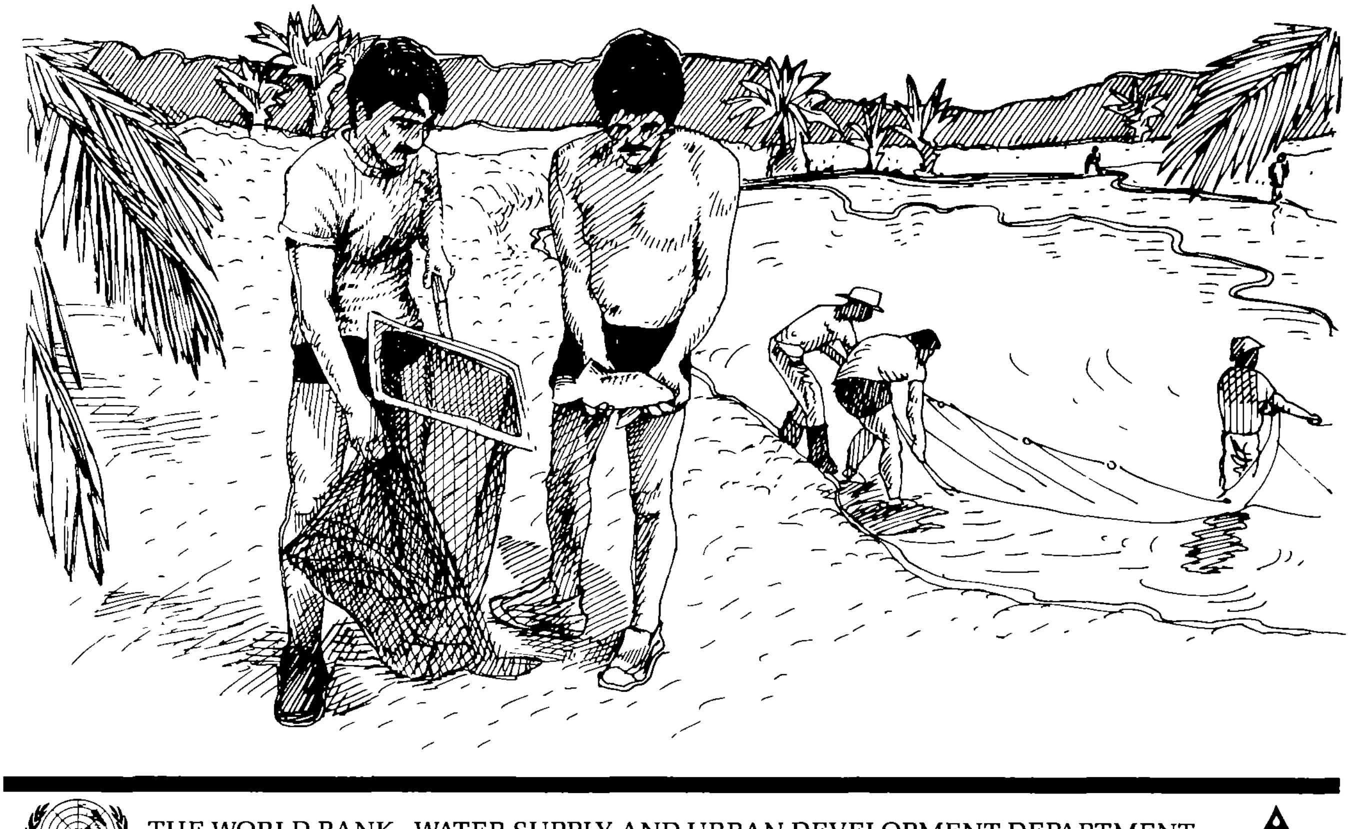
APPLIED RESEARCH AND TECHNOLOGY (WUDAT)—TECHNICAL NOTE NO. 3
INTEGRATED RESOURCE RECOVERY PROJECT

Aquaculture With Treated Wastewater:

A STATUS REPORT ON STUDIES CONDUCTED IN LIMA, PERU

Compiled and Edited by
Sandra Johnson Cointreau



UNITED NATIONS

THE WORLD BANK—WATER SUPPLY AND URBAN DEVELOPMENT DEPARTMENT
A Joint United Nations Development Programme and World Bank Contribution to the
International Drinking Water Supply and Sanitation Decade

1981 1990

INTEGRATED RESOURCE RECOVERY REPORT SERIES
GLO/84/007

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ABSTRACT

Fish culture is one means of producing a large amount of protein material in a relatively small amount of space. This study has shown that significant quantities of protein for either human consumption or livestock feed could be produced from wastewater-based aquaculture, which could be integrated with sewage stabilization lagoon systems. Reuse of treated sewage to fertilize the microbial food chain for aquaculture presents one of the most economic resource recovery options for cities in developing countries. Wastewater-based fish and prawn culture research, development, and demonstration efforts were conducted in Lima, Peru, through the sponsorship of the UNDP/World Bank Integrated Resource Recovery Project and the GTZ, the agency through which the Federal Republic of Germany provides technical assistance to developing countries.

Fish and prawns were cultured in wastewater stabilization lagoons operating as polishing lagoons in series with primary and secondary ponds. Some of the fish ponds were operated as batch-type (receiving make-up water only) rather than flow-through ponds. The fish fed on the natural food chain fertilized by the nutrients in the treated wastewater; no supplemental feed was added.

The hypothesis being tested was that fish and prawns would grow in wastewater-based ponds and be acceptable for human consumption either directly or indirectly (for example, fish may be used as a protein source for livestock or a second generation of fish ponds). It was found that the environmental conditions in the ponds were satisfactory for the survival and growth of tilapia and carps, particularly in the cycle-end polishing ponds. Although the prawns grew satisfactorily, they did not survive unanticipated large fluctuations in water quality due to shock loadings, which may be common under uncontrolled conditions. The experience in Lima (and at other sites discussed in the literature) indicates that ammonia is a key water quality constraint for fish growth and production, and that total ammonia should not exceed 2.0 mg-N/l.

Raw fish examined in this study had no parasites on the gills or skin, or in the muscle. Furthermore, the bacteria load of the muscle portion of raw fish was acceptable for human consumption. However, higher bacteria levels within the digestive tract and peritoneal fluid could lead to contamination of food preparation areas during fish cleaning. Experiments in processing the fish through salting and smoking showed promise as a means of minimizing public health risks to consumers. Indirect consumption through crushing the fish and disbursing them to other fish ponds or for use as livestock feed has not been tested.

A demonstration project, where missing parameters will be verified, that is expected to verify aquaculture yield results and elaborate on the health and economic aspects of this study will soon follow.

PREFACE

More and more people in developing and industrial countries alike are recognizing the need for technical and economic efficiency in the allocation and utilization of resources. Resource recovery and recyling provide developing countries with a means of optimizing the use of indigenous natural resources, reducing the need for imports and thus conserving foreign exchange, increasing local employment opportunities, and developing industrialization skills.

In 1981, a global research, development, and demonstration project on integrated resource recovery (GLO/84/007, formerly GLO/80/004) was undertaken by the World Bank as executing agency for the United Nations Development Programme (Division of Global and Interregional Projects). The goals of the project are to achieve economic and environmental benefits through sustainable and replicable resource recovery and recyling of liquid and solid wastes from municipal and commercial sources.

A major goal of the project is to develop and encourage resource recovery as a means of offsetting some of the costs of community sanitation, which may account for more than 50% of total expenditures. Aquaculture in higher-level wastewater treatment (polishing) lagoons offers one method of partially or totally offsetting these costs. This would not only make it possible to achieve high quality standards for effluent discharge for environmental improvement but would also enhance the opportunities for effluent reuse. This note documents research, development, and demonstration studies on fish and prawn culture conducted at the San Juan Lagoons in Lima, Peru. Potential public health risks of fish consumption were examined through microbiological analyses of both raw and processed fish. Financial support was provided by the United Nations Development Programme, Global and Interregional Projects Division. Additional financial support came from the German Agency for Technical Cooperation (GTZ).

An international group of experts as well as a group of Peruvian scientists have participated in the research, advising, monitoring, or analysis. Most of their names appear on the title page of this document, however, the list does not include many others who took part in the project. We are grateful to them all.

Your comments on this note would be welcome, and we would be grateful to receive any case study information from which future editions of the resource recovery series could benefit. Please send your comments to Applied Research and Technology Unit, Water Supply and Urban Development Department, World Bank, 1818 H Street, N.W., Washington, D.C. 20433, USA.

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This note was compiled and edited by Sandra Johnson Cointreau, a consultant to the World Bank, who was responsible for coordinating the studies in Lima. The text is largely gleaned from the reports of the Lima researchers:

Carl Bartone, Maria Lusia de Esparza, and Carmen Vargas de Mayo, Centro Panamericano de Ingenieria Sanitaria y Ciencias del Ambiente (CEPIS), who provided water quality monitoring and sanitary engineering technical assistance to secure relatively steady-state conditions within the ponds for aquaculture;

Julio C. Moscoso, Hugo Nava Cueto, Elena Gil Merino, and Raul Porturas, Universidad Nacional Agraria (UNA), who studied fish culture and fish processing, as well as the microbiology of processed fish;

Norma Noe Moccetti, Manuel Tantalean Vidaurre, Sonia Calle Espinoza, and Maria Teresa Amaya Arroyo, Universidad Nacional Mayor de San Marcos, Centro de Investigacion Instituto Veterinario de Investigaciones Tropicales y de Altura (USM/IVITA), who examined bacteria and parasites in raw fish and prawns, as well as parasites in pond sediment; and

Tula Luna and Mercedes Carrasco, Instituto de Investigacion Tecnologica Industrial y de Normas Tecnicas (ITINTEC), who investigated mesophilic batch digestion of animal manure and collaboration with UNA for special fish culture of tilapia in concrete tanks.

To assist the local research scientists, the UNDP/World Bank Integrated Resource Recovery Project arranged for expert consultants Balfour Hepher and Netty Buras to provide regularly scheduled technical assistance on fish culture and epidemiology, respectively.

Alejandro Vinces, Luis G. Carrillo Macedo, and Javier R. Seminario, Program de Proteccion Ambiental y Ecologia Urbana, Ministerio de Vivienda y Construccion, coordinated the daily activities of the various research groups participating in the project. They were also responsible for operating and maintaining the San Juan lagoons in accordance with water quality information obtained and technical assistance provided by CEPIS.

S. Arlosoroff, Project Manager, and Charles G. Gunnerson, then Senior Project Officer, of the UNDP/World Bank Global Project on Integrated Resource Recovery (GLO/80/004) conceived and directed the overall Lima waste-fed aquaculture program. Mr. Klaus Kresse, Chief of Special Studies, Water Supply and Sanitation of the German Technical Cooperation Agency (GTZ), participated in directing the second phase of the studies.

Eric Perrin, Resident Representative of the UNDP in Lima, helped to obtain the initial government approval for research and thereafter assisted with the administration of research contracts.

In March 1985, the GTZ sponsored an Expert Panel Meeting of specialists to examine Phases I and II of the Lima fish culture studies and recommend a plan for a possible Phase III: Balfour Hepher (Israel, fish culture using wastewater), Netty Buras (United States, epidemiology of waste-fed fish culture), Roger Pullin (the Philippines, fish genetics and fish culture), Peter Edwards (Thailand, fish culture using human wastes), Dhrubajyoti Ghosh (India, traditional fish culture using wastewater), and Hans Schlotfeldt (Germany, epidemiology of fisheries).

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Chapter 1

BACKGROUND ON WASTE-BASED AQUACULTURE

Fish culture is one means of producing a large amount of protein material in a relatively small amount of space. Economic benefits from fish culture are usually higher than those from traditional agricultural crops. Fine-grained organic matter added to fish ponds provides a suspended attachment surface and food supply for bacteria and protozoa. These microorganisms also take up nutrients from the water. After they build up their protein-rich cell mass, they serve as food for fish. Other organisms, such as phytoplankton and zooplankton, benefit from the growth of bacteria and protozoa, and in turn serve as fish food (9, Hepher).

One of the richest, yet cheapest, sources of organic matter is human excreta. Whether fish ponds receive human excreta directly or through sewage influent, traditional systems and special demonstration facilities indicate high fish yields. In India, for example, production rates from fish ponds utilizing wastewater have ranged from 3 to 9 tonnes/ha/year (9, Hepher).

Poor people in the cities of developing countries have traditionally found ways to conserve resources and recycle wastes as one means of carving out their livelihood. For example, poor urban dwellers on the fringe of Calcutta have been using sewage to enhance fish culture since the turn of the century. The ratio of sewage to water there is about 5:1 (15). There are presently about 4.5 thousand hectares of sewage-based fish ponds surrounding Calcutta -- stocked mainly with carp and tilapia, which supply an annual harvest of about 6,000 tonnes (18).

Direct use of night soil to fertilize fish ponds has been more fully practiced in China than anywhere else. In fact, in 1966, 90 percent of the excreta generated in the country was applied to agricultural lands and fish ponds. In Taiwan there are several thousand hectares of fish ponds receiving excreta. A significant portion of the fish ponds in Indonesia have overhanging latrines that directly discharge excreta into the underlying waters (15).

The polyculture of carp, tilapia, and mullet fish in wastewater treatment ponds has been practiced in Israeli kibbutzim. Fish are often allowed to rest in clean water for several weeks to help depurate them before consumption. Because of the concern over public health risks, Israel now allows only polishing ponds or freshwater ponds receiving secondary-level treatment effluents to be used for fish culture (15). Studies in Israel have shown that adding wastewater to fish ponds can markedly increase fish feed utilization and fish yield -- which rose as much as 75 percent in one set of experiments (22).

In Germany, several hundred hectares of fish ponds (mostly stocked with carp) receive sewage that has been partly treated in sedimentation tanks and then diluted with relatively fresh water (15). Fish yields from the wastewater-fed ponds are reportedly much higher than those from regular ponds in the region (22).

Despite the efforts that have already gone into promoting waste-based aquaculture, much more remains to be done, as indicated recently by a panel of internationally recognized experts who were asked to examine the global state of knowledge on waste-based aquaculture. They pointed out that

> while there has been extensive experience in Asia (mainly in China, Indonesia, Vietnam and others) on use of human excreta in fish ponds and, to a much less widespread extent, on fish culture in sewage in India, most of the experience has been devoid of data generation and scientific examination. Data generated have largely been from traditional systems without flexibility to experimentally adjust and examine variables, replicate studies, and thereby obtain reasonable confidence in the results.
>
> Significant studies have been done primarily in Israel, Thailand, and now Peru to examine individual growth rates, pond yields, and public health consequences of sewage-based fish culture under various comparable experimental configurations. In Thailand, comparable studies have been done to examine excreta-fed fish culture systems. These studies are essential to the eventual formulation of design standards and monitoring criteria for waste reuse (21).

Chapter 2

THE CITY OF LIMA

SETTING

Lima, the capital of Peru, is located on the Pacific Coast of South America. It has a moderate climate, but receives little rain, although there is often a mist in the morning. Cloud cover prevails during most of the winter months (July through September).

Aside from three small river basins that cut through Lima (Rio Rimac, Rio Lurin, and Rio Chillon), the city rests on desert land. Groundwater is deep and fairly well contained by overlying sandy soils interspersed with layers of impermeable clay soils. The regional groundwater flow is from the Andes Mountains westerly to the Pacific Ocean.

The city has a population of about 5 million, of which roughly 50 percent are recent rural immigrants living in precarious housing with limited access roads. About 65 percent of the houses have access to water supply and sewerage or other sanitation infrastructure (8, 16). And about 60 percent of the city's refuse is collected (10).

Epidemiological data indicate that the most common infectious diseases, in order of prevalence, are acute diarrhea (primarily rotovirus, enterotoxigenic and enteropathogenic *Escherichia coli*, and *Campylobacter*), typhoid and paratyphoid fever, intestinal parasite afflictions (primarily ascariasis and giardiasis), and viral hepatitis (8, 35). Acute diarrheal diseases among infants represent the leading cause of child mortality in Peru, and the typhoid and paratyphoid fever rate there is the highest in Latin America (6).

The above-mentioned diseases are believed to be transmitted principally through the consumption of food and water that has been contaminated by excreta (17). These diseases account for nearly 50 percent of the total number of illness cases in Lima (35). Contamination occurs through numerous channels, most notably via raw vegetables that have been irrigated with untreated wastewater, inadequate sanitation, pigs raised at clandestine refuse dumps, and flies that have been in contact with exposed refuse heaps (10).

It is estimated that some 80 percent of the wastewater from cities in developing countries is being used raw or partially treated for irrigation (33). With Lima's high rate of population growth and the lack of rainfall in the region, the city's underlying groundwater supply has dropped considerably. Thus potable water is precious and reuse is the norm. Since much of the reuse is uncontrolled and includes numerous taps on the city's raw sewerage system (10), measures to provide wastewater treatment prior to reuse could go a long way toward reducing public health risks from excreta-related infectious diseases.

HISTORY OF DEVELOPMENTS

The concept of full-scale reuse of treated wastewater has been steadily developing in Lima for more than two decades -- beginning in 1959 with a presentation of how to treat Lima's wastewater in stabilization lagoons by Alejandro Vinces, formerly President of the Parks Service (SERPAR), to the National Congress of Sanitary Engineering.

Beginning in 1961, SERPAR supervised the development of 21 stabilization lagoons in San Juan de Miraflores, a municipality in the southern zone of Lima's metropolitan area (6). Supreme Decree No. 105-67-DGS of 1967 authorized SERPAR to create forestland by irrigation with treated effluent from the San Juan lagoons (36).

More than 500 ha of land (most of which was desert, and part of which was closed sanitary landfill) are presently being irrigated with effluents from the San Juan lagoons -- of which about 1,280 ha consist of woodland and 220 agricultural land, with another 1,300 ha being planned for greenbelts with low irrigation requirements (6). Pilot plots of various fruits (e.g., pineapples and papaya), vegetables (e.g., corn and platanos), and flowers (e.g., roses and hibiscus) have been created on-site (10, 36). Part of the irrigated land is farmed by 16 families who have set up irrigation systems to use the partially treated wastewater to grow cow fodder and vegetables (6). Figure 1 shows the location of plots cultivated by these families, as well as larger plots cultivated by agricultural cooperatives.

The movement toward wastewater reuse has been paralleled by a growing concern about air pollution and the need to preserve greenbelts of forestland to filter and oxygenate the air (36). Air pollution, coupled with cool damp climatic conditions during many months of the year, lowers human resistance to respiratory infections. A review of Peru Ministry of Health statistics shows that tuberculosis and other respiratory infections are among the 10 leading causes of morbidity in Lima and constitute about 20 percent of the total number of cases of disease reported there (35). As a step toward improving environmental quality, Legislative Decree No. 143 of 1981 created the Program of Environmental Protection and Urban Ecology to authorize the cultivation and preservation of greenbelts (36).

Research and development studies have been an integral part of SERPAR's efforts to promote wastewater reuse. With local funding as well as external support from the International Development Research Center -- Canada (IDRC) and the Panamerican Health Organization (PAHO), CEPIS has studied the mechanisms of treatment occurring in the lagoons and the levels of water quality achievable through various sequences of lagoons and periods of retention (6, 37, 38, 39).

A special multisectoral commission formed in Lima in 1973 has been investigating the possibility of using a 5,000-ha site (called San Bartolo) south of Lima for the full-scale demonstration of wastewater reuse (figure 1). In 1980, a mission from Israel established the prefeasibility of irrigation with treated wastewater at San Bartolo. In 1981, SERPAR engaged

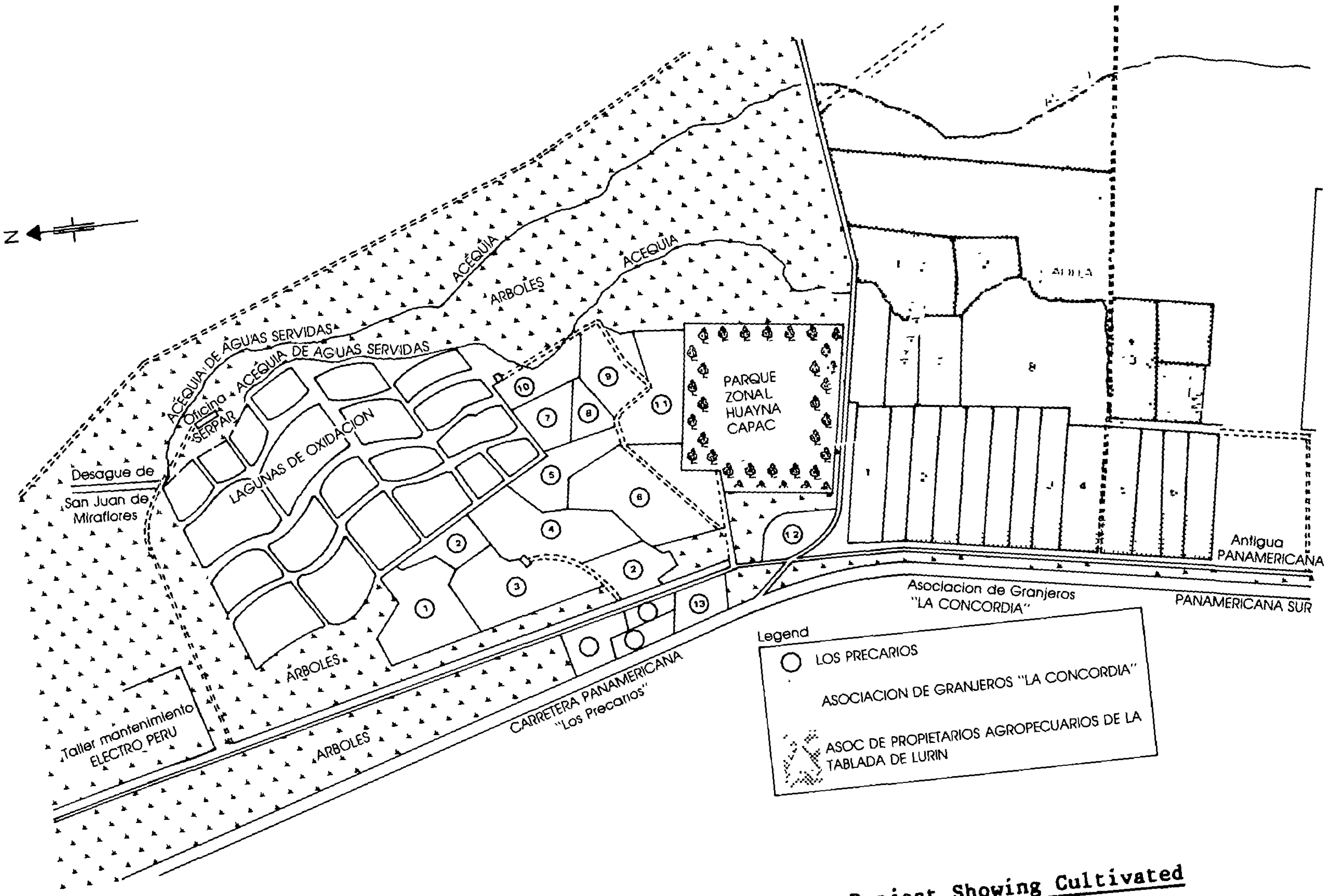


Figure 1. <u>Map of the San Juan Effluent Reuse Project Showing Cultivated Household Plots</u>

<u>Source:</u> (6)

the National Agrarian University (UNA) to further evaluate costs and benefits of the San Bartolo project (10). The concept was also studied from an engineering perspective by contractors to the Lima Water Supply and Sewerage Agency (SEDAPAL) as part of a master plan for water supply and sewerage for Lima (17).

In 1983, the Inter-American Development Bank (IDB) and SEDAPAL approved and allocated funds for an in-depth study of costs and benefits and for the development of preliminary engineering designs. A group of consultants carried out this study in late 1984 and early 1985, and they recommended the reuse of 2.5 m^3/s of treated effluents to irrigate some 5,000 hectares (34).

If a reuse system is to be successful in the long term, it must limit the accumulation of salts and metals in the soil column, especially in a desert climate such as Lima's. To accomplish this, wastewater treatment should be capable of removing a high percentage of suspended solids and of precipitating dissolved solids. Also, longer retention times are recommended to enhance the die-off or removal of potentially pathogenic micro-organisms, and thereby minimize public health risks (1, 39). Aquaculture could be included in the final stages of treatment with a view to generating revenues from the sale of the fish or plant byproducts that could offset the costs of obtaining a higher quality wastewater effluent (9, Hepher).

In a modest experiment conducted during the mid-1970s, an attempt was made to grow tilapia fish within secondary wastewater treatment ponds at San Juan (6). However, the water quality was found to be unsuitable to support fish growth (10).

Beginning in 1983, external support from the World Bank, UNDP, and GTZ made it possible to incorporate tilapia fish and giant prawn culture studies into the overall scheme and operation of the lagoons -- with lagoons being resequenced to obtain higher levels of treatment. After initial harvests showed good growth and survival of fish, several varieties of common carp were added to examine the effects of polyculture.

These aquaculture studies were performed by local research teams at UNA, CEPIS, and the University of San Marcos Institute of Veterinary Investigations (USM-IVITA) under the umbrella of SERPAR initially, and then under the Program of Environmental Protection and Urban Ecology (6, 27, 28). As part of the aquaculture studies, bacteria and parasites were monitored in lagoon effluents and sludges, as well as in raw and processed fish.

In cooperation and in parallel with these studies, but with financial support from local industries, the Institute of Technical Investigations and Standards Setting for Industry (ITINTEC) has studied mesophilic anaerobic digestion of animal manure and provided some of the slurry to UNA for use in aquaculture of tilapia in tanks supplied with potable groundwater (24).

Furthermore, a study sponsored by the U.S. Agency for International Development (USAID) was conducted in 1984-85 to evaluate the cost-effectiveness and public health risk of growing duckweed (Lemnaceae) as a poultry feed (10). Floating macrophyte populations have the potential to improve effluent water quality from the ponds since they are capable of stripping nutrients and heavy metals from wastewaters (6).

Chapter 3

THE WASTEWATER STABILIZATION SYSTEMS IN LIMA

STRUCTURE

The wastewater treatment facility in San Juan de Miraflores has operated continuously since 1964. The facility consists of 21 waste stabilization ponds occupying 20 ha. The ponds are divided into two batteries: the upper battery consists of ten ponds, and the lower battery eleven ponds. Final effluents from each of the two batteries are used for the irrigation of farmland and forestland (6).

Wastewater in the upper battery receives primary and secondary treatment. Up until the start-up of aquaculture studies, the ponds in the lower battery were operated at the primary and secondary treatment level. See Figure 2 for a layout of the lagoons prior to the aquaculture studies (37).

The wastewater influent at the San Juan facility is residential sewage from about 108,500 people living within three slum neighborhoods. The average flow is 360 lps, and the average organic loading is 250 to 350 kg/ha-day of 5-day biological oxygen demand (BOD_5). At these average loadings, the primary ponds were operated in the facultative mode, while the subsequent ponds were aerobic (6).

STUDY METHODS AND MEASUREMENTS

Pond Management

For purposes of the aquaculture studies, the sequence of ponds within the lower battery was modified into three series:

- Series 1 consisted of four wastewater stabilization ponds (referred to as P1, S1, T1, and C1) of which the third- ("tercera") and fourth- ("cuarta") level ponds were used for aquaculture;
- Series 2 consisted of five wastewater stabilization ponds (P2, S2, T2, C2, and Q2) of which the third-, fourth-, and fifth- ("quinta") level ponds were used for aquaculture; and
- Series 3 consisted of two levels of treatment ponds (P3 and S3) and was not used in the experiments (6).

Figure 3 illustrates this three-series pond arrangement within the lower battery. Pond S2 was initially a primary pond and ponds T1, C1, T2, C2, and Q2 were initially secondary ponds prior to the initiation of research at San Juan.

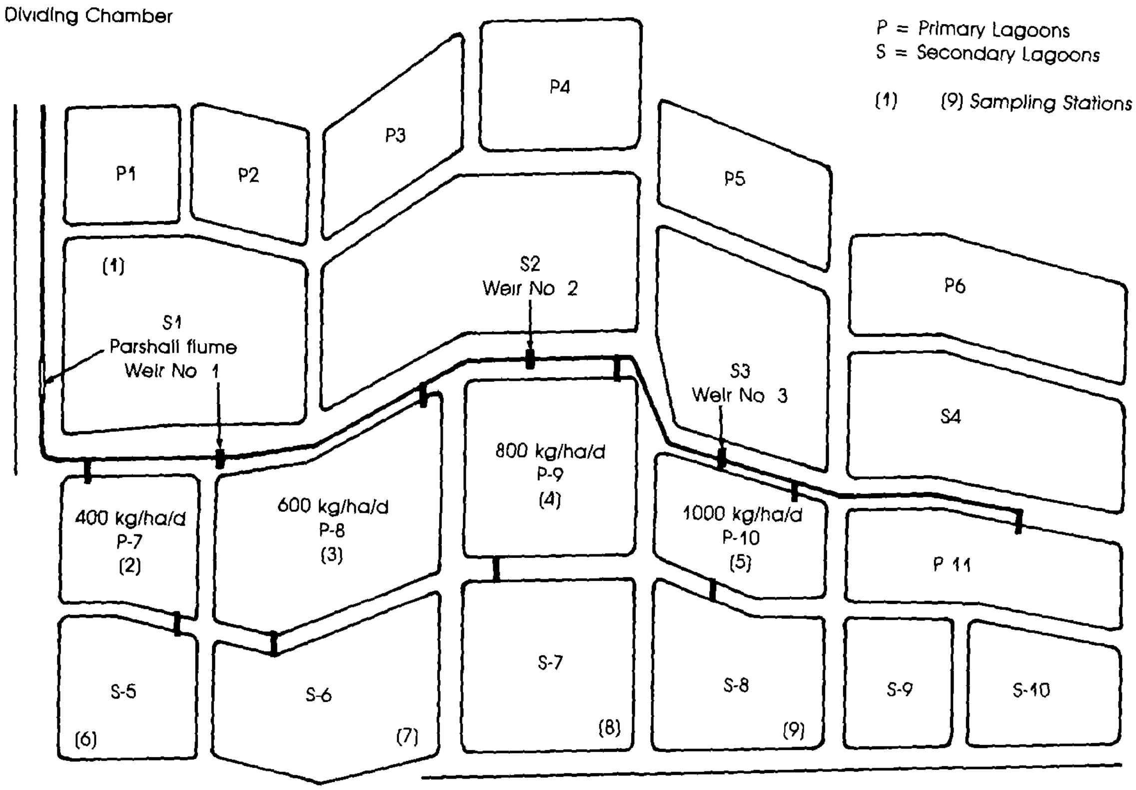


Figure 2. <u>Map of San Juan Lagoons Showing Levels of Treatment Prior to the Aquaculture Studies</u>

<u>Source:</u> (37)

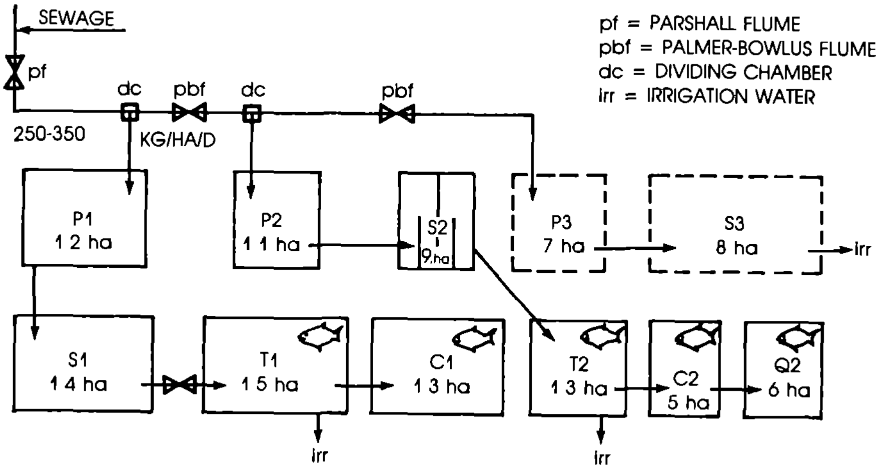


Figure 3. Pond Arrangement during Aquaculture Studies

Source: (6)

During harvest activities at the end of Phase I, it was realized that the existing means of draining the ponds were entirely inadequate for fish culture. Therefore, for Phase II, sluice gates were built to facilitate quick and adequate drainage for harvesting (27). Otherwise, the ponds were not altered. Because of the time constraints under the UNDP/World Bank Integrated Resource Recovery Project, the sediment that had accumulated at the bottom of the ponds could not be removed. As a result, the fish may have been subjected to more contamination than they would have been otherwise.

SERPAR, under the technical guidance of CEPIS, operated the Series 1 and 2 ponds within the lower battery in accordance with CEPIS's flow measurements and water quality parameters and UNA's monitoring of fish survival and growth. The primary, secondary, and third-level ponds (P1, P2, S1, S2, T1, and T3) were operated as continuous-flow ponds with an average depth of 1.3 m, while the fourth- and fifth-level ponds (C1, C2, and Q2) were operated as batch-flow ponds with an average depth of 1.0 m. Within the batch-flow ponds, only enough treated wastewater was added to compensate for evaporation and infiltration losses (6).

Selection of Species

The choice of fish for stocking fish ponds depends on the temperature and water quality of the given location in relation to the conditions considered suitable for the various species being considered. Fish that are readily sold, bring a good price on the market, reproduce easily, and grow fast are ideal. In order to maximize utilization within a pond, culture of more than one compatible species (i.e., polyculture) might be desirable.

In the Lima study, tilapia were selected for stocking because of their hardiness in oxygen-stressed aquatic environments and their recognized good taste. Tilapia is a native freshwater fish of Africa. Originally exported to Southeast Asia as an ornamental fish, it is now the region's most important pond-cultured fish for food consumption (25). Experience in Thailand, India, Taiwan, and Israel has clearly shown tilapia to be a hardy species able to survive and grow well in waste-fed pond systems (15, 22). The species of tilapia selected for these aquaculture studies (*Oreochromis niloticus*) is recognized for performing well in fish culture and for being easily caught by seining (22).

Prawns were selected for stocking because of their popularity with consumers and potential market value. The market price of prawns in Lima, for example, typically runs 10 times higher per kilogram than the price of freshwater table fish (9). The variety selected (*Macrobrachium rosenbergii*) is a tropical species that is popular for culturing because it is more tame and less cannibalistic than other species and has a faster growth rate, shorter larval period, and wider range of temperature tolerance (27). No reference in the literature was found to indicate whether prawns could be cultured in wastewater-based systems; but experiments have shown that they benefit when organic matter, including organic manure, is added to their pond water (9, Hepher).

Common carps (Cyprinus carpio) have a long history of being raised in ponds heavily laden with human excreta, notably in China (15). The two varieties selected for stocking in the Lima study are the "big belly" variety, which is scaled, and the "mirror carp" variety, which is only partly scaled (22). They are able to tolerate harsh aquatic conditions better than the other varieties.

The species of tilapia selected feeds principally on phytoplankton, whereas the common carp is an omnivore that feeds on protozoa, zooplankton, and bottom fauna such as insect larvae, worms, and molluscs (22). Since tilapia and common carp do not compete for food and generally occupy different zones of the pond, they are well suited for polyculture.

Stocking

Stocking activities occurred in two separate stages of aquaculture.

Phase I. Tilapia, which had been previously imported to Peru from Israel, were spawned. Juveniles were nursed to the fry stage at UNA.

Prawn juveniles were also imported from Israel and arrived in April 1983; however, only half survived the long journey and another importation was required -- this time from Panama.

Just before the ponds were stocked, bioassays were conducted in which fish and prawns were separately held in cages (about 10 individuals in each) within selected ponds for one week. The bioassays showed good survival under the pond water conditions upward of 90 percent for tilapia and 75 percent for prawns).

Stocking (tilapia in T1, C1, and C2 and prawns in Q2) occurred in June 1983. The stocking rate for tilapia was 12.2 kg/ha in T1, 5.2 in C1, and 44 in C2, while 450 prawns were placed in Q2. Aquaculture took place over the next three (winter) months and the tilapia and prawns were harvested in late September 1983 (27).

Phase II. Some of the tilapia from the Phase I activities were allowed to spawn. At the UNA laboratories, the fry were fed on Mesterolona, a synthetic androgen, for the first month after hatching in order to induce sex reversal and obtain a male population.

Some of the first prawns to arrive for Phase I had been selected for broodstock. After spawning in the summer of 1984, their larvae were reared in specially designed UNA facilities and the resulting juveniles nursed in UNA tanks for a month.

Common carp fry were imported from Panama. The fry were late and did not arrive in Lima until February 1984.

Stocking for monoculture of tilapia in Q2 occurred in September 1983 and in T2 in October 1983. The stocking rate of Q2 was appreciably higher than that for T2 (205 kg/ha in Q2 versus 150 kg/ha in T2). Prawns were stocked in C2 in October 1983 at a stocking rate of 4.6 kg/ha -- C2 was selected because its water quality was expected to be better than that in Q2 during Phase I operations. Aquaculture proceeded over the summer months, and tilapia and prawn were harvested from Q2, T2, and C2 in April 1984.

Stocking for polyculture in C1 began with tilapia in February 1984; common carp were stocked in March 1984 and prawn in April 1984 (27).

Flows

The total sewage flow to the lower battery where aquaculture studies were conducted was measured continuously from May 1983 to April 1984. Stevens type-F water-level recorders were located over Parshall or Palmer-Bowlus flumes and monitored by CEPIS. The influents to ponds P1 and P2 were controlled by two dividing chambers calibrated to provide about 350 Kg/ha-day of BOD5 to P1, and 250 Kg/ha-day to P2 (6).

Water level in all the aquaculture ponds was measured once daily by CEPIS. Precalibrated triangular weirs gave instantaneous flow measurements of effluents from P1, S1, P2, and T2. To complete the information needed to calculate flow balances, CEPIS also closed inlets and outlets of all ponds for a 24-hour period and measured losses attributable to infiltration or evaporation (6).

Environmental Observations

CEPIS staff recorded daily observations of meteorological conditions, pond appearance (e.g., color, odor, scum, floating matter), conditions of dikes, and presence of birds, insects, fish, or prawns. A meteorological station nearby also provided data on daily air temperature, wind speed and direction, evaporation, solar radiation, hours of sunshine, precipitation, and cloud cover (6).

Water Quality Measurements

Every day at about 10 a.m. and 2 p.m., CEPIS measured temperature and dissolved oxygen (DO) in the aquaculture ponds. Polargraphic analysis (Yellow Spring Instruments, Model 57) with electrodes positioned at 20, 40, and 60 cm were used. At the same time, surface pH levels were measured using an Orion Research, Model 301 pH meter (6).

On a rotational basis (at least twice for each aquaculture pond), CEPIS also recorded temperature and DO profiles every 2 hours for periods of 3-5 days. Sets of temperature thermistors and DO electrodes positioned at 20, 40, and 60 cm were used with a galvanic cell (Precision Scientific DO Analyzer) and recorder (Esterline Angus, Model MS 401BB). Cycles of pH were also recorded for each aquaculture pond (6).

From May 1983 to April 1984 CEPIS collected water samples daily at about 10 a.m. in all of the aquaculture ponds. The samples, which were in plastic 1-liter bottles, were acidified immediately with 1 ml of concentrated sulfuric acid and taken directly to the CEPIS laboratory for analysis of total ammonia nitrogen (NH_3 and HN_4^+) by distillation. Un-ionized ammonia levels were computed using the Ferrara-Avci equations with concurrent daily measurements of total ammonia, pH, and temperature (6).

During the same period raw sewage was sampled weekly and all pond effluents biweekly. Composite samples were obtained in two plastic 2-liter bottles using a 24-hour automatic sampler. One sample, fixed with 2 ml of concentrated sulfuric acid, was analyzed for total ammonia (it was also analyzed for total organic nitrogen and soluble chemical oxygen demand [COD] during Phase I of the studies). The second sample was packed in ice during the 24-hour sampling period and transferred immediately thereafter to the laboratory, where it was analyzed for soluble (filtered) BOD_5 in pond effluents and total BOD_5 in raw sewage (6).

During Phase I, CEPIS also measured detergents (MBAS), total orthophosphate phosphorus, and occasionally total nitrate nitrogen in raw sewage. In Phase II, total alkalinity and phenolphtalein alkalinity were measured (6).

Between June 1983 and June 1984, CEPIS took water samples for bacteriologic analysis from all ponds and raw sewage. Samples were collected monthly in sterilized 125-ml glass bottles. CEPIS analyzed samples for total coliforms and fecal coliforms (E. Coli) using multiple tube methods. From March to June 1984, Standard Plate Count (SPC) and Salmonella Most Probable Number (MPN) were determined. Salmonella isolates were sent to the National Reference Laboratory for Enterobacteria in Peru for serotype identification and antibiotic sensitivity tests (6).

Water samples for parasitological analysis (protozoa and helminths) were taken quarterly in clean plastic bottles of 1- to 4-liter capacity (6).

All measurements discussed above were performed according to the standard methods outlined in Table 1. Methods of preservation and holding times are described in Table 2. Data were processed on CEPIS's microcomputer, with consistency tests and verification routines applied to all data observations, and statistical summaries provided regularly to UNA for correlation with fish growth results (6).

Sediment Quality Measurements

Between March and April 1984, monthly samples of sediment sludge were taken from T2, C2, and Q2, and between May and July 1984, monthly samples of sediment sludge were taken from P1, S1, T1, and C1. A 2" diameter PVC sharp-edge corer was used by CEPIS to collect the samples (6).

Subsequently, 50 g of sediment from each sample were mechanically mixed with 450 ml of buffer solution and were tested immediately for SPC and for total coliforms and fecal coliforms MPN. Initially the sludge was also analyzed for qualitative isolation of Salmonella. Since results were

Table 1. Components of the Experimental Program and Analytical Techniques

Parameters	Units	Frequency	Analytical Method
I. NONBIOLOGICAL			
A. Meteorological			
1. Wind velocity	Km/h	Daily	Items 1-5 obtained from a nearby meteorological station
2. Wind direction	Degrees	Daily	
3. Air temperature	°C	Daily	
4. Evaporation	mm	Daily	
5. Solar radiation	$gm\text{-}cal/cm^2\text{-}day$	Daily	
6. Infiltration	mm/day	Once	Change in level
B. Hydraulic			
1. Average flow	l/s	Daily	Flow recorder
2. Maximum hourly flow	l/s	Daily	Flow recorder
3. Maximum daily flow	l/s	Daily	Flow recorder
4. Water balance	mm	Monthly	By calculation
5. Level fluctuation	mm	Daily	Field measurement
6. Depth	m	Once	Field measurement
C. Physical factors			
1. Water temperature	°C		Electrometric (in situ)
a) Profile (20, 40, 60 cm)		Twice daily	
b) Diurnal		Hourly during 3-5 days	
2. Pond appearance	Qualitative	Daily	Field observation
3. Odor	Qualitative	Daily	Field observation
4. Scum and floating matter	Qualitative	Daily	Field observation
5. Vegetation on dike	Qualitative	Daily	Field observation
D. Physical factors			
1. pH	Units		
a) surface (20 cm)		Twice daily	pH meter
b) Diurnal		Hourly during 3-5 days	
E. Chemical factors			
1. Dissolved oxygen	mg/l		Galvanometric/Winkler
a) Profile (20, 40, 60 cm)		Twice daily	
b) Diurnal		Every hour during 3-5 days	
2. Chemical oxygen demand	mg/l	Bi-weekly in the first phase	Volumetric (potassium dichromate)
3. Alkalinity	mg/l	Weekly	
4. Nutrients			
a) Organic nitrogen	mg/l	Weekly	Volumetric (Kjeldahl)
b) Ammonia nitrogen	mg/l	Weekly	Photometric (Nessler)
c) Nitrate nitrogen	mg/l	Occasionally	Cd reduction and diazotation)
d) Orthophosphate Phosphorus	mg/l	Weekly	Photometric (Ascorbic acid)
5. Detergents (MBAS)	mg/l	Weekly	Spectrophotometric methods
F. Biochemical factors			
1. BOD 20° C, 5 days	mg/l	Weekly	Electormetric method
2. BOD 20° C, 1,2,3,5,7 days	mg/l	Weekly in Phase I aquaculture ponds	Electrometric method
3. Primary productivity	$mg\text{-}O_2/m^2\text{-}hr$	Occasionally	Galvanometric cell
II. BIOLOGICAL			
A. Microbiological			
1. Total Coliforms	MPN	Monthly	Multiple tubes (Lauryl triptose)
2. Fecal coliform	MPN	Monthly	Multiple tubes (E.C.)
3. Salmonella	MPN	Monthly	Multiple tubes (selenite/Novobiocine)
4. Protozoa/helminths	Identification	Quarterly	Concentration; flotation with Zn sulfate and sedimentation with formalin-ether

Source: (6)

Table 2. Preservation of Samples and Sample Holding

Parameter	Volume required (ml)	Container[a]	Preservative	Holding time
Alkalinity	100	P, G	Cool, 4° C	24 hours
BOD	1,000	P, G	Cool, 4° C	6 hours
COD	50	P, G	H_2SO_4 to pH 2	7 days
Dissolved oxygen				
Probe	300	G only	Determined on site	No holding
Winkler	300	G only	Fix on site	4-8 hours
MBAS	250	P, G	Cool, 4° C	24 hours
Nitrogen				
Ammonia	400	P, G	Cool, 4° C H_2SO_4 to pH 2	24 hours
Organic	500	P, G	Cool, 4° C H_2SO_4 to pH 2	7 days
Nitrate	100	P, G	Cool, 4° C H_2SO_4 to pH 2	24 hours
pH	25	P, G	Cool, 4 ° C Determined on site	6 hours No holding
Orthophosphate	50	P, G	Filter on site Cool, 4° C	35 hours

a. P = plastic, G = glass

Source: (6)

inconclusive, later samples were assayed for Salmonella spp. and enumerated by the multiple-tube method. In addition, diluted samples of sludge (200 g sediment mixed with 1,800 ml buffer solution) were analyzed for parasites (6).

Primary Productivity Measurements

CEPIS conducted primary productivity measurements four times in aquaculture ponds T1, C1, C2, and Q2 on a rotational basis during hours of greatest sunlight. Pairs of light and dark bottles filled with pond water from depths of 10, 20, 30, and 40 cm were suspended at those depths for one hour. Initial and final DO concentrations were measured in the field using a specific electrode (Yellow Spring Instruments 5720A probe) or in the laboratory, as needed when DO levels were especially high, using the Winkler method. Net photosynthesis was estimated as mg/l of net oxygen production and converted to mg/m^2-hr of oxygen by integrating through the water column (6).

Aquaculture Evaluation Techniques

UNA performed all of the stocking, sampling, harvesting, and evaluation associated with the aquaculture project. Throughout the aquaculturing periods, fish and prawns were sampled monthly by seining, to measure weight and length of individuals. A minimum of 3-5 percent of the stocked number from each pond was removed per sample (27).

Harvesting

At the end of each growing period, each pond was drained and individuals harvested by seining to determine survival and growth. The following parameters were subsequently calculated from the data on weight, length, growth period, stocking number, and harvest number:

- condition factor (as a function of weight and length),
- daily growth rate (as a function of weight and time),
- daily growth rate (as a function of length and time),
- stocking rate (as a function of weight and pond surface area),
- density (number of individuals per hectare of pond area),
- yield (final minus initial stocking rate),
- productivity (as a function of weight gained per hectare per day),
- final survival (final number of individuals versus initial number per pond),
- intraspecific competence (as a function of productivity for a low-density population versus productivity for a high-density population),
- interspecific competence (as a function of productivity for a monoculture pond versus a polyculture pond),
- carrying capacity (maximum total weight of cultured species that the pond can sustain), and
- critical standing crop (maximum total weight of cultured species that the pond can sustain without showing a reduction in growth rate) (27).

Food Processing

Following the Phase II harvest, fish were studied at the UNA laboratories to determine the yield of traditionally edible sections. First, some of the fish were segmented into head, gills, gut, skin, bones, and tail. Each part was weighed and the percentage of the total weight occupied by each was calculated. Edible yield for several kinds of cuts (e.g., fillet, dressed, and semidressed) was determined as a percentage of total weight (27).

Two samples of fish were transported as quickly as possible after harvest to the UNA laboratories for processing:

1. Fish from Q2 were washed, cut along the back (butterfly cut), washed again, dipped into a brine solution (36.3 percent salt for 30 minutes), drained, layered within salt in a salt:fish ratio of 1-1/2:1, washed and drained after 3 days, then packed in polyethylene and refrigerated.

2. Fish from T1 were washed, cut along the back, washed again, dipped into a brine solution, drained, smoked at 30°C for 12 hours, then smoked at 60° C for 2 hours, cooled, packed, and refrigerated (27).

Bacteria Measurements

Bacteriology studies on raw fish from all the aquaculture ponds were performed by USM-IVITA during Phase II. A random sample was taken from the monthly seinings performed by UNA. Samples destined for microbiological examination were placed in plastic 45-liter containers and transported live. At USM-IVITA they were removed from the water and placed live in a refrigerator, where they remained living (although subdued) until analysis. Meticulous sterile laboratory procedures were followed in skinning and cleaning the fish, so that no contamination on the skin, fins, gills, or within the digestive system would enter the muscle samples (28).

The first examinations were carried out on juveniles before they were placed in ponds, and monthly examinations were performed thereafter. Muscle and digestive tract content (DTC) were examined monthly for the presence of bacteria in fish, and at harvest time peritoneal fluid was also examined (28).

The MPN was determined monthly on individuals. Muscle sample size in the final stages was increased to 65 g to see if large samples would yield positive results.

At the time of harvest, SPC (on nutrient, McConkey, and mFC agar) was also determined -- again, 65-g muscle samples were used. At the same time, enrichments (in selenite broth and tetrationate broth) were prepared for isolation (on *Salmonella*-*Shigella* agar and mFC agar) of *Salmonella*. Serological tests were performed for plate colonies that showed biochemical reactions comparable to those of *Salmonella*. Those that had a positive serological reaction were taken to the National Reference Laboratory for Enterobacteria for identification.

At harvest, enrichments were also prepared for the isolation of M-Enterococcus from muscle and DTC, and colonies were confirmed by microscopic examination (28).

Because the full array of analyses was not performed every month, the microbiology data are not complete and at this time provide only an indication of the public health aspects of the aquaculture undertaken (9, Buras).

For purposes of reference, USM-IVITA also examined MPN and SPC of muscle, peritoneal fluid, and DTC samples of tilapia harvested from small aquaculture tanks supplied with clean well water and fertilized by anaerobically digested animal manure (24, 28).

Bacteriology studies on raw and processed fish harvested from T1 and Q2 were performed by UNA. During processing, the tilapia were washed and filleted by standard commercial-home procedures. As a result, the knife used to cut open the fish and clean it was also used to do the filleting, and no antiseptics were used for disinfection. Any contamination on the skin and within the digestive tract of the fish could therefore be transferred to the muscle of the fish (10). Homogenized samples of fillets with skin, fillets without skin, and processed fish were evaluated for MPN, SPC, Salmonella, and Streptococcus fecalis (9, Buras; 27).

Parasite Measurements

Parasite examinations were performed by USM-IVITA on fish sampled monthly and at harvest during Phase II. Muscle, gills, and DTC were examined.

Gills were placed in a glass jar with physiological serum and then stirred vigorously. The resulting liquid was observed for monogenea. Then the liquid was centrifuged in test tubes to obtain sediment. Each sample of sediment thus derived and of DTC separately obtained was further centrifuged and processed so that any flotable solid matter (namely, parasites) could be concentrated and then collected on a slide for examination under the microscope (the overall process followed is known as the Faust Flotation Method) (28).

Portions of muscle were pressed between two glass plates and examined under a stereomicroscope for metacercaries and plearocercoids. In the event of a positive result, the parasites would have been separated from the muscle by digestion with pepsin (29).

Fish Toxicology Measurements

CEPIS undertook a one-time sampling of six tilapia fish from C1 in April 1984. A homogenized sample of all muscle tissue was prepared, of which 100 g were analyzed for water content, lipid content, and fixed solids. Three 10-g samples were prepared by acid digestion and extraction for mercury analysis by wet vapor atomic adsorption spectrophotometry. Three 5-g samples were analyzed for lead, chromium, and cadmium using atomic absorption spectrophotometry (6).

Subsequently, 2g of lipids were extracted from 500g of the homogenized sample for qualitative analysis of organochlorinated and organophosphorus pesticide using methods prescribed by the U.S. Food and Drug Administration (FDA). A portion was also prepared for analysis by gas chromatography using AOAC methods for identification and measurement of organochlorinated pesticides (6).

Chapter 4

FINDINGS

HYDRAULICS AND LOADINGS

CEPIS determined a daily water balance for each lagoon and computed hydraulic loadings. Table 3 summarizes the flows entering each pond, pond area, depth and volume, estimated infiltration rates, and nominal hydaulic detention time (6).

The hydraulic detention times shown in Table 3 are somewhat overestimated with respect to real detention times, since considerable short-circuiting occurs in the ponds. According to earlier tracer studies conducted by CEPIS at San Juan, real detention time is probably about two-thirds the number of days shown (6).

Table 3 also shows the daily estimate of average BOD_5 and the resulting average surface loading rate per pond. To calculate the average BOD_5 loadings in the ponds, the average flow for each pond was multiplied by the corresponding BOD_5 concentration interpolated from weekly sampling results (6).

PHYSICAL-CHEMICAL WATER QUALITY

As part of the water supply and sewerage master planning effort, the water quality of the main interceptors was analyzed for a number of parameters, including heavy metals (16). The results indicate that the overall quality of wastewater in the main interceptor of the south is markedly better than that of interceptors in the north. This difference reflects the limited industrial development in the southern region of Lima, where the San Juan lagoons are located (16).

Earlier studies undertaken by CEPIS at San Juan (lagoons located in the southern portion of Metropolitan Lima) to develop stabilization pond design relationships showed a high correlation of removed 5-day biological oxygen demand (BOD_5) load versus applied BOD_5 load for the primary and secondary ponds of San Juan, a strong correlation between total BOD_5 and soluble BOD_5, and a relationship between COD and BOD_5 (37, 38).

During the aquaculture studies, raw sewage loadings were lower than anticipated (kg-BOD_5/ha-day was 333 in P1 and 201 in P2, versus expected loads of 350 for P1 and 250 for P2). CEPIS found the average loads removed in the primary facultative ponds were 307 kg-BOD_5/ha-day in P1 (92.2 percent removal) and 188 kg-BOD_5/ha-day in P2 (93.5 percent removal). The loads removed in downgradient ponds were: 70 percent in S1, 45.8 percent in T1, 66.4 percent in S2, 47.5 percent in T2. These were lower removals than CEPIS had experienced in earlier studies and were attributed to the reduced bacterial biomass in advanced polishing ponds. In any case, multicell pond systems

Table 3. Hydraulic and Organic Loads on Ponds. Average Values, May 1983 to April 1984

Pond	Inflow (lps)	Area (Ha)	Average depth (m)	Volume (m^3)	Infiltration rate (mm/day)	Hydraulic detention (days)	Influent BOD_5 (mg/l)	Surface BOD_5 load[a] (KG/Ha-day)
P1	29.7	1.20	1.3	15,600	7.6[b]	6.1	155.7[c]	333.2[c]
S1	28.8	1.44	1.6	34,040	3.7	9.3	12.6	21.6
T1	28.1	1.49	1.3	19,370	6.1[b]	8.0	11.7	18.0
C1	2.0	1.30	1.0	13,000	13.1[b]	-[d]	20.9	2.5
P2	15.3	1.10	1.3	14,300	19.2[b]	10.8	155.7[c]	201.0[c]
S2	12.8	0.88	1.3	11,440	14.1	10.3	10.6	15.5
T1	11.4	1.30	1.3	16,900	18.0	17.2	10.4	6.7
C2	1.2	0.49	1.3	6,370	10.0[b]	-[d]	18.7	3.9
Q2	0.6	0.53	1.0	5,300	10.0[b]	-[d]	7.8	0.9

a. Average of computed daily loads.
b. Estimated (37).
c. Total BOD_5, other values are soluble BOD_5.
d. Operated as batch-flow ponds.

Source: (6)

should be designed so that primary ponds serve mainly for BOD removal, while polishing ponds are used principally for pathogen control (6).

Table 4 summarizes the average daily DO, temperature, pH, and total ammonia nitrogen levels for the entire study period, May 1983 to April 1984. Tables 5 and 6 show the averages and ranges of these same parameters for Phases I and II, respectively. The CEPIS report provides graphic presentations of daily measurements, as well as raw data, monthly statistical summaries, plots of monthly averages, and diurnal patterns for DO and temperature (6).

Table 7 provides average weekly data on BOD_5, total ammonia nitrogen, organic nitrogen, orthophosphate phosphorus, total alkalinity, and detergents (MBAS). More detailed information (e.g., detailed statistical information, raw data tables for each pond effluent, and probability distribution curves for each parameter) is available in the CEPIS report (6).

In February 1984 water quality changed dramatically as a result of an accidental upset in the operation. Adjacent farmers tampered with pond inlet sluice gates, without authorization, in order to let large quantities of wastewater through to their irrigation channels. A shock load of secondary effluent entered the T2 pond and thereafter was felt (but to a lesser extent) downgradient in C2 and Q2. Weekly data revealed that soluble BOD_5 levels in T2 shot up to 44.9 mg/l compared with the average 18.7 mg/l. Surface loading after the incident reached 20 kg-BOD_5/ha-day in T2 compared with the average load of 6.7 kg-BOD_5/ha-day (6).

Dissolved oxygen is a key parameter in aquaculture. As expected for waste stabilization ponds, phytoplankton photosynthesis caused supersaturated DO conditions during most afternoons; and nighttime biomass respiration led to a reduction in DO so that levels were near zero by the early morning. Nighttime DO levels were lower and remained low for more prolonged periods in the tertiary ponds. As a result, the fish probably experienced oxygen stress for short periods each day, a factor that could have limited their growth potential. As Table 4 indicates, however, the overall average DO levels in the San Juan aquaculture ponds were sufficiently high (well above 2 ppm and often reaching 10-15 ppm in the afternoon) for the survival and growth of fish and prawns (6).

Earlier CEPIS studies had shown primary and secondary ponds to have only limited nitrification potential (37,38). Batch operation of the aquaculture ponds showed 1.5-2.1 mg-N/l total ammonia nitrogen (NH_3 + HN_4^+), while continuous-flow tertiary ponds averaged effluents of 8.3-11.6 mg-N/l. During aquaculture studies (see Tables 4 and 7), total ammonia decreased progressively through both series of ponds. This was due to biological uptake followed by sedimentation of organic nitrogen, control of surface loading rates through batch operation, and possibly to leaching into pond subsoil (6).

Table 4. Environmental Conditions in Aquaculture Ponds: Averages of Daily Samples, May 1983 to April 1986

Pond	DO (mg/l)			Temperature (^oC)			pH	Total ammonia (mg-N/l)	Un-ionized ammonia (mg-N/l)
	20 cm	40 cm	60 cm	20 cm	40 cm	60 cm			
Morning									
T1	2.6	1.9	1.6	22.2	22.2	22.1	7.8	11.6	0.4
C1	4.6	3.6	3.1	23.5	23.5	23.5	8.3	2.1	0.2
T2	4.3	2.7	1.8	24.2	24.2	24.2	8.1	8.3	0.7
C2	5.3	4.3	3.7	23.4	23.4	23.4	8.6	1.8	0.3
Q2	4.2	3.0	2.3	23.8	23.8	23.7	8.5	1.5	0.3
Afternoon									
T1	7.1	5.3	3.8	23.2	23.0	22.8	8.2	11.6	1.0
C1	11.4	9.3	7.1	25.4	25.2	24.9	8.7	2.1	0.5
T2	16.1	12.9	5.5	27.1	27.0	26.1	8.5	8.3	1.4
C2	13.4	11.1	7.4	25.2	24.9	24.5	9.1	1.8	0.6
Q2	12.8	9.6	5.8	26.0	25.8	25.0	9.0	1.5	0.5

Source: (6)

Table 5. Range and Averages for Physical and Chemical Parameters of Aquaculture Ponds Recorded in Phase I, April to September 1983

Pond		Temperature ($^{\underline{o}}$ C)		DO ppm		pH		Total Ammonia (ppm)	Un-ionized ammonia (ppm)
		Morning	Afternoon	Morning	Afternoon	Morning	Afternoon		
T-1	Average	20.5	21.5	2.9	6.9	7.9	8.1	13.5	0.79
	Maximum	23.0	24.5	11.8	21.0	8.5	8.9	19.6	2.70
	Minimum	18.8	19.2	0.2	0.3	6.8	6.8	6.3	0.30
C-1	Average	21.1	22.1	5.5	11.3	8.3	8.6	0.8	0.12
	Maximum	24.8	27.0	19.0	20.0	10.0	10.6	3.6	0.98
	Minimum	17.8	19.0	0.5	1.5	6.6	6.5	0.17	0.01
C-2	Average	21.2	22.2	7.4	11.9	8.7	9.0	0.53	0.20
	Maximum	26.6	27.5	15.8	20.0	9.0	9.2	5.80	0.40
	Minimum	19.2	20.0	0.1	0.6	7.0	7.0	0.36	0.004

Source: (27,6)

Table 6. Ranges and Averages for Physical and Chemical Parameters of Aquaculture Ponds Recorded in Phase II, October 1983 to April 1984

Pond		Temperature (° C)		DO ppm		pH		Total ammonia (ppm)	Un-ionized ammonia (ppm)
		Morning	Afternoon	Morning	Afternoon	Morning	Afternoon		
C-1	Average	26.2	28.5	5.3	12.3	8.5	8.8	2.84	0.75
	Maximum	27.8	31.4	14.6	20.0	9.1	9.8	6.10	1.93
	Minimum	25.0	26.6	0.6	1.4	7.5	7.4	0.85	0.18
T-2	Average	24.8	27.5	4.4	16.6	8.1	8.7	7.17	1.38
	Maximum	28.0	33.0	21.0	21.0	9.2	10.0	16.90	3.89
	Minimum	20.0	20.5	0.2	1.0	7.0	7.0	0.78	0.10
C-2	Average	24.8	27.2	4.8	15.5	9.3	9.1	1.74	0.60
	Maximum	28.5	31.5	16.6	20.0	10.2	10.6	5.43	2.05
	Minimum	19.9	20.8	0.3	3.0	7.2	7.4	0.20	0.02
Q-2	Average	24.4	27.0	4.4	14.4	8.7	9.1	1.02	0.40
	Maximum	28.8	31.1	16.5	21.0	10.4	10.5	5.80	2.66
	Minimum	18.5	19.8	0.2	2.8	7.5	7.6	0.20	0.01

Source: (27, 6)

Table 7. Environmental Conditions in Ponds: Average of Weekly Samples, May 1983 to April 1984

Pond	BOD_5	NH_4-N	Org-N (mg/l)	PO_4-P	MBAS	Alkalinity
Influent	155.7	31.4	18.1	3.10	1.38	270.5
P1	12.6	23.5	12.1	-	1.62	215.5
S1	11.7	16.6	12.0	-	1.46	196.9
T1	20.9	10.7	9.8	1.03	1.12	189.8
C1	16.5	2.6	10.7	0.55	0.94	141.0
P2	10.6	19.8	14.4	0.11	1.55	241.4
S2	10.4	16.8	9.8	0.16	1.78	185.6
T2	18.7	8.8	9.0	0.53	1.13	135.9
C2	7.8	2.5	10.5	0.45	0.85	116.6
Q2	6.8	1.6	10.8	0.78	0.98	121.2

Source: (6)

Ammonia (specifically, un-ionized ammonia, NH_3) can be toxic to fish. Toxicity is most severe when pH is high (particularly in the late afternoon when pH rises as a result of photosynthesis activities), because more of the total ammonia is in the un-ionized form. Furthermore, the toxicity of un-ionized ammonia is proportional to temperature (fish are 50-100 percent more sensitive to NH_3 at (30°C than at 20°C) (6). Therefore, it was recommended that aquaculture not take place in ponds exceeding a maximum allowable un-ionized ammonia concentration of 1-1.5 mg-N/l (9, Hepher).

As indicated in Table 4, the average levels of un-ionized ammonia in the ponds studied were generally acceptable. But, after the accidental shock loading into the Series 2 ponds, un-ionized ammonia levels went up -- significantly in T2, but to a much lesser extent in downstream ponds C2 and Q2. Immediately after the incident, the daily values of un-ionized ammonia in T2 exceeded 2 mg-N/l, while in C2 and Q2 daily NH_3 concentrations rose to 1 mg-N/l (6).

Heavy mortalities of tilapia occured in T2, whereas tilapia in Q2 appeared unaffected; in addition, all of the prawns in C2 died. Thus it appears that tilapia will grow and survive if total ammonia is maintained at less than 2 mg-N/l, average un-ionized ammonia is less than 0.5 mg-N/l, and short-duration levels of un-ionized ammonia do not exceed 2 mg-N/l (6). Prawns are obviously a much more sensitive species, but not enough information is available from the studies to indicate how stringent their un-ionized ammonia criteria should be.

Detergents can be toxic to fish, the lethal level depending on the fish species. Experiments carried out in Israel have suggested that the combined concentration of alkyl-benzene-sulfonates (ABS) and soft long-chain benzene sulfonate (LAS) should not exceed 1-1.25 ppm in aquaculture ponds (9, Hepher).

Detergent removal rates at the San Juan lagoons were found to be limited (largely because of foaming at pond outlets). But fortunately the detergent levels in the raw sewerage were low (1.0-1.5 mg/l MBAS) (9, Hepher). As Table 7 indicates, detergent levels in C1, C2, and Q2 were not at detrimental levels, while those in T1 and T2 were on the borderline of being stressful to fish.

MICROBIOLOGICAL WATER QUALITY

Earlier studies by CEPIS have clearly shown that the removal of fecal coliforms increases with the time that wastewater is retained in the lagoons. In the continuous-flow tertiary ponds, fecal coliforms died off to levels below 10^4/100 ml. For primary ponds, the constant die-off rate for fecal coliforms averaged 0.740 l/days, and for secondary ponds it averaged 0.934 l/days. Salmonella exhibited similar die-off rates of fecal coliforms (39).

Measurements of total coliforms and fecal coliforms during the aquaculture studies showed comparable results. Figures 4 and 5 demonstrate a steady decline in both total coliforms and fecal coliforms in successive ponds. Overall fecal coliform removals averaged 99.99 percent in Series 1 ponds and 99.999 percent for Series 2 ponds. Raw data, statistical summaries, and plots of individual observations for the entire study period are available in the CEPIS report (6).

Geometric averages for fecal coliforms in the fish ponds, expressed in MPN/100 ml, were: 2.9 x 10^4 in T1; 2.3 x 10^3 in C1; 1.7 x 10^4 in T2; 5.1 x 10^2 in C2; and 1.6 x 10^2 in Q2. During the period of excessive loading in February 1984, a peak value close to 10^6 MPN/100 ml occurred in pond T2 (6).

In earlier CEPIS studies, fecal coliforms proved a reliable indicator of the removal of *Salmonella* from the San Juan ponds (39). A few samplings during the aquaculture studies confirmed expected reductions in *Salmonella* on the order of 99.99 percent. Serotyping showed survival of *S. paratyphi B* in effluents of T1 and C1 (6).

In previous studies, all serotypes of *Salmonella* encountered within the ponds were resistant to the majority of antibiotics tested (39). These results are of some concern with respect to wastewater treatment and reuse, because resistant bacteria of one type can transfer their resistance to bacteria of other types through conjugation. Moreover, bacteria that are resistant to antibiotics also tend to be resistant to other physical and chemical factors, such as chlorination (9, Buras).

Table 8 shows the frequency of positive identification of parasites in each pond effluent (6). The results support earlier studies performed by CEPIS showing that parasites are largely removed from the water column during primary treatment.

SEDIMENT QUALITY

Total coliforms and fecal coliforms in pond effluent and sediments were assessed during May-June 1984. Figures 6, 7, 8, and 9 show the comparative results, which indicate consistently higher coliform levels in the sediments than in the pond effluents. Concentrations in sediments were two or more orders of magnitude greater on the average than in effluents (6).

Figure 10 shows *Salmonella* concentrations in pond effluents versus Series 1 sediments. The serotypes identified are shown in Table 9 (6). Note that some of the *Salmonella* in the higher-level ponds could have been added by water fowl attracted to the ponds by the fish (21).

Standard Plate Count results for raw sewage, pond effluents, and sediments are shown on Figure 11 (6).

USM-IVITA examined the pond sediments for parasites. They found significant numbers within the primary pond sediments, as was expected, since parasites are known to settle within 3-6 days (23, 33). In addition, they found amoeba cysts within the sediment of Q2 -- probably because these persisted in sediments remaining within the pond from earlier times when Q2 was a highly loaded secondary pond and there had been a known breakthrough of parasites from the preceding primary pond (6, 10).

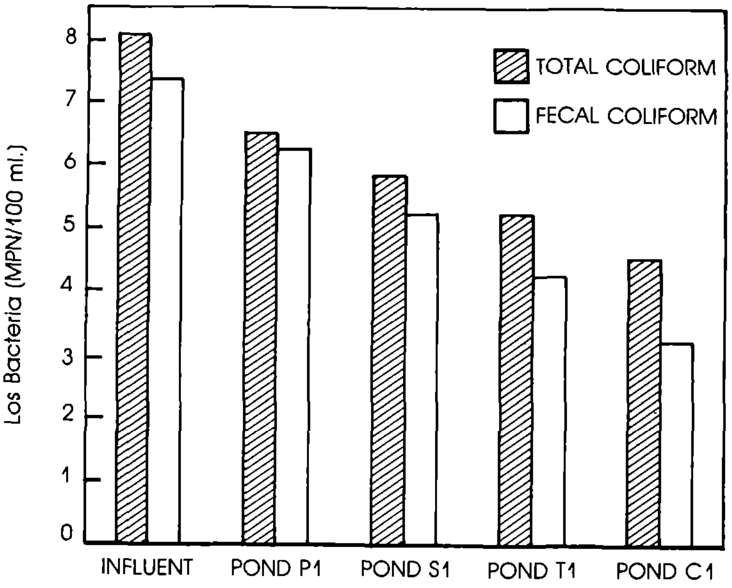


Figure 4. Concentration of Indicator Bacteria in Pond Effluents, Series 1: Geometric Average of Monthly Measurements, March 1983 - June 1984

Source: (6)

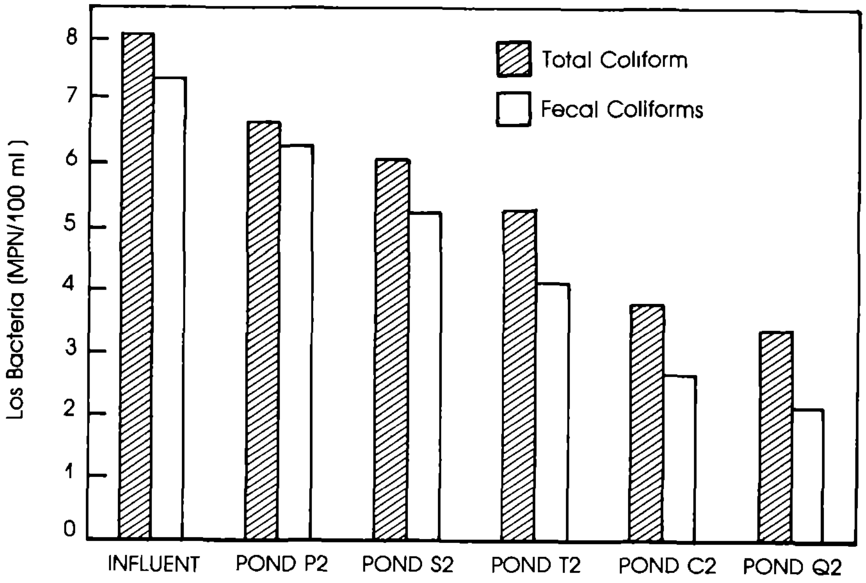


Figure 5. Concentration of Indicator Bacteria in Pond Effluents, Series 2: Geometric Average of Monthly Measurements, March 1983 - April 1984

Source: (6)

Table 8. Frequency of Positive Identification of Enteric Protozoa and Helminths, May 1983 to April 1984

Organism	Raw sewage	Series 1 effluents				Series 2 effluents				
		P_1	S_1	T_1	C_1	P_2	S_2	T_2	C_2	Q_2
Protozoa										
Giardia lamblia	8/8	1/6	0/4	0/5	0/3	0/3	0/3	0/3	0/2	0/1
Entamoeba coli	8/8	1/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Entamoeba histolytica	1/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Endolimax nana[a]	6/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Iodamoeba butshili[a]	8/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Chilomastix mesorili[a]	1/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Helminths										
Ascaris lumbricoides	5/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Trichiuris trichiura	3/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1
Hymenolepsis nana	7/8	0/6	0/4	0/4	0/5	0/3	0/3	0/3	0/2	0/1

a. Normally inhabiting the human intestine.

Source: (6)

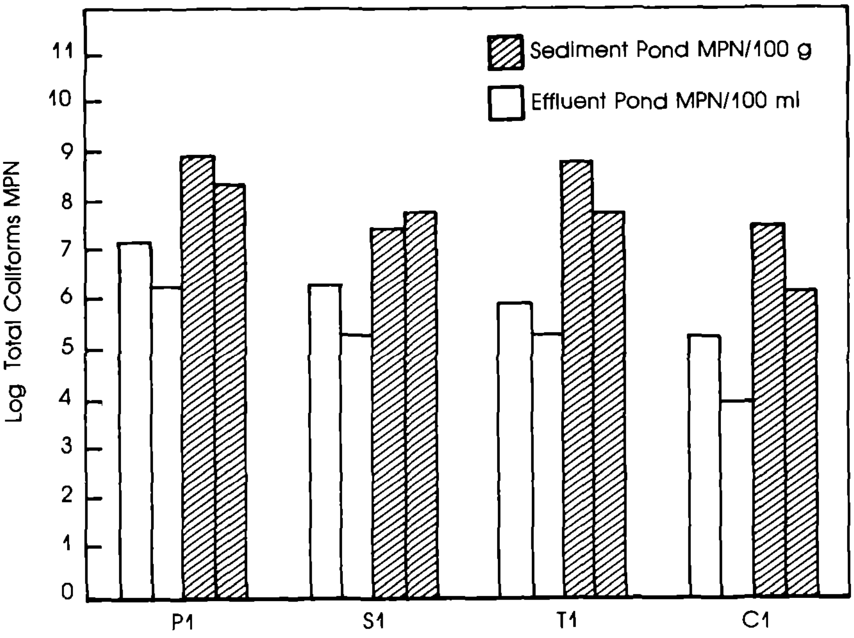


Figure 6. Total Coliforms in Pond Effluents and Sediments, Series 1: May-June 1984

Source: (6)

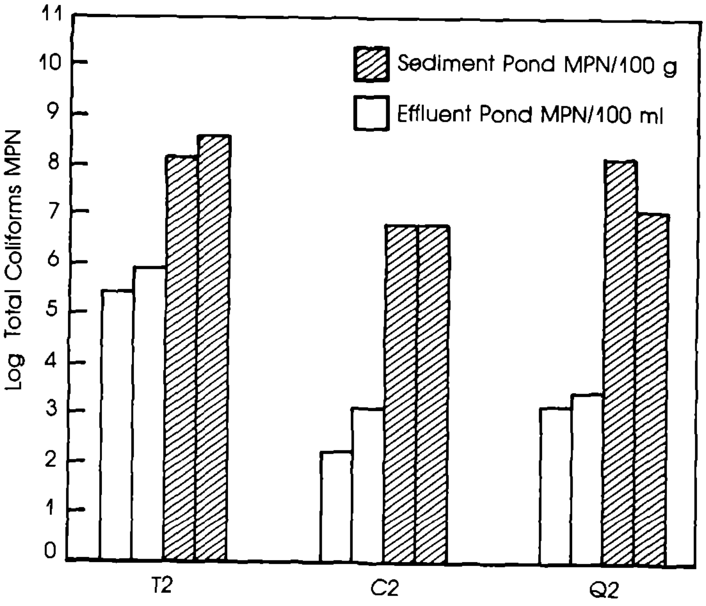


Figure 7. Total Coliforms in Pond Effluents and Sediments, Series 2: May-June 1984

Source: (6)

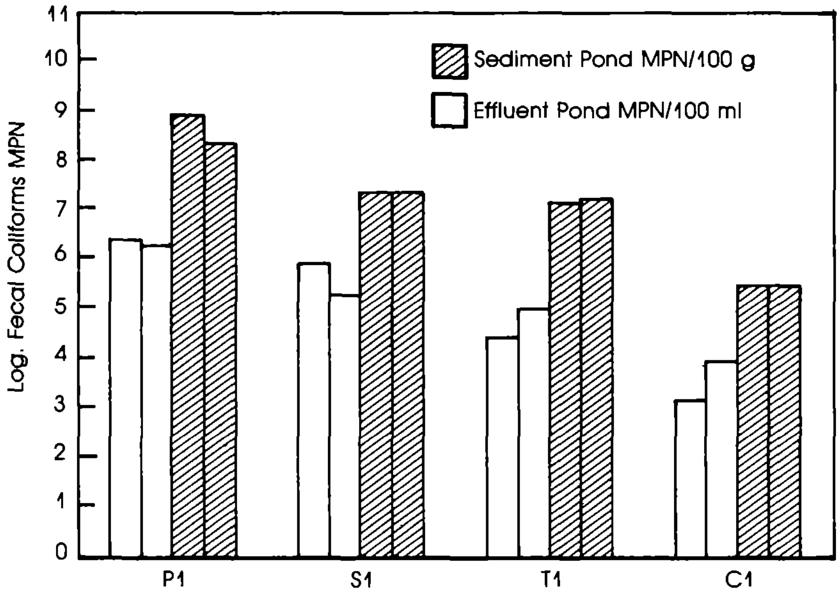


Figure 8. Fecal Coliforms in Pond Effluents and Sediments, Series 1: May-June 1984

Source: (6)

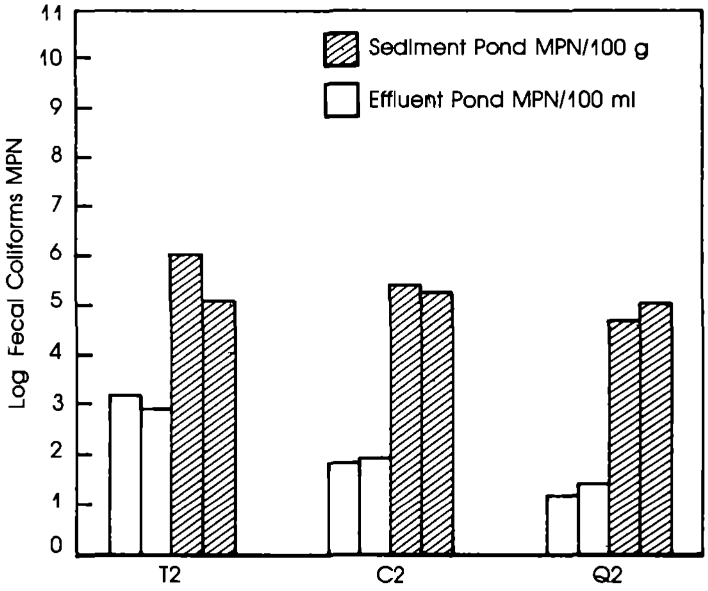


Figure 9. <u>Fecal Coliforms in Pond Effluents and Sediments, Series 2: May-June 1984</u>

<u>Source</u>: (6)

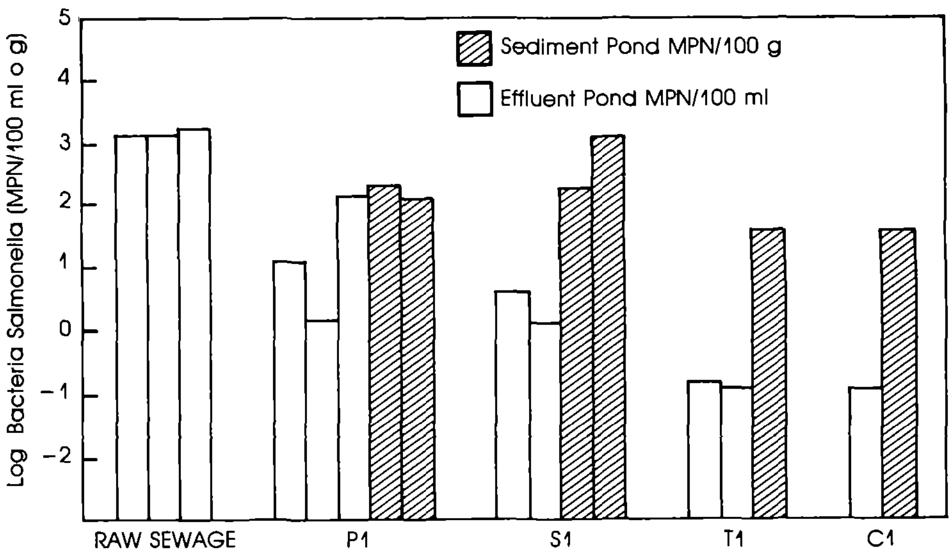


Figure 10. Salmonella sp. Concentration in Pond Effluents and Sediments: May-June 1984

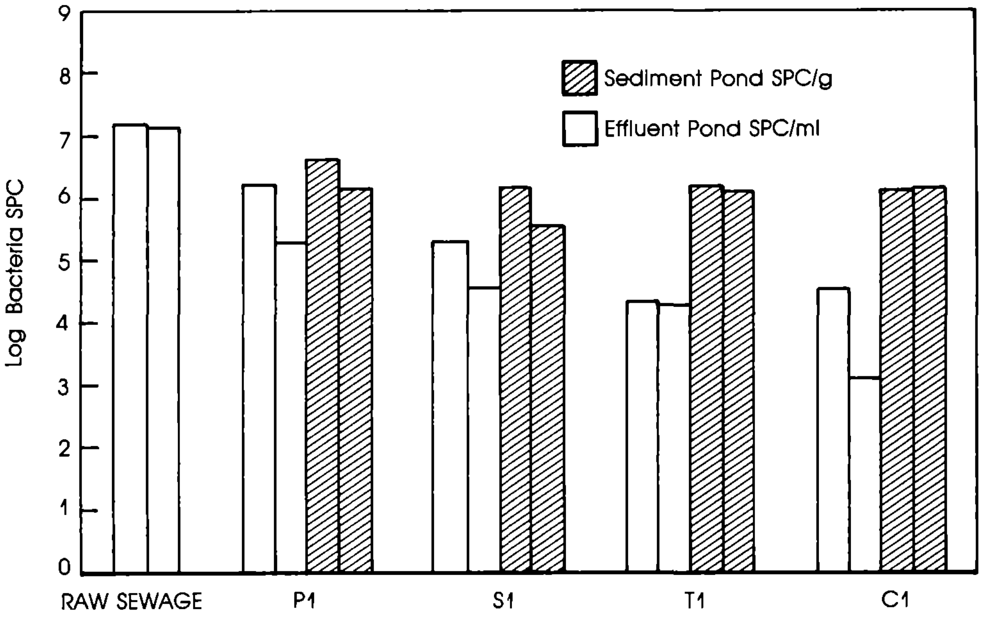
Source: (6)

Table 9. Frequency of Positive Identification of Salmonella Serotype, March to June 1984

Serotype	Serogroup	Raw sewage	Pond effluents				Pond sediments			
			P_1	S_1	T_1	C_1	P_1	S_1	T_1	C_1
S. paratyphi B	B	3/5	3/3	2/2	1/2	1/5	1/2	2/2	1/2	0/2
S. typhimurium	B	2/5	0/3	0/2	0/2	0/5	0/2	0/2	0/2	0/2
S. 4,5,12:-:-NM	B	0/5	1/3	0/2	0/2	0/5	0/2	0/2	0/2	0/2
S. montevideo	C_1	1/5	0/3	0/2	0/2	0/5	0/2	0/2	0/2	0/2
S. oranienburg	C_1	1/5	0/3	0/2	0/5	0/2	0/2	0/2	0/2	0/2
S. newport	C_2	1/5	2/3	0/2	1/2	0/5	1/2	1/2	0/2	0/2

Note: Preliminary results from the National Reference Laboratory for Enterobacteria.

Source: (6)



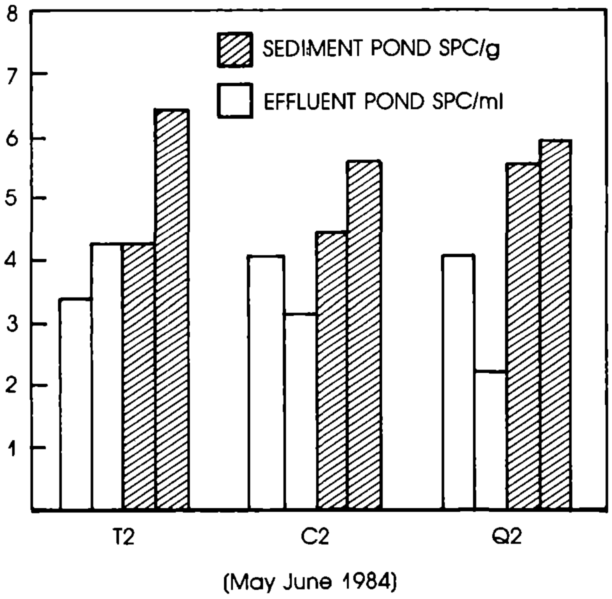


Figure 11. SPC in Pond Effluents and Sediments: May-June 1984

Source: (6)

AQUACULTURE RESULTS

The main objective in Phase I of the aquaculture studies was to assess whether survival and growth of fish and prawns appeared likely. In Phase II, the objectives were to further assess growth and to begin assessing optimum carrying capacity and stocking density of the ponds. Phase I consisted only of monoculture studies, whereas polyculture studies were started during Phase II.

Phase I took place during the winter season (June through October 1983). Survival and growth of tilapia during Phase I were good (see Table 10), despite the relatively cool temperature of the water (it averaged about 22°C). About 83 percent of the fish survived in the most contaminated pond, T1. Survival was 97 percent in C1 and 84 percent in C2. Final growth rates were 0.85 g/day for T1, 1.17 g/day for C1, and 0.79 g/day for C2 (27).

The fish densities in T1 and C1 were similar; however, tilapia in T1, which started at 7.7 g, reached an average weight of 106 g, whereas tilapia in C1 reached an average weight of 147 g even though it started from a smaller size (2.4 g). The difference is clearly attributable to the poor quality of water in T1; in particular, oxygen levels in T1 were often sufficiently depressed to stress the fish and cause them to eat less (27).

Although the quality of water in C1 and C2 was similar, C2 was much more densely stocked (4569 fish/ha in C2 versus 2,006 fish/ha in C1). C2 showed the highest stocking rate (746 kg/ha) and highest growth rate (3.8 g/day) about midway into the aquaculture period. However, fish experienced a negative growth rate in the final stages, probably because the pond reached its "critical standing crop" -- that is, the maximum weight of fish that could be supported by the natural food in the pond. At harvest time, fish in C2 weighed an average of 89 g, whereas those in C1 reached 147, even though those in C1 were initially smaller (27).

Prawns at San Juan exhibited good growth rates (0.4 g/day), which were comparable to those of prawns raised in clean water ponds just a few miles away at La Molina (see Table 11). Apparently water temperature was a more significant factor in prawn growth than differences in the quality of water in the freshwater and wastewater ponds tested. Prawns raised in clean water ponds of much warmer temperatures (at Satipo) experienced a much higher growth rate (1.2 g/day) (27).

Harvesting conditions led to a significant mortality among the prawns. The pond had previously been used for secondary treatment and been subjected to very high loadings, which had left a thick layer of sludge. There were unanticipated difficulties in draining the pond; and, after partial drainage, the embankment had to be cut down to allow complete drainage. The prawns were subjected to shallow pond conditions and a significant deterioration in water quality just prior to the harvest. As a result, all of the prawns died and only 10 percent of the dead animals could be recovered from the thick sludge (27).

Table 10. Summary of Measurements and Calculated Parameters per Controls and Final in the Monoculture of Tilapia in Phase I

Pond	Tertiary (T-1)				Final	Quaternary (C-1)				Final	Quaternary (C-2)				Final
Control	Initial	1st	2nd	3rd	Ave.	Initial	1st	2nd	3rd	Ave.	Initial	1st	2nd	3rd	Ave.
Average length, cm	7.3	10.6	13.6	17.0	17.0	6.0	10.1	12.0	18.5	18.5	17.0	12.5	14.3	16.4	16.4
Average weight, g.	7.7	27.0	55.7	106.0	106.0	2.4	26.7	100.4	147.2	147.2	8.2	48.9	154.2	89.0	89.0
Condition factor	1.97	2.26	2.21	2.15	-	1.92	2.59	5.81	2.32	-	2.39	2.50	5.27	2.01	-
Growth rate, mm/day	-	1.13	1.00	0.60	0.84	-	1.50	0.67	0.95	1.09	-	1.61	0.64	0.48	0.92
Growth rate, g/day	-	0.66	0.95	0.89	0.85	-	0.71	2.63	0.76	1.17	-	1.19	3.76	1.63	0.79
Density, fish/ha	1,600	1,503	1,411	1,324	-	2,128	2,107	2,086	2,066	-	5,455	5,125	4,839	4,569	-
Stocking Rate, kg/ha	12.3	40.6	78.6	140.3	-	5.1	56.3	209.4	304.1	-	44.5	250.6	748.2	406.6	-
Productivity, kg/ha/day	-	0.97	1.26	1.10	1.11	-	1.50	5.47	1.55	2.43	-	6.06	17.69	8.48	3.55
Yield, kg/ha	-	-	-	-	128	-	-	-	-	299	-	-	-	-	362
Duration, days	-	29	59	115	115	-	34	62	123	123	-	34	62	102	102
Survival, %		93.94	88.19	82.75	82.75	-	99.01	98.03	97.08	97.08	-	94.47	89.20	84.22	84.22

"-" = not applicable or not measured

Source: (27)

Table 11. Summary of Growth Data in Prawn Assays
Phase I

Place	San Juan	La Molina	Satipo
Average size (cm)	5.86	6.53	8.90
Average weight (g)	3.91	6.22	7.60
Growing period (days)	88	94	60
Daily growth rate (g/day)	0.405	0.533	1.23
Daily growth rate (mm/day)	0.315	0.274	1.12
Date of control	Sept. 6	Sept. 15	July 21
Average water temperature (^{o}C)	21	21	29

Source: (27)

Phase II took place during the summer season (October 1983 through April 1984), during which time pond temperatures averaged about 27°C. During the first 3 months after stocking, the growth rate of tilapia in T2 was similar to that of tilapia in Q2 (27). (Note that water quality in all the ponds during Phase II was better than during Phase I.)

With the accidental shock load to T2 in February 1984 and the resulting changes in dissolved oxygen and ammonia levels, significant mortalities occurred. Only about 40 percent of the tilapia in T2 survived (27). On the other hand, about 88 percent of the tilapia in Q2 survived -- this rate is comparable to the survival measured in Q2 during Phase I (27). This high survival is due to the batch operation of Q2, which significantly reduced toxic ammonia loads (6). When the fish were harvested, they looked healthy and vigorous (9, Buras). (See Table 12 for harvest results for Phase II.)

Polyculture within pond C1 was unaffected by the accident in the Series 2 ponds. Monthly control samples indicated good growth for tilapia (2.70 g/day), big belly carp (3.55 g/day), and mirror carp (7.24 g/day). In fact, tilapia grew much faster in the polyculture ponds -- probably because water quality remained constant in C1 in contrast to that of T2 and Q2. The results clearly indicate that tilapia were not competing with carp for food (see Table 13).

None of the prawns in C2 survived (27). Die-off could have been brought about by a combination of factors, since water quality in C2 was very poor even before the accidental shock loading occurred. In fact, NH_3 and pH were at their worst levels one month prior to the accident, possibly because of the increased algal activity of the early summer. Therefore, the possible causes of the die-off of the prawns during Phase II include:

- excessive total ammonia concentrations because of accidental shock loadings of raw sewage to pond series;
- excessive NH_3 concentrations because of increased phytoplankton photosynthesis and, hence, higher pH in the summer months;
- low nocturnal DO because of shock loading of raw sewage; and
- low nocturnal DO because of high benthal (sludge) oxygen demand and high summer temperatures.

Tilapia had been sexed prior to being placed in Q2. Nevertheless, there were some females and they spawned. The pond thus yielded a large production of fingerlings at the time of harvest, almost equivalent in total weight to the weight of large fish. The fingerlings that were caught were placed in another pond for stocking subsequent fish culture operations. The stocking rate for this pond had been higher during the fourth month of aquaculture than at the time of harvest. Apparently the fish experienced a negative growth rate in the final month because of the unexpectedly high density caused by the small fish (27).

Table 12. Summary of Measurements and Calculated Parameters per Controls and Final in the Monoculture of Tilapia, Phase II

	Quinquenary (Q-2)													Tertiary (T-2)							
	Large fish								Small fish					Large fish							
CONTROL	Init.	1st	2nd	3rd	4th	5th	Har-vest	Fin. Avr.	3rd	4th	5th	Har-vest	Fin. Avr.	Init.	1st	2nd	3rd	4th	5th	Har-vest	Fin. Avr.
Average length, cm	16.9	18.9	21.5	24.1	26.7	27.4	27.7	27.7	10.8	12.3	13.6	14.0	14.0	18.3	19.5	22.2	24.7	24.9	24.9	27.1	27.1
Average weight, g	96.0	135.0	214.0	286.0	369.0	377.0	355.0	355.0	29.8	39.2	46.5	51.7	51.7	140.0	182.0	253.0	343.0	308.0	300.0	368.0	368.0
Condition factor	1.97	2.00	2.16	2.04	1.93	1.83	1.67	-	2.37	2.10	1.86	1.88	-	2.28	2.45	2.30	2.27	1.99	1.94	1.84	-
Growth rate, mm/day	-	0.34	0.78	0.95	0.77	0.22	0.09	0.51	-	0.44	0.42	0.16	0.35	-	0.38	0.82	0.89	0.06	0.002	0.033	0.0044
Growth rate, g/day	-	0.68	2.39	2.58	2.43	0.25	-0.79	1.23	-	0.27	0.03	0.18	0.23	-	1.36	2.12	3.22	-1.03	-1.08	1.02	1.14
Density, fish/ha	2,129	2,085	2,041	1,997	1,954	1,911	1,868	-	-	-	-	13,824	-	1,064	1,042	1,021	1,000	663	441	424	-
Stocking rate, kg/ha	205	282	437	572	722	720	663	-	-	-	-	715	-	149	190	258	343	204	132	156	-
Productivity, kg/ha/day	-	1.4	4.7	4.8	4.4	-0.06	-2.03	2.18	-	-	-	2.57	2.57		1.3	2.1	3.0	-4.1	-10.3	0.4	0.03
Yield, kg/ha	-	-	-	-	-	-	-	458	-	-	-	-	276	-	-	-	-	-	-	-	7.0
Duration, days	-	57	90	118	152	182	210	210	-	34	64	92	92	-	31	64	92	126	133	199	199
Survival, percent	-	97.93	95.87	93.80	91.78	89.76	87.74	87.74	-	-	-	-	-	-	97.93	95.96	93.98	62.31	41.45	39.84	39.84

"-" = not applicable or not measured
Source: (27)

Table 13. Summary of Measurements and Calculated Parameters per Controls in the Polyculture of Carps and Tilapia in Pond C-1, Phase II

FISH SPECIES CONTROL	Nile tilapia					Mirror carp				Big belly carp			
	Initial	1st	2nd	4th	Final Average	Initial	1st	2nd	Final Average	Initial	1st	2nd	Final Average
Average length, cm	2.8	12.3	17.7	22.8	22.8	10.8	18.1	29.2	29.2	7.0	13.0	19.6	19.6
Average weight, g	0.6	41.0	137.1	240.7	240.7	19.2	130.0	511.8	511.8	4.8	47.4	168.2	168.2
Condition factor, K	2.3	2.2	2.5	2.0	-	1.5	2.2	2.1	-	1.4	2.2	2.2	-
Growth rate, mm/day	-	4.1	1.6	1.8	2.3	-	4.2	3.8	2.7	-	3.5	2.3	2.7
Growth rate, g/day	-	1.8	2.8	3.6	2.8	-	6.5	13.2	7.2	-	2.5	4.2	3.6
Density, fish/ha	1,900	-	-	-	-	7.7	-	-	-	1,358	-	-	-
Stocking rate, kg/ha	1,197	-	-	-	-	1.5	-	-	-	6.5	-	-	-
Duration, days	-	23	57	86	86	-	17	68	68	-	27	46	46

"-" = not applicable or not measured

Source (27)

Primary productivity is a measure of the natural food available for fish within a pond. The larger the fish, the more absolute amounts of food it requires to maintain body weight and the potential for growth. When available food is just sufficient to maintain the fish but not allow growth, the pond has reached its "carrying capacity" for the type, number, and size of individuals living within it (22). The primary productivity of the aquaculture ponds was measured during June to October 1983 (Phase I) by CEPIS. The overall average in the ponds was 1,645 mg-O_2/m^2-hr, but rather large fluctuations occurred now and then -- at one point, for example, productivity rose from 220 to 3,345 mg-O_2/m^2-hr in Q2 within just 2 days. Even so, the average daily value of 12.6 g-O_2/m^2-d indicated high productivity even under less favorable winter conditions (6).

After the harvests of Phase II, researchers at UNA processed tilapia by both the wet salting and smoking methods. Color, texture, and taste of both salted and smoked tilapia were considered good and competitive with other locally available products (27).

FISH MICROBIOLOGICAL ASSESSMENT

When fish are grown in wastewater, bacteria present in the water column enter their digestive tract. From there it can pass into the bloodstream and end up in the various organs of the fish. However, the numbers of bacteria in the water column must be high before the bacteria will end up in the fish muscle (which is the typically edible portion). The numbers and kinds of bacteria reaching the muscle depend on the immune system of the fish, the degree to which the fish is exposed to high concentrations, and the general health of the fish at the time of excessive exposure (9, Buras).

The primary purpose of the microbiology studies in Lima was to assess whether the fish under consideration contained microorganisms that could be pathogenic to humans. These bacteria would not typically be pathogenic to fish but they could stress the fish. Any type of bacteria can cause the immune system of the fish to become blocked and therefore increase the fish's vulnerability to infection by its own pathogens (9, Buras).

Aquaculture experiments in Israel and the United States suggest that the numbers of bacteria in the digestive tract may often be equal to, or higher than, the numbers in the surrounding water (10, 20). Peritoneal fluid would typically be the next most contaminated part of the fish (9, Buras). In Israel, only when fish received inoculations of 10^6 E. coli per fish was E. coli subsequently detected in fish muscle. In pond water, concentrations of E. coli had to be higher than 10^6/100 ml to cause muscle contamination. On the other hand, only 10^4/ml of Salmonella or Streptococcus fecalis was necessary to cause muscle contamination (7). In the San Juan aquaculture ponds, E. coli were present on the order of 10^2 - 10^4/100 ml and Salmonella on the order of 10/ml.

In MPN tests during the early months of aquaculture, relatively high levels of total coliforms were present in the DTC of raw tilapia from the T2 pond (1.8 times 10^7/100 ml). Fecal coliform levels in the digestive tract of T2 tilapia were also high (1.4 x 10^6/100 ml). Total coliform and fecal coliform levels found in the DTC of tilapia were markedly lower in ponds of higher water quality; for example, the levels in tilapia harvested from Q2 were 1.1 x 10^4/100 ml and 2 x 10^3/100 ml, respectively. Coliform counts were somewhat higher in the DTC than in water samples (28).

After the accidental excessive loading occurred in the Series 2 ponds, total coliform and fecal coliform levels in T2 fish rose (2.2 x 10^8/100 ml in both cases). By harvest time, several months later, these levels had decreased significantly (2.5 x 10^5/100 ml and 1.4 x 10^3/100 ml, respectively). Similarly, total coliform and fecal coliform levels in Q2 fish rose after the accidental excessive loading (1.8 x 10^8/100 ml and 7x10^5/100 ml, respectively), and were reduced at harvest time (8x10^4/100 ml in both cases) (28).

Fecal coliforms were not recovered from most MPN tests on muscle, and total coliforms were present only in low concentrations-less than 50/100 ml (28). These results are compatible with those from studies conducted in the United States, South Africa, and Israel, all of which found levels of fecal coliforms to be either zero or extremely low (less than 25 per 100 g, even when levels in the DTC were 10^5 per 100 g) (9, Buras; 20; 29).

Studies conducted in Israel showed that when fecal coliform concentrations in muscle are very low or nonexistent, contamination by other bacteria may be found. MPN tests for coliforms were therefore deemed inadequate as a means of indicating pathogenic contamination in aquaculture studies (9, Buras). For this reason, SPC tests (as well as MPN tests) were performed in the latter stages of the Lima aquaculture studies.

SPC procedures revealed a significant population of bacteria, including enteric bacteria, in the peritoneal fluid and muscle of raw tilapia samples from both T2 and Q2. An average of 3.1 x 10^2 bacteria per gram of muscle were found in tilapia grown in T2, and 4.5 x 10^2 bacteria per gram of muscle were recovered in those grown in Q2. In C1, 2.3 x 10^2 bacteria per gram of muscle were recovered in the carp, whereas no bacteria were recovered from muscle of the tilapia grown there (28). Pond water samples averaged 10^4 SPC/ml (6).

Among the bacteria recovered by SPC procedures from the DTC of tilapia in T2 and Q2 were *E. coli*, *Citrobacter freundii*, *Klebsiella sp.*, *Proteus vulgaris*, and *Enterobacter cloacae*. *Salmonella* bacteria were isolated from the DTC in some of the T2 and Q2 tilapia samples, and *Streptococcus fecalis* was found in the DTC of some of the T2 and C1 big belly carp samples (28).

All samplings of gill, muscle, and the DTC of raw tilapia, carp, and prawns were negative for protozoa cysts and helminths (28).

On the basis of the numbers of bacteria found in muscle, the quality of fish grown in the San Juan lagoons was determined to be *acceptable* for human consumption (9, Buras and Hepher). According to the international standards for freshwater fish allowable for human consumption, the SPC for three of the five fish sampled should not exceed 1 times 10^5/100 g and the fecal coliform count should not exceed 4/100 g (10).

The main public health concern is that in the cleaning and preparing of fish in home kitchens or restaurants, people may spread the contamination from the digestive tract or peritoneal fluid to foods that can be eaten raw (including fish muscle, which is often consumed raw in Peru in a marinated salad known as "ceviche"). For this reason, controlled fish processing and packaging was recommended as a means of minimizing public health risk. SPC bacteriology tests on tilapia from T2 indicated that smoking reduced total bacteria levels. Surprisingly, salting did not reduce total bacteria, presumably because the salt used was contaminated (27).

In a few trials of depuration, fish were placed in clean water for 3 to 7 days. However, the data from the subsequent microbiological studies are inconclusive. Oddly, coliform bacteria were recovered from the muscle of T2 fish after depuration, but not before. No significant decrease in bacterial content within the DTC of the fish occurred after depuration (28). Assuming that the depuration water was free of pathogens, the unexpected contamination in the fish could have resulted from handling stress (9, Hepher). According to another theory, the relatively high pH levels of the aquaculture ponds could have suppressed the growth of most types of bacteria (except those resistant to pH, such as Streptococcus fecalis) until the fish were placed in fresh water (10).

UNA and ITINTEC grew tilapia in clean water tanks in which digested animal manure was used to fertilize the production of natural fish food (24). USM-IVITA conducted microbiological studies on a sampling of the partially grown fish. Total coliform counts and overall bacterial presence were somewhat higher in these tilapia than in those from the wastewater treatment ponds: total coliforms measured by MPN reached 2.5 x 10^7/100 ml in the DTC and 1.3 x 10^1/100 ml in muscle; SPC showed levels up to 4.2 x 10^2 bacteria/ml in muscle; fecal coliforms were found in the DTC but less than 20/100 ml were found in the muscle (28). As in the fish raised at San Juan, bacteria counts in the DTC were somewhat higher than those found in the water column.

TOXICOLOGICAL ASSESSMENT

A few samples were analyzed for trace metals and pesticides, but the results do not indicate any accumulation of toxic substances in tilapia grown in San Juan ponds. A combined sample of six fish collected for toxicology measurements yielded 1,057 g of homogenized muscle tissue for analysis. The results of this analysis are shown in Table 14. Gas chromatographic analysis for organochlorinated pesticides showed only one minor peak of low concentration (0.001 micro g/g), which was not identifiable but appeared similar to Aldrin and could be an isomer (6).

Table 14. Results of Analysis on Fish Muscle Sample (1,057g), May 1984

Analysis	Result
Bromatological analysis	
Water content	80.3%
Lipid content	0.4%
Fixed solids	1.2%
Trace metal analysis	
Mercury	0.3 ug/g
Lead	0.26 ug/g
Cadium	not detected (<0.01 ug/g)
Chromium	not detected (<0.1 ug/g)
Pesticide analysis (qualitative)	
Organophosphorus	negative (<0.2 ug/g)
Organochlorinated	negative (<0.2 ug/g)

Source: (6)

Chapter 5

CONCLUSIONS

The principal finding of the aquaculture studies is that fish grew in all of the ponds. However, the growth rate appeared favorable only in the fourth- and fifth-level ponds.

During the low temperature months (Phase I), the critical standing crop -- the weight of fish at which the pond can no longer support an increase in daily growth rate -- of the fourth-level ponds (C1 and C2) was 250 kg/ha. The carrying capacity -- the maximum weight of fish that the pond can sustain -- was 550 kg/ha (27).

During warmer months (Phase II), the critical standing value of the fifth-level pond (Q2) was 600 kg/ha and the carrying capacity was 1,350 kg/ha. The improved values were largely due to the higher level of natural food produced at warmer temperatures. Also, the water quality was better (27).

Polyculture began at the end of the Phase II summer months. As water temperatures declined from 26°C to 21°C, the daily growth rate for tilapia did not rise as high as the rate for carp (27).

Prawns raised during Phase I exhibited a good growth rate for the low water temperatures, but poor harvesting conditions led to mortalities. During Phase II, poor water quality led to mortalities. Despite the problems encountered, the researchers believe that the potential for prawn culture under more controlled conditions appears good (27).

The bacteria loads of fish harvested from aquaculture ponds were acceptable for human consumption (in terms of number of bacteria and fecal coliform count within the muscle portion). Improved processing would ensure that home kitchens did not become contaminated by bacteria from the fish digestive tract (9, 27).

The key water quality parameter for fish growth and production appears to be ammonia. The following maximum ammonia concentrations are recommended (6):

Total ammonia ($NH_3 + NH_4^+$)	2.0 mg-N/l
Average un-ionized ammonia (NH_3)	0.5 mg-N/l
Short duration NH_3 diurnal peaks	2.0 mg-N/l

Dissolved oxygen did not present problems under typical operating conditions, even with normal diurnal variations and heavy benthal deposits (6).

Detergent levels at San Juan did not present problems for fish culture, as the aquaculture ponds were generally maintained below 1 mg-MBAS/l of ABS detergents (6).

Fecal coliforms were effectively maintained below 10^4 MPN/100 ml, and therefore no fecal coliforms were recovered from the muscle of fish (6, 9, 28).

Complete protozoa and helminth removal was achieved in the primary and secondary treatment ponds; therefore parasites were not a problem in the aquaculture ponds (6).

Good treatment pond design and operation are vital for pathogen removal. Among the factors that should be considered are: the use of baffled outlet structures to prevent pathogen breakthrough on floating solids; proper position of inlet and outlet structures; and proper pond shape to reduce short-circuiting due to thermal stratification (6).

Ponds in which aquaculture is to be conducted need to be specially designed to facilitate regular sludge removal and fast and effective harvesting by seining (27).

Pond management must consider the human element. Fish ponds need to be adequately protected from external interference, such as people stealing fish or sabotaging the pond structure so that water quality is disturbed (6).

The fish grown in San Juan's treated domestic wastewater did not contain significant levels of heavy metals or pesticides. However, further study would be needed to determine the levels of toxic substances in wastewater containing industrial loads (6).

Chapter 6

RECOMMENDATIONS

In March 1985, a panel of experts on fish culture and epidemiology was convened in Lima, Peru, for 5 days to closely examine the Lima aquaculture studies vis-à-vis the global state-of-knowledge. At the conclusion of the meeting, the panel put forth a number of recommendations, and plans are now under way to implement them under the UNDP Integrated Resource Recovery Project, with financial support from the GTZ. Following are the principal points emphasized in the panel's review of the Lima studies:

> The Phase I and II studies at San Juan provided indicative data on water quality requirements, choice of fish species, potential growth rates and yields, and public health aspects of sewage-based fish culture. However, to derive design standards and monitoring criteria for extension of the technique, more investigative studies are necessary. New fish ponds are needed to enable replicable and statistically significant results, as well as to demonstrate appropriate design configurations.
>
> Economics received little attention in Phases I and II, but studies will be crucial in setting future priorities. Culture of high value products, such as large tilapia (ca. 500 g) and prawns, requires careful management and more expensive inputs than culture of carp and smaller tilapias. Options for choice of products range from high value foods for direct human consumption to fish seed (for grow-out elsewhere) and fish biomass (irrespective of individual size) as an animal feed ingredient.
>
> The following economic studies should be undertaken to enable a fuller assessment of the potential of wastewater-fed aquaculture: (1) Macroeconomics - fish and fish products supply, demand and markets (local, national, regional, and global); (2) Microeconomics of the proposed wastewater-fed culture systems with various end products; (3) Socioeconomics of the same systems and possible beneficiaries, with consideration of possible income and/or nutritional improvement for the urban and rural poor. Socioeconomic studies are particularly important and should include product preference, markets, food preparation habits, health status, health risks from aquaculture and presently available alternative food items, nutritional status, cost-benefit analyses, and comparisons with other re-use options such as irrigated vegetable production.

> Phases I and II provided a valuable insight into fish growth in wastewater lagoons, but because of the limited number of ponds used in the study and their design for only wastewater treatment, further studies are needed to provide bio-engineering design criteria for wastewater reuse through aquaculture.
>
> A series of experimental ponds designed specifically for aquaculture is required to permit scientific assessment of the various parameters involved in the use of wastewater in aquaculture. A scientific experiment with statistically meaningful data would involve a minimum of 4 treatments in triplicate - a total of 12 experimental ponds. These ponds must be large enough to obtain meaningful data for testing in larger pilot scale/demonstration ponds but small enough to permit an adequate number to be used for a scientifically valid study. An experimental pond size of 400 m^2 is recommended.
>
> A smaller number of larger pilot-scale/demonstration ponds is required to test the results of the experimental studies on a more realistic commercial scale. Data obtained from these ponds would be used for an economic assessment of the various options of wastewater reuse in aquaculture. The ponds would also be used as a demonstration of economically viable wastewater reuse options. It is recommended that at least 2 ponds of 2,500 m^2 be constructed for this purpose.
>
> Ponds would also be needed at the facility for servicing the experimental work.
>
> Construction of a service reservoir is recommended to ensure adequate water quality control. Also, a well should be constructed on the site to provide a supply of clean water to give flexibility in the quality of water supplied to the facility (21).

The panel of experts also provided detailed suggestions on the types of experimental programs to be conducted using the recommended aquaculture facility. The following are some of their recommendations: test several stocking densities (from 0.1 to $2/m^2$) of monosex nile tilapia and then blue tilapia; include several different portions (from 0 to 20 percent) of common carp with the optimum monosex tilapia stocking density; increase the size of the harvested fish through use of supplemental rice bran at several portions (from 0 percent to 6 percent body weight/day); repeat the above tests for different water quality levels (in ponds receiving influent equivalent to third-level to fifth-level treated wastewater). They further advised that more attention be given to measuring natural food within the ponds: the phytoplankton, zooplankton, and benthos (21).

They also noted that tests for Standard Plate Count and Most Probable Number should continue to be performed on the digestive tract, peritoneal fluid, and muscle of the cultured specimens. Because only a few pathogens were recovered from fish muscle, it was recommended that sample sizes be substantially increased and that additional bacteria (e.g., *Campylobacter jejuni* and various vibrios) be investigated. Initially, serological examinations of fish sera would be performed (versus direct virus isolation) to investigate the presence of viruses. In addition, the panel recommended that fillets of processed fish be tested for the presence of *Clostridium botulinium* and *Staphylococci* toxins (21).

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