final report

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Feasibility Study on Using Integrated Aquaculture to Treat Wastewater from the Meat Processing Industry

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CONTENTS

1 Executive Summary

This feasibility study was done to treat Abattoir Wastewater at TMC using an Integrated Aquaculture approach and culture fish of commercial importance. The work was undertaken in 3 stages, namely 1) A preliminary assessment of pond water quality; 2) A series of laboratory investigations; and 3) Fish culture trials.

Following a preliminary assessment, configurational changes for the onsite wastewater ponds were recommended. These were believed to facilitate proper discharge of water in the ponds in order to allow sufficient hydraulic retention time and facilitate biological nutrient removal in the wastewater. The need for adequate aeration was also highlighted as important within the primary settling ponds to promote bacterial ammonification and nitrification. The preliminary assessment also suggested that it was imperative to achieve low levels of nutrient in pond water before fish culture could begin.

Laboratory investigations primarily focussed on micro algae's ability to remove nutrient from wastewater and fish's ability to tolerate varying nutrient levels, particularly ammonia. Results suggested that micro algal growth; primary criteria for nutrient removal; was directly dependent on the euphotic depth of the water (extent of penetration of sunlight into water governed by turbidity). However, it was also found that better micro algal growth and nutrient reduction in ponds could be achieved by promoting mixing within the pond water enabling better exposure of the growing micro algae to sunlight. As the pond water was rich in organic carbon and nitrogen (especially in the primary settling ponds), aeration was found necessary to enhance microbial activity. Laboratory investigations on nutrient tolerance by fish species, showed that Silver Perch, Paradise fish, Goldfish and European Carp were able to tolerate high ammonia levels in pond water. On this note, they were considered as candidate species of commercial importance for TMC pond fish culture trials.

Field fish culture trials in ponds were conducted using Silver Perch and Goldfish fingerlings in two storage ponds at TMC ensuring levels of ammonia and phosphorus in the pond water were acceptable for aquaculture. High mortality rates in both species

resulted during the trial due to presence of toxic blue green algae, indicating presence of high levels of P in the water. It must be noted that, in freshwater environments phosphorus is generally a limiting nutrient, controlling the algal growth. Therefore there is a current need to adopt suitable methods to reduce P in pond sediments, so as to maintain acceptable aquatic environment to enable future fish culture using pond water.

In order to continue with investigation, feasibility of fish culture using abattoir water, an alternate pond with fresh water (with no influence from sediment P) was chosen for a subsequent fish culture trial. Fish culture was conducted successfully with minor mortality even after regular addition of abattoir water from the pond receiving CAF effluent. Good growth rates were obtained from the fish groups tested. The final standard length and wet weight of the three fish species were significantly higher ($P < 0.05$) than the initial ones. It is interesting that growth of goldfish (SGR = 1.54 %/d) and silver perch juveniles (SGR = 1.26 %/d) were better than the carp juveniles (SGR = 0.76 %/d, Table 2).

Based on the feasibility study, it is believed that worlds best aquaculture practice using abattoir wastewater in ponds could be developed. This would begin with a recommendation on the wastewater flow through the ‘aquaculture ponds’ that promote stable growth of plankton and fish, details on stocking densities of fish, periodic monitoring of plankton and the fish community and harvesting methods for fish.

2 Background

Abattoir operations result in generation of large quantities of wastewater containing high organic and nutrient load. Meat and Livestock Australia (MLA) has engaged SARDI through the Environmental Biotechnology CRC (EBCRC) to investigate the feasibility of conducting aquaculture using abattoir wastewater in an attempt to improve water quality and generate an additional revenue stream. The feasibility study was conducted at Tatiara Meat Company (TMC), which processes approximately 40,000 lambs per week resulting in effluent discharge of around 1 ML daily. The discharged effluent passes by gravity through a series of ponds from where it is taken to an adjacent field 15 ha field for flood irrigation of Lucerne. Currently, during periods of high rainfall and low irrigation requirements for the Lucerne the ponds merely act as storage for wastewater.

3 Investigation Rationale

The basis of the investigation is to recommend best methods and practice in order to:

- Facilitate the utilisation of nutrients from the wastewater for aquaculture
- Enhance algal growth by improving light penetration and reducing turbidity in the ponds
- Provide habitat for Aquatic organisms such as zooplankton and fish species that contribute to improving water quality
- Evaluate fish survival and growth performance

4 Key Objectives

- Preliminary data analysis and defining an acceptable aquatic environment
- Wastewater quality profiling using historical and laboratory data
- Establish nutrient removal abilities of micro algae and determine nutrient tolerance of fish species using pond water to choose candidate fish for aquaculture trials.
- Conduct fish culture trials in cages and in pond conditions
- Recommend best aquaculture practice using abattoir wastewater in ponds

5 Literature review

It is increasingly being recognised that organic wastewater is not necessarily a pollutant but a nutrient resource that can be recycled through integrating farming practices. Livestock waste is as an excellent fertiliser for enhancing the biological productivity of ponds (Gopakumar et al., 2000; Kumar et al, 2000; Kumar, 2002; Kumar and Sierp, 2003). Fingerlings of six carp species (Sharma and Das, 1988) produced 6792 kg/ha in one year. Sharma and Olah (1986) recorded production rates of 18.4 kg/ha/day by recycling the pig manure in a fish poly-culture pond. There is limited research in meat and livestock effluent and integration of aquaculture. Most of the work has been limited to primary algal production. Evans et al. (2005) has described successful performance of a high rate algal pond system to treat abattoir wastewater highlighting an avenue for establishing polyculture. Poly-culture method may be adopted for situations where species like common carp may be able to use the planktonic and benthic food resources resulting from the primary production. Nutrient removal from sewage was shown by Kim et al. (2003) using an artificial food web system consisting of phyto and zooplanktons. Wastewater-grown zooplankton has already been found to be an excellent nutrition source for raising Silver perch (Kibria et al., 1999) demonstrating the potential for utilising meat processing wastewater. Benefits from polyculture system include significant reduction in nutrient load and increase in dissolved oxygen levels in the final water discharged (Olah, 1990).

6 Description of Treatment System

6.1 Abattoir Water Pre-treatment

Abattoir wastewater typically contains fat, blood, and portions of internal viscera, bones, skin and hair. This is generated from a number of areas in the slaughterhouse and processing areas where boning, wash down, sterilisation, and rendering occur. Therefore the wastewater is rich in soluble and insoluble organics and is characterised by high BOD, nutrients (Nitrogen and Phosphorus), suspended solids (SS), fats, oil and grease (FOG). Abattoirs commonly have pre-treatment steps such as Cavitated Air Floatation system (CAF) before the wastewater is discharged into pond systems. This step reduces a large proportion of FOG and suspended solids through polymeric chemical coagulation and flocculation.

Table 1 shows characteristics of untreated abattoir wastewater. But the wastewater discharged into the ponds would still be rich in organics and nutrients (up to 150 mg/L ammonia and around 5 mg/L of phosphate) requiring further secondary biological treatment. This treatment step assists in improving water quality that is suitable for discharge into a public waterway or constructed wetland as required by the local council.

There is also a pressing need to manage pond nutrients that otherwise may lead to toxic algal blooms and other problems.

Table 1. Water quality in untreated abattoir wastewater.

Parameters	Influent* (Average)
BOD	90
SS	98
FOG	30
PH	7.1

* Source: TMC Pond 3 water data (May 2005) in Milestone Report 1.

6.2 Pond Infrastructure

The pond infrastructure adjacent to the abattoir was created by TMC with an objective of cleansing the abattoir wastewater for onsite reuse, irrigation of Lucerne and to meet effluent permit requirements. A series of 11 ponds are meant for primary sedimentation (Ponds 1, 2 and 3), sludge storage (Pond 5) and for surplus wastewater storage (Ponds 4, 6, 7, 8, 9, 10 and 11). A layout of the ponds is shown in Figure 1. The Ponds are 1.5 m deep, clay lined and interconnected. Water flow between individual ponds is mostly by gravity. While water flow between ponds 1, 2 and 3 occurs by gravity, water is pumped from pond 3 to pond 4 from where gravity flow continues into rest of the ponds. There are two aerators in Pond 1 to aid mixing and boost the dissolved oxygen level in the wastewater. Approximately 1 ML of pre-treated wastewater is received by the pond system daily from the abattoir's CAF plant.

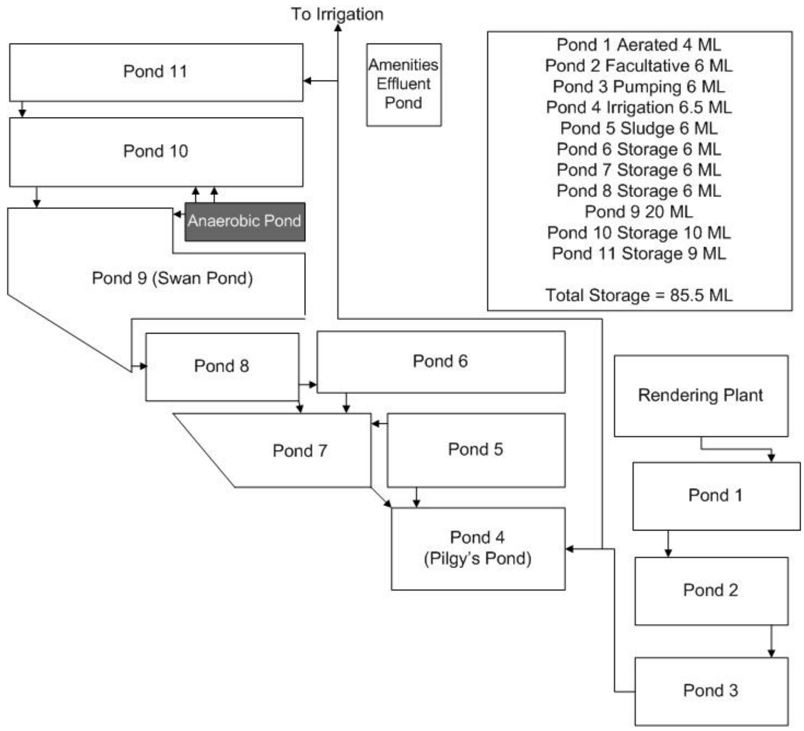

Figure 1 Layout showing the wastewater ponds and water flow directions at TMC abattoir.

6.3 Overview of Study

The proposed aquaculture feasibility study goes through several stages beginning with a preliminary assessment of the ponds and its associated water quality, and a review of company's historical pond water quality data. This is followed by nutrient investigations using micro algae and fish, leading to recommendations through individual stages to ensure an acceptable aquatic environment is achieved in the pond prior to fish culture trials. The study pathway is shown as a flow chart (Figure 2)

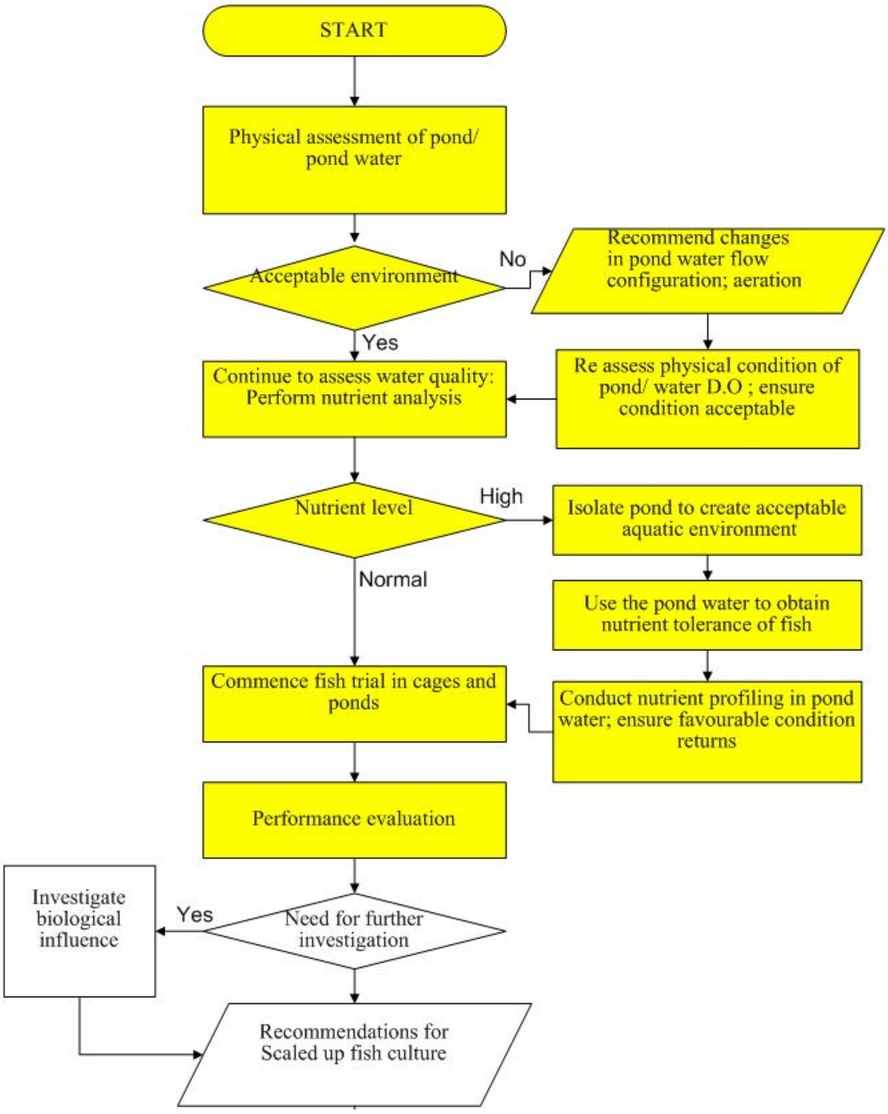

Figure 2 An overview of events showing water quality management and fish culture trials during feasibility study using Integrated Aquaculture approach to treat wastewater from the TMC, Bordertown, SA.

7 Preliminary Appraisal

This appraisal involved pond infrastructure and pond water quality. This task was facilitated by an initial guided tour organised by TMC taking SARDI staff through abattoir activities to provide an overview on the wastewater generation process, pre-treatment steps and water discharge into ponds. The objective of this assessment is to review pond operation factors, analyse primary and historical water data and recommend operational changes to achieve an acceptable aquatic environment. This section briefly summarises activities and outcomes achieved during milestones 1 and 2.

7.1 Operational Factors

Direct physical factors influencing pond water flow such as piping configuration, hydraulic retention time of wastewater received, and aeration in the pond were investigated. It was found that there was short-circuiting of wastewater flow between some ponds (ponds 1, 2 and 3). The purpose of the pond system is to provide effective retention time to enable remediation of wastewater. Therefore configurational changes (bottom discharge and top feed in ponds) were recommended for better transfer of wastewater between ponds and maximise its retention time. When considering ponds 1, 2 and 3 as a single entity the overall hydraulic retention time of wastewater would be 14 d. Adequate aeration could promote favourable biological changes and create a niche environment for growth of micro algae.

7.2 Pond Water Quality

The quality of water from primary ponds and all storage ponds was monitored. Water quality is best expressed in terms of quality indicators; therefore onsite measurements were conducted to determine turbidity, pH, and dissolved oxygen (DO). Samples were also analysed for nutrients (Ammonia and Soluble P) and algal counts. The DO levels were close to zero in most of the pond water, which is attributed to the presence of COD (organic carbon and ammonia). The need for aeration was important to promote carbon assimilation, ammonification, and nitrification by aerobic bacterial community. This is currently limited by the availability of DO, although pH was stable around the acceptable biological range in favour of these bacterial communities. It was therefore recommended that the TMC management consider installing new aerators. However once the pond is stabilised the oxygen needed for bacterial respiration is provided by algal photosynthesis. In turn algae easily absorbs the carbon dioxide produced by bacteria produced from the organic carbon source in the CAF effluent. It was also noted that the suspended solids which contribute to the dark colour of the water (as indicated by turbidity between 200-

400 NTU) was interfering with the effective light penetration in the pond water. This reduces the euphotic depth (depth of water that receives sunlight) in the water; a factor considered essential for algal growth in open pond water. Algal enumeration during investigation revealed relatively lower algal counts for a natural water body and showed mixed species including *Chlorella*-like species and a motile *Chlamydomonas*-like species. Analytical results clearly indicated higher ammonia levels in all pond water, which could affect fish survival and growth. This situation presented the assessment team an opportunity to consider isolation of these storage ponds to stabilise the ponds, reduce ammonia levels and enable fish culture trials to continue. Economic value in the stabilised ponds comes from the ability to harvest algae from time to time where the harvested algae are of commercial value.

7.3 Historical Water Data

The historical data from TMC includes a number of physical and chemical parameters for pond 3 water monitored on a monthly basis since March 2001. Graphical trends of critical parameters have been presented using the historical data (Figure 3). Suspended solids level seems to be constant during the monitored period, suggesting that the solids were carried over into ponds down stream of pond 3. This might be partly due to insufficient hydraulic retention time and dissolved oxygen level available in pond 3 to promote sustained biological activity. The installation of CAF (June 2001) plant seems to have an effect on the TDS (and hence electrical conductivity) of the effluent as seen by their downhill trend from the last quarter of 2001 and onwards. This may have resulted from a change in management practice such as a reduction in application of a particular type of chemical compound following the installation of CAF. It also suggests there may be less influence from dissolved salts. There is also a 4-fold reduction in the ammonia and BOD level during the same period, indicating favourable biological changes may have occurred following the installation of CAF. Review of the historical data from the current feasibility study shows there are opportunities to establish the upstream ponds 1, 2 and 3 as stabilisation ponds for an effective and economical means of wastewater treatment.

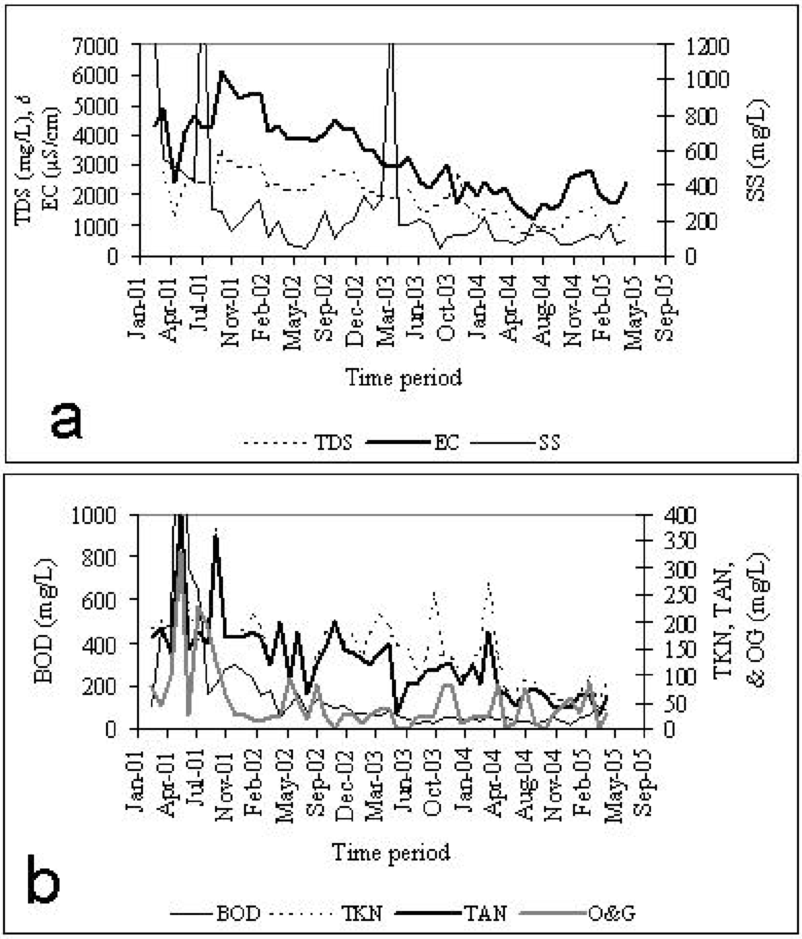

Figure 3. Graphical representation of TMC pond water quality trends.

8 Nutrient investigations

This section deals with key activities undertaken during Milestone 3, and involves biological nutrient removal (Nitrogen and Phosphorus) and nutrient tolerance (specifically Ammonia) by fish. TMC Pond wastewater (ponds 1, 2 and 3) was relatively higher in ammonia, (100- 150 mg/L), which was far in excess of the upper tolerance limits of most fish species. Thus there was a need for nutrient reduction in the pond wastewater prior to aquaculture trials. Micro algae presented an excellent opportunity to utilise the surplus N and P present in the pond water. *Chlorella vulgaris*, (a commercially significant micro algae), was chosen for this purpose following observation of *Chlorella* like algae present in all pond water samples from TMC. The nutrient tolerance (ammonia) by fish was also critical in order to make an appropriate choice from candidate species of commercially significant fish and to ensure that right aquaculture environment exists in the pond prior to mass culture of fish.

8.1 Nutrient Reduction

The laboratory study was conducted using wastewater collected from TMC ponds 1 and 2 (containing ammonia level in excess of 100 mg/L) to culture *Chlorella*. The experiment was conducted with a light intensity of 4,500 lux, and 16:8 hour light: dark photoperiod at an ambient temperature of around 18°C. The micro algal growth and nutrient removal were monitored as a function of mixing and aeration. Culture samples were also analysed to determine the algal counts, ammonia, phosphorus, pH, and DO. It was observed that the extent of micro algal growth was dependent on turbidity present in the culture (Figure 4).

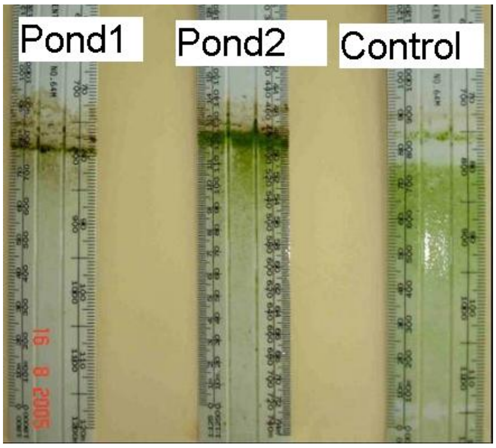

Figure 4. Depths of Micro Algal growth relative to Light penetration and Turbidity.

Turbidity was found to influence the culture's euphotic depth, i.e. the level of penetration of light into culture media (in this case pond wastewater). Higher growth of micro algae found in water from pond 2 was due to a higher euphotic depth resulting from lower turbidity. Further, it was observed that higher algal growth and nutrient removal were also promoted by mixing. This could be attributed to a better exposure of algae to sunlight while maintained in suspension than in sediment. Aeration during the experiment was noted to enhance the microbial ammonification (ammonia production through decomposition of organic N) and aerobic nitrification (ammonia consumption by bacteria). Therefore adequate aeration may be beneficial at all times in the upstream TMC ponds (ponds 1, 2 and 3) receiving CAF effluent that is rich in organic N. Microbial nitrification results in the oxidation of ammonia to nitrate, a compound that is also a nutrient for the micro algae. It was noted that higher micro algal growth rate was also accompanied by a higher ammonia removal rate. The experiments demonstrated that micro algae are able to grow and utilise ammonia at levels well above 100 mg/L, even though the level was affecting the survival of fish. Following this, it was recommended that ponds with water containing higher level of nutrients N and P (particularly ponds 6 and 10) be isolated in order to promote micro algal growth and facilitate ammonia reduction to a level favourable for subsequent aquaculture trials.

8.2 Nutrient Tolerance

Short-term nutrient tolerance (ammonia) was studied in fish using wastewater from TMC (Pond 3). The experiment was evaluated using the observed fish mortality rates and the results were used to choose the candidate fish species for pond aquaculture trials. Fish mortality results showed that Molly and Swordtails have poor survival ability under high ammonia concentration while goldfish (Comets), paradise, silver perch, and European carp showed better tolerance (up to 10 mg/L). However, the ammonia levels ideal for growing fish is in the range of 2-3 mg/L. As paradise fish is commercially more valuable, it would be targeted in a large-scale aquaculture setting.

9 Fish culture trials

Aquaculture trials were conducted in cages suspended in ponds at TMC using a choice of fish species including carp, goldfish and silver perch. Three experiments were conducted (9/3/06 to 18/5/06) in the established facilities as described in the Milestone report 4. The objective of these experiments was to evaluate the aquaculture potential utilising the pond wastewater at TMC.

Three sets of experiments conducted included:

- A Trial experiment in wastewater pond
- A Fish culture experiment in freshwater storage pond

- A Fish culture experiment in plastic tanks

9.1 Pond aquaculture

9.1.1 Introduction

The water quality in pond 10 was noted for its high ammonia and phosphate content. After allowing a greater retention time in isolation, the water quality tended to improve gradually. This was observed by a change in colour of the water from red to green, indicating the establishment of the microalgal community. An overall reduction in the ammonia concentration was achieved (7.17 mg TAN/L by Feb 06). Temperature and pH values were in the appropriate range (20.4 °C and 8.87 in March, respectively) for the culture of carp and goldfish. Therefore, the infrastructure in Pond 10 was used for fish rearing experiment between March to May 2006. The objective of this experiment was to determine fish survival and growth rate in the wastewater ponds under ambient conditions.

9.1.2 Materials and methods

9.1.2.1 Net-cages and fish rearing

Six net-cages (L x W x D = 1.0 x 0.5 x 0.6 m^3) were set up in the Pond 10 (Figure 5). Nylon mesh (0.5 cm) was to ensure the zooplankton could move into the net cages via water exchange between the cages and pond. A mesh lid was made for each cage to protect the fish from predators. After measuring standard length (SL) and live wet weight (W) on-site, 15 carp juveniles were randomly assigned and put into 3 cages (5 fish in each cage, in triplicate), whilst 21 goldfishes were placed into another 3 cages (7 fish in each cage, in triplicate). No commercial feed was used; fish were only allowed to feed on zooplankton or other live food produced in the Pond 10. Detailed infrastructure descriptions are available in the Milestone Report No. 4.

9.1.2.2 Fish measurement

Standard length (SL) and wet weight (W) were measured on-site after being anaesthetized in a 0.3% 2-phenoxyethanol solution. The carps used in this experiment are

96-139 mm in SL and 24.1-70 g in W, and the goldfish 56-76 mm in SL and 8.4-22.4 g in W.

Figure 5 Fish net cages set up in TMC Pond 10 on 9th March 2006. Three cages were used for carp juveniles and another three for goldfishes.

9.1.2.3 Data collection

Sludge sample was sampled from Pond 10 and total phosphorus content analysed. Fish mortality, total ammonia-N (TAN) level, pH and temperature of Pond 10 were monitored. The data is presented in Table 2.

9.1.3 Results

9.1.3.1 Fish survival

Mortality of carps occurred in the third week, indicating carp could not tolerate the nutrient levels in Pond 10. However the gold fish could tolerate the similar conditions and survived. In the middle of April 2006, large quantity of effluent was pumped in Pond 10 by accident resulting in total mortality of goldfish.. As a result of this the experiment was terminated on 13 March 2006.

Water quality was measured at the beginning (9/3/06) and end (13/3/06) of the experiment (Table 2)

Table 2. Water quality parameters of Pond 10 in TMC.

	Mar. 9th 2006	**Apr. 13th 2006**
T °C	20.4 °C	18.3 °C
pH	8.87	8.79
TAN	5.56 mg/L	5.39 mg/L

The sludge from Pond 10 contained 10 g PO_4-P /L total phosphorus.

9.1.4 Discussion

Relatively high ammonia level and high pH value (8.87 – 8.79, see Table 3) could be attributed to fish mortality. It appears that carps are more sensitive than goldfish in terms of the high nutrient loading water like TMC's. We noticed that the microalgae density was more that 5000 cells per ml when the experiment commenced, which is common in waste water ponds (Kibria et al., 1999). The high pH value resulted from microalgae blooms. Ammonia toxicity can be increased due to elevated pH levels (Emerson et al., 1975). Also in water, ammonia exists in two forms, unionized ammonia (NH_3) and ionized ammonium (NH_4^+). The proportional amounts of unionized ammonia (NH_3) is toxic to fish especially in relation to pH and temperature (Emerson et al., 1975). Therefore, pH has a significant impact on ammonia tolerance and it is a parameter that can be easily controlled in an integrated system. It is mediated by algal growth, and pH manipulation is not envisaged as an experimental treatment. The management of algae blooms is recommended in the TMC system.

The other reason for fish mortality was the high concentration of soluble phosphorus (PO_4-P), which was 11.4 mg/L in Pond 10 (MLA milestone report 4). Our result shows that the sludge in TMC pond is extremely high in phosphorus. The phosphorus tends to bind to the sludge on the bottom of the waste water ponds by accumulating of the organic particles in the TMC (see Milestone report 4) ponds which is contributing to the high phosphorus content in the pond water resulting toxic algal bloom (Hepher, 1958). According to the literature, the upper phosphorus concentration limit for effective pond fertilization is 0.5 mg/L (Eren et al., 1977). Once P saturation occurs in the sediments, its content in the water level increases and reaches undesirable high levels often resulting in the appearance of toxic algal blooms in ponds (Boyd, 1990 &1995).

9.1.4.1 Pond Sediment

Analysis of a sample from pond sediment (Pond 10) showed a high percentage of phosphorus was found in the non-reactive form. This could be explained for a pond scenario as follows. When nutrient-rich water enters the pond, the nutrients are absorbed by the suspended particles and settle to the pond bottom by sedimentation. In an unmanaged pond, P accumulates within the pond sediment until saturation followed by a gradual increase in soluble orthophosphate with P accumulation (Figure 6) over saturation levels (Kumar et al., 2002). Sediment is also usually considered as a major sink of orthophosphates in fish ponds (Boyd 1971).

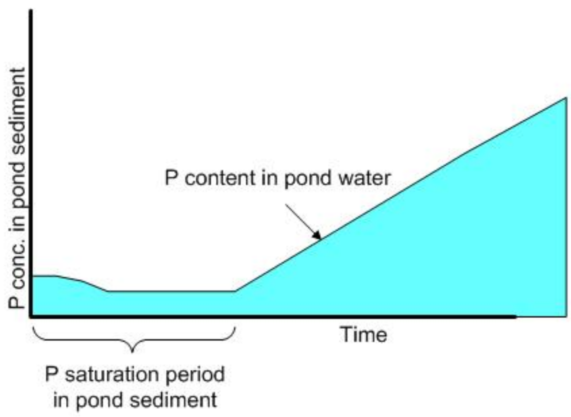

Figure 6. An illustration of phosphorus levels in an unmanaged pond over time.

The growing algae take up the nutrients, but when the algae dies it settles down and adds significant organic carbon load onto the sediments. After this, the sediment P is again made bioavailable by chemical reduction of sediments and remobilisation of P, leading to unavoidable algal blooms.

Zooplankton and fresh water mollusc (bivalves) are potential species, which can be introduced into the TMC system, which will have a good impact on algal density. The poly-culture of fish with fresh water molluscs is recommended for the future research, as well as zooplankton culture experiments.

Currently, the TMC pond bottoms are covered by thick sludge formed by the accumulation of waste particles. As we pointed out in last report the integrated aquaculture production cycle should begin in a clean-bottomed pond and annual cleaning of aquaculture ponds is strongly recommended to maintain low levels of sediments on the bottom.

9.2 Aquaculture in Fresh Water Storage (FWS) pond

9.2.1 Introduction

As part of investigation of fish culture in abattoir wastewater, an experiment was conducted in a Fresh Water Storage (FWS) pond partially mixed with abattoir wastewater using Carp, Goldfish and Silver Perch juveniles. This FWS pond is currently used for holding water overflowing from a Reverse Osmosis (RO) plant and has no nutrient loading. Wastewater from Pond 1 was added to maintain desirable nutrient level. The objective of the experiment was to evaluate aquaculture performance utilising nutrient from wastewater for primary production. The experiment was conducted for a period of 70 days (between March 2006 and May 2006).

9.2.2 Materials and methods

9.2.2.1 Net-cages and fish rearing

The net-cages set up in FWS pond were similar to those in Pond 10. A mesh lid was also made for each cage to protect the fish from its predators (Figure 7). After measuring

Standard Length (SL) and live Wet Weight (W) on-site, 14 goldfish were randomly assigned into two replicate cages (7 fish in each cage, in duplicates); while 8 carp juveniles were placed into another two replicate cages (4 fish in each cage, in duplicates). Likewise 38 silver perch juveniles were randomly assigned into two replicate cages (19 fish in each cage, in duplicates) in the same pond. No supplementary feeding of commercial pellets was provided to fish during the experimental period. The fish were only allowed to feed on the zooplankton or other live food produced within the FWS pond.

Figure 7. Fish net cages set up in TMC FWS pond on 9th March 2006.

9.2.2.2 Quantification of Fish Growth

Standard length (SL) and wet weight (W) of fish were measured after being anaesthetized in a 0.3% aqueous solution of 2-phenoxyethanol. The average SL and W are presented in

Table 2. The size of the fish chosen in this experiment was based on abundance of the natural food supply primarily produced in the FWS pond.

Fish growth was determined by the calculating the absolute growth rate (AGR) as mm/d or g/d and specific growth rate (SGR) as %/d (Hopkins, 1992). AGR was calculated as: AGR = $(SL_f - SL_i)/\Delta t$ for standard length or AGR = $(W_f - W_i)/\Delta t$ for wet weight of fish, and SGR was determined as: SGR = $100(\text{Ln}SL_f - \text{Ln}SL_i)/\Delta t$ for standard length or SGR = $100(\text{Ln } W_f - \text{Ln } W_i)/\Delta t$ for wet weight of fish, where SL_f and SL_i are the final and initial fish standard length (mm) and W_f and W_i are the final and initial fish wet weight (g), respectively, Δt: is the time interval (d).

9.2.2.3 Data collection

During the course of experiment, daily fish mortalities were monitored and recorded. Standard Length (SL) and Wet weight (WW) of fish were determined before and after the experiment. Mean wet weights of initial and final measurements were computed in order to calculate the AGR and SGR. Mean values of both initial and final measurements of SL and W of fish were compared using a one-way ANOVA using SPSS 10.1 (*SPSS, Richmond, USA*) followed by *Duncan* multiple-comparison tests to determine significant differences between means ($P < 0.05$). At the end of the experimental period, random samples of fish were anaesthetized in a 0.3% aqueous solution of 2-phenoxyethanol and dissected in the laboratory. This was conducted to collect the stomach and gut contents and assess under dissecting and compound microscopes to study feeding pattern and establish the dietary constituents of fish while they were reared in the tanks. During the experimental period, water quality and environmental parameters including ammonia, pH and temperature were also monitored in the culture water. Ammonia was measured as Total Ammonia Nitrogen (TAN) and expressed as Mean ± Standard Deviation (SD) (n = 3).

9.2.3 Results

9.2.3.1 Fish growth

One goldfish died in each cage (85.7 %, survival rate). All the carp and silver perch survived. Good growth rates were obtained from all the fish groups. The final standard length and wet weight of the three fish species were significantly higher ($P < 0.05$) than the initial ones. It is interesting that growth of goldfish (SGR = 1.54 %/d) and silver perch juveniles (SGR = 1.26 %/d) were better than the carp juveniles (SGR = 0.76 %/d, Table 3).

Table 3. Initial and final measurement of fish and growth rate. "*" indicates significant difference (P < 0.05) comparing to the initial measurement.

	Goldfish		Carp		Silver perch	
	SL (mm)	W (g)	SL (mm)	W (g)	SL (mm)	W (g)
Initial	59.56 ± 2.43	11.66 ± 1.07	111.38 ± 0.53	39.69 ± 2.95	35.87 ± 1.01	1.03 ± 0.21
Final	89.58 ± 3.18 *	34.42 ± 0.19 *	128.13 ± 0.88 *	67.56 ± 3.27 *	48.13 ± 0.41 *	2.49 ± 0.19 *
AGR (mm/d or g/d)	0.43	0.33	0.24	0.40	0.18	0.02
SGR (%/d)	0.59	1.54	0.20	0.76	0.41	1.26

Goldfish recoded higher growth rate (AGR and SGR) compared to carp or silver perch. The colour of the goldfish after the experiment was also much brighter than it was at the beginning of the experiment (Figure 8), indicating that the fish are obtaining carotenes and other compounds from the live feed present in the FWS pond that contribute to the bright skin colour.

9.2.3.2 Water quality monitoring

Water quality parameters in the pond water including temperature, pH and TAN are presented in Table 4. Although the pH values showed that the water system was alkaline, the total ammonia nitrogen measured during the experiment was less than 2.5 mg/L, which was well below critical concentration affecting the survival of fish.

Figure 8. Goldfish after 70 days rearing in the FWS pond in TMC. Showing the brighter colour of the fish.

Table 4. Water quality parameters of FWS pond in TMC.

	Mar. 9th 2006	**Apr. 13th 2006**	**May 18th 2006**
T °C	20.4 °C	18.3 °C	15.0 °C
pH	8.87	8.79	8.58
TAN	0.00 mg/L	2.44 mg/L	0.55 mg/L

9.2.3.3 Gut contents

A gut content analysis was conducted to observe the feeding pattern of the fish and determine the relative abundance of its prey in the cultured water. From the goldfish digesta, it was observed that Ostracoda (Crustaceans) or so-called seed shrimp (*Newnhamia* sp.) comprised the major part of the digesta (Figure 9), with minor contribution (less than 5%) from the backswimmer, *Notonecta* sp (Family Notonectidae). Seed shrimp has a bivalved carapace (0.7-0.8 mm) which encloses the whole animal. Ostracoda occur as free-swimming animals in ponds, dams and temporary freshwater pools (Ingram et al., 1997). They swim upside-down near the water surface.

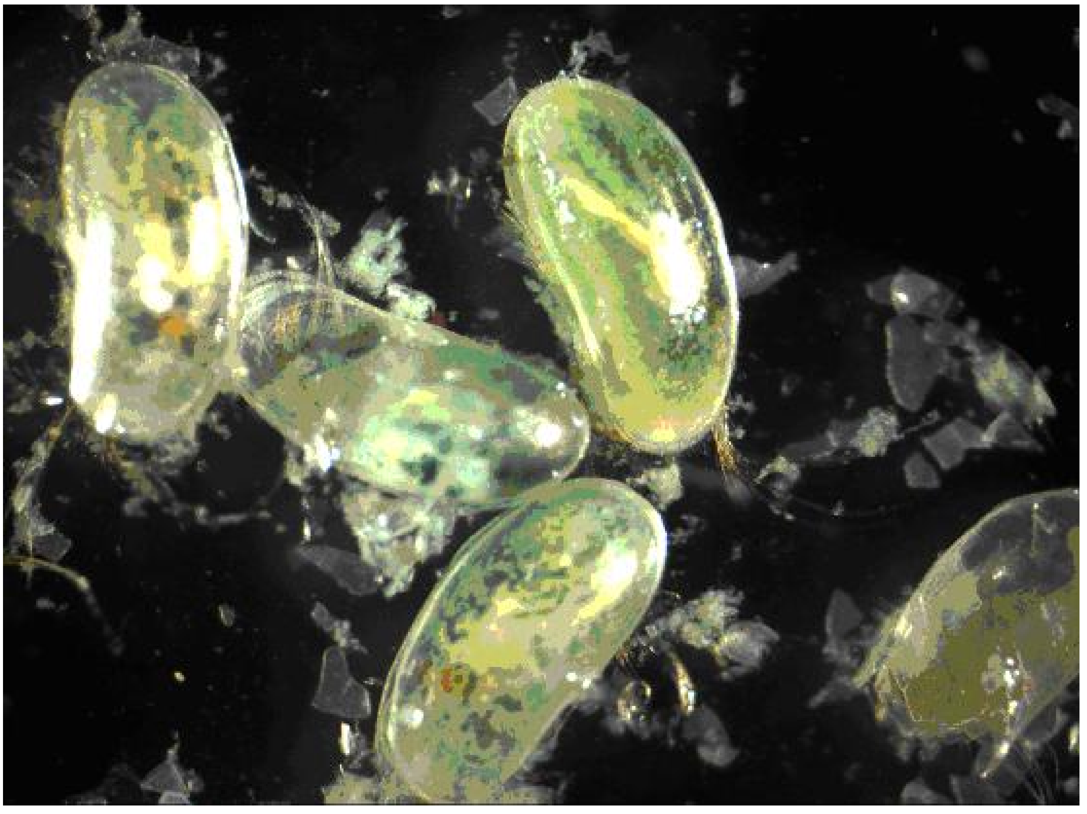

Figure 9. Seed shrimps (Newnhamia sp., Family Notonectidae) taken out of the goldfish gut. Size of this animal is between 0.7 and 0.8 mm.

Body parts of the backswimmers were noted in the gut contents. Neither other species of zooplanktons, such as Daphnia, Monia and Copepod nor any plant material was found in the goldfish digesta.

In carp juveniles the gut contents mainly comprised body parts of the backswimmers. A few seed shrimps were also found, but no other species of zooplanktons, such as Daphnia, Moina and Copepod or any plant material was found in the carp's gut content.

While the major constituent of the gut contents from silver perch was seed shrimp, there was no evidence of backswimmers in the contents. Likewise Daphnia, Moina and Copepods were not found in the gut contents from silver perch juveniles.

9.2.4 Discussion

FWS pond stores clean water with no nutrient loading (ammonia concentration was <0.01 mg/L) at the beginning of the experiment). The desirable nutrient load was maintained using effluent from pond no 1 (with low phosphorus concentration in the water) enabling better control on aquaculture system resulting better fish growth and survival performances.

Zooplankton inoculation in the FWS pond was carried out on 30th January 2006 (Milestone report 4). However, few zooplankton were observed in the pond or in the gut contents of the three species. One of the reasons may be the inoculation was not sufficient to enable zooplankton to establish dominance in the FWS pond. Further experiments with zooplankton inoculation and dynamics of zooplankton in the TMC pond should be carried out.

The gut content studies indicated there were no zooplankton (Daphnia, Monia and Copepod) with seed shrimps and backswimmers being the main components. The abundance of seed shrimp in the fish gut contents is the main reason why goldfish and silver perch showed greater growth than carps. Because the seed shrimp is small (0.7-0.8 mm) the carp were unable to catch and consume them.

The first experiment indicated that direct use of the wastewater in the TMC ponds might not be safe for growing fish. However the system established in FWS pond, with regular addition of effluent provided suitable environment for fish culture. Thus the wastewater added in the FWC pond is not only compensation of water lost by the evaporation but also nutrient input for the primary production.

Very good survival rate and growth rate of fish were obtained with the latter experiments and fish appeared in a healthy condition. In addition, the goldfish were more colourful than it in the beginning of the experiment, indicating potential opportunity of the TMC system for rearing colourful ornamental fish. Since the fish were constrained in the cages which suspended in the pond, the carps may not get sufficient food comparing to the other two species due to the different habitats requirement. This could explain why the growth rate of carp was less than goldfish and silver perch.

Seed shrimps may be the source of carotenes and other unknown compounds that contribute to skin colour of goldfish, which could be a good food source for ornamental fish. Further research should be done to value the seed shrimps as food source for ornamental fish industries, and the advantages of TMC pond system to culture ornamental fishes.

9.3 Aquaculture using Open Tanks

9.3.1 Introduction

A parallel experiment was conduced in three tanks at TMC between the Pond 1 and FWS pond. Different sizes of carp juveniles were used in this experiment. The experiment lasted for 50 days and started in March 2006 and ended in May 2006. The objective this experiment was to evaluate fish growth and survival performance in mesocosm (small scale) under natural conditions.

9.3.2 Materials and methods

9.3.2.1 Fish rearing and water supply of the fish tanks

Three round tanks, with a capacity of 1900 L, were set up at TMC between the Pond 1 and FWS pond (Milestone report 4). The FWS pond water was pumped into the tanks to provide a working volume of 1500 L. Another 15 L of Pond 1 water was added every three days to supply the nutrient to the fish in the tanks.

After measuring standard length and wet weight on-site, 12 carp juveniles were sorted into three weigh groups; large, medium and small fish. The three groups of fish were randomly assigned into 3 tanks (4 fish in each tank, in triplicate).

No artificial feeding (commercial pellets) was provided to the fish. The fish were allowed to feed on zooplankton primarily produced in the tanks and by the pumping of FWS pond water during the experiment period.

9.3.2.2 Quantification of Fish Growth

The fish growth was determined by calculating absolute growth rate (AGR) in weight as g/d and specific growth rate (SGR) as %/d (Hopkins, 1992). AGR was calculated as: AGR = $(W_f - W_i)/\Delta t$ for wet weight of fish, and SGR was determined as: SGR = 100(Ln W_f - Ln $W_i)/\Delta t$ for wet weight of fish, where W_f and W_i are the final and initial fish wet weight (g), respectively, Δt: is the time interval (d).

9.3.2.3 Data collection

Daily mortality was recorded. At the end of the experiment when the final measurement of standard length and wet weight were completed, fish were anaesthetized in a 0.3% 2-phenoxyethanol solution. Fish were taken back to the laboratory and dissected. The gut contents were collected and assessed under a dissecting and compound microscopes in the laboratory to find out what fish feed on in the tanks. During the experiment ammonia level, pH and temperature of the three tanks were monitored.

The data collected are presented in Table 5 and 6. Mean weights of initial and final measurements were calculated. Total ammonia-N (TAN) level is given as Mean ± SD (n = 3).

9.3.3 Results

9.3.3.1 Fish growth

There was no fish mortality occurred through out the experiment (100 %, survival rate).The growth rates were calculated as AGR and SGR in wet weight (W) and are presented in Table 5. The growth rate of the carp juveniles in this experiment is less than the carp juveniles in the FWS pond experiment according to the SGRs.

Table 5. Initial and final measurement of fish and growth rate in AGR and SGR. SL: standard length; W: wet weight.

	Tank 1				**Tank 2**				**Tank 3**			
Fish number	1	2	3	4	1	2	3	4	1	2	3	4
Initial W (g)	56.7	18.5	5.6	4.1	60.6	7.6	7.0	4.5	76.5	9.7	5.1	4.1
Final W (g)	61.6	20.3	7.3	5.6	63.1	9.7	8.3	6.6	77.0	11.7	6.3	5.0
AGR (g/d)	0.0495				0.04				0.023			
SGR (%/d)	0.22				0.18				0.1			

9.3.3.2 Water quality monitoring

The total ammonia-N (TAN) was monitored during the experiment. The dynamics of the changes in TAN during the experiment is charted in Figure 10.

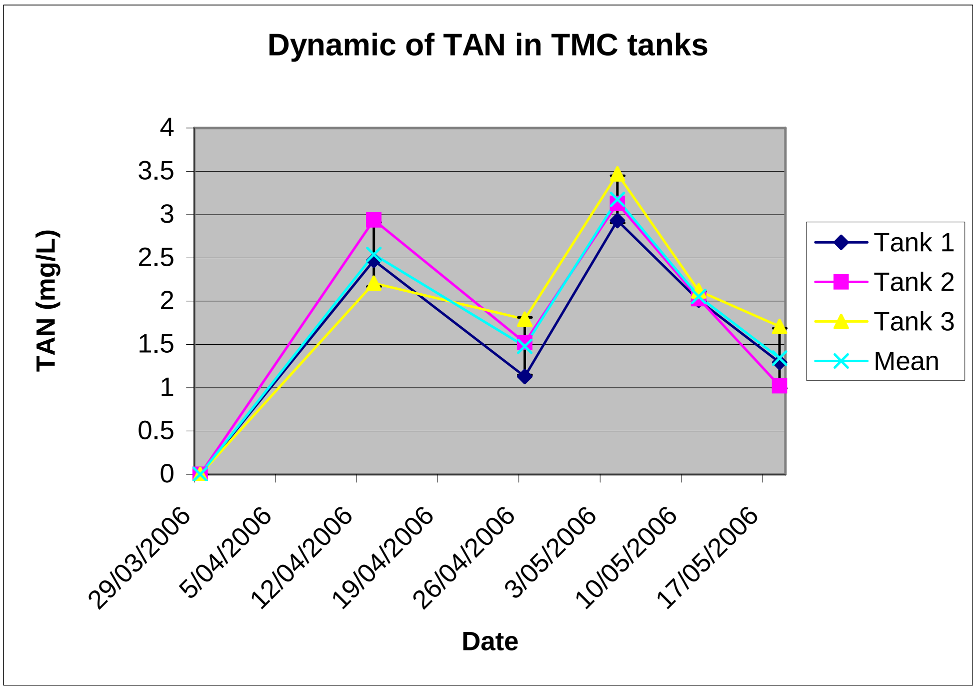

Figure 10. Dynamic of TAN changes in TMC tanks.

9.3.3.3 Gut content Analysis

In the gut contents of carp juveniles, body parts of the backswimmer comprise the major part of the ingested food. A few seed shrimps were found in the contents, but not many. The feeding rate was less than the carps exhibited in the FWS pond. Similarly, there was no other zooplankton (Daphnia, Monia and Copepod) found in gut contents.

9.3.4 Discussion

High survival rate in fish in this experiment was obtained (as in FWS pond experiment), indicating that the partial addition of wastewater into the aquaculture system is a safer way to utilise the TMC wastewater. However, slow growth was obtained in tanks compared to the FWS pond experiment. This was probably because there is insufficient live food in the tank system. Perhaps more time was needed to allow the tanks to build up in food supply. Thus, tank system can be used for fish culture.

10 Technical Constraints and Recommended Suggestions

10.1.1 Accumulation of Phosphorus in pond sediments

Currently, there is a need to remove high P sediment in TMC storage ponds. Although, several options are available to meet this need, a suitable choice may arise out of a cost-benefit analysis. Options include physical removal of the sludge by pumping, draining and re-filling the pond with clean water and subject it to chemical treatment using a commercial product (such as Phoslock™). Figure **Error! Reference source not found.**11 illustrates these situations in unmanaged and managed ponds and associated implications.

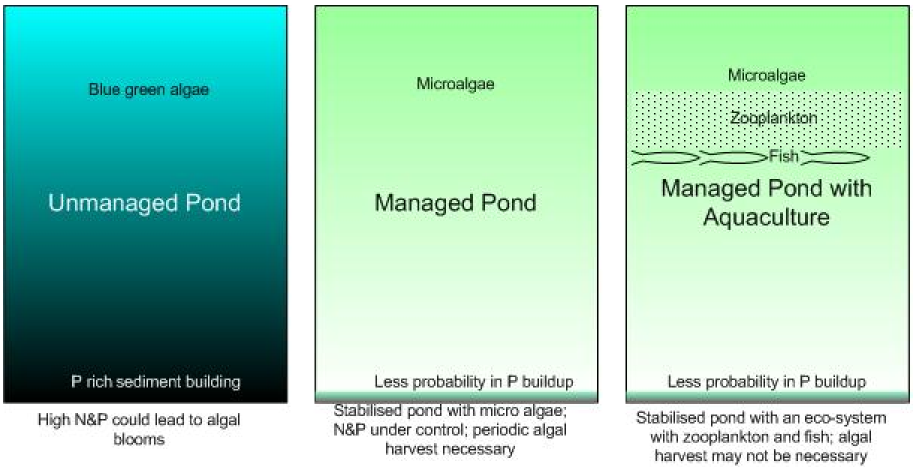

Figure 11. Pond system with nutrient inflow in managed and unmanaged situations.

The problem of blue-green algae blooming is a growing concern due to the high phosphorus levels locked up into pond sediments in the TMC ponds. The pond bottoms are covered by thick sediments that have been growing due to cumulative accumulation of the settling matter. Once saturation of Phosphorus occurs in these sediments, its content in the pond water level increases and reaches undesirably higher levels often resulting in the appearance of toxic algal blooms.

10.1.2 Water Quality Monitoring

There is a need to adopt a water quality management strategy especially prior to and during the pond aquaculture practice. As the TMC management already has a monthly monitoring schedule to test its wastewater from pond 3, this could be extended to ponds used for aquaculture. This monitoring strategy helps to avoid fish mortality resulting from change in management that affects wastewater quality. Water quality monitoring on a regular basis is critical where there increasing level of contaminant loading. Monitoring also aids in assessing the effectiveness of any new management practices or infrastructure. Pathogen monitoring is also an important factor to consider suitable depuration methods for fish. It is recommended that some of the common human pathogenic bacteria be monitored using indicator organisms like *E.coli*.

10.1.3 Field-scale Aquaculture Practice

Based on the current feasibility study, World's best aquaculture practice could be developed for utilising abattoir pond wastewater. This would begin with a recommendation on the wastewater flow through the 'aquaculture ponds' that promote stable growth of plankton and fish, details on stocking densities of fish, periodic monitoring of plankton and the fish community and harvesting methods for fish. The integrated aquaculture production cycle should begin in a clean bottom pond, as fish culture would start better with cleaner water. Wastewater could then gradually be added in metered doses to act as source of water and nutrient.

10.1.4 Polyculture for Improved Pond Water Quality

Research focussing on the growth of zooplankton in the wastewater pond should be conducted to efficiently manage the micro algae density in the pond. Zooplankton growth in the wastewater ponds is an important source of food for fish, especially for the juveniles. The Zooplankton acts as an agent, to utilise the nutrients from the wastewater by grazing the microaglae and serving as live feed for fish. However, the current situation is that few zooplankton (Daphnia, Monia and Copepod) can be found either in any of the TMC ponds. Further research of the dynamics of zooplankton growth in the wastewater pond should be conducted, because zooplankton play a very important role in the process of waste water treatment and integrated aquaculture.

Fresh water molluscs (bivalves) are also potential species, which could reduce high densities of micro algae, thereby reducing the level of soluble phosphorus in the pond water. In addition, the bivalves can graze the organic particles in the pond bottom and contribute to reduction in the level of accumulated sludge. Further research should be conducted using bivalves polycultured with fish in the TMC ponds.

11 Recommendations for Commercial Aquaculture Development

The current R&D program mainly focussed on commercial aquaculture development. The feasibility study clearly indicates that there is good potential for commercial aquaculture development for raising meal and ornamental classes of fish in an abattoir wastewater environment. The following recommendations are presented to facilitate the large-scale pond wastewater aquaculture at TMC.

- Design and Establishment of an Integrated Aquaculture system infrastructure that affords favourable aquatic environment by managing the nutrient budget.
- Scale up of Pond Fish culture to utilise the bulk of abattoir wastewater with particular reference to Species density and practice of Poly culture.
- Development of Ornamental and Meal-quality fish of commercial significance. Major aquaculture species would be considered for production of fishmeal to target formulated feed market especially in the backdrop of an increasing demand from Aquaculture sector.
- Large-scale production of Micro algae and zooplankton for development of formulated feed, biofuel and biomaterial.
- Seed shrimps can be developed commercially as a potential live food for fish fingerlings, especially for ornamental fish demanding better colour performance. There is a need to undertake further research to understand the life cycle,

reproduction, mass culture and methods for their large-scale production in pond systems.

Table 7. Milestones Update

No.	Milestone	Completed %
1	Preliminary wastewater analysis to identify an acceptable aquatic environment (Report 1)	100
2	Wastewater profiling at TMC using existing and new data (Report 2)	100
3	Laboratory trials to establish optimum microflora and fish species for TMC ponds. (Report 3)	100
4	Establish on-site infrastructure for demonstration facility. (Report 4)	100
5	Evaluation of fish culture cage trials (Report 5)	100

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