to oxidise the pyrites even after filling the pond with water.

# d. Application of lime.

Application of lime should be done carefully just to overcome the extreme acidic conditions. Higher pH values can favour the formation of harmful metal oxides in culture ponds.

# e. Fertilisation of ponds.

Application of higher doses of fertilizer, particularly the phosphate fertilizers is suggested in higher doses to overcome the low levels of phosphates in pond water. To prevent the fixation of phosphates by pond sediments application in dissolved form is recommended.

## f. Selection of culture systems.

In acid sulphate soil areas semi-intensive type of culture systems which need minimal or no aeration is recommended.

g. Maintenence of oxygen level.

Maintain the minimal "safe level" for oxygen concentration. Avoid the use of artificial aeration as far as possible.

#### h. Monitoring the water quality.

More emphasis should be paid to monitor closely the redox potential and pH of the pond sediments and water. Eh-pH relationships could be maintained with reference to the Eh-pH stability diagrams for metal systems to minimize the

risk of formation of harmful metal oxides.

i. Monitoring of metals in cultured shrimps.

Regularly monitor the iron concentration in cultured shrimps. This will provide warning on the accumulation processes related to metals.

j. Monitoring of brown/black gill syndrome and soft shelled condition in culture ponds.

Regular monitoring of brown/ black gilled condition and soft shelled condition can also be used to identify the initiation of problems in shrimp culture on these soils.

## k. Culture period

Since the metal concentration in shrimp tissues shows a close correlation with the time that shrimps are exposed to acid sulphate soil conditions, adjustment of the culture period to about 10 weeks or less is recommended in acid sulphate soil areas.

#### 9.3. Future research requirements.

The present work provides information on several important aspects of the utilization of acid sulphate soils for aquaculture which have not previously attracted adequate attention. Several aspects of the work merit further attention and research and in particular, future studies should place emphasis on bridging the gap between research results and their application to aquaculture management. In order of priority, the following research is recomended.

a. Detailed studies on manipulation of the pond environment to minimize the oxidation of pyrites and formation of hydrated oxides in the sediment.

b. Investigations of the pond oxygen budget and the effects of pond aeration and Eh-pH relationships on the pond environment.

c. Studies on the utilization of organic manure and lime to manipulate the EhpH relationships in the pond environment.

d. Studies on fertilizer application rates, application frequencies and phosphate fixation by pond sediments to optimise the use of fertilizer and to enhance the natural productivity in ponds.

e. Investigations on shrimp parasites and pathogens in relation to the acid sulphate pond environment.

f. Studies on the changes in the physical nature of iron precipitates with time in ponds and their relationship to the accumulation of iron in gills of cultured shrimps.

g. Studies on ionic balance and other physiological processes of cultured shrimps in relation to acid sulphate pond conditions.

h. Studies on the sensitivity of other shrimp species to the acid sulphate pond environment with aview to finding potential alternate species of culture.

i. Further investigations into economically feasible methods of removing iron from the pond environment.

j. Assessment of the environmental impact of iron, aluminium and manganese from acid sulphate sediments used for shrimp culture.

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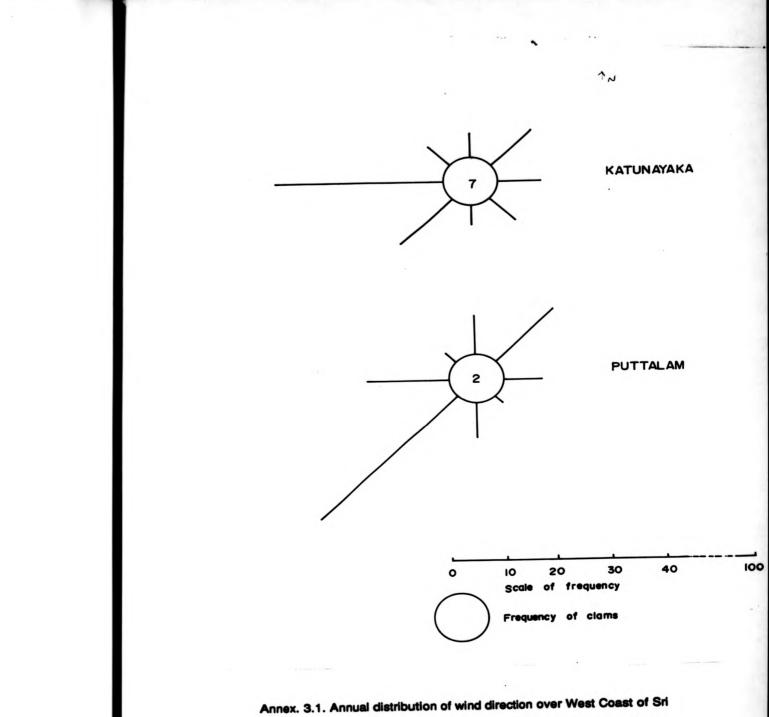
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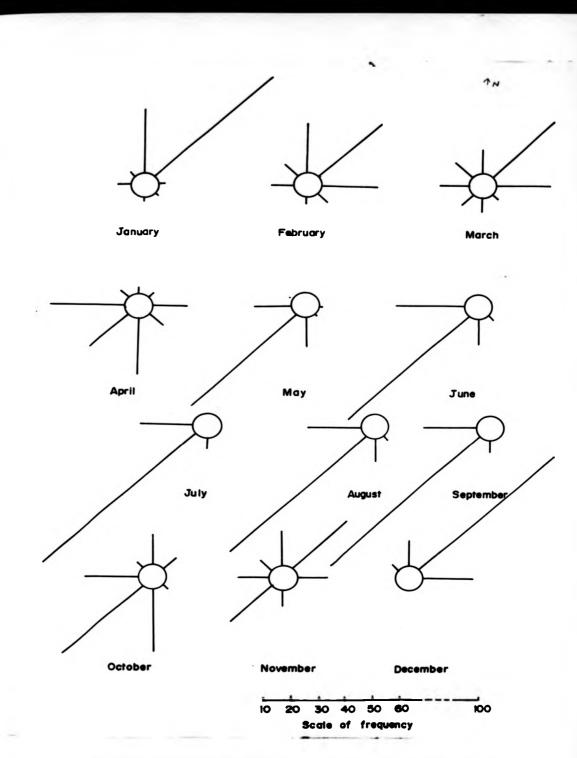
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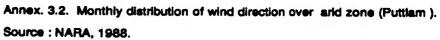
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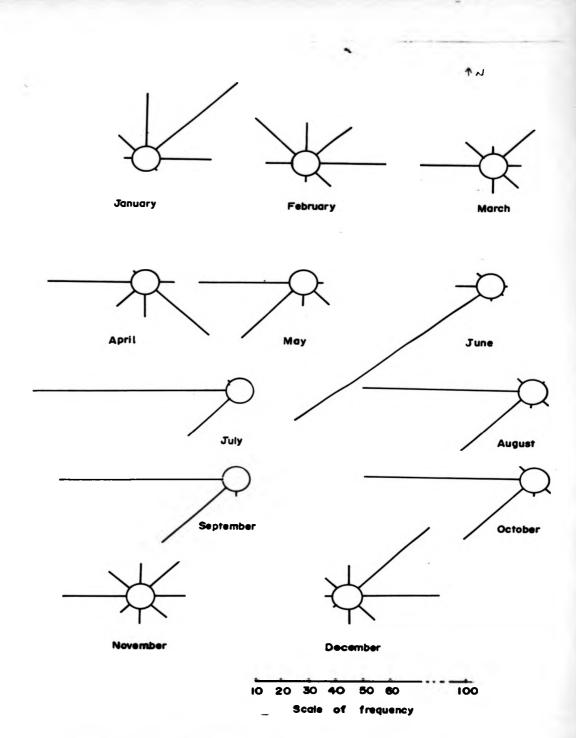
# ANNEX



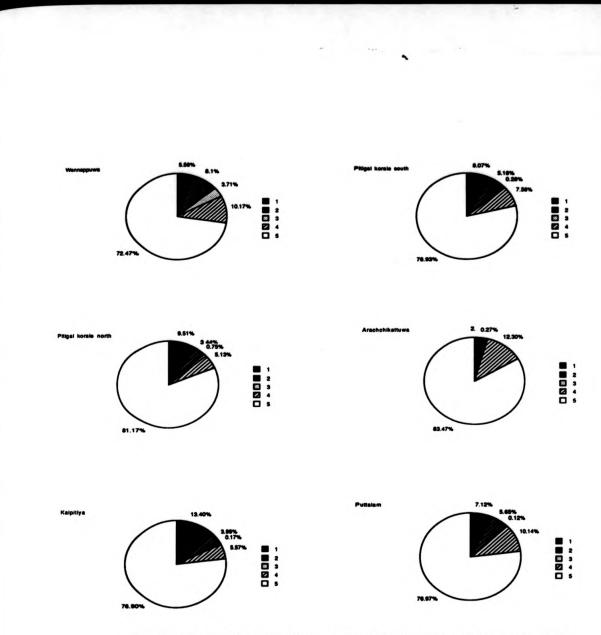
Annex. 3.1. Annual distribution of wind direction over West Coast of Sn Lanka. Source : NARA, 1988. (A) wet zone (Katunayaka), (B) and zone (Puttlam).



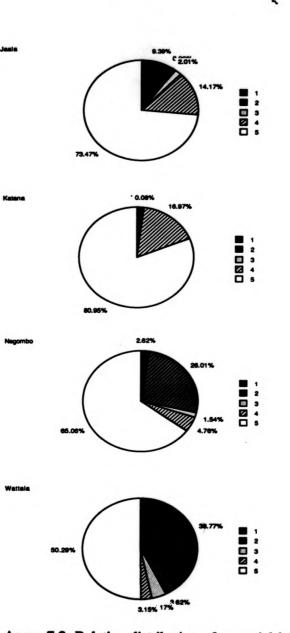


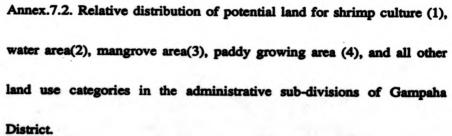


Annex. 3.3. Monthly distribution of wind direction over wet zone (Katunayaka). Source : NARA, 1988.



Annex. 7.1. Relative distribution of potential land for shrimp culture (1), water area (2), mangrove area (3), paddy growing area (4), all other land use categories in the administrative sub-divisions of Puttlam District.





Annex. 7.3. Water bird census on the West Coast of Sri Lanka (Adapted from Hoffman, 1988).

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	NO. OI	No. recorded in	COAStal	
	species	swamps		
		₽-C	С-и	
bes	1	90	12	
icans	1	1	-	
morants	4	907	122	
ons	12	1563	632	
rks	5	29	20	
.805	2	274	-	
ks	4	15217	212	
Ls/Coots	15	877	237	
ers	13	31782	168	
ls/ Terns	10	16753	00	
	77	67493	1403	

Annex. 7.4. Livestock statistics for the administrative sub divisions of Puttalam district (Puttalam Kachchery, 1989)

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Division	Cattle	Buffaloes	Goats	Sheep	Total
Wennapuwa	6525	2171	2437	0	10863
Piti.north	100080	2171	2435	160	14846
Piti south	7640	2429	4260	0	14149
Arachikattu	23790	2280	5055	225	31350
Puttallam	12875	1169	4715	260	19019
Kalpitiya	9593	165	10676	55	20489

Annex.8. History of Shrimp Farming Development in Sri Lanka.

1 History and present status.

Shrimp culture in the Western Coastal areas of Sri Lanka commenced in the mid 1980's. The first recorded production from shrimp farming was for 1984 and amounted to 10 mt. Within 4 years the production rose to 669 mt (1988) and shows the every sign of increasing further. The revenue from cultured shrimps in 1988 was approximately SL Rs. 174 million. The total area of land utilized by shrimp farms by the end of the year 1988 was estimated to be in the range of 800 to 1050 ha (NARA, 1988).

### 2. Technology

Shrimp farming in Sri Lanka can be categorized broadly into three levels of operation. The first, those companies involved in large scale operations with large sums of money invested in the technology of shrimp culture. These companies usually have some form of joint collaboration with foreign personnel or companies, and are technologically independent. The second group are those farms that primarily depend on local technology with a sizeable investment, though not as large as those with foreign participation. The third group are those farmers which operate small scale farms usually not greater than 5 ha in extent run by individual investers.

## Annex.8 continued

Among the differences between the first two categories and the third is that small farms (by and large) are constructed on private land whilst the larger operations use state land obtained through leasing and other means.

Pond size are typically in the range 0.4 to 1.0 ha.Pond preparation consists of liming and filling the pond with water after the drying process. Drying of the pond is carried out for two to three weeks after the harvest, but some of the ponds cannot be fully drained as they are situated below the mean water level of the water source. Tilling is carried out by 30% of the farms, after the draining of the pond using a harrow. Liming is mostly carried out during pond preparation at a rate of 200 to 500 kg/ha. Chemical fertilizers (urea and triple super phosphate) are used by some farms during the initial pond preparation in very small amounts (urea 10-25 kg/ ha, TSP 10-15 kg /ha). Organic fertilizers are presently not used. The water is pumped to a depth of 20 cm and left to season for 10 to 14 days. The pond is then filled to approximately 1 m. Stocking commences approximately 1 month after the pond preparation commences.

The major species of shrimp cultured is the tiger shrimp *Penaeus monodon* although some farms have experimented with the Indian shrimp *Penaeus indicus* and the banana shrimp *Penaeus merguiensis*. In the case of the latter two species the results were not encouraging because their low survival rates. In the initial stages of shrimp farming in Sri Lanka seed supply was a major problem for small scale operators and this severely affected the planning of their culture cycle. However in recent times the supply of seed through the many hatcheries that operate has stabilised, and is no longer a cause for concern.

After purchase, post larvae (PL 20 to 35) are stocked directly in the ponds with minimal acclimatization. Stocking densities vary between 20,000 and 50,000 /ha. At the initial stages of the cycle, water is pumped into the ponds in order to maintain a constant water level. This amounts to filling the pond with about 5 cm per day, which is lost to seepage and evaporation. At the later stages of the culture cycle, after the second month, more frequent exchange is carried out depending on the water quality. A few farms regularly exchange water once in two days between 10-15% of the total volume. Water is released through pumping or using the sluice gate.

Feeding is carried out twice a day using the stipulated rations of the manufacturer based on the initial number of post larvae stocked. Feeding trays are rarely used, hand broadcasting being the main method. A recent trend to supplement part of the formulated diets with bivalves (*Meretrix* sp.) has been introduced by some of the farms. The mussels are usually boiled and the shells removed. In the third month the amount of feed distributed per day Annex. 8 continued

approximates to 24 kg of formulated feed and 40 kg mussel meat/ ha, at the farms which use mussel meat. Farms that do not use mussels, feed the stock at rates varying from 5 to 8% of the body weight per day.

No regular monitoring practices for water quality are carried out. Most observations with respect to water quality are based on "gut feeling". However, regular sampling for growth is carried out usually atweekly intervals. The yields are in the range of 750 to 1600 kg/ha/crop.

Harvesting is carried out between 3 to 4 months after stocking. The accepted harvesting size averages 32 pieces/kg. Most of the farms operate 2 culture cycles per year.

The pond is partially drained, a drag net is used for harvesting. This net is usually rented from local fisherman. After the use of the dragnet the pond is emptied of all the water. Hand-picking of shrimps on the bottom is then carried out. It should be noted that some farms do not use a dragnet, instead they simply empty the pond and hand-pick the shrimps. The harvest is usually stored in barrels with ice and immediately transported to the buying centre. Annex.9. Cluster analysis.

Introduction

Cluster analysis is a multivariate statistical technique widely used in geochemical data analysis. This technique is aimed to group data together on the basis of their similarity in terms of their composition.

1. SAS statistical package and cluster analysis.

The statistical package SAS has the capability to differentiate a given set of data into clusters considering their similarities. SAS package also provide cluster means, standered deviations of the means and centroid distances (distance between two clusters).Centerod distances are helpful in comparing clusters obtained for different sets of data. In the present work the centroid distance for 2 clusters differentiated for water (109.0) is lower than the centroid distance among 2 clusters obtained for sediments (118.5). This indicates that clusters obtained for sediment are relatively more differentiated than those obtained for water.

#### 2. Goodness of clusters.

The goodness or the validity of the grouping can be assessed using several statistical procedures. Howarth and Larsen (1983) have described values referred to as B, W, and T in assessing goodness of clusters.

Dispersion of all values about the grand mean (T):

The value of T which is fixed for a given set of data gives the dispersion of all values obtained around the grand mean. The relative value of T is valuble in comparing the dispersion of clusters obtained for 2 different sets of data.

#### Annex. 9 continued

Dispersion of all values about their respective groups (W): The scatter of values obtained for each group around their group or cluster mean is reflected by the value of W.

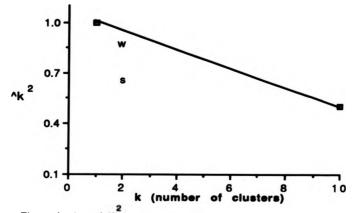
Dispersion between the groups (B):

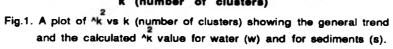
Dispersion of group mean (cluster mean about the grand mean is represented by B value.

The good grouping criteria can be obtained with the help of the relative values obtained for B and W. Relatively higher values in W and lower values for B in a particular clusters obtained provide a good indication of clustering. It is apparent from the values obtained for W and B in the present study that the grouping of clusters appear to be reasonable. However these values still do not quantify the validity of the clusters indicated.

3. Determination of Wilk's lambda (^).

Wilk (1932) has derived a test statistic, Wilkk's lambda, to assess and quantify the goodness of clusters obtained in cluster analysis. This test statistic (^) is derived using the ratio of determinants of W (IWI) and T (ITI). The value of(^) will decrease inversely with  $k^{2}$  (k= no of clusters obtained). The general trend is that if  $^{k^{2}}$  is plotted against k, will decrease from a value of 1.0 to k=1 to 0.5 at k=10 (Fig.1). Statistically valid clusters can be partitioned if the calculated values for  $^{k^{2}}$  fall below this general trend.







# Annex. 9 continued

The  $^k^2$  values calculated for clusters obtained for water (0.88) and sediments (0.66) fall below the general trend described above. It may also be noted that the  $^k$  observed with respect to sediments falls well below the that observed for water. This indicates that the clusters observed for sediments are statistically more significant than those observed for water.

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