Effectiveness of a commercial probiotic for water and sludge management on an inland shrimp aquaculture farm in Thailand

by

Michele-Lee Moore
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ABSTRACT

Shrimp aquaculture, particularly the culture of *Penaeus monodon* (black-tiger shrimp), has expanded rapidly throughout Asia in recent decades. Thailand has emerged as the world's leading producer of black-tiger shrimp, placing it at the forefront of a competitive international market that currently gives high value to seafood products. In order to meet this intense demand, new technologies are continually being developed and new farms are being established in areas not previously used for aquaculture. One of Thailand's most recent innovations includes low-salinity shrimp farming, which allows these farms to extend into freshwater areas, and as a result new environmental concerns regarding the industry have arisen. One of the critical issues for shrimp aquaculturists today involves the management of wastewater and the large volume of organic sludge being created within the ponds during rearing periods and later being released into surrounding waterways. Commercially prepared microbial solutions (or probiotics) have been marketed as bioremediation tools for maintaining water quality and reducing the accumulation of organic material in pond sediments, despite a paucity of information available about their effectiveness.

The purpose of this study was to document the techniques of application of a probiotic (EM-1) by a shrimp farmer and investigate the additive's efficacy in improving water quality and minimizing the output of organic sludge. Unfortunately, the manufacturer's application protocol was not followed and possibly, as a result, no significant differences were found for *in situ* water quality variables, biological oxygen demand (BOD), and total percent organic matter, and thus, the effluents released through the crop cycle and for the final harvest were not improved by the probiotic treatment. Final measures of BOD exceeded

Thai government standards (<10 mg/L) in all of the ponds. The results indicate that probiotics are not currently an effective management tool for inland shrimp farmers, however, the lack of success in the treatment ponds was mainly due to the application methods adopted by the farmer. While future research needs to explore the possibilities of different combinations of bacteria or different quantities of probiotics in the treatment of ponds, efforts also need to focus on the development of education and training programs for growers utilizing probiotics to ensure the success of this low-cost waste management tool.

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1. INTRODUCTION

1.1: Nature of the problem

Aquaculture is defined by the Food and Agriculture Organization (FAO, 2000; pg. 3) as "the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants" where farming implies firstly, that humans have manipulated the rearing process in order to enhance production through regular stocking, feeding, or protection from predators, and secondly, that the stock being cultivated is owned by either an individual or a corporation. While the intensive operations that are recognized as aquaculture systems today have not been in existence for more than a few decades, the practice of culturing species is thousands of years old, with records dating back more than 2500 years ago (Landau, 1992). Although aquaculture has provided seafood in the past, capture fisheries have prevailed as the world's predominant supplier. In 1989, the worlds' capture fisheries collected approximately 99 million metric tonnes of aquatic species (FAO, 1991). Declining wild stocks, which has been attributed by many analysts to overfishing, reduced catches to 92 million metric tonnes by 1999 (FAO, 2001b). The decreasing supply of capture fisheries worldwide, combined with advances in aquaculture technology that led to more intensive operations, has resulted in the aquaculture industry experiencing explosive growth in recent decades. Between 1991 and 1999 the quantity of aquatic organisms produced by aquaculture nearly tripled from more than 13 million metric tonnes to greater than 33 million metric tonnes (FAO, 2001a).

One of the most important species on the global market that is produced by aquaculture is *Penaeus monodon* (Fabricius), (black-tiger shrimp) (Bhaskar *et al.*, 1998; Csavas, 1993) with culture production exceeding 575 thousand metric tonnes (FAO, 2001a). Only a few decades ago, consumers in most developed countries rarely ate black-tiger shrimp. However, in the 1980's seafood distributors began marketing frozen shrimp at grocery stores and restaurants— particularly "jumbo" or black-tiger shrimp (Tibbetts, 2001). At the same time, consumer awareness was increasing regarding the need to change health-risk diets and they sought healthy alternatives to standard products (Tibbetts, 2001). The successful marketing of shrimp coincided with the realization that wild shrimp stocks were being rapidly depleted around the world. This combination ultimately increased pressure on the aquaculture sector to fill in the anticipated gap between demand and supply. At the same time many developed countries were embracing the nutritional values of high-protein seafood. As a result, the farming of *P. monodon* began to expand rapidly and spearheaded a lucrative transition in which the poorer countries of the Southern Hemisphere became the primary producers of internationally traded seafood.

Even with the swift, large-scale adoption of shrimp farming in a growing number of tropical developing countries, the world demand for shrimp began to exceed supplies. This prompted shrimp farmers to intensify their methods and increase the density of their stocks, thereby improving overall production efficiency. Larval hatcheries and artificial feeds were among the technologies developed that enabled shrimp farming to remain a profitable business. Since the majority of countries involved in shrimp aquaculture are typically less developed countries, the economies of these regions receive a much needed boost when their shrimp products are supplied to the international market which currently places high value on seafood. In Asia, many countries were encouraged by the fact that international financial organizations such as the World Bank and the Asian Development Bank were offering support, and ponds were developed (whether practical or not) in a variety of habitats including salt pans, rice paddies, sugar fields, agricultural land, and coastal mangrove forests (Patmasiriwat *et al.*, 1998; Phillips *et al.*, 1993). Owing to the rapid expansion and intensification of these farms, and the vast improvements the shrimp commodities brought to local economies, very few regulations or controls were enforced in any aspect of the industry (Csavas, 1993). Unfortunately, knowledge regarding how the farms should be managed was limited due to the lack of experience in this newly emerging industry; hence, social and environmental problems ensued (see for example: Bailey, 1988). The development of farms was particularly quick along the coastlines of Thailand and the industry here was especially culpable in embracing growth with little information regarding its impacts. However, awareness was finally raised in Thailand regarding the environmental impacts of shrimp farming when farmers building ponds along the coastline destroyed approximately 16-32% of the total mangrove area of the country and complaints from various NGO's and researchers were lodged (Dierberg and Kiattisimkul, 1996).

Many of the environmental issues within aquaculture are similar to those in its terrestrial counterpart, agriculture. Many farmers add fertilizers and various chemicals that promote the growth of shrimp and prevent disease (Phillips *et al.*, 1993), neutralize waters (Boyd and Massaut, 1999) and improve the growth of oxygen-producing phytoplankton (Paez-Osuna, 2001). In an attempt to maximize profits, farmers also tend to overstock the shrimp and overfeed with artificially-derived nutrition pellets (Flaherty and Vandergeest, 1998). Unfortunately, the large quantities of additives and feed exceed the requirements of the shrimp, which results in the surplus sinking to the bottom of the pond and accumulating throughout the grow-out period (Phillips *et al.*, 1993). This accumulation— in addition to the inevitable production of shrimp feces and the molting of shrimp exoskeletons— leads to a considerable build-up of organic material, known as sludge.

To ensure healthy environments for current and future crops, farmers began to exchange water during the cycles with outside sources, and disposing of accumulated sludge after the crop harvest (Dierberg and Kiattisimkul, 1996). Using high-pressure hoses, the polluted water and sludge are released into adjacent waterways (Flaherty and Karnjanakesorn, 1995). With numerous farms and intensive rearing practices, the receiving waters for shrimp ponds (which are typically at the mercy of several other sources of pollution as well, e.g. industry, agriculture) become even more severely degraded. In this manner a cycle has been created within the shrimp industry whereby water quality is further degraded by the very practices that are promoted to improve the state of the resource. The shrimp farmers, however, focus on the value of their crops and do not always have the financial luxury of being concerned with the ramifications that such practices may have in the future.

Due to the contamination in coastal areas and the lack of remaining suitable sites many scientists felt the industry within Thailand had peaked in the mid-1990s and would then decline (e.g. Dierberg and Kiattisimkul, 1996). However, the problems in the coastal regions coincided with an increased level of competition in rice production from Vietnam, India, Bangladesh, and Pakistan (Flaherty *et al.*, 1999). Shrimp farmers began to experiment with growing *P. monodon* at lower salinities, allowing the industry to expand further inland to freshwater areas. With the movement of the shrimp ponds from coastal areas to inland regions, a host of new problems has emerged including the salinization of freshwater and rice paddy areas (Braaten and Flaherty, 2001), the possible contamination of inland water canals (e.g. Corea *et al.*, 1995), and the potential of human health problems that arise from the use of poorly sanitized water (e.g. Wu *et al.*, 1999).

Although the removal of sludge has been recognized as a problem for the ecosystems surrounding the ponds, little research has been completed on methods for minimizing the quantity and improving the quality of effluent from shrimp aquaculture ponds. The Thailand Department of Fisheries did announce in 1991 that it was forbidden to drain saltwater into public freshwater systems or farming areas (Flaherty *et al.*, 1999). However, no single well-developed regime governing aquaculture and ensuing development exists, since land, water, environmental, and fish and game laws all affect shrimp culture and all have conflicting objectives (Flaherty *et al.*, 1999). The ineffectiveness of enforcement agencies in protecting the health of ecosystems surrounding aquaculture areas, including the people living within them, is mainly due to a bureaucratic tangle of contradictory goals regarding the need for expansion for exporting versus ensuring long-term stability, which results in virtually no monitoring taking place and numerous violations (Bailey, 1998; Dierberg and Kiattisimkul, 1996). The mis-management of aquatic resources is a global concern— particularly in regions dependent on agriculture and aquaculture. Finding an affordable solution that lessens the organic load and the resultant deterioration of water quality is critical for the long-term sustainability of shrimp aquaculture, and for the entire population that depends on the water that is being degraded. Concerns arise involving both the water quality and the health of the shrimp produced in the degraded environments. Diminished supplies and poor water quality have been inextricably linked to human health (e.g. Wu *et al.*, 1999), to socio-economic disruption (e.g. Postel, 1996), and to further ecosystem damage (e.g. Dierberg and Kiattisimkul., 1996).

Some scientists have developed models that suggest potential solutions for the reduction of sludge, including: lower stocking densities, sedimentation ponds to settle suspended sediments, optimization of feeding strategies, and biofiltration methods using bivalves to filter particulate matter (e.g. Paez-Osuna, 2001; Nunes and Parsons, 1998; Thongrak *et al.*, 1997; Kinne *et al.*, 2001). Studies testing these strategies often yield inconsistent results however. Since the implementation of new rearing techniques typically requires financial investment, success must be guaranteed before farmers will consider it a worthwhile venture.

One solution developed recently involves a type of biotechnology called bioremediation or probiotics. Probiotics can be defined as solutions of live microorganisms that may benefit a "host" by modifying the microbial communities within or surrounding the host, thereby improving the overall quality of its environment (Verschuere *et al.*, 2000). Advocates of the use of bacterial amendments claim that the rate of organic matter degradation is enhanced, levels of Dissolved Oxygen are increased, nitrite, ammonia, and carbon dioxide are decreased, the amount of blue-green algae is reduced, and "off-flavouring" of the shrimp is prevented (Boyd, 1990). However, little research has been done to test microbial products and the effectiveness of the probiotics in improving water quality or reducing organic matter and biological oxygen demand during a crop rearing cycle. Moreover, no investigation has been undertaken to assess the effectiveness of the probiotic application methods of small-scale Thai shrimp farmers. It is not yet clear how widespread the practice of applying probiotics is, but if proven effective, the microbial applications could be an economical means by which farmers can reduce the organic load entering nearby waterways. The handling of sludge produced by aquaculture is becoming increasingly important as the industry continues to grow, since a large number of farms will only result in a larger volume of sludge being generated and invariably, a poorer aquatic environment. Deteriorating water quality is not only a concern from an environmental conservation standpoint, but also in terms of the impacts the poor conditions may have on aquaculture production within the ponds. Investigating the efficacy of a microbial additive would evaluate the usefulness of this mode of biotechnology application for conservation as it would assess the effectiveness of reducing the amount of sludge that is produced in a shrimp cycle and improving overall pond water quality.

1.2: Purpose of Study

This research focuses on water and wastewater management pertaining to inland shrimp aquaculture in rural Thailand. The purpose of the project is to investigate the efficacy of probiotic technology in reducing the production of organic wastes and biological oxygen demand in shrimp farm effluent. The specific objectives are:

- To review the potential impacts of intensive, inland, low-salinity shrimp aquaculture on the health of the natural environment and the possible implications for human health;
- To document the pond water quality and the total organic content of the sludge on a typical inland shrimp farm;
- To investigate the effectiveness of a commercial probiotic (EM-1) for improving pond water quality and decreasing the organic content of the sludge;
- To document the probiotic application techniques of a small-scale shrimp farmer and evaluate the effectiveness of the bioremediation method, which could allow small-scale Thai shrimp farmers to reduce the impact of their activities on the organic load and water quality.

Using water quality parameters as well as the measurements of BOD and total percent organics as indicators of organic load, this research assesses whether the commercial microbial culture improves the general pond water quality and reduces the accumulation of sludge in a typical inland shrimp farm. The data obtained on the effectiveness of the microbial product provide essential information by which effluent quality can be improved.

1.3: Outline of Thesis

This thesis has been organized into six chapters. Chapter 2 reviews the development of shrimp aquaculture and documents both the environmental impacts of this industry and the health issues surrounding aquaculture. The concept of bioremediation in aquaculture with probiotics is also described, including highlighting the benefits this biotechnology may have on the health of entire ecosystems (humans included). Finally, one particular commercial product (EM-Effective Microorganisms) is introduced. Chapter 3 describes the field site and outlines the methodologies utilized in the application of EM and the water and sludge sampling. Chapter 4 presents the results and analysis of the data collected, while Chapter 5 discusses the implications of these findings, both with respect to the objectives of this project and on a broader scale. Chapter 6 summarizes the goals and major findings of the research conducted, describes how these results may assist resource managers in shrimp aquaculture and provides recommendations for future investigations.

2. BACKGROUND

Shrimp aquaculture has evolved to utilize more intensive inland farming techniques, which has led to the deterioration of water quality and an overuse of chemicals to protect the growth of crops in most shrimp producing regions. This chapter discusses this evolution, firstly in the context of aquaculture worldwide, and secondly with particular reference to shrimp culture in Thailand. Literature regarding the impacts that shrimp farming practices have on the natural environment and human health, is then reviewed. The concept of probiotics/microbial additives, and their use as a water and wastewater management tool is then introduced. The gaps in aquaculture and probiotic research conducted to date are identified, which leads to the present research.

2.1: Shrimp aquaculture development

Often referred to as the founder of fish farming, Wen Fang was one of the first people to build ponds and keep records of fish growth and behaviour during the Shang Dynasty in China in 1135 B.P. (Landau, 1992). Other historic accounts of aquaculture development include laws that were passed in the Indo-Pacific region to protect fish farmers from thieves approximately 3500 years ago (Iversen, 1976). While the complete control over entire rearing cycles in aquaculture was slow to develop for most species, the culturing of carp proved to be one exception (Barnabé, 1990). One key player in this development was Fan Li who wrote about his carp culture practices in the Treatise on Fish-breeding, dated back to 475 B.P. (Landau, 1992). Aquaculture initially spread throughout Asia and Europe and today involves four major types of organisms: molluscs, crustaceans, fish and algae (Barnabé, 1990). Technological developments in the industry have progressed significantly in the past four decades, and the volume of aquatic species raised for food has grown considerably in the past 15 years. In 1987, 10 635 187 mt (metric tonnes) of foodfish was produced by aquaculture throughout the world (FAO, 1998). By 1996 the amount of foodfish produced globally through aquaculture had more than doubled to 26 384 583 mt (FAO, 1998). Today, roughly 9 out of every 10 oysters and the same ratio of Atlantic salmon, 4 out of every 5 mussels, 3 out of 4 scallops and 1 out of every 4 shrimp consumed are the product of aquaculture (New, 1999). Although consumption of farmed foodfish is occurring worldwide, the majority of species are cultured in tropical and semi-tropical regions, with Asia producing nearly 89% of the world's total by weight (New, 1999).

One of the most lucrative food species produced by aquaculture is the black-tiger shrimp (*Penaeus monodon*). Of the world's total aquaculture production in 1998, 575 842 mt were cultured *P. monodon*, with a value exceeding US$3.6 billion, a higher value than any other single cultured species (FAO, 2001a). Environmental degradation and disease outbreaks, however, have resulted in many countries experiencing 'boom and bust' cycles in shrimp aquaculture and non-uniform success in the industry (Szuster and Flaherty, 2002). Thailand has emerged and maintained its position as the world's largest single producer of farmed shrimp for more than ten years, producing 39% of the total cultured black-tiger shrimp (FAO, 2001a). Thailand's Department of Fisheries Marine Shrimp Culture Research Institute (2002) claims that more than 54 countries are now importing Thailand's cultured shrimp (Table 2.1).

The majority of black-tiger shrimp producers within Thailand today are small-scale and use intensive practices--a result of evolving culture techniques that began with large, traditional and extensive open systems which progressively lead to closed intensive farms, and finally to inland, low-salinity, intensive operations (Figure 2.1).

Table 2.1 List of Thailand's shrimp importers (Department of Fisheries Marine Shrimp Culture Research Institute, 2002).

Continent	Country
North America	Canada Haiti Jamaica Panama United States of America
South America	Puerto Rico
Asia	Brunei Cambodia Cyprus Hong Kong India Indonesia Israel Japan Laos Lebanon Malaysia Maldives Myanmar People's Republic of China Philippines Democratic People's Republic of Korea Saudi Arabia Singapore Taiwan
Australasia	Australia New Zealand Papua New Guinea
Europe	Belgium Croatia Denmark Finland France Germany Hungary Italy Netherlands Norway Poland Romania Russian Federation Slovenia Spain Sweden Switzerland United Kingdom
Africa	Kenya Mauritius South Africa
Islands	French Polynesia Guam New Caledonia Turks & Caicos Islands

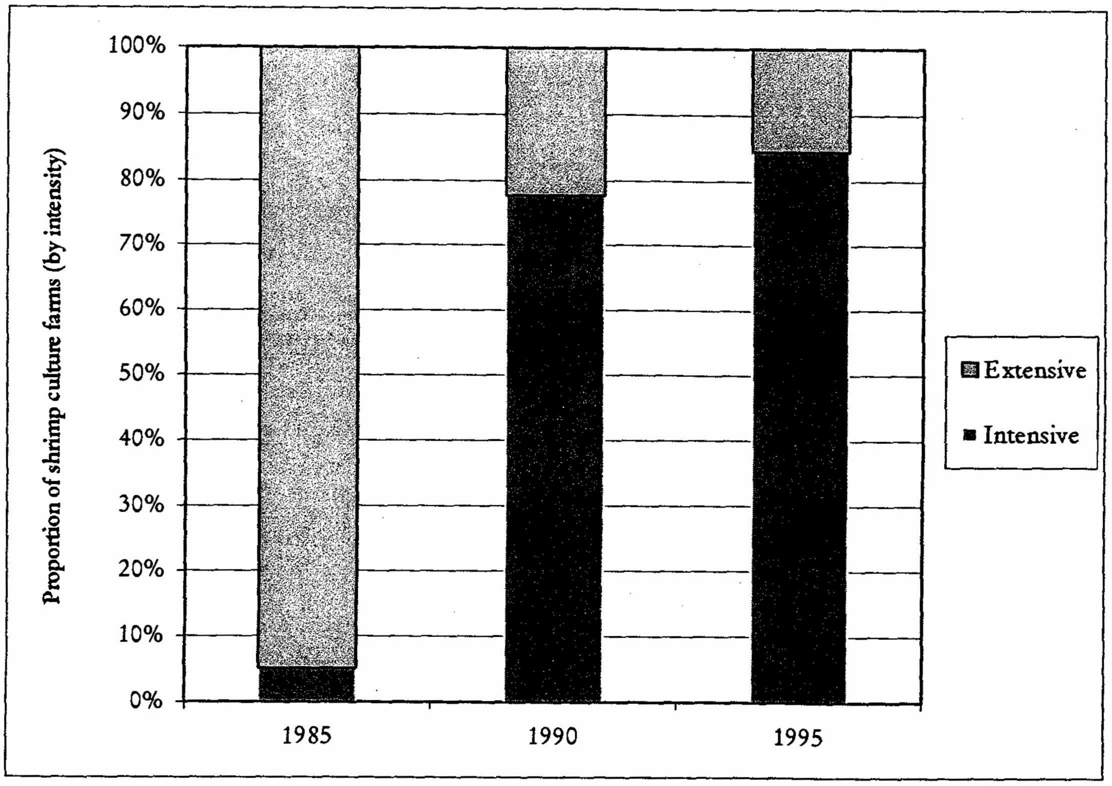


Figure 2.1 The proportion of extensive and intensive shrimp farms in Thailand from 1985-1995 (Modified from: Sirirattrakul, 2000).

2.1.1: Traditional and extensive shrimp farms

Traditional shrimp culture ponds were large, ranging from a few hectares to several hundred, and were open to the sea with no gates to control the flow of the water (Patmasiriwat *et al.*, 1998). Later, alterations were made when farmers constructed dikes to create enclosures that trapped naturally growing juvenile shrimp and nutrients (Patmasiriwat *et al.*, 1998). This development produced a type of farming known as extensive culture, which initially emerged on the coast where seawater flooded the low-lying areas through tidal action, thereby providing adequate water exchange (Dierberg and Kiattisimkul, 1996). Mangrove swamps originally provided the most preferred locations for pond construction since these areas often had gentle slopes, adequate tidal range, and abundant wild shrimp "seed", and they typically provided local communities free access to government land

(Menasveta, 1997). Mangrove ecosystems have also provided breeding grounds and nursery areas for fry and growing larvae of many commercially important finfish, crustaceans and molluscs (Phillips *et al.*, 1993), which allowed farmers to practice polyculture in these areas and achieve greater yields with certain combinations of fish and other species (Landau, 1992).

By the 1970's, the market demand for various shrimp began to exceed the supply and the Thai government began to promote the conversion of the extensive ponds more to intensive monoculture brackish water farms (Patmasiriwat *et al.*, 1998). *Penaeus monodon* were used almost exclusively at this point, due to desirable features such as a rapid growth rate to a large size, low mortality rate, and lack of cannibalism (which occurred in some other species) (Flaherty and Vandergeest, 1998), which maximized profit earning potential.

2.1.2: Intensive shrimp farms

Facilitated by rapid developments in technology such as artificial feeds, fertilizers and hatcheries, Taiwan was the first country in Asia to transform extensive shrimp farms into intensive farms in the 1980's (Patmasiriwat *et al.*, 1998; Phillips *et al.*, 1993). Intensive ponds required greater initial investment for the construction of mud ponds, control structures such as gates, water supply and drainage channels, and aerating devices (Patmasiriwat *et al.*, 1998; Flaherty and Karnjanakesorn, 1995). One of the biggest changes was in the development of the nursery stage--where shrimp stock from hatcheries replaced the use of wild fry. In response to the latest innovation in the industry, Thailand's Department of Fisheries built its own hatchery to provide young fry to small-scale farmers, thereby further encouraging the industry to expand (Patmasiriwat *et al.*, 1998; Flaherty and Karnjanakesorn, 1995). Intensive production systems also depended on high stocking densities, specially formulated feed pellets, strict water management and the maintenance of healthy stock, all of which stimulated shrimp growth (Patmasiriwat *et al.*, 1998). Some intensive farms were established on a broad commercial scale but most were owned by small-scale producers operating ponds simultaneously, each ranging from 0.16-1.0 ha (found to be the optimal size for efficient farm management, and lower overhead and investment costs) (Sasson, 2000; Kongkeo, 1997; Csavas, 1993).

The technological developments in Asia's shrimp culture industry coincided with Thailand's ratification of the 200 mile exclusive economic zone, which resulted in the loss of approximately half of the previous fishing grounds since Thai boats could no longer fish beyond the territorial sea limits (Menasveta, 1992). Driven by the limitation of fishing sites, and the high costs associated with limited coastal land availability, Thailand soon followed Taiwan's lead to convert to intensive culture (Patmasiriwat *et al.*, 1998). Despite an overall reduction in farm and pond area, the changes in the farming practices enabled production to increase 20-fold between 1977 and 1992 (Dierberg and Kiattisimkul, 1996).

The Taiwanese shrimp industry crashed in 1987-90, mainly due to poor water quality conditions and outbreaks of disease (Patmasiriwat *et al.*, 1998). The reduced production of shrimp resulting from this crash created a rise in world prices sustained by the consuming nations (Japan, U.S., and Western Europe), and ultimately, provided Thailand with an opportunity to increase shrimp production rapidly for major financial gain (Patmasiriwat *et al.*, 1998, Flaherty and Karnjanakesorn, 1995). Japanese investors urged Thailand to produce *P. monodon* for continuous year round supply, moving their capital investments from Taiwan into the the Upper Gulf of Thailand (Kongkeo, 1994). Steady support from the government allowed for research and development for technologies appropriate to the Upper Gulf of Thailand and provided a boost to production in the form of loans, infrastructures, and marketing (Sasson, 2000). Inspired by Thailand's success, other countries created similar plans to encourage the continued development of the shrimp farming industry (Dierberg and Kiattisimkul, 1996). Similarly to Taiwan, however, Thailand and numerous other countries also eventually experienced crashes in their production cycles, mainly due to disease outbreaks (Patmasiriwat *et al.*, 1998). However, with Thailand's large size and land availability, the shrimp industry was able to move from the northern Inner Gulf, to the East, the South, and across the Gulf to the Andaman Sea (Patmasiriwat *et al.*, 1998). Migration, the continuous progression in farming methods, "seed" supply and technology, and support from the government all enabled Thailand to increase production despite localized crashes (Patmasiriwat *et al.*, 1998). The movement of farms along the coastline, however, often involved the conversion of mangrove areas to shrimp ponds. Until that point, the mangrove areas were owned by the Thai government and had little commercial value. The Thai Royal Forestry Department granted concessions for locals to utilize the mangroves for subsistence, providing fuelwood, building materials, charcoal, and other household needs (Bailey, 1998; Flaherty and Vandergeest, 1998). As coastal areas became more valuable due to the profitability in shrimp farming and the extent of land required for extensive culture systems escalated, the people in the coastal communities often ended up excluded from areas to which they previously had access (Bailey, 1998). In addition to being displaced, the coastal communities who were used to the diversity and productivity of the resources in the tropical coastal zone also had to adjust to the shrimp monocultures being introduced (Bailey, 1988). Essentially, aquaculture transformed indigenous mangrove and coastal habitats from a multi-use/multi-user resource to a privately owned, single purpose resource (Bailey, 1988). The coastal residents lost mangrove products and suffered from declining fish catches of species that were previously associated with the mangroves (Phillips *et al.*, 1993; Bailey, 1988).

The trend towards greater intensification and expansion of aquaculture has not been embraced worldwide due to concerns about environmental impacts (Phillips *et al.*, 1993). Many countries in South America and Asia still produce shrimp from extensive and semi-intensive systems with reduced stocking densities and levels of feeding (Phillips *et al.*, 1993)(see Table 2.2). For example, in India the government policy supports the development of semi-intensive methods rather than capital intensive techniques (Phillips *et al.*, 1993), while Brazil has refrained from the use of chemicals to treat diseases in shrimp ponds (Thapanachai, 2003).

Table 2.2. A comparison of the management practices of extensive, semi-intensive and intensive shrimp aquaculture farms (based on Patmasiriwat *et al.*, 1992; MacIntosh and Phillips, 1992).

	Extensive	Semi-Intensive	Intensive
Pond Size	8-16 ha	3-5 ha	1 ha or smaller
Stocking Density	$<10/m^2$	$10\text{-}20/m^2$	$>50/m^2$
Water Management	Tidal	Daily water exchange, with pumps	Closed, with some water exchange with pumps
Fry sources	Wild	Wild or hatchery	Hatchery
Feed	Natural	Supplemented with dry or wet feed	Artificial

2.1.3: *Inland, low salinity intensive shrimp farms*

Initially, due to the marine nature of the *Penaeus* spp., shrimp farms were limited to areas where saline water was available--that is, coastal zones. The water quality along the coast eventually began to deteriorate, mainly due to farm intensities and poor farm management (Flaherty and Vandergeest, 1998). Thus, many people believed that the industry had peaked and with few sites left to exploit, would quickly decline in productivity (e.g. Dierberg and Kiattisimkul, 1996). However, the emergence of a new technique--one that involved low-salinity culture methods--allowed farmers to establish further inland than ever before (Flaherty and Vandergeest, 1998).

The literature available to-date suggests that Thailand in fact, is the only country to have developed intensive *P. monodon* ponds in inland areas (Kongkeo, 1997). A survey by Flaherty and Vandergeest (1998) in 1996 and 1997 showed that inland shrimp farmers were culturing shrimp in salinity levels between 5 and 15 ppt (parts per thousand), significantly lower than the 15-45 ppt range on coastal farms. Producers operate with little water exchange, both for the purpose of protecting against external sources of disease or pollution, as well as for the maintenance of salinity levels (Flaherty and Vandergeest, 1998). Farmers may bring in truckloads of salt water or bags of salt crystals to add to ponds at the beginning of a grow-out period (Flaherty and Vandergeest, 1998). Alternatively, saltwater that intrudes into farm areas during the dry season (causing brackish conditions) may be stored in reservoirs and the salinity becomes concentrated through evaporation.

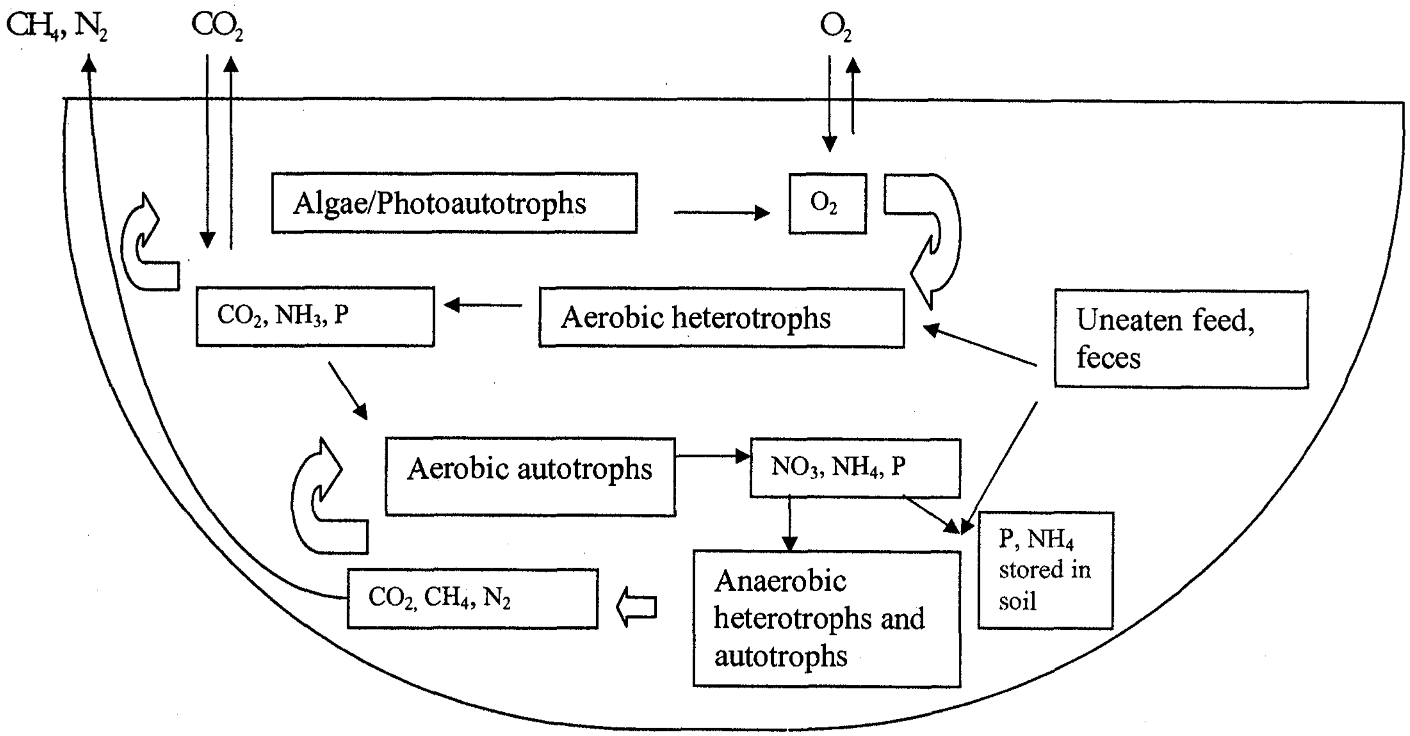
Low-salinity culture was developed purely through the serendipitous experimentation of small-scale farmers (Flaherty *et al.*, 1999). In this sense, shrimp farming has helped to develop and foster respect for the resourcefulness of farmers and has represented a small step of success. Speed and openness are key characteristics of Thailand's economy--every opportunity is welcomed and the learning process is accepted as being instantaneous (Phongpaichit and Baker, 1996). For this reason, the industry has survived and will likely continue to adapt with new practices.

2.1.4: Environmental impacts: the creation of organic-laden effluent

In order to maximize crop sizes and profits, aquaculturists commonly use high stocking densities, excessive quantities of artificially-derived nutrition pellets for feed, fertilizers to promote the growth of oxygen producing phytoplankton, and antibiotics and pesticides to control diseases (Flaherty and Vandergeest, 1998). Such unnatural inputs can lead to considerable amounts of material that is mostly organic accumulating in the pond sediments, a buildup known as sludge (Phillips *et al.*, 1993). Dead phytoplankton, uneaten feed, and feces are decomposed by naturally occurring heterotrophic microorganisms – that is, microbes that break down organic materials (Boyd and Tucker, 1998; Atlas and Bartha, 1998). These microbes convert organic matter into basic molecules such as carbon dioxide, water, ammonia and phosphate (Figure 2.2) (Boyd and Tucker, 1998). During the decomposition process, microbes consume Dissolved Oxygen, creating a biological oxygen demand (BOD) in the pond system and depriving the shrimp of the oxygen required for growth.

Pond soil pores, entirely saturated with water, are unable to take up Dissolved Oxygen (Boyd, 1995), causing pond soils to be oxygen-limited environments. Since Dissolved Oxygen concentrations are already low in a pond bottom (compared to the water surface where gas exchange can readily occur with the surrounding atmosphere), bacterial growth is limited. With limited bacteria available to decompose organics, an accumulation of particles will occur, especially when the rate of organic input is higher than the rate of microbial decomposition (Gaudy Jr. and Gaudy, 1980). The few bacteria that are present will use up any available oxygen, typically leading to anoxic conditions. Anoxic conditions kill most aquatic organisms (Boyd, 1990), and the decomposition of these organisms creates an additional oxygen demand (Atlas and Bartha, 1998). As a result, oxygen-limited shrimp ponds often lead to the demise of aerobic bacterial communities and, perhaps more importantly to farmers, of commercial shrimp crops.

To ensure pond water quality from crop to crop, the sludge that accumulates on the pond bottom is often removed after one or more rearing cycles (Dierberg and Kiattisimkul, 1996). For most grow-out ponds, the water, including the large loads of organic waste, is released directly to adjacent waterbodies (Flaherty *et al.*, 2000). Many farmers in Thailand clean their ponds using high pressure hoses that scour out the bottom (Flaherty and Karnjanakesorn, 1995). Although the practice of "flushing" of shrimp pond sediment is illegal in Thailand, the failure to control this form of environmental degradation relates to several socio-economic factors including: lack of funds for enforcement, lack of intergovernmental agency cooperation, and limited compliance by small-scale farmers (Flaherty and Karnjanakesorn, 1995). The tendency to utilize this disposal method is exacerbated by the fact that the pesticide and antibiotic residues, large volume, and high salt content of these sediments renders them unsuitable for fertilizers, and therefore proffers little economic value (Dierberg and Kiattisimkul, 1996).



Note: Aerobic heterotrophic microorganisms oxidize organic matter to carbon dioxide, ammonia and phosphorus using O_2. Algae (photoautotrophs) fix CO_2 into organic matter to produce O_2 or to react with any lime (calcium carbonate) to contribute to the alkalinity of the system. Ammonia is nitrified to nitrate by aerobic autotrophs (organisms that break down inorganic matter) and ammonium and phosphate are adsorbed onto soil particles to be stored in the sediment. Anaerobic heterotrophs ferment organic matter in low oxygen conditions to produce carbon dioxide, methane and gaseous nitrogen which may be lost in the atmosphere or stored in the sludge layer creating anoxic conditions (Modified from Boyd and Tucker, 1998; Gaudy Jr. and Gaudy, 1980).

Figure 2.2 The fate of organic matter and nutrients in a typical aquaculture pond.

Most shrimp farmers today use what is referred to as a "closed system", where little water exchange is required from external sources during the grow-out period of the shrimp, thus reducing the introduction of viruses and other toxic organisms (Kongkeo, 1997). As a result, food, fecal, and chemical products become a problem for the surrounding environment only at harvest when the pond is drained (Beveridge *et al.*, 1994; Beveridge and Phillips, 1993). Due to the increased intensity of farming, however, that final pulse is extremely high in organics and chemicals and presumably is equally, if not more, detrimental than farms that exchange water throughout the crop rearing cycle (Dierberg and Kiattisimkul, 1996).

With the disposal of sediments directly into adjacent waterbodies, a marked increase in phosphorus, ammonia, BOD (biological oxygen demand), COD (chemical oxygen demand) and settleable solids occurs immediately following the harvest (Beveridge and Phillips, 1993). The nutrient enrichment from the organic load can cause plankton blooms and eutrophication in the receiving bodies of water (Tookwinas, 1996). When a farmer degrades the very water that will later be pumped back into the ponds for another crop of shrimp, a situation is created known as "self-pollution". The reality remains, however, that the initiative of one farmer to develop a farm with less environmental impact does not necessarily benefit that farmer unless that style of farm management is widely adopted in the entire area (Thongrak *et al.*, 1997).

When shrimp farms originally began to be moved inland, the Thai government and many NGO's believed the move meant that pressure was being relieved from the remaining mangrove sites (Flaherty *et al.*, 1999). However, while the movement provided a solution to one problem, it created an entirely new set of issues involving the impacts of intensive culture in inland areas. While degraded water quality is common in all shrimp farming areas, the situation is especially problematic in inland regions where the small waterbodies--such as canals--that receive organic-laden effluent have a lower assimilative capacity than large bodies of water, such as in coastal areas (Flaherty *et al.*, 2000; Corea *et al.*, 1995). Another concern regarding shrimp farms in freshwater areas is the seepage of brackish pond water into nearby orchards or rice paddies (Flaherty *et al.*, 2000). The seepage or disposal of saline effluents into freshwater areas may also indirectly cause soil salinization (Braaten and Flaherty, 2001).

In response to the copious amount of research that revealed the extent of degradation of the environment by shrimp aquaculture (whether in coastal or inland regions), the government produced a list of stringent regulations. Farmers engaged in shrimp farming operations covering more than 8 ha are required to be registered and licensed, and to comply with the following restrictions: discharge waters must not exceed a 5-day BOD of 10 mg/L, pond sludge may not be released into natural water sources or public areas, saltwater must not be discharged into public freshwaters, and farmers must utilize an effluent treatment pond with an area that occupies no less than 10% of the culture area (Dierberg and Kiattisimkul, 1996). Herein lies the problem. Most farms in Thailand are small-scale (under 8 ha) and since they are not required to register, they are not monitored for their management regimes.

2.1.5: Monitoring water quality and organic effluent

Some analysts suggest that when assessing the impacts of wastes it is more important to focus on the protection of the receiving waters rather than the effluent quality (Beveridge and Phillips, 1993). However, logistics require that in order to minimize impacts, attention must be paid to the quality of the effluent. The key management variables affecting the quality and quantity of the effluent produced are stocking rate, feeding rate, and water management— practices that are all at the farmers' discretion.

The BOD test prescribed by the Thai government is commonly used in wastewater and water quality management in order to determine the depletion of oxygen by microorganisms growing in the water (Mitchell, 1974). The BOD measurements assess the rate of organic degradation based on the oxygen utilization in the water (Mitchell, 1974). Therefore, the BOD test is a useful indicator of the organic content of water and the level of water quality. In addition to understanding the level of organic accumulation in the water column, it is also important to analyze the organic content of the pond sediments. The dry-ash method measures the organic matter concentration in sludge samples. Although other techniques exist for analyzing organic content of soils, research has determined that all methods produce similar results and that the dry-ash method is the most suitable for aquaculture applications (Boyd, 1995a).

Other water quality parameters considered useful for monitoring the health and impacts of shrimp aquaculture ponds include *in situ* measurements such as Dissolved Oxygen, temperature, pH, salinity, conductivity, and Secchi depth. The presence of Dissolved Oxygen is fundamental in supporting aquatic life that require aerobic conditions, and therefore is the most widely used water quality parameter (Tchobanoglous and Schroeder, 1985). For fresh to brackish-water aquatic life, minimum Dissolved Oxygen should measure approximately 5.0 mg/L (Lamb, 1985).

Water temperature can affect the natural productivity of an aquatic ecosystem by directly influencing the level of solubility for Dissolved Oxygen, but may also affect all other water quality variables (Boyd and Tucker, 1998). Other parameters affected may include chemical and biological reaction rates, gas and mineral solubility, and growth and respiration rates of aquatic organisms, including microbial organisms (Moriarty, 1997; Tchobanoglous and Schroeder, 1985). Temperature may also indirectly affect disease outbreaks in aquaculture ponds since low Dissolved Oxygen concentrations--which are common in high water temperatures--may stress the shrimp and suppress immune system functioning, thereby making the shrimp more vulnerable to disease (Boyd and Tucker, 1998). Therefore, monitoring the distinct temperature ranges of the shrimp ponds can provide a crucial link in understanding the health of the system.

Many biological systems are strongly affected by pH ranges, which in turn are strongly affected by the organic ions in the water (Tchobanoglous and Schroeder, 1985). The pH value of a pond expresses the levels of acidity or alkalinity in the pond water by measuring the hydrogen ion concentration (Boyd and Tucker, 1998). Shrimp are particularly sensitive to extreme changes in pH and are best supported in levels of 7-9 (Boyd and Fast, 1992).

Salinity measures the total concentrations of all dissolved ions in the water, particularly calcium, magnesium, sodium, potassium, bicarbonate, chloride, and sulfate (Boyd and Tucker, 1998). Salinity may interact with other water quality variables as increased concentrations will decrease the solubility of gases, including Dissolved Oxygen (Boyd and Tucker, 1998).

Secchi disk measurements indicate the depth of visibility or the amount of turbidity in a water column. A high concentration of suspended solids in the ponds can interfere with the passage of light and surface oxygen causing a significant impact on the phytoplankton and microbes that depend on both of these conditions for survival and growth within the ponds (Lamb, 1985). If light is not able to penetrate into a pond because of high turbidity caused by suspended particles, only a small proportion of the pond will receive enough light for photosynthetic organisms and therefore, the pond will have low Dissolved Oxygen or an oxygen deficit (Boyd and Tucker, 1998; Moriarty, 1997). Recommendations for aquaculture ponds have stated that Secchi disk visibility should measure between 30-45 cm (Boyd and Tucker, 1998).

2.1.6 Environmental impacts: the creation of chemical-laden effluent

The widespread use of chemicals in aquaculture, although a relatively new practice, has rapidly expanded through all areas of the industry. Examples of chemicals that are sold include products that claim to supplement the nutrients required to develop the exoskeleton (shell) of the shrimp after moulting, those that safeguard against diseases and improve the immune system of shrimp, and those that promote growth. Unfortunately, a system for prescribing or distributing chemotherapeutants does not exist. Consequently, growers may base their decisions about their applications on water temperature, culture conditions, site characteristics, or any other factor they deem appropriate (Weston, 1996). Knowledge of the chemical products is typically limited to the information provided on labels and poster advertisements. Feed and chemical companies also inundate these same farmers with promotions of new additives (Flaherty *et al.*, 1999). Thus, the potential for misapplication and overuse of chemicals is high for farmers who lack proper training and who fear the financial repercussions of a crop failure (Flaherty *et al.*, 1999; Weston, 1996).

Once applied, chemicals may leave the ponds through effluent, seepage through pond bottoms, and through the removal and disposal of sludge following harvests (Flaherty *et al.*, 2000). As a result, the contamination of soil, water, and other crops has become a grave concern, in addition to the adverse effects impacting human health (Flaherty *et al.*, 1999).

2.2: Human health issues within aquaculture

Typically, aquaculture products are marketed and consumed in countries that are distant from the nations in which they were produced. With the transnational distribution of shrimp products, and the fluid nature of water (which does not acknowledge political boundaries) the impacts of the aquaculture industry affect humans and the natural environment on a global scale. Some of the emerging issues are the health of the shrimp that humans are consuming and the socio-economic outcomes of trading a product on the international market from developing to developed countries (Weston, 1996). In order to understand the global and local repercussions of poor environmental management practices, the relationship between biophysical and socio-economic factors needs to be examined within the aquaculture industry, including the reliance of human health on the environment and the sustenance it provides.

2.2.1: *Implications of degraded water quality on human health*

Water delivers nutrients to the seas and their complex food webs, is critical to the survival of economically and culturally important fisheries and aquaculture practices, protects wetlands with their capacity to filter out pollutants, provides habitat for a rich diversity of aquatic life, maintains salt and sediment balances and offers natural beauty to the planet (Postel, 1996). When development plans fail to account for these values, the benefits and services of water are lost (Postel, 1996).

The deterioration of the quality of the world's most precious resource— water--has been a serious concern for several decades. Since waterways are naturally self-cleansing, they have been used for centuries as depositories for waste (de Villiers, 1999). The natural processes such as sedimentation, aeration, mixing, dilution and bacterial processing are effective at decomposing wastes and cycling nutrients back into the environment (de Villiers,

1999) and have therefore fooled humans into believing the capacity of water to cope with pollution is infinite. However, the ever-increasing human population growth has led to intensive use and multiple sources of anthropogenic-based pollutants, resulting in severe degradation of aquatic systems. Diminished supplies and poor water quality have been inextricably linked to human health concerns (e.g. Wu *et al.*, 1999), to socio-economic disruption (e.g. Postel, 1996), and to further ecosystem damage (e.g. Dierberg and Kiattisimkul., 1996).

Agriculture, aquaculture, industries and household sewage are the largest contributors to aquatic contamination, releasing copious amounts of organic waste each day. The World Health Organization (WHO) (1995) reported in 1995 that every eight seconds a child dies from a water-related disease. Each year more than 5 million human beings die from illnesses resulting from unsafe drinking water, unclean domestic environments, and inadequate excreta disposal and treatment facilities (WHO, 1995). The nutrient loading that occurs with the release of organic-laden wastes--the type of effluent that is discharged from shrimp aquaculture ponds--results in eutrophication, an ideal environment for toxic algal blooms to occur. Scientists have reported that a global increase in the frequency, magnitude and geographic extent of Harmful Algal Blooms (HABs) has occurred over the past two decades, leading to toxic and anoxic conditions (Henrickson *et al.*, 2001). The blooms provide suitable "culture media" for the spread of pathogenic organisms such as *Vibrio cholerae* (Rapport, 1999). Sediments may also act as reservoirs for certain pathogens, which is especially dangerous in areas where numerous disturbances may cause sediments and the embedded pathogens to be re-suspended in the water column (Henrickson *et al.*, 2001). An example of a disturbance in the case of shrimp aquaculture may include the fast-flowing, high volume of effluent pumped from the ponds during a harvest.

While water quality conditions are deteriorating on a global scale, the problematic effects that emerge from poor water quality tend to be more concentrated in developing countries, mainly due to the fact that this is where the majority of the world's population growth is occurring today (Saeijs and van Berkel, 1995). Socio-economic constraints in developing nations also limit the sanitation and water treatment facilities, which has led to the spread of waterborne infectious diseases to a much more severe degree than in developed nations.

Research has revealed that exposure to water inhabited by pathogenic organisms directly through bathing or drinking, and indirectly through seafood consumption increases the risk of innumerable diseases, illustrating the inextricable link between environmental and public health (e.g. Henrickson *et al.*, 2001; Lipp and Rose, 1997). Since as early as the 1960's, researchers in Bangladesh have reported an association between algal blooms and the presence of *V. cholerae* (Islam *et al.*, 1990). Other scientists have discussed that the pathogenic organisms inhabiting tropical aquatic systems include those that cause cholera, typhoid fever, Bacillary dysentery, amoebic dysentery and helminthiasis (worm diseases) (Windle-Taylor, 1978). Evidence linking poor water quality and health issues was presented by Esrey *et al.* in 1991 in a series of studies that found improved water supply and sanitation led to a significant decrease in morbidity due to diarrhea, ascariasis, dracunculiasis, schistosomiasis, and trachoma. One of the most thorough studies regarding the effects of water quality on human health was conducted by Lonergan and Vansickle (1991) who found that in addition to the quality of the water supply causing diarrheal illness in Malaysia, behaviour patterns were also strongly related to the incidence of diarrhea. Behaviours are typically based on social/cultural constructs and can affect the type of infrastructure designed for the supply of water and child and household hygiene practices (Lonergan and

Vansickle, 1991). Lonergan and Vansickle's (1991) findings are a perfect example of the complexities involved in solving health issues.

2.2.2: *Pesticide and antibiotic use in aquaculture and human health implications*

One effect of shrimp farmers utilizing poor-quality water is that the conditions are suitable for the proliferation of shrimp disease (Dierberg and Kiattisimkul, 1996). A high risk of disease exists caused by outbreaks of harmful viruses, bacteria, fungi and other pathogens (Graslund and Bengtsson, 2001). Shrimp aquaculturists have experienced problems with numerous viral diseases in the past decade including White spot disease, Yellowhead disease, monodon baculo virus disease and hepatopancreatis parvo virus disease (Graslund and Bengtsson, 2001; Jory, 2000). These infections cause mass mortalities among the shrimp crops, which lead to severe economic losses.

Some shrimp infections can cause illnesses in humans who ingest the organisms. Consequently, food safety monitoring has become an industry unto itself, particularly in developed countries in recent decades. Infections from shrimp can be passed to humans via three different modes. The first method is the ingested pathogen can colonize the gastrointestinal tract having adverse effects, such as *Salmonella* sp., or *E. coli* (Shapiro and Mercier, 1994). The second mode of infection occurs when bacteria travel from the gastrointestinal tract to other tissues (e.g. Hepatitis A virus) and a third type involves toxins that are released when pathogenic organisms multiply (E.g. *Vibrio* spp.) (Shapiro and Mercier, 1994).

In response to the risks posed by the innumerable outbreaks of infection, and as a measure to save their crops, shrimp farmers began to utilize chemicals such as pesticides to kill pathogenic organisms (Table 2.3). Troisi (2002) estimated that approximately 3 million tons of pesticides are used each year worldwide. Rather than using the chemicals merely for treatment purposes, aquaculturists quite often use the treatments prophylatically as a form of insurance and prevention (Brown, 1989). Frequently, a distinction is not made between the different chemicals and their purpose. For example, chlorine is used to disinfect the ponds to kill bacteria and viruses prior to the stocking of the pond with shrimp seed, but is also used as an algaecide, a herbicide, or to regulate the pH of the pond water (Graslund and Bengtsson, 2001).

Table 2.3 Chemicals used in Southeast Asian shrimp farming as documented in multiple research studies (Modified from: Graslund and Bengtsson, 2001).

Azinophos-ethyl	(Richardson, 1992)
Benzalkonium chloride	(Vijayakumaran, 1997)
Calcium carbonate	(Primavera, 1993)
Calcium hydroxide	(Primavera *et al.*, 1993)
Calcium hypochlorite	(Funge-Smith and Briggs, 1998)
Calcium oxide	(Boyd, 1995b)
Calcium sulfate	(Richardson, 1992)
Chloramphenicol	(Primavera *et al.*, 1993)
Chlorpyrifos	(Richardson, 1992)
Copper sulfate	(Vijakakumaran, 1997; Primavera, 1993)
Diazinon	(Richardson, 1992)
Dichlorvos	(Richardson, 1992)
Dimethylammonium bromide	(Primavera *et al.*, 1993)
EDTA (ethylenediaminetetraacetic acid)	(Richardson, 1992)
Endosulfan	(Richardson, 1992)
Erythromycin	(Primavera, 1993)
Formaldehyde	(Dierberg and Kiattisimkul, 1996)
Furazolidone	(Primavera, 1993)
Glutaraldehyde	(Boyd, 1995b)
Malachite green	(Dierberg and Kiattisimkul, 1996)
Malathion	(Richardson, 1992)
Nicotine	(Richardson, 1992)
Nitrofurans	(Vijayakumaran, 1997)
Oxytetracycline	(Weston, 1996)
Ozone	(Dierberg and Kiattisimkul, 1996)
Potassium Permanganate	(Boyd and Massaut, 1999)
Povidone iodine	(Funge-Smith and Briggs, 1998)
Rotenone	(Kongkeo, 1997)
Sapogenin glycosides	(Boyd and Massaut, 1999)
Tea seed cake	(Vijayakumaran, 1997)
Zeolite	(Vijayakumaran, 1997)

One problem that occurs with the excessive use of chemicals and the accumulation of residues is the resultant interference with sediment microbial activity. As described previously, microbes inhabit pond bottoms where they break down organic matter. A high deposition of pesticides and antibiotics in sediments both within and downstream of the shrimp ponds can result in inhibited microbiological activity which directly affects the rate of organic decomposition (Amaraneni, 2002). Thus, farm managers have created a cycle, whereby the use of degraded water leads to disease problems that necessitate therapeutic treatments (chemicals being one option), which, if not used properly, further contribute to the deterioration of water quality and the health of the entire ecosystem. Determining the extent of the impact that chemical use by aquaculturists has on the environment is extremely difficult due to the lack of information regarding the quantities and concentrations used (Graslund and Bengtsson, 2001). Also, when chemicals are used for therapeutic control the responsibility is shared between the chemical company, the farmer, and the food manufacturer. The more individuals and groups involved, the more difficult and labour-intensive it becomes to create and enforce regulations that ensure all three groups are held accountable (Shapiro and Mercier, 1994).

The persistence of chemical residues within the environment is another issue that arises with the intensive administration of chemicals. The distribution and fate of residues has yet to be thoroughly studied in aquaculture areas (Haya *et al.*, 2001), although a few studies have shown some alarming results. Weston (1996) stated that up to 95% of the oxytetracycline (one of the most widely used antibiotics) applied on a farm was not taken up by the shrimp, thus accumulated in the sediments and was released from the ponds as effluent. Studies have shown that the drug does not appear to be microbially degraded (Robins-Brown *et al.*, 1979) thus, it may persist indefinitely in sediments. Following the release of the pond sludge into nearby waterways, the chemical residues and any newly-adapted resistant bacteria begin to affect non-target aquatic organisms. Bioaccumulation is a phenomenon that occurs when a chemical is stored in the fatty tissues of living organisms, where it undergoes little degradation (Smith, 1992). The accumulation creates an increased concentration of the chemical compound, which is then passed up the food chain where again the chemical is retained and accumulated in organisms at upper trophic levels--known as biomagnification (Begon *et al.*, 1996). Thus, species downstream of the shrimp farms—from insects to fish to birds, and mammals including humans--could be exposed to pesticide-ridden effluent. All populations in that ecosystem risk ingesting food contaminated by the aquaculture farms and experiencing the effects of biomagnification (Branson and Southgate, 2001). Species--both native and migratory--that consume the non-target species that have been affected by drug residues, and any humans consuming the shrimp produced by aquaculture, now also carry higher concentrations of the residues. Various effects then occur such as mass mortality, chronic changes in behaviour, lower survival rates, and morphological and physiological changes in various organ systems (Amaraneni, 2002).

For example, Cheng *et al.* (2001) found that shrimp exposed to high levels of nitrate accumulate the nitrate in their heart, gills, muscle and hepatopancreas in concentrations higher than the ambient levels. Research has found links between pesticides and developmental abnormalities in amphibians, such as extra legs, or stumped or fused legs (Ouellet *et al.*, 1997), and compromised immune function in dolphins, seals, and whales (Repetto and Baliga, 1996). For many years, humans have been aware of the effects of aquatic environmental contamination in seafood, but information on the hazards and public health aspects of the actual fish and shellfish harvested from warm water areas is sparse (Howgate, 1998). The data that do exist are based mostly on aquaculture in developed countries when the reality is that the industry is predominantly found in developing countries (Howgate, 1998).

Pesticides also create complications for the people responsible for chemical application, as they risk side effects from long-term exposure. The United Nations estimated that approximately two million poisonings and 10 000 deaths occur each year from pesticides, and three quarters of those occur in developing countries (Quijano *et al.*, 1993). Scientists have found elevated cancer risks and disruption of the human body's reproductive, immune, endocrine, and nervous systems when people have been exposed long-term to pesticides (Troisi, 2002). Even if developed countries regulate the residue levels in imported seafood products, the developing countries do not have the economic means to enforce stringent regulations and thus, still work with and consume products that may not meet international standards. Amaraneni (2002) found that pesticide levels in fish produced in aquaculture farms in Kolleru Lake, India exceeded safety standards and should not have been consumed. Other reports have stated that pesticide users face great hazards and suffer from a range of pesticide-related problems including liver, lung and kidney damage, seizures, reproductive problems, and even death (PANUPS, 1993).

Along with attempts to eradicate using pesticides for the pathogenic organisms that cause diseases, farmers also began to employ techniques to ensure shrimp were resistant to infections through the use of antibiotics. While antibiotics have revolutionized the control of diseases, one of the biggest concerns is the increasing resistance of bacteria to the drugs. Antibiotics cause microbes to either die or to adapt and survive, a mechanism known as "selective pressure" (WHO, 2002; Moriarty, 1999). Those that survive and carry resistance genes can transmit this resistance to other bacteria, including different species, through extra chromosomal pieces of DNA called plasmids (Brown, 1989). When antibiotics are used incorrectly— either for too short of a time period, at dosages that are too low, at inadequate potency, or for the wrong disease— the likelihood that bacteria will adapt and survive rather than be killed is much greater (WHO, 2002). Since bacteria are ubiquitous, the resistant strains easily spread to other organisms such as shrimp and humans as a consequence of direct transmission, or indirectly through the transfer of resistance genes from environmental bacteria to human pathogens (Miranda and Zemelman, 2002). The transference of resistance then threatens the overall effectiveness of antibiotics for treating diseases in other species, including humans (Troisi, 2002). Tendencia and dela Pena (2002) found a higher number of antimicrobial resistant strains in the receiving environments of shrimp pond effluent than in the pond itself. Miranda and Zemelman (2002) also determined that the level of resistance expressed by the majority of bacteria is far higher than what is needed to actually resist a therapeutic dose of an antibiotic, suggesting that the cycling of resistance in microbes is much more severe than scientists initially projected.

2.3: Bioremediation using probiotics

The occurrence of shrimp disease has continued to increase in Thailand due to the poor water quality that has been created by released effluent, the spread of antibiotic resistance, and the contamination by pesticides. As a result, researchers and innovative aquaculturists have been exploring other options to control microbial populations including natural methods to "fix" nature; that is, putting the ecosystem back into a self-sustaining, healthy balance without the use of synthetic chemicals. The scientific community and farmers seem to have realized that aquatic animal disease is the end result of a series of linked events and therefore, the treatment of diseases needs to go beyond solely considering the pathogen (Subasinghe *et al.*, 1998).

One method involves probiotic technology, a form of bioremediation, which introduces commercially prepared solutions of microorganisms into the pond environment in an attempt to balance the number and type of microbes in a pond system (Atlas and Bartha, 1998). The term probiotics is often used to refer to live intestinal bacteria that regulate the colonization and promote a beneficial balance within the gut surfaces of the host (the shrimp) (Skjermo and Vadstein, 1999). The term probiotics, however, can also be applied to include any bacteria able to regulate the colonization of any surface within the hosts' environment (Skjermo and Vadstein, 1999). For the purposes of this discussion the term probiotics refers to any live microbial solution which may benefit the shrimp. The positive effects may include the modification of water and sediment microbial communities, insuring improved use of feed by aiding digestion, the enhancement of the shrimps' response towards disease, or the improvement of the overall quality of the surrounding environment (Verschuere *et al.*, 2000; Moriarty, 1999).

It is not fully understood yet how probiotics work and what mechanisms may be behind the microbes improving the health of shrimp or their environment. Some suggest the added bacteria outcompete pathogenic organisms for nutrients, or for adhesion sites within the intestinal tract of shrimp so that the disease carrying organisms are not able to infect or survive in the shrimp (Sonnenholzner and Boyd, 2000; Fuller, 1989). Others suggest the effects involve enhanced nutrition through the production of vitamins and by aiding the shrimps' digestion (Holzapfel and Schillinger, 2002; Verschuere *et al.*, 2000; Garriques and Arevalo, 1995) or the production of antibiotics and inhibitory substances that act antagonistically towards pathogenic bacteria (Burgess *et al.*, 1999; Fuller, 1989; Verschuere *et al.*, 2000). The presence of microbes within shrimp may also alter the shrimps' metabolism by increasing enzyme activity or stimulating antibody levels, thereby improving the shrimps' immunity to pathogens (Fuller, 1989).

The basic premise underlying probiotic technologies is based on the fact that farmers disinfect ponds with chlorine and remove sludge (the habitat of microbes) between crop cycles, and also add chemicals detrimental to bacterial survival throughout the grow-out periods (Potts and Boyd, 1998; Garriques and Arevalo, 1995). Inevitably, without the presence of any functional bacteria, the process of organic degradation cannot occur (Visscher and Duerr, 1991). With the addition of a probiotic, an attempt is made to re-balance the microbial communities of the water and sediment within the ponds that were destroyed by previous farm management activities. The addition of a probiotic solution should ensure that sufficient bacteria are present to decompose the organic matter of the sludge and release nutrients for use for oxygen-producing phytoplankton, thereby improving the overall quality of water in the pond (Moriarty, 1999; Visscher and Duerr, 1991). The philosophy of this type of biotechnology recognizes that the root of the water quality problem is not the water or the shrimp, but actually the bacterial populations. The implementation of these products is a direct result of the growth of inland shrimp aquaculture ponds. Using probiotics in a coastal environment would be completely ineffective since the sheer size of the water body would make it extremely difficult to modify microbial populations. The smaller, closed inland ponds are much more suitable for this type of technology. The parameters typically measured in a study of bioremediation efficacy should include the rate of microbial respiration or oxygen consumption, and the disappearance of the total organic load (Atlas and Bartha, 1998). Consequently, this study will focus on the BOD of pond water and total organic matter content of sludge when determining the effects of a probiotic solution.

A number of benefits from the use of probiotics have been described in various fields of environmental restoration, agriculture and human health studies (e.g. Atlas and Bartha, 1998; Abe *et al.*, 1995; Barbes and Boris, 1999 respectively). Due to the successful applications of probiotics in these fields, a great potential exists for probiotics to be an effective management tool in shrimp aquaculture. Bioremediation through the use of microorganisms has become increasingly popular as an economical means to reduce organic contamination ever since the approach gave positive results in the 1989 Exxon Valdez oil tanker spill in Prince William Sound, Alaska (Atlas and Bartha, 1998). Biological processes have also been an integrated part of sewage wastewater treatment for the past century (Mitchell, 1974). If the bacteria are non-pathogenic and indigenous to aquaculture areas, and would normally be present had chemicals not killed the communities, it can be assumed that their presence in the water and in shrimp would not cause adverse side effects in any of the consuming species.

2.3.1: *Probiotics in aquaculture products and the human health implications*

In addition to improving the health of the shrimp and their pond environments, probiotics have also been used in attempts to improve human health. Nobel-prize winner Metchnikoff (1907) was the first to propose the use of probiotics for human use when he suggested that the longevity of Bulgarian peasants was the result of consuming fermented milk products containing bacteria that positively influenced their colons. An increase in the elderly sector of world populations, immuno-compromised patients such as those with AIDS, organ transplants, or chemotherapy, along with antibiotic resistance have all contributed to the loss of normal colonizing bacteria within certain populations of humans (Sanders, 2000). Thus, some infections that at one time were readily treatable with antibiotics or were not serious enough to warrant treatment are now a true threat to people's health (Sanders, 2000) and people are seeking alternatives to chemical therapies. Very few well-controlled clinical trials have been able to provide evidence of the purported positive effects of probiotics or probiotic-containing food, since many risk factors and the complex relationships with diet choices are difficult to control (Holzapfel and Schillinger, 2002; Sanders, 2000). However, some examples of probiotics in health studies include research by Monique *et al.* (1986) who found that increased yogurt consumption reduced the risk of breast cancer in women, and Peters *et al.* (1992) who found calcium intake through yogurt significantly reduced the risk of colon cancer. Several cases have also been stated for *Lactobacillus* and its ability to aid humans in relief of constipation, to produce antitumour activities, and to result in anticholesterolaemic effects (Holzapfel and Schillinger, 2002; Fuller, 1989). Most studies conducted to date have focused on *Lactobacillus* cultures and dairy products most likely related to the natural presence of fermented bacteria common in dairy products (Sanders, 2000).

Probiotic bacteria are found worldwide in a variety of traditional food products, dietary supplements and medical foods (Sanders, 2000) so the addition of aquaculture products on the market that have been treated with probiotic applications would not be a radical change. However, the health potential of species included in the probiotic solutions of aquaculture has not been well documented. In order for shrimp to transfer the positive effects of the probiotics when consumed by other organisms, it is essential that the microbes can grow and survive within or upon the shrimp (Svensson, 1999). Since the emphasis is on a preventative, natural method of reducing disease, the concept of probiotics within human diets is emerging as more than a mere trend (Sanders, 2000). The inclusion of probiotics in human and animal diets could diminish the reliance upon pharmaceutical therapies (Elmer, 2001; Sanders, 2000; Barbes and Boris, 1999). However, more research needs to be conducted on the specific strains and quantities of bacteria in order to determine the effectiveness of probiotics (Elmer, 2001; Sanders, 2000). Also, more information needs to be provided to consumers regarding the probiotic content of their food (Sanders, 2000).

2.3.2: A review of probiotic research in aquaculture

Despite advancements in the field of biotechnology, results of probiotic utilization have been varied in shrimp aquaculture studies (e.g. Shariff *et al.*, 2001; Sonnenholzner and Boyd, 2000; Chiayvareesajja and Boyd, 1993). Rengpipat *et al.* (1998) found positive results using a probiotic solution of *Bacillus* species. *P. monodon* survival and growth was significantly higher when treated with the probiotic in laboratory experiments (Rengpipat *et al.*, 1998), but the findings were not confirmed in outdoor, earthen pond trials. Also, the data showed that water quality factors were not affected by the treatment, but biological oxygen demand (a critical measurement in water quality studies) was not measured (Rengpipat *et al.*, 1998). While improving the health of the shrimp through natural means is imperative for the industry to continue, the management of aquaculture ponds needs to focus on the improvement of the health of the entire system involved. If the purpose is to enhance the entire microbial population, it stands to reason that a single strain such as *Bacillus* would not be as effective as a mixed culture of species in maintaining a balanced system (Verschuere *et al.*, 2000).

Ehrlich *et al.* (1998) also found a potential to reduce sediment volume through bacterial augmentation, but the experiment was conducted over a trial period of 8 days in a lake receiving multiple point sources of pollution (textile factory, agriculture and residential waste). Although the study provided initial data to indicate the potential of bacterial solutions in the reduction of sludge, the data cannot be accurately extrapolated to aquaculture pond conditions where crops are grown for approximately 110 days. In terms of water quality, data collected were limited to algal growth and nitrification.

Prabhu *et al.* (1999) found beneficial results for using a commercial probiotic in shrimp ponds in India. However, the probiotic was applied only four different times (selection of the four times was not clearly justified) and data were collected for water quality parameters and analyzed for difference between "before" and "after" values within each pond after each application, rather than an overall analysis between treatment ponds and a control.

Devaraja *et al.* (2002) found positive results for bacterial growth and shrimp production in ponds that were treated with microbial products, but the probiotic solutions used in the study were imported (point of origin is not noted). By importing microorganisms from other countries a risk emerges as non-native species or strains may be introduced to the area, creating a potential for further environmental problems. Also, the impacts of the probiotic product on water quality and sludge were not quantified by this study.

Sonnenholzner and Boyd (2000) considered an application of probiotics that may have prevented the need for sludge removal, by studying whether probiotics could affect the decomposition rates of organic matter in pond bottoms where the crops were harvested, and the water was drained prior to the experiment. Again, however, evaluations were limited to 7-9 days and the methodology only considered the reduction of sludge, but not the improvement of water quality. Based on the state of the aquatic environment in shrimp producing areas, both characteristics of the environment need to be studied.

Chiayvareesajja and Boyd (1993) claimed that bacterial augmentation did not significantly reduce total ammonia nitrogen (TAN) in pond water, although a closer inspection of the results indicates that TAN was visibly lower (even if not at the significant level) in the treatment water. Other water quality or sludge variables were not included in the discussion of this study. It was not clear from this experiment where the pond water came from or what type of aquaculture was involved (fish or shrimp). Also, samples of the ponds were treated and observed in a laboratory separate from any actual aquaculture production. Therefore, the results cannot be accurately applied to an outdoor pond situation where shrimp are concurrently growing.

Shariff *et al.* (2001) found that microbial products were not effective in improving water quality or the carbon, nitrogen and phosphorus contents of sludge in Malaysian shrimp ponds where sludge was not removed from a previous crop. Their suggestion that cleaning the pond bottom prior to stocking with shrimp should be maintained, however, does not provide a viable solution for the waste management issues of the aquaculture industry since cleaning the pond only transfers the problem into the broader aquatic environment. Shariff *et al.* (2001) also noted that unacknowledged sources have reported record shrimp production in Thailand and Indonesia after applying a probiotic solution, which indicates the inconsistency of results in this field and the need to further confirm the potential efficacy of probiotics.

Yusoff *et al.* (2002) performed a study in six shrimp ponds in Malaysia to analyze phytoplankton succession in probiotic treated ponds, using phytoplankton dominance and succession as an indicator of water quality. In addition to the groups of phytoplankton species studied, Yusoff *et al.* (2002) measured *in situ* water quality variables and found that the use of a bacterial product did not significantly improve water quality, although a general decline was observed in the BOD. Interfering with these results, however, is the fact that the ponds were treated with dolomite and tea seed cake 5 days after stocking. The addition of chemical treatments defeats the purpose of a natural alternative for waste management.

A review of the published literature on the subject of sludge management in shrimp aquaculture revealed that investigations have not been made into the effectiveness of a microbial additive in inland, low-salinity shrimp ponds in rural Thailand where a large proportion of the world's shrimp is produced. The reality is that farmers will adopt their own application techniques based on their assessment of pond conditions. Therefore, a laboratory experiment that closely follows the manufacturer's instructions may not be the most accurate means to determine the effectiveness of probiotic solutions in improving pond water quality. Hence, this study attempts to employ and document the techniques of a typical, small-scale, inland farmer in utilizing a microbial additive, and to investigate the impacts of the biotechnology in improving water quality in a pond that is currently inhabited by a *P. monodon* crop.

2.4: EM-1 (Effective Microorganisms)

Numerous microbial additives are marketed in the Chachoengsao region of Thailand. This study, however, is focused on one commercial product called EM-1 (Effective Microorganisms). EM-1 was developed by Dr. Teruo Higa at the University of Ryukyus Okinawa, Japan in late 1979 when he attempted to increase the productivity of conventional organic farms (Kyan *et al.*, 1999). Dr. Higa's original solution of EM contained more than 80 species of microorganisms (Kyan *et al.*, 1999). Over time, various species were eliminated if they were deemed unnecessary to the process or if a particular species could not survive well (Lancaster, pers. comm., May 5, 2003) Today, the EM-1 solution in Thailand consists of a mixed bacterial culture including lactic acid bacteria (*Lactobacillus* spp.), photosynthetic bacteria (*Rhodopseudomonas* spp.), yeasts (*Saccharomyces* spp.), Actinomycetes and nitrogen-fixing bacteria.

While the cycling of organic nutrients is well understood in scientific circles (e.g. Atlas and Bartha, 1998) very little scientifically valid information is available from any of the microbial additive companies other than the fundamental mechanism of bacteria utilizing the organic material for sustenance. Little explanation is offered pertaining to why particular organisms were chosen and how they improve sludge content and water quality within aquaculture ponds. Also, the particular species of the genera listed above that are included in the microbial solutions are not clearly identified or readily cited in any of the literature published by any of the companies, especially EM-1, or on the labels of the products sold to the public. Further communication with EM Tech in the United States revealed that although each country produces its own solution, the species tend to be the same throughout the world (Lancaster, pers comm., May 5, 2003) The lactic acid, yeast and phototrophic species used would include: *Lactobacillus plantarum, L. casei, L. fermentum, L. salivarius, L. delbrueckii, Saccharomyces cerevisiae, Rhodobacter sphaeroides, R. capsulatus, and Rhodopseudomonas palustris* (Lancaster, pers comm., May 5, 2003). Research has shown that the *Lactobacillus* spp. and *Saccharomyces cerevisiae* are commonly used in probiotic products already (e.g. Senne and Gilliland, 2003; Kumprechtova *et al.*, 2000; Uma *et al.*, 1999), however little research has been published on the probiotic effects of the remainder of the species within the EM-1 solution.

EM-1 does claim to use naturally occurring microorganisms indigenous to the countries the product is sold in (Kyan *et al.*, 1999), which eliminates the concerns of introducing exotic species or genetically modified organisms bred for certain characteristics. Some of the specific claims published by Kyan *et al.* (1999) in the users' guidelines state that:

EM reduces capital outlay for aquaculturists by 50%; prawns are healthy with a shining shell; the quantity of sludge produced is reduced, ammonia, methane, and hydrogen sulfide are eliminated; prawns are disease free which saves the cost of antibiotics; and the water will remain clean and will not require repeated exchange.

2.5: Summary

This chapter has outlined the development of the black-tiger shrimp aquaculture industry, specifically in the inland areas of Thailand. The chapter also examined the far-reaching effects of shrimp farming on the environment, with an emphasis on the problems arising from poor sludge management. The direct and indirect impacts that the aquaculture industry may have on human health were also explored. One alternative farm management practice currently being promoted in Thailand involves microbial additive, or probiotics, to reduce the build up of sludge, improve pond quality and shrimp health. A discussion was included that reviewed studies that utilized bacterial solutions in shrimp aquaculture ponds, illustrating that research thus far has not been conclusive regarding the effects of probiotics in improving pond conditions, although the potential clearly exists for its benefits. EM-1 (Effective Microorganisms) is one of the products presently in use in Thailand, despite the fact that knowledge about its contents and their effects are limited to a farmer's own understanding of biological processes. To assess the effectiveness of microbial additives as an approach to sludge management, specifically on inland shrimp farms, the product must be tested under field conditions using techniques typical of a small-scale farmer. The research presented in this thesis attempts to document the methodology of EM applications on an inland shrimp farm and to investigate the efficacy of the probiotic solution in improving pond water quality and reducing the accumulation of organic sludge.

3. STUDY AREA AND METHODOLOGY

This thesis presents a field study that investigates the effectiveness of a commercially prepared probiotic to improve water quality and minimize the accumulation of sludge on an inland shrimp farm. The study took place on one inland, low-salinity farm located in Bang Khla, Chachoengsao Province, Thailand. In this chapter, the logistics behind the site selection are discussed and the biophysical and socio-economic characteristics of the study area are described. The farm management practices are then presented, followed by a description of the sample collection and measurement methodology.

3.1: Site Selection

This study could have been conducted in any number of different regions within the Central Plains of Thailand. However, only four provinces within the Central Plains have been exempt from a ban imposed by Thailand's Department of Fisheries on inland shrimp farms. Therefore, it was believed that selecting a study location within one of those four provinces would provide a more accurate representation of the sites where legal shrimp farming operations may continue to develop in the future.

The field site was selected during a preliminary research trip to Thailand in December 2001 and was granted approval by the farmer. The selection was purposive as the farmer was a graduate from a recognized university and was familiar with water quality research. Therefore, conflict or lack of mutual understanding between the farmer, the researcher, and the associated academic institutions was not an issue. The exact location of the farm and the shrimp farmer's name are kept anonymous since some management practices, although common in Thailand, do violate government regulations.

3.2: Study area

This study took place within the Central Plains region of Thailand, a low-lying, flat area surrounded by mountains, and well irrigated by several major river systems, including the Chao Praya River, and the Bang Pakong River (Figures 3.1, 3.2). The abundant water supply has enabled the Central Plains to become an extremely productive agricultural centre for Thailand. As stated, the study site is located in Chachoengsao Province, located in the southeastern part of the Central Plains (Figure 3.2). Irrigation within Chachoengsao Province is primarily provided by water from the Bang Pakong River.

3.2.1: The biophysical environment of the study area

The Bang Pakong River basin is approximately 18,758 km^2 in area and 240 km in length and empties into the Gulf of Thailand (Figure 3.3) (Szuster, 2001; Thanomsak *et al.*, 1999). The town of Bang Khla falls within the Bang Pakong's east bank sub-basin, which covers 912 km^2 (Figure 3.2)(Department of Land Development, 1998). Weather in the entire basin area tends to follow three distinct tropical seasons including the "cool" season of November to January, the extremely hot and dry season between February and May, and the wet/monsoon season from June to October. Soils within this region are predominantly clay-based and the combination of these conditions with the climate has resulted in extremely fertile land, which has lead the region to be Thailand's dominant agricultural centre, known as the "Rice Bowl". Other agricultural and horticultural activities in the area include fruit orchards (e.g. coconut, mango) and intensive livestock production (e.g. chicken, swine) (Szuster and Flaherty, 2002).

Widespread shrimp disease and deteriorating environmental conditions in coastal areas have resulted in an increasing shortage of suitable coastal land for shrimp aquaculture. Driven by this factor and the reality that much higher potential profits can be found in shrimp farming as opposed to rice farming, low-salinity shrimp farms have become a much more dominant activity in the Central Plains in the past decade (Flaherty *et al.*, 2000; Flaherty and Vandergeest, 1998). The Department of Land Development estimated in 1998 that approximately 22 455 hectares of the Central Plains were utilized for low-salinity shrimp farming, and 18 530 of those hectares were located within the Bang Pakong River basin. The Bang Pakong east bank sub-basin contains approximately 4 494 hectares of pond area (Department of Land Development, 1998). Although the Thai government passed and upheld a ban on low-salinity shrimp farming in fresh water areas in 1998, this only applied to areas outside of the Bang Pakong River basin. Since the Bang Pakong River experiences seasonally brackish conditions due to salt water intrusions from the Gulf, low-salinity shrimp farming was legally permitted to continue its expansion into both the freshwater and seasonally brackish areas of Chachoengsao, Prachinburi, Chonburi and Nakhon Nayok provinces of the Central Plains (Szuster, 2001)(Provinces labelled 16, 19, 20, and 17 respectively in Figure 3.2). However, despite the seasonal "brackish" nature of the permitted farming areas, research has shown that the discharge of effluent from shrimp ponds significantly heightens the salinity levels of the receiving canals (Braaten and Flaherty, 2001). The problem is worsened by the fact that pond discharge occurs year round, not just when the river is naturally brackish (Braaten and Flaherty, 2001).

Shrimp farms in the Bang Pakong River basin have been estimated to consume more than 335 million m^3 of water per year, representing 10.7% of the total agriculture water use in the area (Kasetsart University, 2000; Land Development Department, 1998). The shrimp farms in this area have also been estimated to produce more than 604 million m^3 of effluent per year (Szuster and Flaherty, 2002). In particular, the Bang Pakong east bank sub-basin contributes approximately 146 million m^3 of effluent to the total annual production in the basin area (Szuster and Flaherty, 2002).

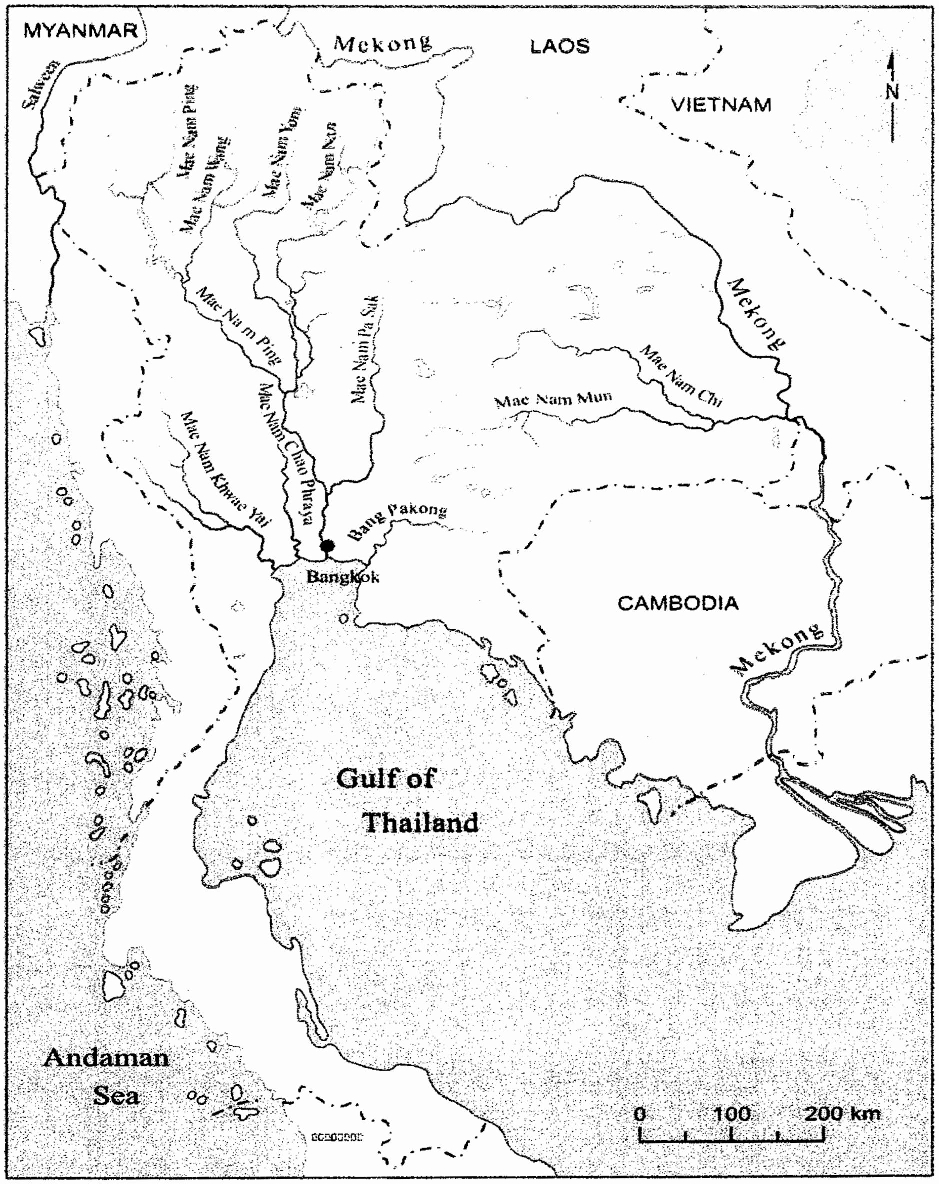


Figure 3.1 Map of Thailand displaying the several major river systems (courtesy of O. Heggen, 2003)

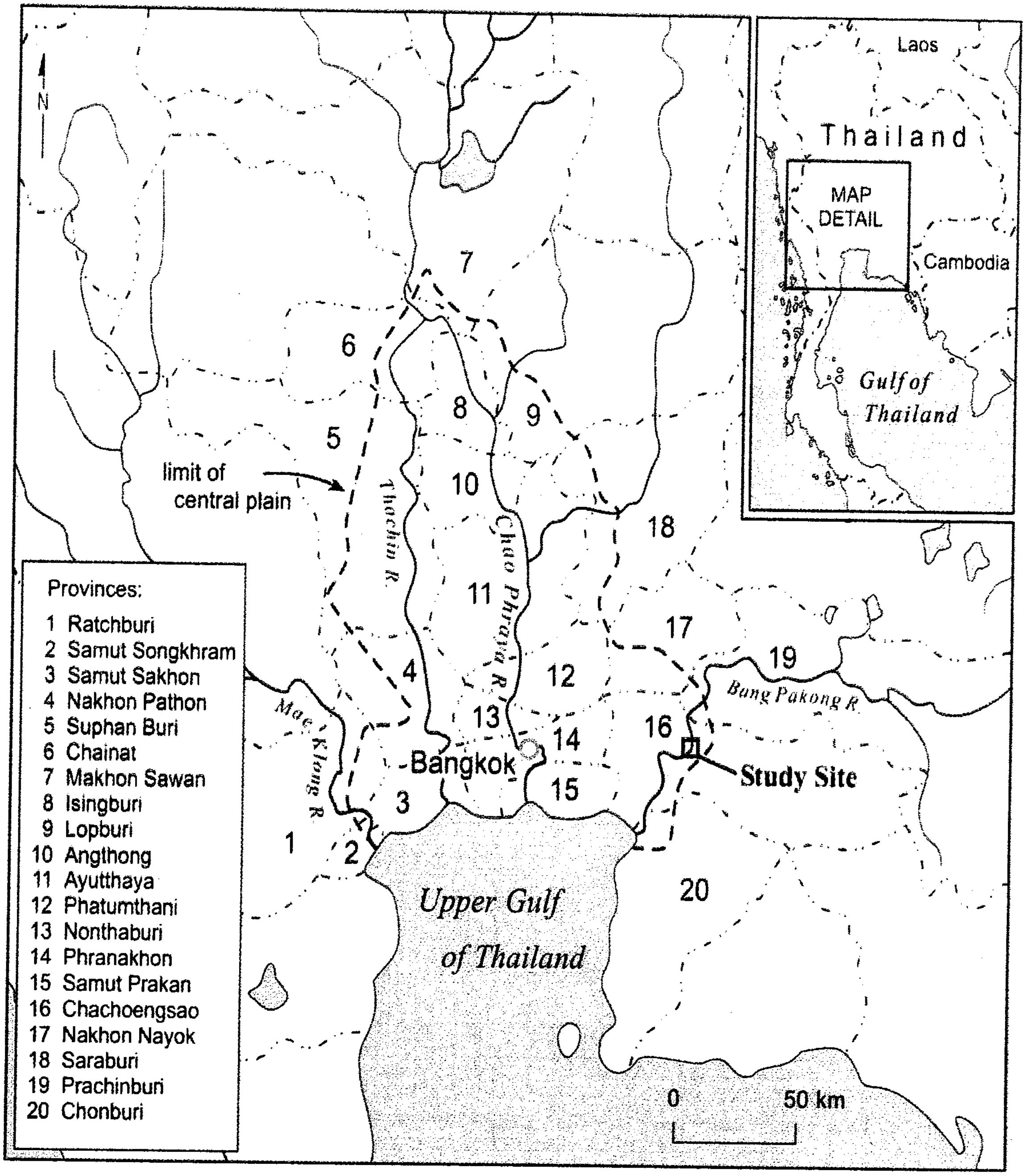


Figure 3.2 Map illustrating the limits of Thailand's Central Plains region. The study site was located in province 16 (courtesy of O. Heggen, 2003).

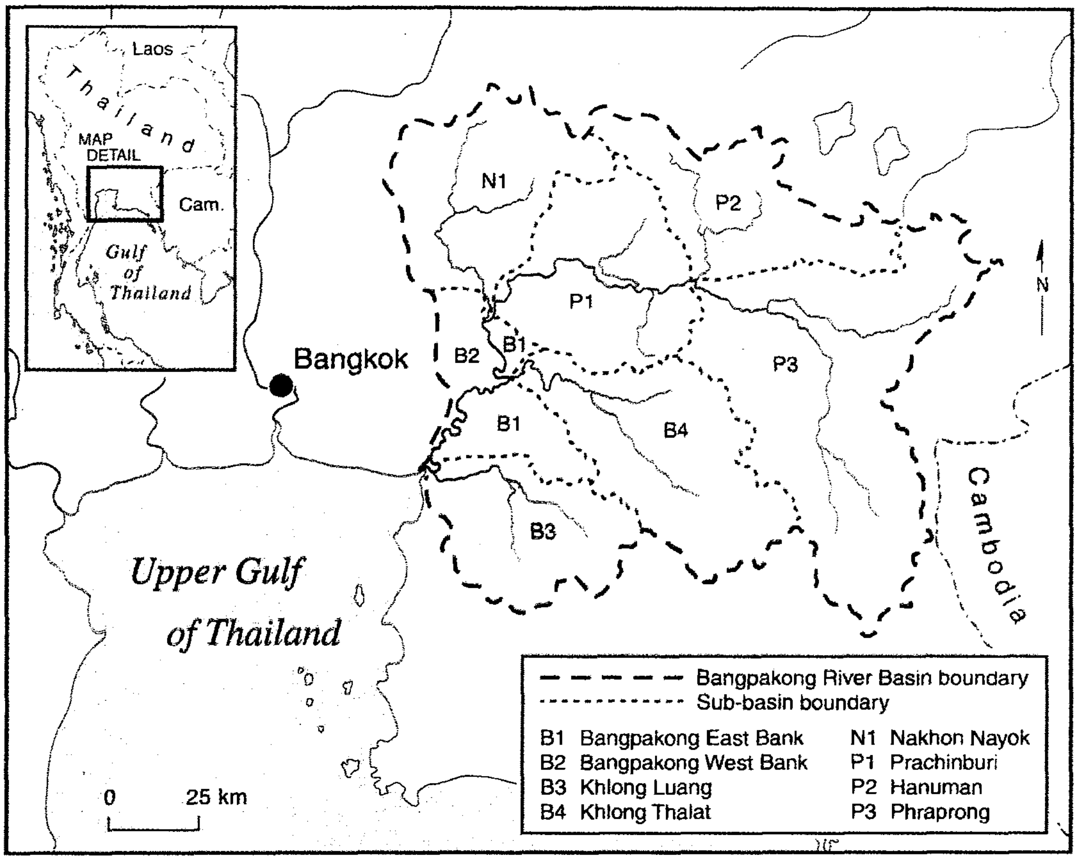


Figure 3.3 The Bang Pakong River Basin and Sub-basins (courtesy of O. Heggen, 2003)

3.2.2: The socio-economic background of the study area

Unlike many areas in Thailand, the economy of Chachoengsao Province relies very little on tourism. The Central Plains region, or the "Rice Bowl", is closely connected to the Thai identity and their "peasant" culture, providing their diet staple in addition to contributing to the Central region's historical importance to Thailand's rice export economy (Flaherty *et al.*, 1999; Phongpaichit and Baker, 1996). The rice paddies are mainly planted and harvested through manual labour, rather than mechanized methods and are supported by an irrigation system that was developed over a century ago.

Shrimp farming has the potential to be much more lucrative with estimated profits as high as 15 times the income obtained in rice production (Flaherty *et al.*, 1999). Although the initial capital investment is quite high, farmers can easily recoup their investment in one year (Flaherty *et al.*, 1999). Hence, the switch to shrimp production made economic sense to farmers and proliferated throughout rice paddies in the region shifting the dependence of the people from agriculture to aquaculture revenues. A study conducted by the Royal Thai Government states that approximately 72% of the low salinity shrimp farms in the Central Plains region lies within the Bang Pakong River Basin (Ministry of Science, Technology, and Environment, 1998).

Bang Khla farmers tend to rely on local seafood markets in Chachoengsao and Chonburi provinces and processing plants in Samut Sakhon for the sale of their shrimp, although the capital city of Bangkok and its potential export facilities are a mere 90 minutes away by automobile. The majority of the shrimp farms in the area are small-scale operations that have been producing crops for the past 10 years. Families often supplement their income from shrimp farming with other businesses, including restaurants, aquaculture feed and additive stores, and fruit stands.

3.2.3: Field site and Farm Management

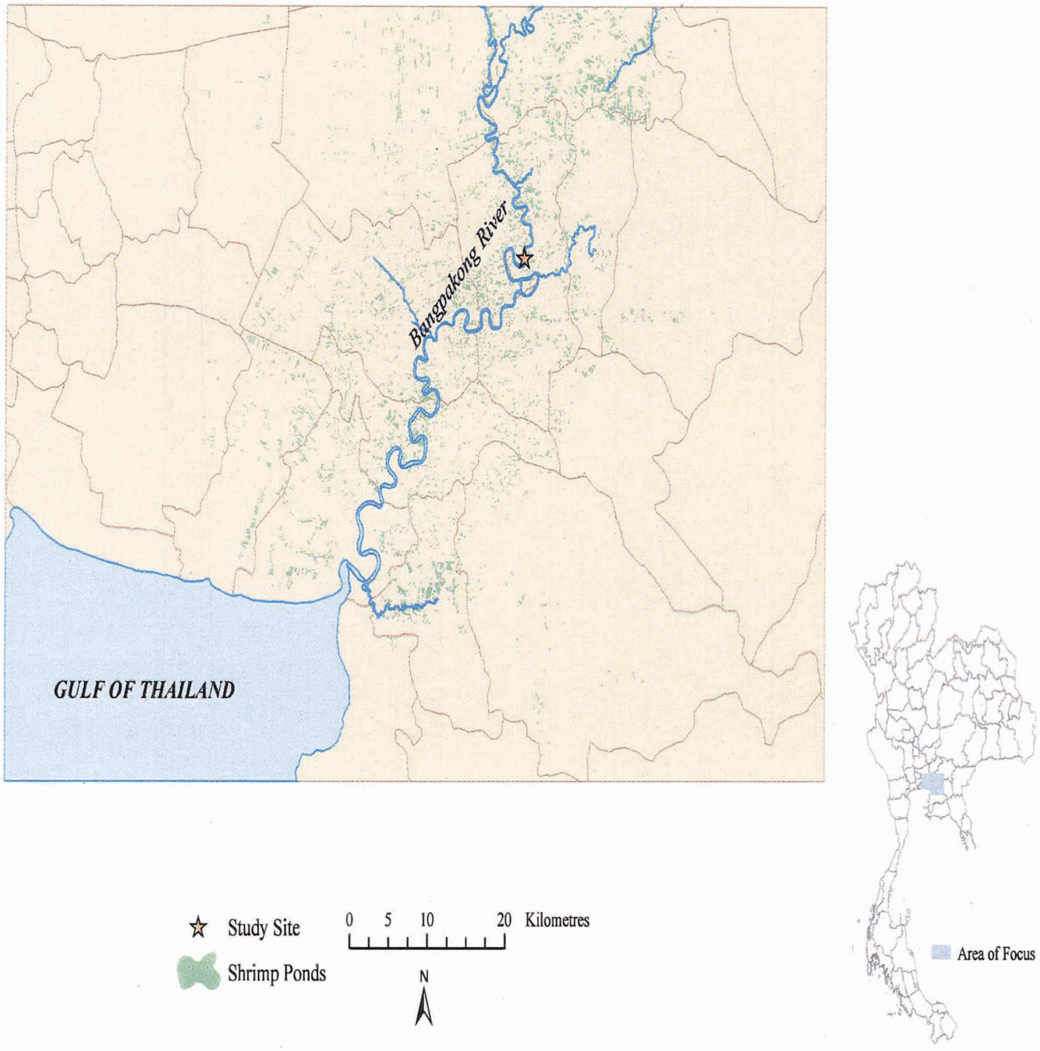


Figure 3.4 The southeastern portion of the Central Plains region of Thailand highlighting the study site location in Chachoengsao along the Bang Pakong River amidst the dense concentration of shrimp ponds (courtesy of S. Jiaraniawiwat and J. Miller, 2003).

The farm focused on in this study is located approximately 50 km inland from the mouth of the Gulf of Thailand coastline and 1 km from the bank of the Bang Pakong River in the east bank sub basin (Figure 3.4). The farm has been in operation for approximately 10 years. At the time of this study there were three shrimp ponds and one reservoir with hard-packed clay bottoms that were all approximately 1.5 m in depth. The areas of the ponds were Pond 1- 1620 m^2, Control-1353 m^2, Pond 2- 1638 m^2, and the Reservoir- 1135 m^2. Therefore, pond volumes can be estimated as Pond 1- 2 430 m^3, Control- 2 030 m^3, Pond 2- 2 457 m^3, and Reservoir- 1 703 m^3. Each of the ponds on the farm is surrounded by coconut palms, and a wetland area is located between the reservoir and the Bang Pakong riverbank (Figure 3.5).

Shrimp were stocked within the ponds at 80-100 postlarvae per m^2. Crop cycles typically last approximately 110-120 days on the farms in this area, although the shrimp may be harvested earlier in the case of disease. A canal that irrigates the entire farm is the receiving area for output during harvest and draining (Refer to "main canal" Figure 3.5). The canal may also provide the water input for the reservoir, although since this farm was located only 1 km from the bank of the Bang Pakong the farmer frequently pumped water directly from the river with a diesel motor pump and plastic hose. In the hot season when the river tends to be brackish, the water would be partially saline, but at the time of this study the water being pumped in was freshwater (0.2 ppt— parts per thousand). The canal is one of many in an extensive canal system that supplies all of the neighbouring farms. The canals flood during heavy rains with both rainwater and overflow from the Bang Pakong, but are otherwise fairly dry until a pond is harvested and drained into them.

Farmers in the Chachoengsao area do not ship in salt supplies for the water, but allow brackish water from the Bang Pakong to concentrate in their reservoirs. While it is preferable to concentrate the salt for the entire dry season, the reservoir does not contain enough water to fill all of the shrimp ponds on any given farm. Thus, it is common for water to be held-in the reservoir for only a few days before ponds are either filled, or topped off for water exchange throughout the shrimp cycle. Since rainwater is the primary source of water in the reservoir throughout the crop season, the water tends to be only fresh water after the original concentrated water has been used.

In an effort to reduce the amount of sludge entering the waterways, farmers in this region try to follow a three-crop cycle management system where sludge is left on the bottom of the grow-out ponds to dry in the sun and break down after harvest for two consecutive shrimp crops. After a third crop is harvested, the sludge that has accumulated is pressure-hosed out of the bottom of the ponds into the adjacent canal. At the time of the study, all three of the ponds were on the second crop of this cycle.

At the beginning of the crop season, ponds were aerated for 3 hours each day (3:00 - 6:00 am) using diesel engine-powered paddle wheel aerators that were placed diagonally across each pond. Aeration practices increased progressively throughout the season as the shrimp grew and water quality declined. From week 7 to the end of the crop season the ponds were aerated thrice daily (1:00-4:00 am, 2:00-3:30 pm, and 7:00-9:30 pm).

Shrimp were fed 0.4 kg of pelleted feed twice daily for the first three weeks of growth. Feed portions were increased as the shrimp grew and were determined by monitoring the consumption of pellets placed on feed trays. The feed trays were nets attached to poles that could be lowered into the pond and raised to observe the size and health of the shrimp and the rate of feed consumption. After the feeding rate was determined by this method, feed was then manually applied to the ponds using a "broadcast" method, which involves tossing handfuls around the entire perimeter of the pond. This feeding technique is common on most shrimp farms in Thailand. By week five of the study and onwards the shrimp were fed 0.5-1.5 kg of feed four times per day. If the shrimp appeared diseased on any of the checks, the farmer decided whether or not to harvest early. Chemical disinfectants and antibiotics were not used on this farm during this study, as the farmer has already discontinued their use at his own discretion.

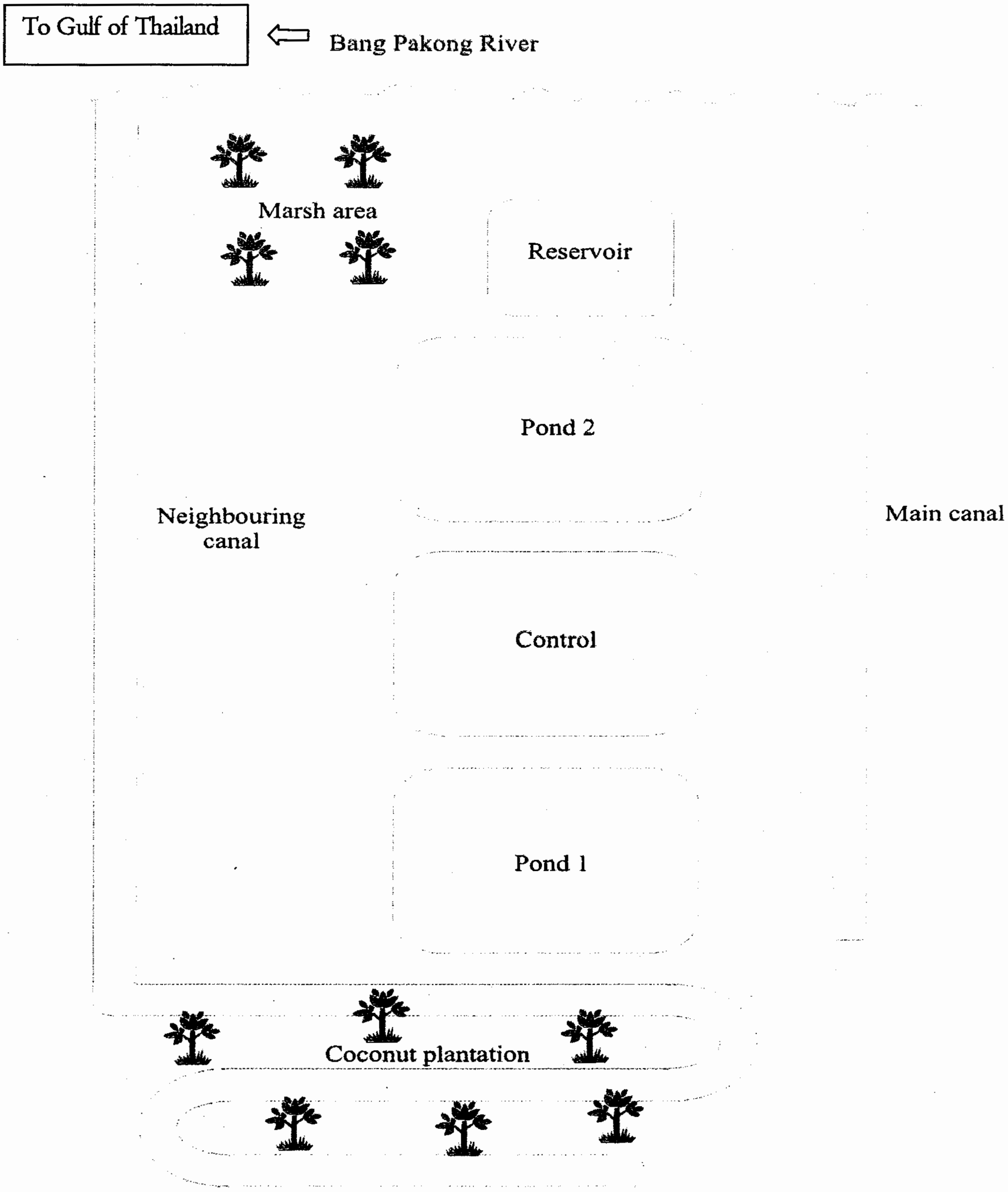


Figure 3.5 Schematic diagram of shrimp farm layout

Throughout the grow-out cycle freshwater was added to compensate for any evaporation and seepage losses, or to provide an exchange with the pond water if quality was determined to be poor by the farmer. The freshwater consisted of rainwater collected in the reservoir during this season and on average measured 0.2 ppt for salinity. Previous research by Braaten (2000) has documented the pattern for water exchange on a farm in this area. From day 1 after stocking with the shrimp until day 40, 36 ± 5.5 cm of water are added to the pond for filling and topping up purposes while no water is taken out (Braaten, 2000). From days 40-60 very little exchange takes place as water quality within the ponds is still acceptable (Braaten, 2000). From days 60-110 of the grow-out 103 ± 12 cm of water are added to the pond, while 76 ± 20 cm of water are removed (Braaten, 2000). The exchange of water indicates that the term "closed system" has become a misnomer in the inland shrimp farming industry.

Although EM solutions were readily available at the majority of local feed and aquaculture supply stores for approximately $B60/L (60 baht≈ CAN$2.20) the solution for this study was purchased directly from the Thailand distributor located in Bangkok. Application of EM-1 was based on a combination of the farmer's past experiences with the product and the general instructions from the EM manual. For the first seven weeks, 5 L of stock EM-1 were mixed with 5 L of molasses and 100 L of water and stored in a sealed container at ambient temperature for 5-7 days to create a single batch of "Extended EM-1" solution as recommended by the EM instructions manual (Kyan *et al.*, 1999). The solution is said to be ready when the pH is below 4.0, but in this case the pH was not closely monitored and was applied regardless of the pH levels. Each treatment pond received an entire batch (110 L) of extended EM-1 on a weekly basis. It is important to note that each single batch of extended EM-1 solution was created from the same 10 L bottle of stock EM ensuring that variance in batch or variance in the age of the stock culture was not different between treatment ponds. After week 7, the 5 L of molasses were no longer added to the EM-1 as the farmer believed that it interfered with the pond pH levels. Consequently, the 5 L of stock EM-1 was poured directly into the pond on a weekly basis for the remainder of the crop cycle. During week 7, 15 kg of rice husks were added to each pond. Rice husk treatments are a common practice in this area that attempt to provide food for the microbes breaking down organics (a similar concept to EM-1) and to maintain water quality.

3.3: Sampling Collection Methodology

The shrimp crops for all three ponds began on July 14, 2002. Each pond was very similar in terms of the amount of sun and shade received throughout the day. The water used to fill the ponds was from the same source, each pond was exposed to the same level of aeration and feed, and stocking was from the same batch of shrimp seed. The similarity in conditions helps to ascertain that any differences in water quality measurements can be attributed to the treatment rather than to confounding effects.

Hourly sampling was conducted for a 24-hour period at the beginning of the crop cycle to determine the optimal sampling hour for *in situ* measurements based on diurnal fluctuations (Figure 3.6). The optimal time was selected based on the lowest Dissolved Oxygen values, which can be significantly inflated by phytoplankton photosynthesis. Typically, Dissolved Oxygen increases throughout the ponds during daylight due to photosynthesis effects, and decreases at night, reaching a minimum by dawn (Boyd and Tucker, 1998). In this pond system the lowest Dissolved Oxygen values were found at 6:00 am. However, with the operation of the mechanical aerators between the hours of 3:00-6:00 am and feeding taking place at 7:00 am, it was determined that the optimal sampling time would not follow natural diurnal cycles. Rather, it was decided with the farmer that weekly sample collections would begin at 8:00 am.

Data were collected weekly for the duration of the crop cycle for parameters that would indicate the effectiveness of the EM additive. The choice for weekly sampling was made for practical reasons. No rules for sampling frequency in aquaculture seem to exist (e.g. Boyd and Tucker, 1998), and so the limitations dictated by available storage space in the laboratory facilities determined sampling frequency. A simple random design was employed, with each pond divided into an imaginary grid with 1 m^2 squares, from which three random samples were selected from each pond each week. The choice of three sampling locations was deemed suitable for statistical purposes by Boyd and Tucker (1998) who studied 14 ponds ranging in area of 0.04-10 ha and found similar areal variation for all. These findings suggest that an average of sample measures from three locations would be representative. Sample sites falling within 1 metre of the aerators were not used due to the excessive disturbance created by the aeration that renders this portion of the pond unrepresentative of the majority of the pond. Although removing this area from the sampling regime adds a "deliberate" element to the design, this was deemed as the best option since complete randomization without the exclusion of the aerator area would not have avoided confounding variation (Quinn and Keough, 2002).

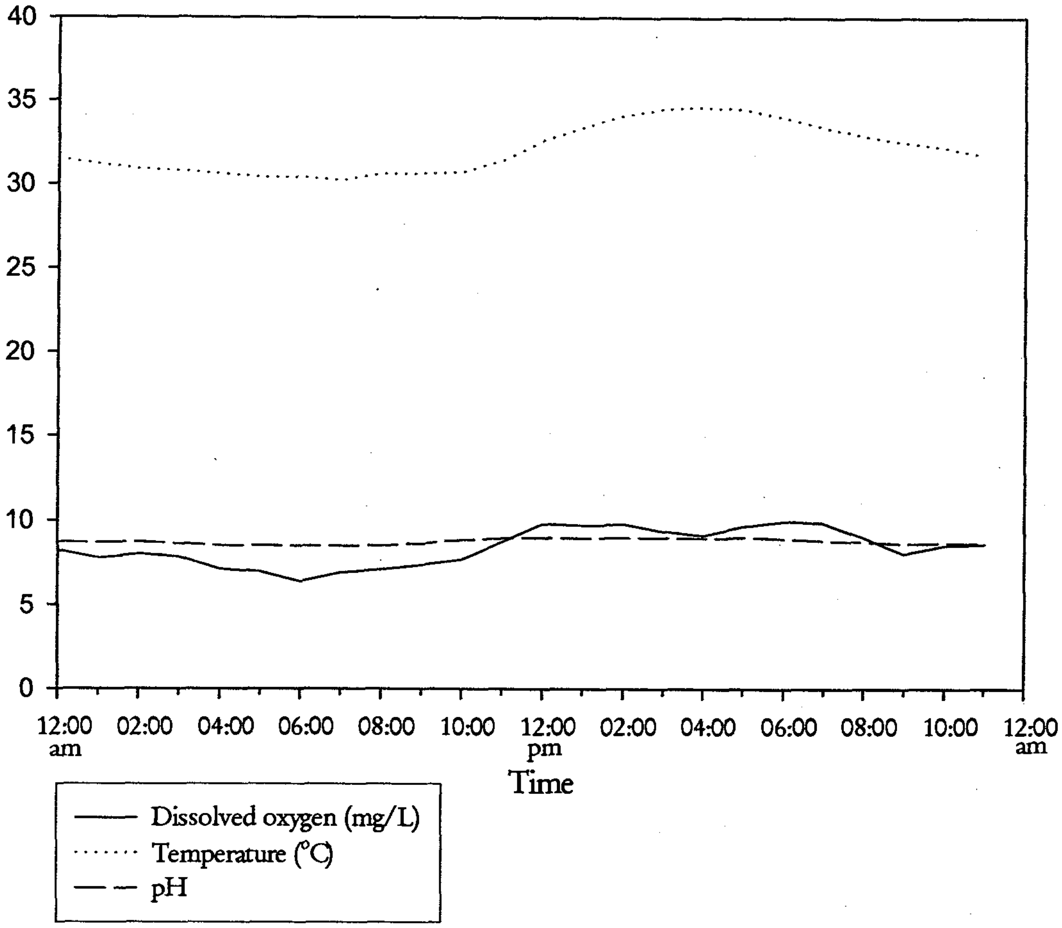


Figure 3.6 A 24-hour cycle of Dissolved Oxygen, temperature and pH found in an inland shrimp aquaculture pond without mechanical aeration.

In order to compare the water quality and organic accumulation in a typical shrimp pond treated with EM-1, the following water quality parameters were measured: *in situ* measures of Dissolved Oxygen (mg/L), pH, temperature (°C), salinity (ppt) and a 3-day biological oxygen demand (mg/L) test. Additionally, the total percent organic content of the sludge layer from the pond sediment was analyzed. Water samples were collected at 0.5 m depth using hand grabs, with 10 L jugs with rubber stoppers that were removed and replaced under water to prevent air from entering the sample. Composite samples of the sludge layer in the entire square metre were collected using an Ekman Bottom Grab Sampler. Within the laboratory, three replicates were measured for each sample to ensure accuracy of analysis. All measurement instruments were calibrated weekly for the field, and daily for the laboratory.

3.3.1: In situ measurements

On each visit, *in situ* measurements of Dissolved Oxygen, temperature, and salinity were taken using a YSI 85 Oxygen, Conductivity, Salinity and Temperature probe and the measurements of pH were determined using a HORIBA pH meter D-21 model. Secchi depth was measured using a weighted disk, 20 cm in diameter that was painted with alternating black and white quadrants.

3.3.2: Biological oxygen demand measurements

The amount of oxygen utilized in the metabolism of biodegradable materials in water is termed the biological oxygen demand (BOD). The measurement is commonly used in water and wastewater testing as an indication of the concentration of oxidizable materials present in water samples and the potential organic load this may represent for receiving waters (Tchobanoglous and Schroeder, 1985). That is, BOD measures the overall effect of the organic component of water or wastewater on oxygen resources without having to identify the specific constituents of the organic load (Lamb, 1985). Investigating the oxygen demand would also reveal whether small-scale farms typically meet Thailand's Department of Fisheries standard for effluent (10 mg/L).

The BOD of a water sample is determined by measuring the Dissolved Oxygen levels in the sample before and after a period of incubation at 20°C in the dark. The measurement determines the change or demand for oxygen by microbes as they decompose the organic matter present in the sample (Best and Ross, 1977). Internationally accepted practice recommends that the incubation period last five days. Polluted samples expected to have high BOD values can be diluted according to APHA (1992) methods to ensure the ultimate BOD is clearly quantified. However, the methodology requires a solution of dilution water that was not readily available during this study and preliminary testing revealed that 5-day incubation resulted in a zero reading on the final day, making accurate BOD calculations difficult to assess. However, the use of five incubation days is actually an arbitrary practice originally developed by the Royal Commission on Sewage Disposal in the United Kingdom where British rivers did not have a flow time to the open sea greater than five days (Tchobanoglous and Schroeder, 1985). Therefore, the theoretical basis for adopting a 3-day methodology was considered acceptable. Moreover, 5-day tests are often deemed to be too lengthy and many research scientists now have adopted shorter incubation periods (e.g. Amarasinghe *et al.*, 1993). Thus, it was determined that 3-day samples would ensure some differentiation in BOD values and allow accurate comparisons of differences in BOD readings.

Dissolved Oxygen, temperature and pH measurements of the BOD samples were made with a HACH Sension 6 portable DO meter that was calibrated daily in accordance with HACH instructions, but was also verified in preliminary tests to have a 94.5% accuracy in Dissolved Oxygen readings compared with the Winkler Titration method (APHA, 1992).

3.3.3: Total percent organic measurements

The flocculent layer, or sludge, was analyzed for the total percent organic content as a method for understanding the amount produced in a typical inland shrimp pond compared to ponds treated with EM-1. The standard ash-weight method for determining the organic content of soils was used in the laboratory (ASTM, 1987). An initial sample was placed in a pre-dried, pre-weighed crucible and weighed on a Sartorius MCI electronic scale, dried for 24 hours at 110°C in a Edusystems Binder oven, weighed again, then combusted at gradually increasing increments reaching 550°C for 8 hours (determined as the optimal combustion time in preliminary laboratory investigations) in a Carbonlite CWF 1200 Muffle Furnace to remove organics. The weight of ash remaining was then used to determine the percent total organic content.

3.4: Summary

This chapter has described the location of the field site for this study, which was conducted on an inland shrimp farm in Chachoengsao Province. The farm was typical of other operations in the area in terms of its small-scale nature and the management practices used. This chapter then outlined the types of data collected and the collection methods. Application techniques for the probiotic were documented, and *in situ* water quality parameters and biological oxygen demand measurements were taken from water samples. The total percent organic content was assessed for sludge samples. The results and analysis of these measurements are further described in Chapter 4.

4. DATA ANALYSIS AND METHODS

This chapter describes the methods selected for analyzing the field data collected throughout the study period. The results of the analyses along with general observations are presented. Interpretations of these findings and their management implications at the farm and regional levels are provided in Chapter 5.

4.1: Methods for data analysis

The first observations reported involve the general outcome of the shrimp crop with respect to the growth, health, and harvest of the shrimp. Local availability of EM-1 and the associated costs are also noted, based on observations at local retail outlets.

In order to better understand the overall results of the study, it is useful to compare the behaviour of the three individual ponds using descriptive statistics and graphical models. Separate line graphs illustrating the trends for each pond throughout the crop season are presented for each of the variables measured. As noted in Chapter 2, some of the water quality parameters measured in this study are known to affect other water quality parameters. For example, higher temperatures result in lower saturation points for Dissolved Oxygen concentrations. To understand the nature of the relationship between the measured parameters, Pearson correlation tests were performed for measurements for all the ponds, after the data were tested for the assumptions of normality.

Since the outputs leading up to the harvest are minimal in a "closed" system, the harvest output is the greatest volume released and therefore, potentially could have the most significant impact on the surrounding environment. For this reason, regardless of the values for the variables measured in each pond throughout the grow-out period, the final conditions of each pond are the most meaningful values. Accordingly, the final measurements for each water quality and sludge parameter are compared between the treatment ponds and the control pond using independent t-tests. From these results, inferences about the quality of the final effluent (which was released two days following these measurements) are drawn.

The overall research question for this study was to determine whether a commercially prepared probiotic could improve water quality and minimize the accumulation of organic sludge in an inland shrimp farm. In order to address this issue, it is necessary to compare all the measured variables between the treatment ponds and the control pond over time, and to investigate any existing interaction effects. The comparison was performed using a Repeated Measures Analysis of Variance (RMAV). The data were tested for conformance to the normality and sphericity assumptions of RMAV. Since the sample size was small, Mauchly's test of sphericity could not be calculated. As a result, F-test degrees of freedom were adjusted using the Greenhouse-Geisser test for a more conservative assessment. All statistical tests were performed at 95% confidence level using SPSS v10.0.

4.2: Results

The shrimp carapace lengths (from the base of the eye stalk to the end of the tail) of 10 randomly sampled shrimp in each pond were measured at weeks 7, 10, and 13. At week 7, Pond 1 and Pond 2 shrimp measured between 4-9 cm, while shrimp from the Control pond measured approximately 7 cm. In week 10, Pond 1 and Pond 2 shrimp measured between 5-10 cm, while the control shrimp were much larger at 13-15 cm. In spite of the original stocking densities (80-100/m^2) being equal between all three ponds, the control pond had fewer shrimp by week 10 but were much more evenly sized, while the density of the treatment ponds was higher but with mixed sizing. By week 13, the shrimp in the two treatment ponds ranged from 5-12 cm, while the shrimp in the control pond measured 15-16 cm. The farmer observed, however, that the shrimp in the control pond and in treatment pond 2 were beginning to show signs of sickness, and suggested that the shrimp carapaces appeared to have been exposed to too much iron in the water, particularly in the treatment ponds. As a result of these observations, approximately 500 kg of shrimp were harvested from each pond on day 98 of the crop cycle, although the farmer indicated that the treatment ponds contained slightly more shrimp at harvest than the control pond.

Observations at local farm supply stores surrounding the study area revealed that a number of additive products are on the market for shrimp farmers. In addition to the microbial solutions for water quality improvement other examples include products that claim to supplement the nutrients required to develop the exoskeleton (shell) of the shrimp after moulting, ones that safeguard against diseases and improve the immune system of shrimp, and ones that promote growth. While buyers' knowledge regarding the effectiveness of these products was limited to the information available on the product labels and company posters used for advertising, several farmers in the area were observed to be utilizing various additives, including EM-1. It was widely reported however, that many farmers were adopting their own application techniques. Rather than purchasing the total amount of EM-1 suggested by the manufacturer, farmers often purchase one 10 L jug and allow the microorganisms to propagate in bins with the prescribed molasses in an attempt to create more of their own solution for free. The prices quoted were typically the same in all the stores regardless of location, although success or failure in haggling (a standard practice in Thailand) may change that value for many of the farmers.

4.2.1 Dissolved Oxygen

Dissolved Oxygen is expressed as a solubility factor of milligrams of oxygen per litre of water (Boyd and Tucker, 1998). Throughout this study, mean Dissolved Oxygen concentrations for all of the ponds ranged between 3.00 mg/L to 13.77 mg/L (Figure 4.1). The solubility of oxygen in water decreases as water temperature and salinity increase (Boyd and Tucker, 1998). In fact, research has determined the expected Dissolved Oxygen solubility concentrations, based on specific temperatures, salinity, and atmospheric pressure. In the samples of this study, which ranged in temperature between 28 to 35°C and in salinity from 0 to 10 ppt at 760 mm Hg, saturation would be reached between 6.58 mg/L and 7.81 mg/L (based on information from: Boyd, 1990). Measurements exceeding these values (such as at week 11) indicate supersaturation may have occurred, most likely due to the mechanical aeration. Measurements below these levels occur when the water is undersaturated, that is, when oxygen is consumed quicker than it can enter the system. Exposure of shrimp to Dissolved Oxygen levels below 4-5 mg/L can reduce growth rates, feeding efficiency and molting frequency (Boyd and Tucker, 1998), although Seidman and Lawrence (1985) found that *P. monodon* could survive continuous exposure to Dissolved Oxygen concentrations between 1.9-4.0 mg/L.

The Dissolved Oxygen concentration trends throughout the crop cycle illustrate a "boom and bust" type of pattern (Figure 4.1). The overall trend demonstrates a pattern whereby Dissolved Oxygen within the ponds would begin to decline as the shrimp and other aquatic organisms consumed oxygen through respiration in amounts greater than the photosynthesizing phytoplankton and mechanical aerators could produce it. When the

Dissolved Oxygen values of the pond declined to levels that would no longer sustain the shrimp or that might have increased the shrimps' vulnerability to disease, conditions often visibly deteriorated (e.g. drastic decline in water clarity, algal blooms). The farmer then added or exchanged water within the ponds to improve water quality, which temporarily increased Dissolved Oxygen concentrations. For example, the farmer reported that following the sample collections of week 7, a depth of approximately 10 cm of the control pond was exchanged due to poor water quality (mean Dissolved Oxygen = 6.39 ± 0.09 mg/L). Improvements in the Dissolved Oxygen values were observed in this particular pond in week 8 (mean Dissolved Oxygen = 7.47 ± 0.27 mg/L)(Figure 4.1). Variation for Dissolved Oxygen values observed throughout the shrimp crop season may also be the result of the natural environment rather than farm management practices. For example, large inputs of freshwater occurred as a result of heavy monsoons during the entire week following week 2 collections, which effectively re-set the Dissolved Oxygen concentrations in all the ponds with a flush of low-saline water (Figure 4.1, Table 4.1).

It is worth noting that the two treatment ponds both exhibited lower mean Dissolved Oxygen concentrations than the control for the final measurements and that treatment pond 1 consistently had lower Dissolved Oxygen concentrations throughout the crop season.

Table 4.1 Mean Dissolved Oxygen concentrations (in mg/L) and standard deviations for each pond in week 2 and week 3 with total mean change (mg/L).

Pond	Week 2		Week 3		Total Mean Change
	Mean	S.D.	Mean	S.D.	
Control	4.03	0.09	9.17	0.29	+5.14
Treatment Pond 1	3.89	1.58	8.36	0.09	+4.47
Treatment Pond 2	3.00	0.46	8.58	0.15	+5.58

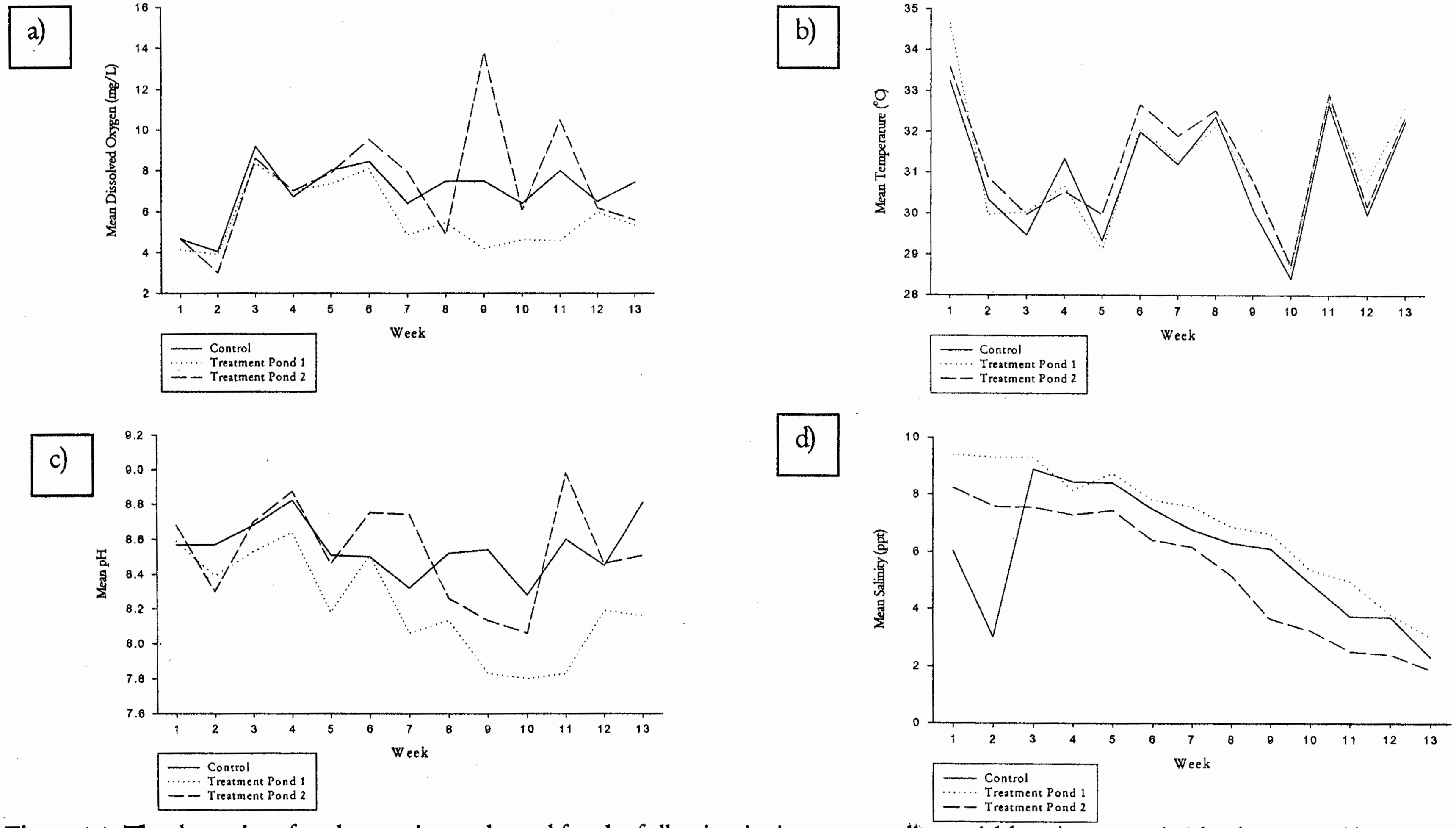


Figure 4.1 The dynamics of each experimental pond for the following *in situ* water quality variables: a) Mean Dissolved Oxygen, b) Mean Temperature, c) Mean pH and d) Mean Salinity.

Dissolved Oxygen solubility is affected by other water quality parameters such as salinity and temperature. The relationships between these water quality variables are discussed in the appropriate sections that follow.

The final values (week 13) for mean Dissolved Oxygen concentrations did not significantly differ between the treatment ponds and the control (*t*= 8.79; df= 1; $p<0.07$)(Figure 4.2). Therefore, it can be inferred that the Dissolved Oxygen concentrations in the discharge released at harvest did not differ significantly between ponds.

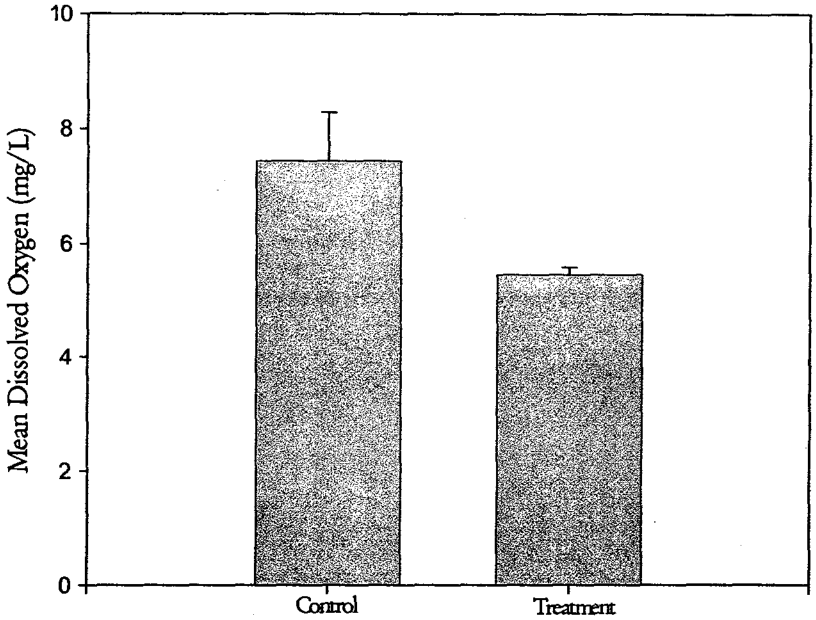


Figure 4.2 Final measurements of mean *in situ* Dissolved Oxygen concentrations (in mg/L) with standard error illustrating no significant difference ($p<0.07$) between the treatment and control ponds.

The *in situ* Dissolved Oxygen concentrations did not significantly differ in the treatment or the week, and neither of those factors interacted over the entire crop cycle (treatment x week: F=0.15; df=1; $p<0.77$, treatment: F=0.10; df=1; $p<0.81$, week: F=1.39; df=1; $p<0.45$) (Figure 4.3). Therefore, it can be inferred that the Dissolved Oxygen concentrations in the water released during the grow-out period for exchange and the effluent released at harvest were equal among the control and probiotic treatment ponds. However, it is worth noting that for 9 out of the 13 weeks (69%) the treatment ponds had lower mean Dissolved Oxygen concentrations than the control.

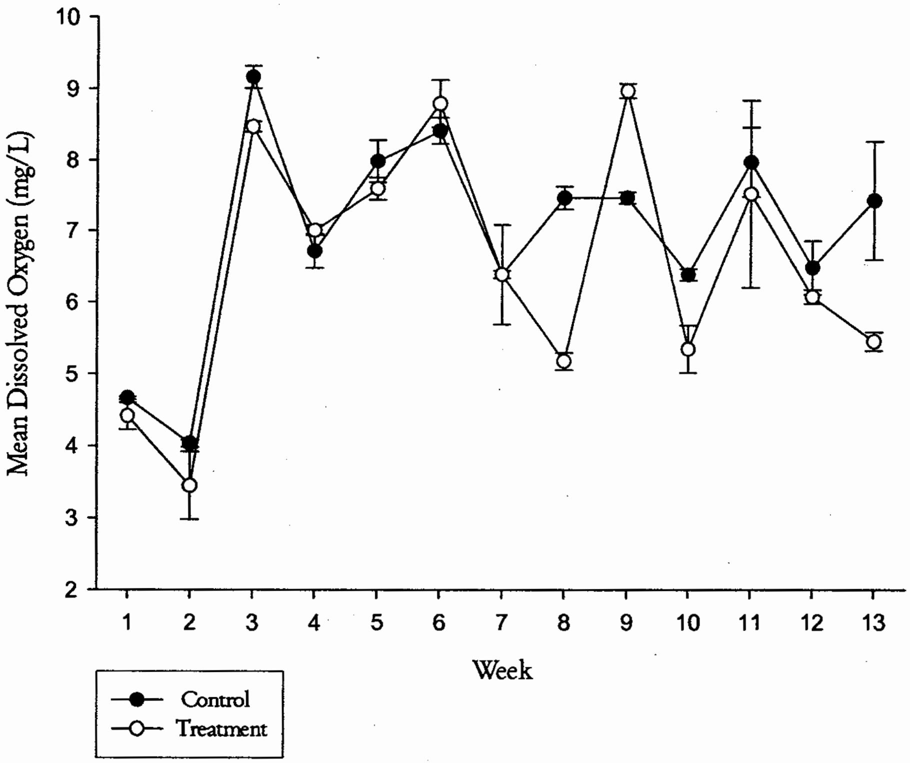


Figure 4.3 Mean *in situ* Dissolved Oxygen concentrations (mg/L) with standard error of the control and treatment ponds over the study period illustrating no significant difference between treatments or weeks, and no significant interaction of the two factors ($p<0.81$, $p<0.45$, $p<0.77$, respectively).

4.2.2: Temperature

The temperature of a pond is the result of prevailing atmospheric temperatures and solar radiation; it is unlikely that either the farmer, or the probiotic treatment could influence the temperature. Inputs from the reservoir and the Bang Pakong River for topping off and water exchange would be expected to be approximately the same temperature, as they are exposed to the same local conditions. Due to the nearly constant mechanical aeration and shallow depth of these ponds, thermal stratification does not occur and temperatures tend to remain uniform throughout the pond (Boyd, 1995a).

Temperature fluctuations did occur week to week within the ponds, ranging from 28.40°C to 34.63°C over the shrimp grow-out period (Figure 4.1). As long as temperatures do not change too rapidly (i.e. more than 0.5°C per minute for changes greater than 5°C), shrimp are able to adapt physiologically (Boyd and Tucker, 1998). These natural fluctuations, however, largely influence the entire pond system. As explained in Chapter 2, monitoring temperature in any water quality study is important due to the effects that temperature has on many other processes functioning within the ponds. Accordingly, temperature and *in situ* Dissolved Oxygen concentrations do follow the expected trend for weeks 1 to 8, with Dissolved Oxygen decreasing when temperature increases (Figure 4.1). This pattern may have occurred for two reasons. Firstly, the saturation point for Dissolved Oxygen concentration would have declined with the increasing temperatures. Secondly, the pattern may have emerged as a result of the relationship between temperature and shrimp metabolism. As water temperature rises, the metabolic rate of the shrimp (and all other aquatic organisms present) will increase, which affects their energy requirements (Alzieu, 1990). As the shrimp consume organic matter in greater amounts to meet their requirements, Dissolved Oxygen is utilized, thereby decreasing the overall concentration in the ponds.

However, from week 9 to the end of the crop cycle the Dissolved Oxygen concentrations seem to have been affected by other parameters more significantly than by temperature. This pattern is particularly evident in week 10 when the temperature reached its lowest point, but the Dissolved Oxygen did not peak (Figure 4.1). In fact, temperature was not significantly correlated with any of the *in situ* water quality parameters (Dissolved Oxygen: $r = -0.09$; $p<0.57$, pH: $r = 0.27$; $p<0.09$, salinity: $r = -0.08$; $p<0.63$, Secchi depth: $r = -0.06$; $p<0.81$)(Table 3)(Figure 4.4).

The mean temperature of the control and treatment ponds did not differ significantly in the final week's measurements ($t=-1.23$; df=1; $p<0.44$)(Figure 4.5). Consequently, it can be inferred that the effluent from each of the ponds would not have significantly differed in temperature.

The mean temperature of the ponds did not significantly differ between the treatment and control ponds over the entire crop cycle, and treatment and week effects did not interact with one another (treatment x week: F=0.63, df=1; $p<0.57$; treatment: F=6.95, df=1; $p<0.23$; week: F=36.87, df=1; $p<0.10$)(Figure 4.6). Therefore, it can be inferred that the mean temperature of the water released during the grow-out period for exchange and the effluent released at harvest were equal among the control and probiotic treatment ponds.

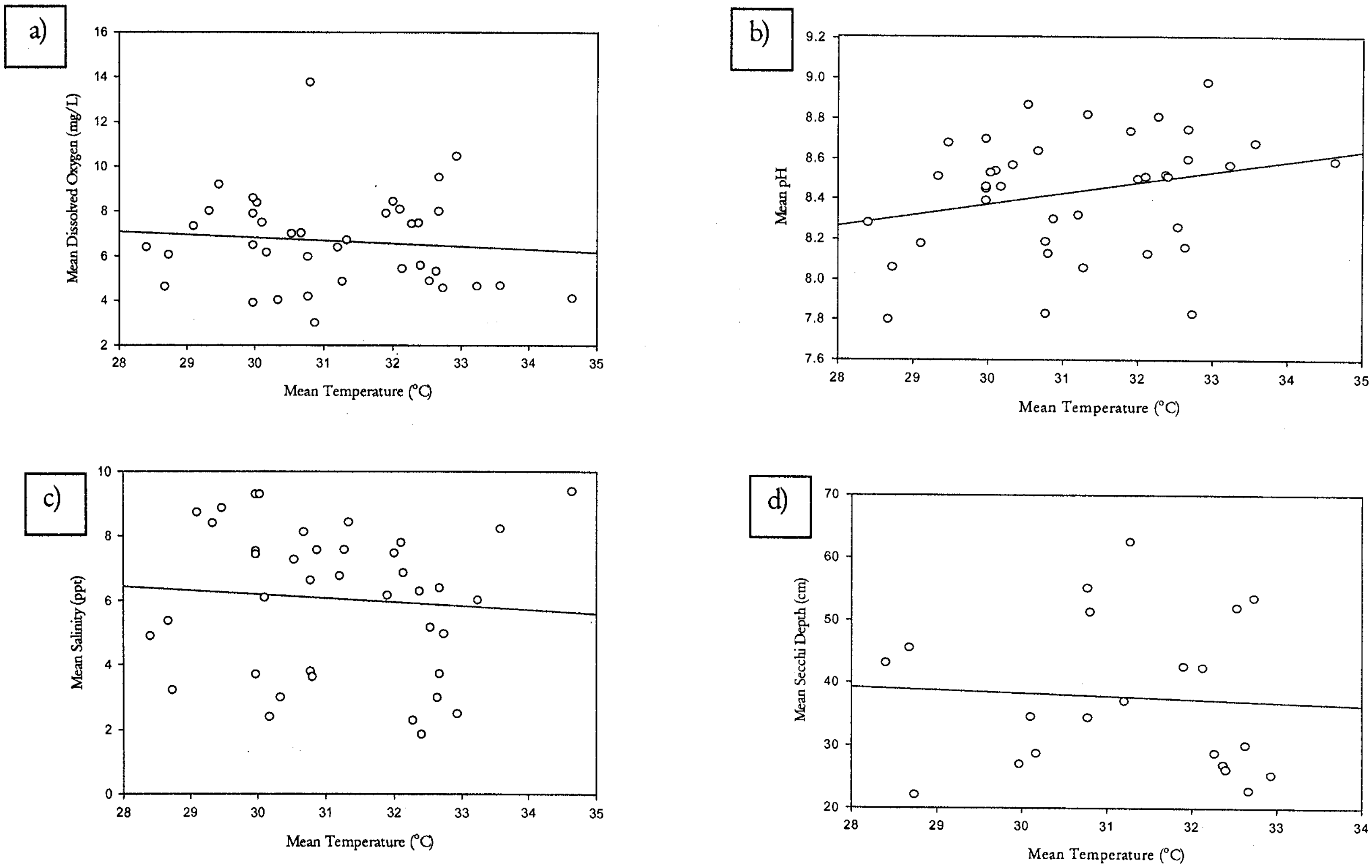


Figure 4.4 Plots illustrating the correlation between mean temperature (°C) and: a) Dissolved Oxygen concentrations (mg/L) ($p<0.57$), b) pH ($p<0.09$), c) salinity (ppt) ($p<0.63$) and d) Secchi depth (cm) ($p<0.81$).

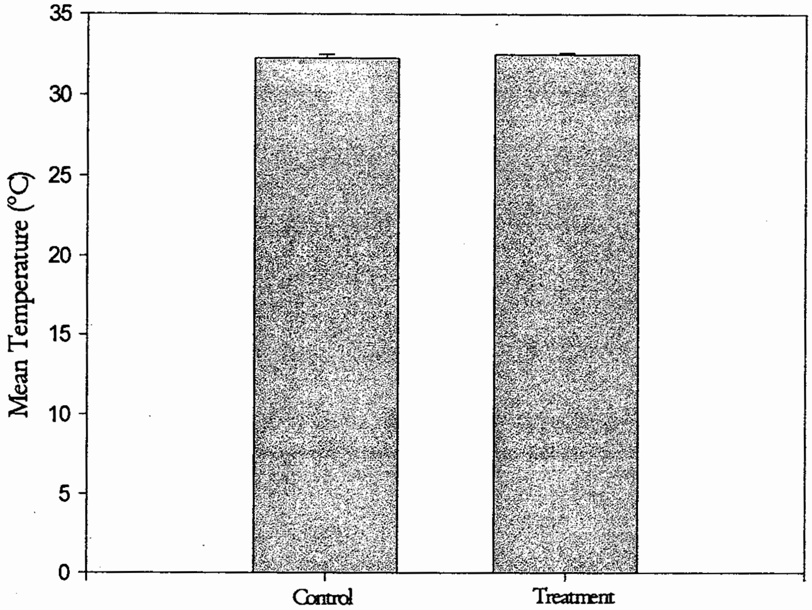


Figure 4.5 Final measurements of mean *in situ* temperature (°C) with standard error illustrating no significant difference ($p<0.44$) between the treatment and control ponds.

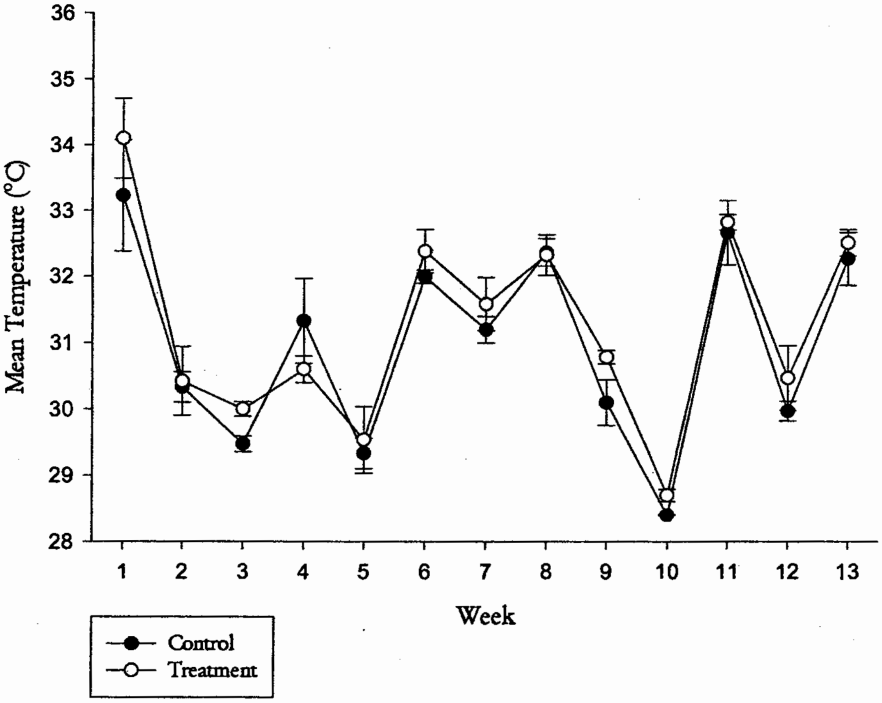


Figure 4.6 Mean *in situ* temperature (°C) with standard error of the control and treatment ponds, depicting no significant difference between treatments or weeks and no significant interactions between the two factors ($p<0.23$, $p<0.10$, $p<0.57$, respectively).

4.2.3: pH

Aquatic organisms can survive fluctuations in pH, but may not survive long bouts of extreme values (highly acidic or highly basic). Drastic fluctuations in pH did not occur in any of the ponds. Differences between the minimum and maximum pH measurements in each pond always measured less than 1.0, with the entire cycle ranging between 7.80 and 8.98 for all of the ponds (Figure 4.1). General trends indicate that treatment pond 1 consistently measured lower for *in situ* pH and both treatment ponds had lower pH values than the control in the final week (Figure 4.1). The pH of most aquaculture ponds will cycle diurnally with changes in photosynthesis and respiration that correspond to changes in daylight. Since the sampling for *in situ* measurements took place at the same time each week, diurnal fluctuations were not expected to be the cause of any variation week to week.

As explained in Chapter 2, pH values are highly interrelated with other water quality parameters. Supporting this theory, the mean pH measurements for all of the ponds were positively correlated with mean Dissolved Oxygen concentrations ($r = 0.38$; $p<0.02$)(Figure 4.7). The mean pH measurements were not significantly correlated with mean *in situ* temperature ($r = -0.09$; $p<0.57$) or salinity ($r = -0.07$, $p<0.68$)(Figure 4.7). However, mean Secchi disk visibility, or depth, was significantly correlated with pH ($r = -0.61$, $p<0.00$)(Figure 4.7).

Although the pH measurements for the final week differed by nearly 0.5 units for the treatment and control ponds, a significant difference between the pH of the treatment and control ponds did not exist ($t = -1.23$; df=1; $p<0.44$)(Figure 4.8). Consequently, it can be assumed that the pH of the effluent released at harvest was equal between the ponds.

Also, the repeated measures ANOVA test found that the ponds were not significantly different for treatment or week throughout the entire crop cycle, and that neither treatment nor week significantly interacted with the other factor (treatment x time: F=0.64; df=1; $p<0.57$, treatment: F=0.43; df=1; $p<0.63$, week: F=1.82, df=1; $p<0.41$) (Figure 4.9). Thus, the mean pH of the effluent released during the crop cycle for water exchange and at harvest was equal among the control and probiotic treatment ponds.

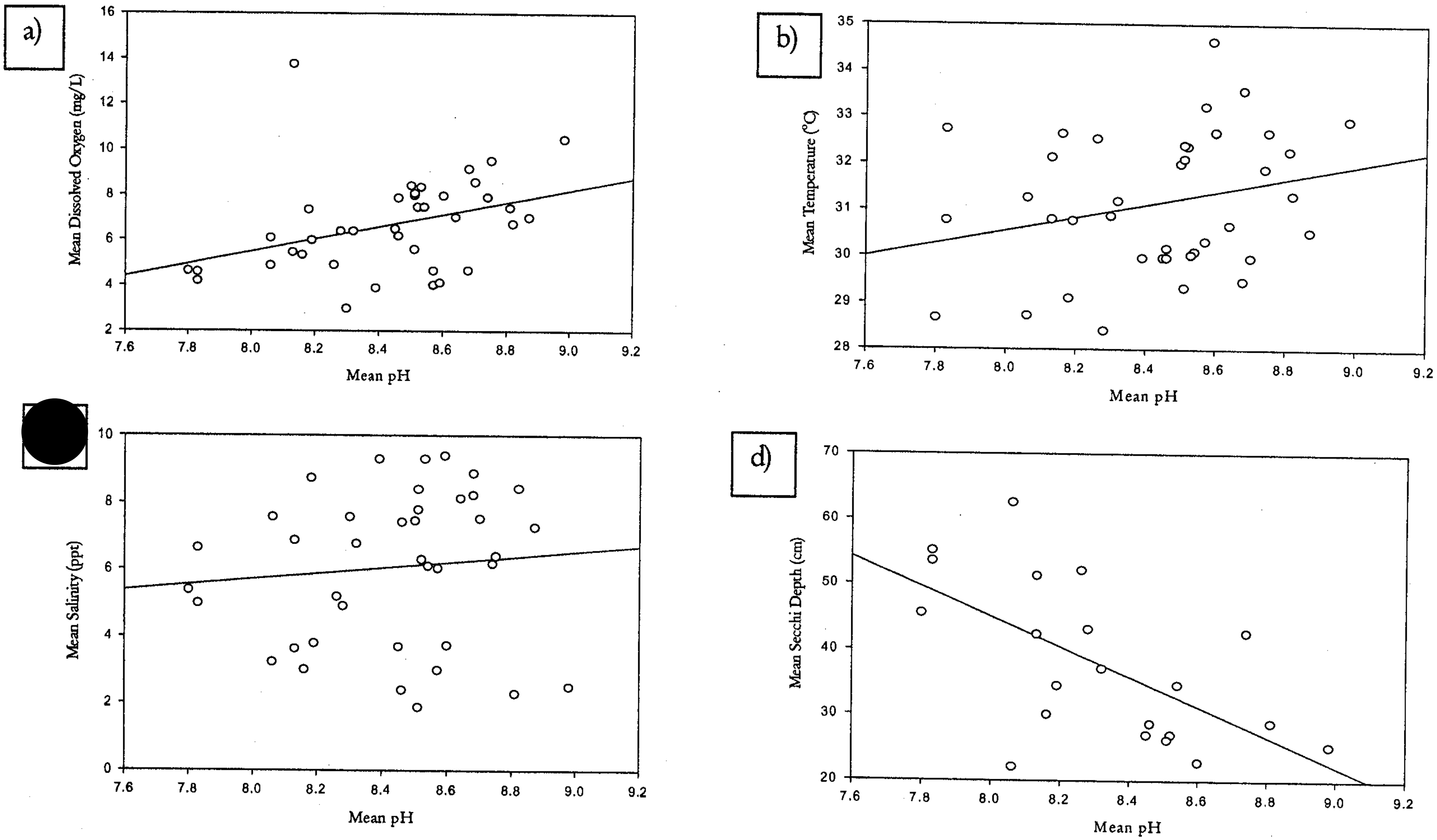


Figure 4.7 Correlation relationships between mean *in situ* pH and the following *in situ* parameters: a) mean Dissolved Oxygen concentrations (mg/L)($p<0.02$), b) mean temperature (°C)($p<0.57$), c) mean salinity (ppt)($p<0.68$) and d) mean Secchi disk visibility (cm)($p<0.00$).

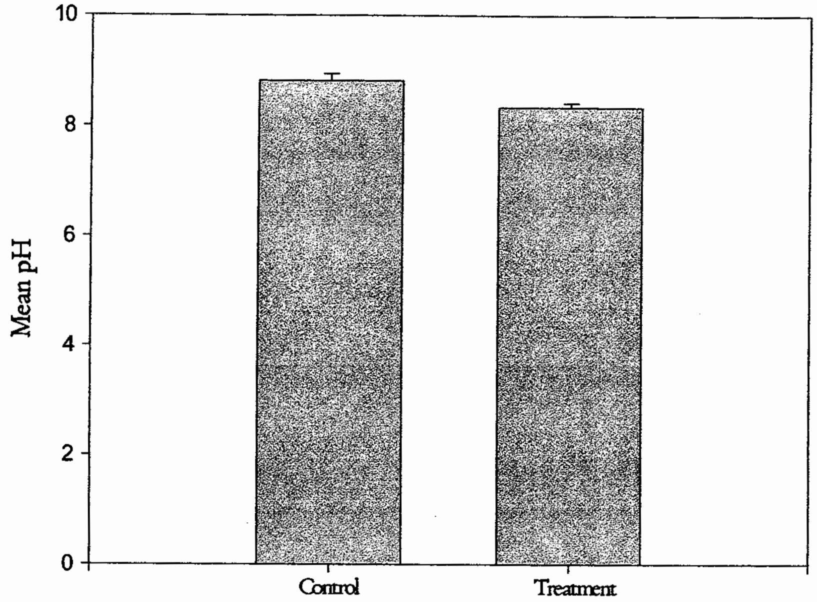


Figure 4.8 Comparison of final mean *in situ* pH values with standard error between the control and treatment ponds illustrating no significant difference ($p<0.44$).

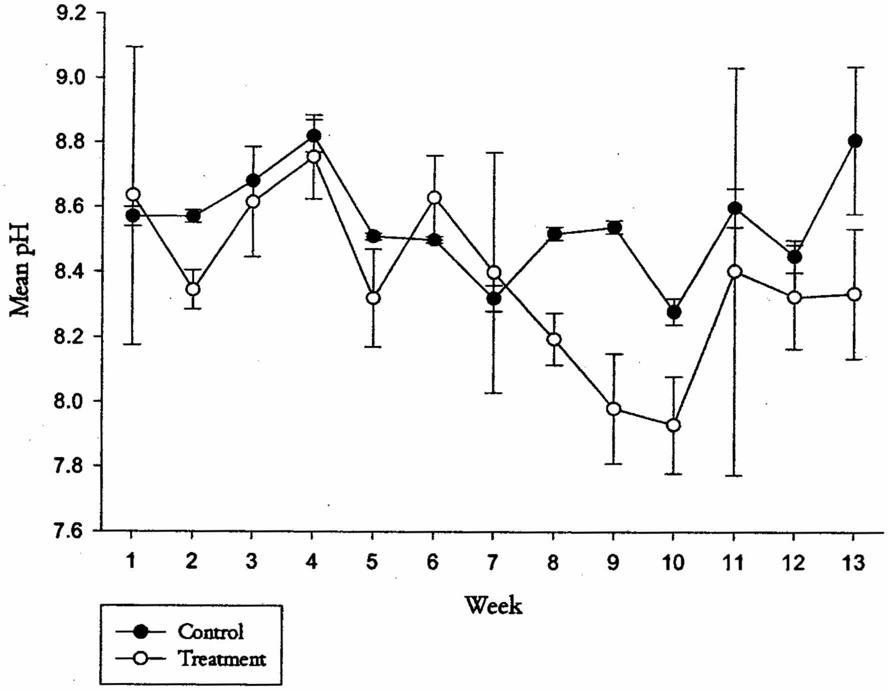


Figure 4.9 Mean *in situ* pH with standard error of the control and treatment ponds, depicting no significant difference throughout the crop cycle between treatments or weeks and no significant interactions between these two factors ($p<0.43$, $p<0.41$, $p<0.57$, respectively).

4.2.4: Salinity

Fluctuations in salinity during a crop season are often the result of high evaporation rates that increase salt concentrations or high precipitation, which causes a decline in salt concentrations. The relationship between rainfall in Bangkok, Thailand and the salinity of the Bang Pakong River is documented in Table 4.2. This study took place during Thailand's wet season and as a result, heavy rains were common and high evaporation rates were infrequent. Accordingly, high/low fluctuations of salinity levels during this particular study were not often observed (Figure 4.1). The other factor influencing the salinity of the ponds involved the addition of freshwater from the reservoir throughout the season. Since all inputs (rainwater and freshwater for exchange) contained minimal salt concentrations, it is not surprising that the salinity of all three ponds would steadily decline throughout the shrimp rearing cycle, with measurements ranging from 9.40 ppt at the beginning of the cycle to 1.87 ppt by the end (Figure 4.1).

Table 4.2 Relationship between rainfall in Bangkok, Thailand and the salinity at the mouth of the Bang Pakong River (Boyd, 1990).

Month	Rainfall(mm)	Bang Pakong salinity (ppt)
January	10.3	31.2
February	30.7	31.2
March	23.7	32.1
April	63.5	32.0
May	185.3	29.3
June	159.8	10.3
July	170.8	7.3
August	198.2	5.6
September	341.8	7.2
October	221.3	18.5
November	44.0	28.2
December	8.0	27.9

Although the very existence of inland low-salinity farming demonstrates that *P. monodon* can be acclimatized to a wide range of salinity levels, salinity is an important water quality variable due to the effect the salt concentration may have on the solubility of other dissolved gases in the water. As already noted, the solubility of Dissolved Oxygen decreases with increasing salinity (Boyd and Tucker, 1998). However, mean salinity levels were not significantly correlated with mean Dissolved Oxygen concentrations ($r = -0.07$, $p<0.68$)(Figure 4.10). As forementioned, mean *in situ* temperature and pH were not significantly correlated with salinity ($r = -0.08$; $p<0.63$ and $r = 0.11$; $p<0.52$, respectively). A positive significant relationship did exist between salinity and Secchi depth ($r = 0.64$, $p<0.00$)(Figure 4.10).

The final measurements of pond salinity did not significantly differ between ponds ($t= -0.14$; df=1; $p<0.91$) (Figure 4.11). Presumably, the mean salinity of the final effluent released at harvest did not significantly differ between ponds.

A significant difference did not exist between the mean pH measurements of the treatment and control ponds over the entire shrimp grow-out period and no significant interaction between these two factors (treatment and week) occurred (treatment x week: F=12.31, df=1; $p<0.18$, treatment: F=0.05; df=1; $p<0.86$, week: F=60.95; df=1; $p<0.08$)(Figure 4.12). Therefore, it is understood that the mean salinity of the effluent released throughout the crop cycle for water exchange and at harvest was equal among the control and probiotic treatment ponds.

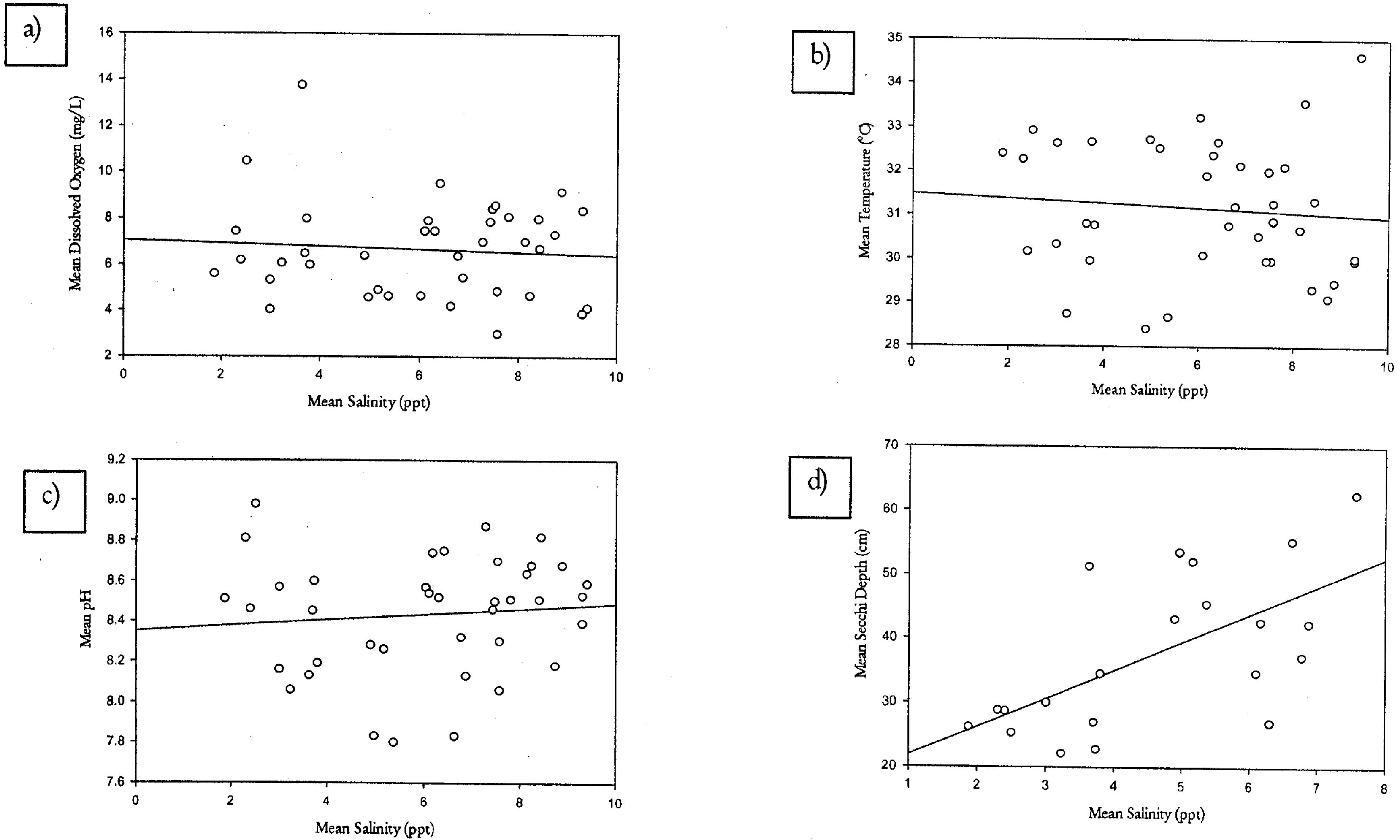


Figure 4.10 Correlation relationships between mean *in situ* salinity (ppt) for the following *in situ* water quality parameters: a) mean Dissolved Oxygen concentrations (mg/L)(p<0.68), b) mean temperature (°C)(p<0.63), c) mean pH(p<0.52), and d) mean Secchi depth (cm)(p<0.00).

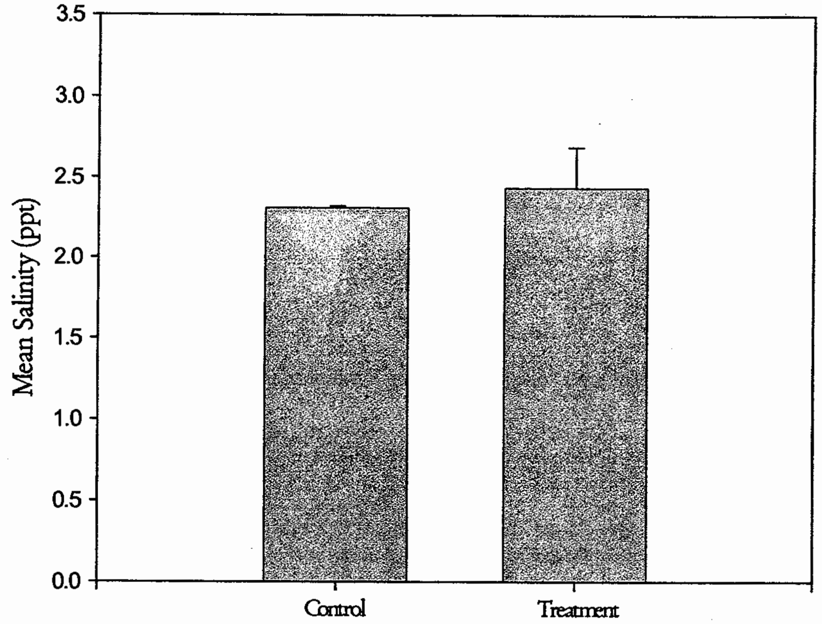


Figure 4.11 A comparison of the final mean *in situ* salinity (ppt) with standard error in the control and treatment ponds, illustrating no significant difference ($p < 0.91$).

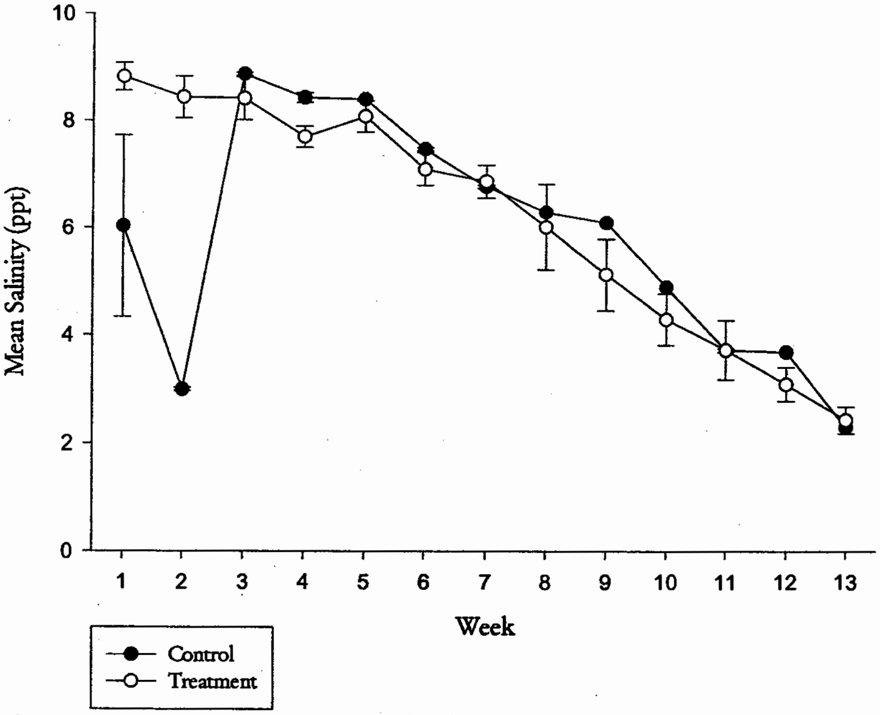


Figure 4.12 Mean *in situ* salinity (ppt) plot with standard errors for control and treatment ponds over time showing no significant difference between treatments or week and no interactions between the two factors ($p < 0.86$, $p < 0.08$, $p < 0.18$ respectively).

4.2.5: Secchi disk visibility

The visibility of water in aquaculture ponds can be affected by suspended material such as soil particles, plankton, and organic detritus (Boyd and Tucker, 1998). The mechanical aeration that takes place on the majority of inland shrimp aquaculture ponds results in a constant mixture and suspension of solid particles. These particles cause turbidity, meaning that light is scattered or absorbed rather than transmitted through the water in a straight line (Boyd and Tucker, 1998), thereby decreasing the amount of available light for photosynthesizing organisms as depth increases. The decreased photosynthetic activity in turn, can decrease the amount of Dissolved Oxygen within the pond. Water exchange can reduce the problem by removing a portion of the suspended particles along with the water and replacing it with water from the reservoir that has the sediment settled out. The constant feed inputs and aeration within the ponds, however, mean that the water exchange effects are only temporary. Moreover, the turbidity problem has then been transferred to the waterways receiving the pond effluent where sedimentation and eutrophication can occur.

Secchi disk visibility decreased within each pond throughout the crop cycle (Figure 4.13). Although legal standards have not been set for disk visibility, experts in the field recommend a depth of 30-45 cm to ensure optimal rearing conditions for the shrimp (Boyd and Tucker, 1998). The control pond and treatment pond 2 began with visibility in the recommended range, and treatment pond 1 possessed a photic zone much deeper than 45 cm. By the end of the crop season, only treatment pond 1 remained within Boyd and Tucker's (1998) recommended range, but this end result may be due to the extremely clear starting conditions rather than any effects of the probiotic (Figure 4.13). General observations of pond clarity collected for this study stated that for weeks 7-9 the treatment ponds looked clearer than the control pond, which was murky and greenish. From week 10 to week 13, however, treatment pond 1 was observed to be much clearer than both the control pond and treatment pond 2. Treatment pond 2 appeared to become more brownish and murky in colour and by week 12 an algal bloom covered the pond surface. These general observations correspond to the Secchi disk visibility measurements for each pond that indicate a greater visibility for the treatment ponds for weeks 7-9 and then greater visibility for only treatment pond 1 for the remaining weeks (Figure 4.13).

A significant correlation between mean *in situ* Dissolved Oxygen concentrations and mean Secchi disk visibility did not exist ($r = -0.21, p<0.36$)(Figure 4.14). The relationship between Secchi disk visibility and the remaining *in situ* water quality parameters were presented in the previous sections.

The final mean Secchi depth measurements did not significantly differ between the control and treatment ponds ($t = 0.05$; df=1; $p<0.97$)(Figure 4.15). Therefore, it can be inferred that the mean Secchi disk visibility was equal in all of the effluent released at harvest.

The mean Secchi disk visibility measurements did not significantly differ for the treatment, the week, or the interaction of the treatment and week factors throughout the entire crop cycle (treatment x week: F=0.94; df=1; $p<0.51$, treatment: F=1.01; df=1; $p<0.50$, week: F=1.34; df=1; $p<0.45$)(Figure 4.16). As a result, it is assumed that the mean Secchi disk visibility of the effluent released throughout the entire shrimp grow-out cycle for water exchange and at the time of harvest was equal for the control and probiotic treatment ponds.

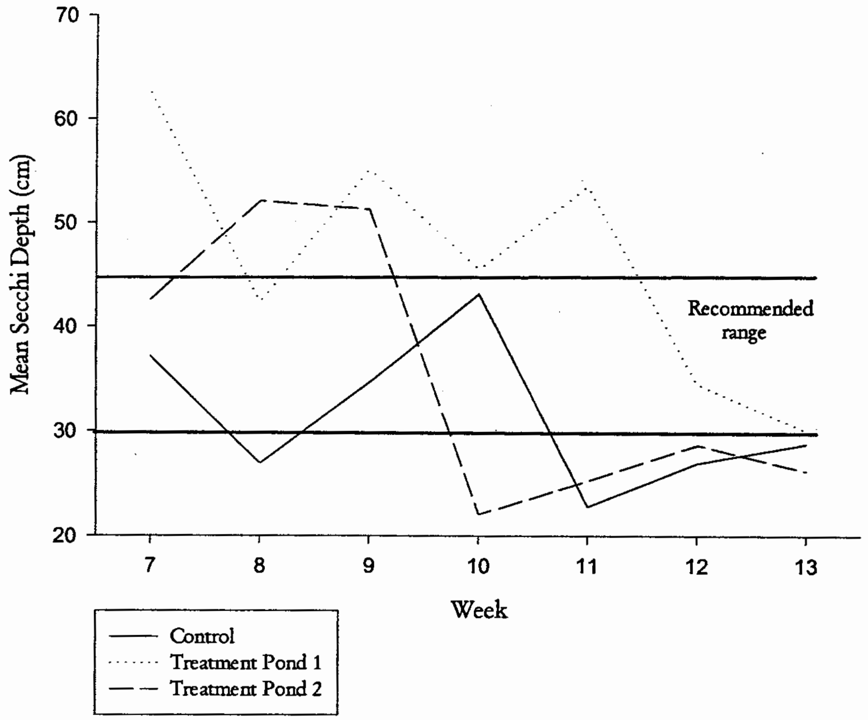


Figure 4.13 Individual pond dynamics for mean *in situ* Secchi disk visibility (cm) for weeks 7 to 13.

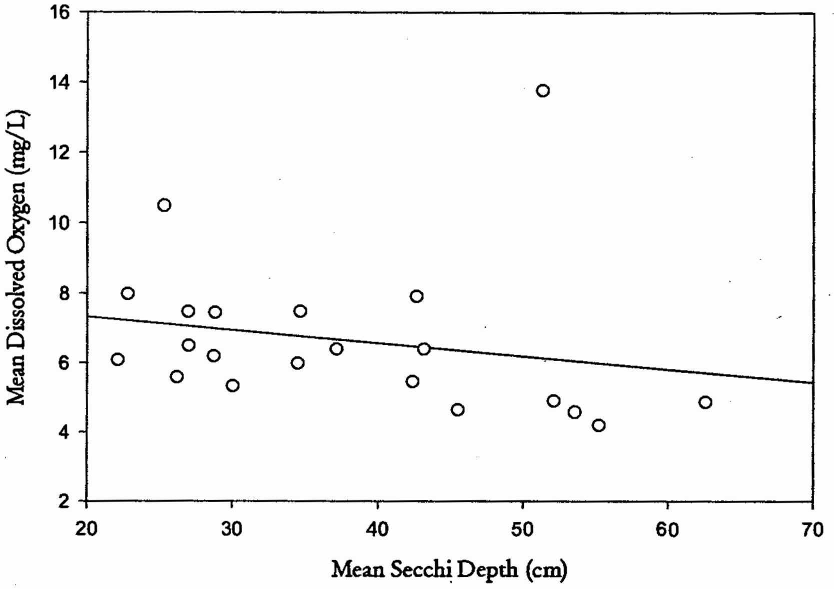


Figure 4.14 Illustration of the correlation between mean *in situ* Secchi disk visibility (cm) and mean *in situ* Dissolved Oxygen concentrations (mg/L) (p<0.36).

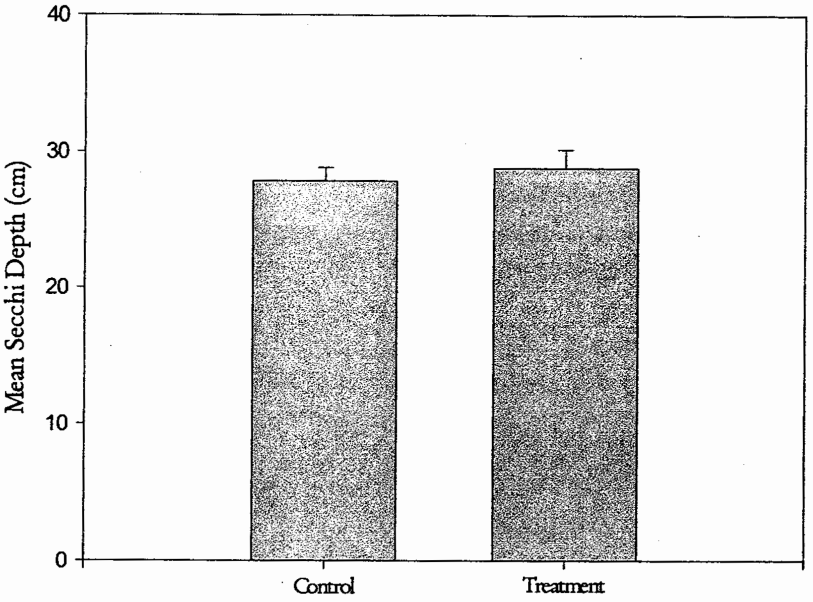


Figure 4.15 Comparison of final mean *in situ* Secchi disk visibility (cm) with standard error for the control and treatment ponds, illustrating no significant difference ($p<0.97$).

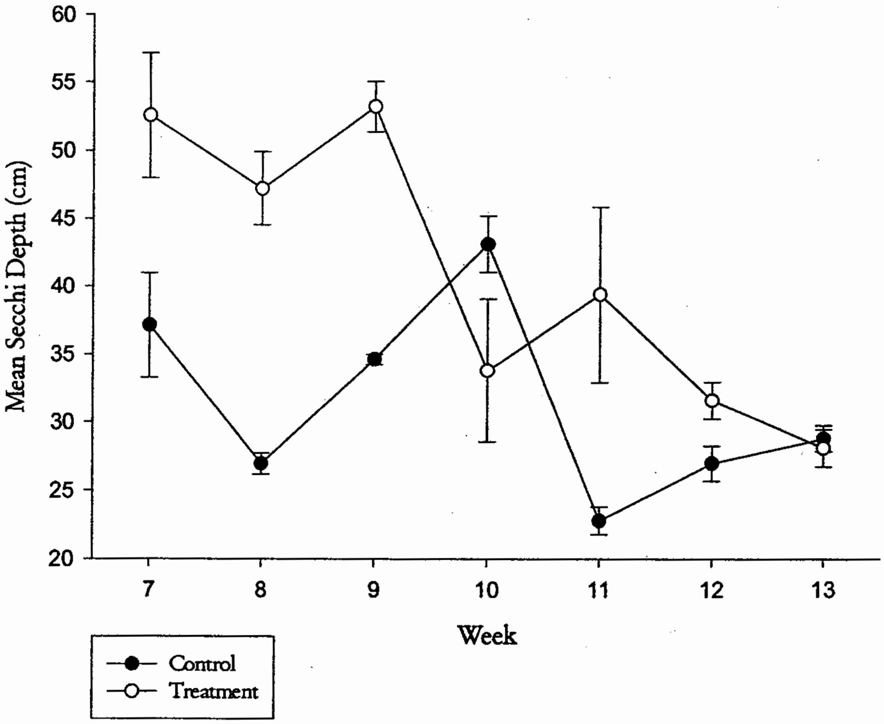


Figure 4.16 Mean *in situ* Secchi depth (cm) measurements with standard error illustrating no significant difference between treatment or week and no significant interaction between the two factors ($p<0.50$, $p<0.45$, $p<0.51$, respectively).

4.2.6: Biological oxygen demand

One of the principal parameters of interest to this study is BOD, as the Thai government has set legal BOD standards for pond effluent. Only treatment pond 1 had starting conditions that met the legal standard of an oxygen demand below 10 mg/L. These initial conditions indicate that the water already in the river does not meet the effluent criteria set by the government of Thailand (Figure 4.17). By the end of the crop season, all three ponds contained water that exceeded the legal limit for BOD in effluent (Figure 4.17). However, with the exception of weeks 4-6, the BOD of treatment pond 1, and ultimately its effluent, was lower than the other ponds (Table 5, Figure 4.17).

Table 4.3 A comparison of the final 3-day biological oxygen demand (BOD)(mg/L) values in week 13 for each pond and the amount the effluent exceeded the Thai government's legal limit of 10 mg/L.

Pond type	3-day BOD (mg/L)	Compared to legal limit (mg/L)
Control	30.3333	+20.3333
Treatment Pond 1	25.9037	+15.9037
Treatment Pond 2	30.1667	+20.1667

Week 1 and week 2 observations noted that the treatment ponds were clearer than the control pond, which was greenish and murky. These observations are supported by the fact that the BOD measurements were lower for the two treatment ponds, which indicates a lower level of organic and oxidizable particles in the water column (Figure 4.17). Additional support for these observations is found with the pond clarity described for Secchi disk visibility. Again, since BOD measurements quantify the amount of oxidizable material within a pond sample through oxygen depletion, the Secchi disk visibility measurements should correspond since visibility would decline with an increase in organic particles within the water column. In fact, mean BOD and Secchi disk visibility are significantly correlated in a negative relationship ($r = -0.70; p<0.00$)(Figure 4.18). Comparing mean BOD to the other *in situ* water quality parameters, however, does not provide a meaningful analysis since the conditions of the water in the BOD samples are dependent upon the conditions within the bottle stored in the laboratory.

The final mean BOD values are not significantly different between the control and treatment ponds ($t = 0.62$; df=1; $p<0.65$)(Figure 4.19). Consequently, it is understood that the mean BOD of the effluent released at harvest did not significantly differ between the control and treatment ponds.

Although graphical trends indicate that the treatment ponds experienced a lower oxygen demand and thus, may have had lower concentrations of oxidizable materials in the water column, mean BOD measurements did not significantly differ between treatments or weeks, and treatment and week effects did not interact throughout the entire crop cycle (treatment x week: F=1.02; df=1; $p<0.50$, treatment: F=4.37; df=1; $p<0.28$, week: F=4.49; df=1; $p<0.28$)(Figure 4.20). Therefore, it can be inferred that the mean biological oxygen demand of water released for water exchange throughout the crop cycle and at the time of harvest was equal among the control and probiotic treatment ponds.

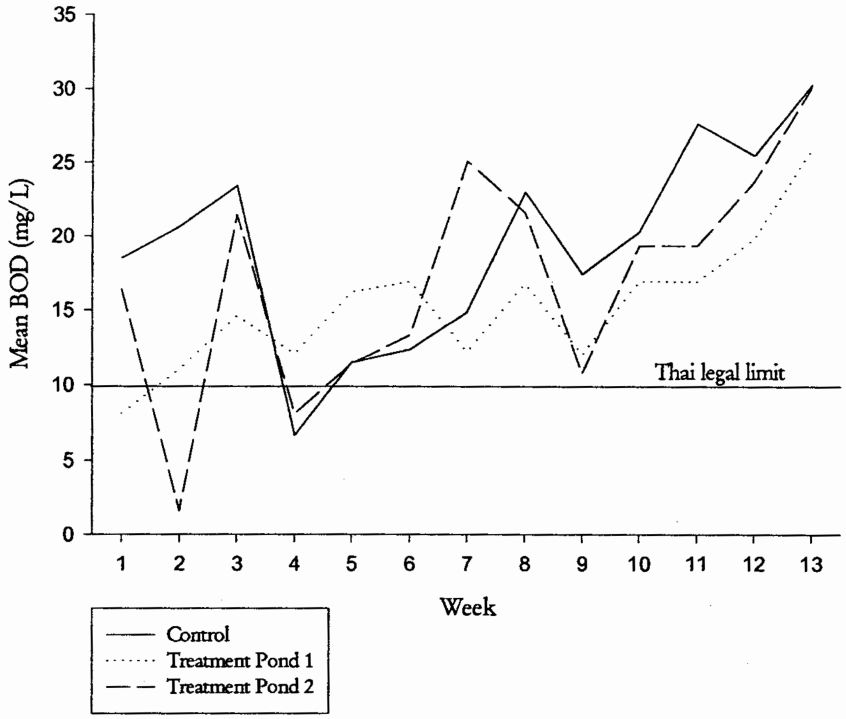


Figure 4.17 Individual pond measurements for biological oxygen demand (BOD)(mg/L) for the entire crop cycle. Note: The decline observed in the measurements for pond 2 in week 2 were the result of laboratory error.

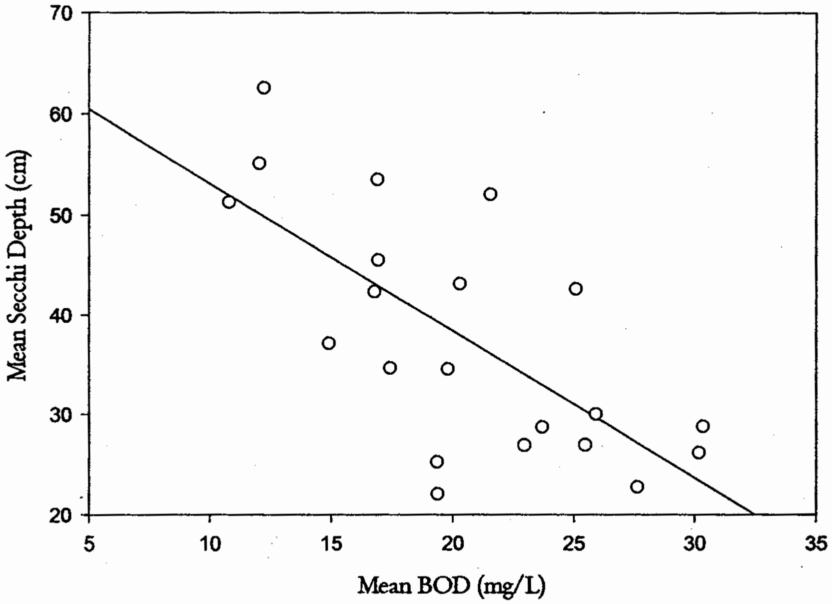


Figure 4.18 Relationship between mean BOD (mg/L) and mean Secchi disk visibility (cm) showing a significant negative correlation ($p<0.00$).

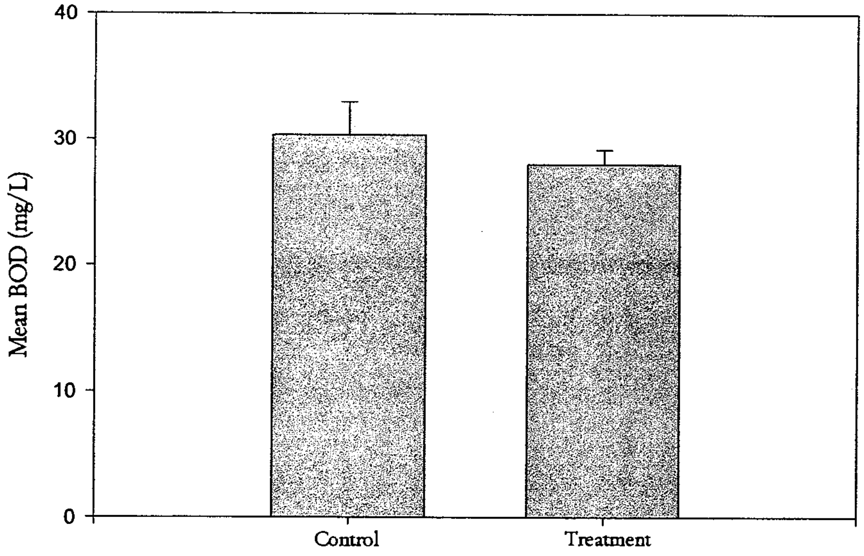


Figure 4.19 Comparison of final mean biological oxygen demand (mg/L) with standard error in control and treatment ponds, depicting no significant difference ($p<0.65$).

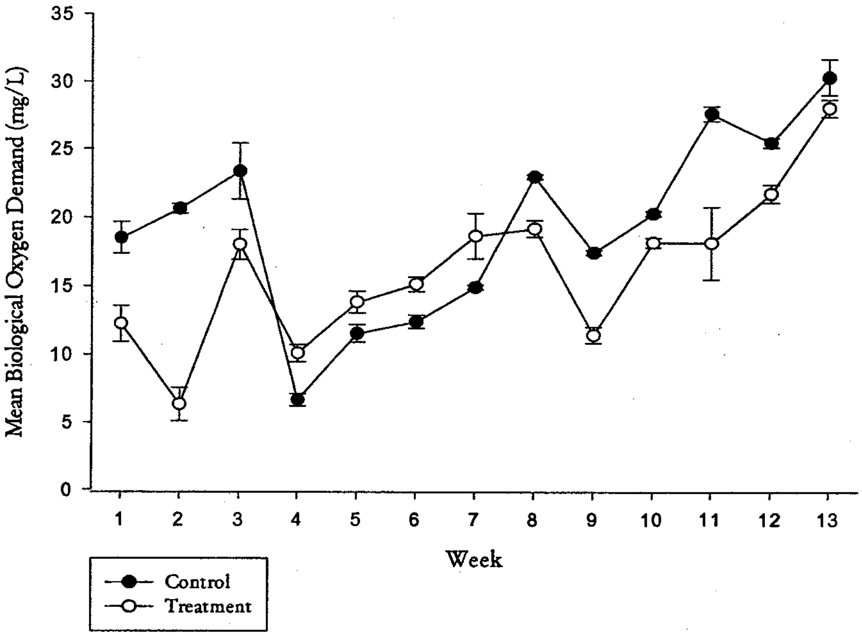


Figure 4.20 Mean biological oxygen demand (mg/L) measurements with standard error illustrating no significant difference between treatment or week and no significant interaction between the two factors ($p<0.28$, $p<0.28$, $p<0.50$, respectively).

4.2.7: Total percent organic content

In the first week of sludge sample collection the initial observations found that the layer of sludge on the bottom of the pond was 5-6 cm thick and had the distinct smell of hydrogen sulfide. These observations correspond with the information provided by the farmer that the sludge from the last crop cycle had not been removed from the pond bottom.

The samples analyzed for total percent organics showed that the sludge was generally within the range of 2-4% organic content (Figure 4.21). All three ponds showed a very similar pattern over time (Figure 4.21). The decline observed in week 5 was due to technical problems within the laboratory and therefore, should not be considered an accurate account of the total percent organic content of the sludge samples that week. Otherwise, the values vary between a narrow percentage range and do not increase substantially throughout the grow-out period. Interestingly, declines observed in the control pond in week 8 and in all ponds in week 12, correspond to the timing of reports of water exchange on the farm.

If organic particles are sinking to the pond bottom rather than remaining in the water column as the observations in the previous variables presented suggest, a relationship between the depth of visibility and the oxygen demand in the water should be observed with the total organic content of the sludge. In fact, a significant positive correlation was found between the mean total organic content and the mean Secchi disk visibility ($r = 0.45$, $p<0.04$), although no significant correlation was found through the analysis with mean biological oxygen demand ($r = -0.14$, $p<0.39$)(Figure 4.22).

A significant difference did not exist between the final mean total organic content between ponds ($t = 1.22$; df=1; $p<0.44$)(Figure 4.23). As a result, it can be assumed that any sludge released at the point of harvest in the effluent and any sludge that remained on the pond bottom for drying out would not be different among the ponds.

Mean total organic content did not significantly differ between the treatment and control ponds for the treatment or week factors, nor did a significant interaction exist between the two factors for the duration of the crop cycle (treatment x week: F=3.26; df=1; $p<0.32$, treatment: F=0.01; df=1; $p<0.93$, week: F=53.82, df=1; $p<0.09$)(Figure 4.24). Therefore, it is understood that the mean total organic content of the sludge present throughout the shrimp grow-out period, the sludge removed by the action of the pond draining and the sludge that remained on the pond bottom following the crop harvest was equal among the control and probiotic treatment ponds.

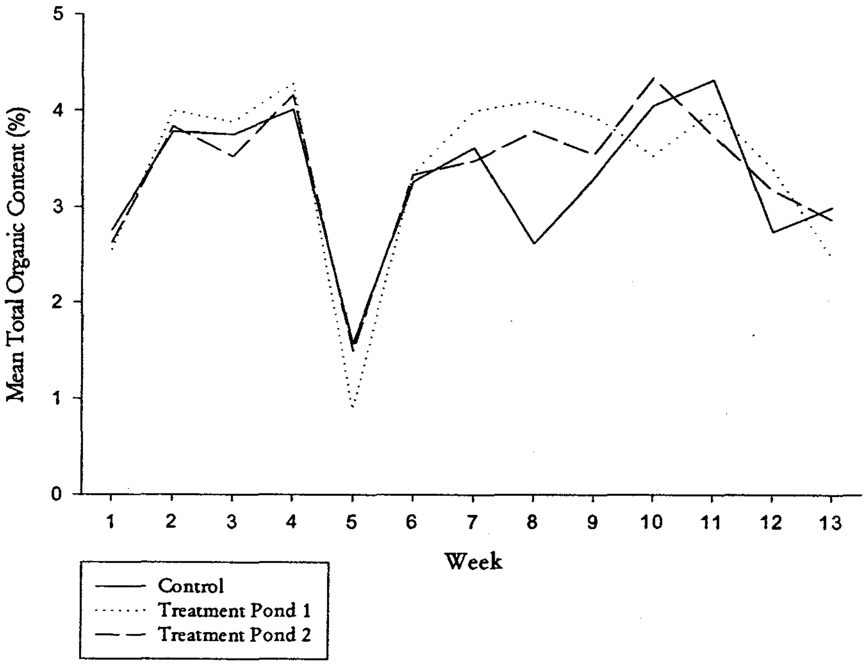


Figure 4.21 Trends indicating the mean total organic content (%) of sludge for each pond throughout the crop cycle.

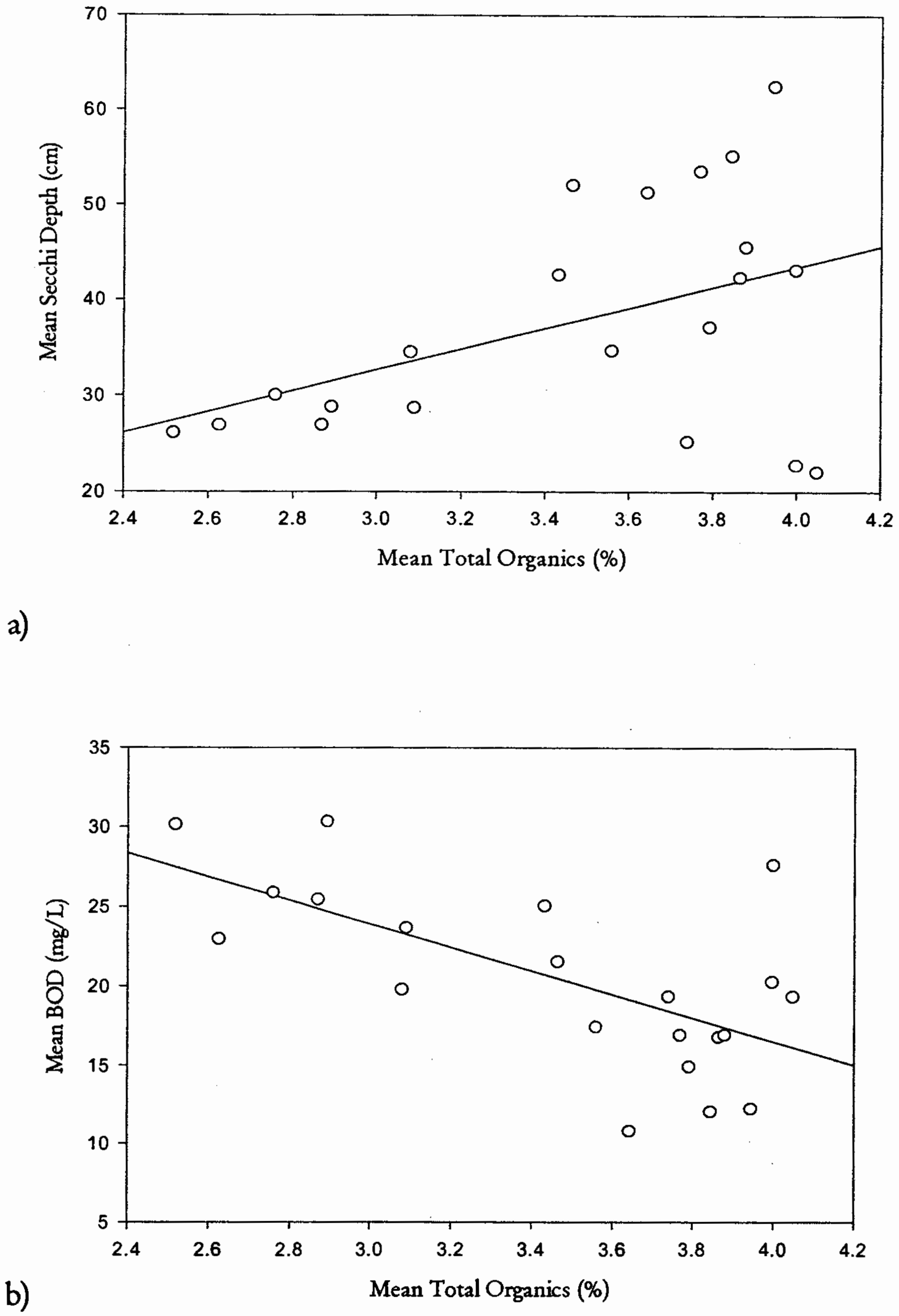


Figure 4.22 Illustration of: a) the significant positive correlation between mean total organic content (%) of sludge and mean Secchi disk visibility (cm) (p<0.04), and b) the relationship between mean total organic content (%) of sludge and mean biological oxygen demand (mg/L) showing no significant correlation (p<0.39).

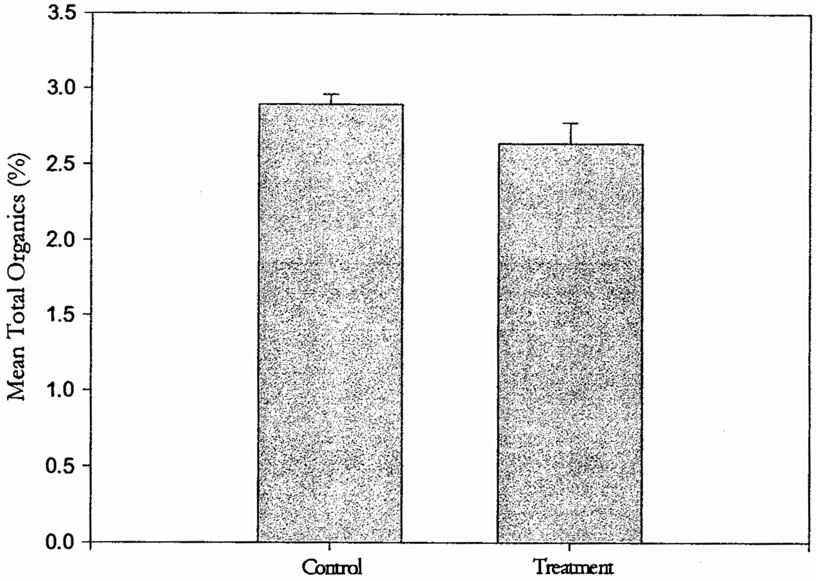


Figure 4.23 A comparison of mean total percent organic content of the final sludge samples with standard error, illustrating no significant difference ($p<0.44$).

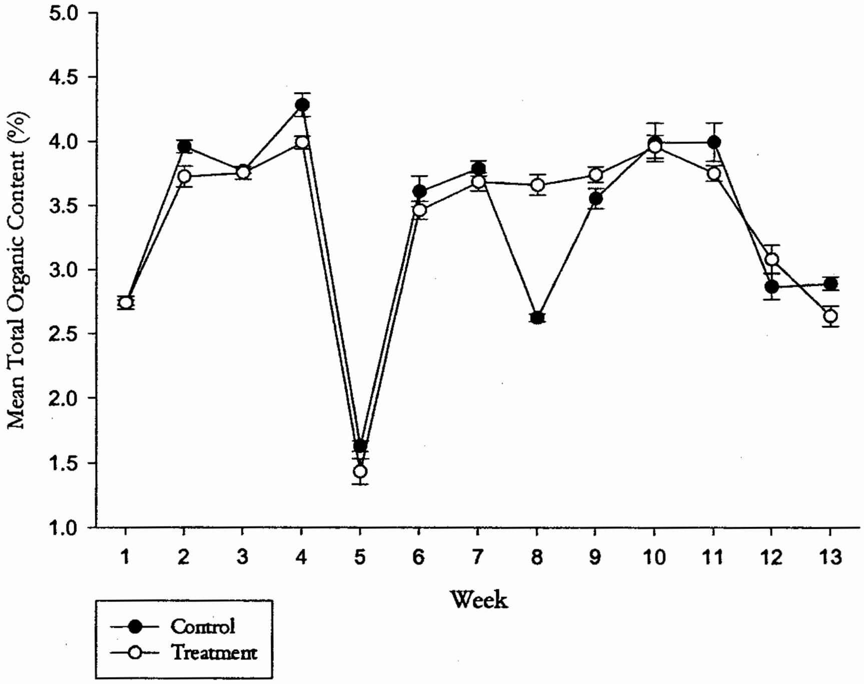


Figure 4.24 Mean total organic content (%) with standard error illustrating no significant difference between treatment or week and no significant interaction between the two factors ($p<0.93$, $p<0.09$, $p<0.32$, respectively).

4.3: Summary

This chapter has presented the results of the study beginning with a description of shrimp health and growth and details of EM-1 prices and local application techniques. The shrimp were found to grow larger but in lower density within the control pond compared to the treatment ponds. Each water quality and sludge variable was described using statistical and graphical analyses. Fluctuations in Dissolved Oxygen concentrations were often related to water exchange practices and heavy rainfall, as well as pH. Secchi disk visibility tended to increase when organic particles sank to the pond bottom, increasing the organic content of the sludge, but this variable was also influenced by the salinity levels of the pond water. The organic content of the sludge was fairly constant throughout the crop cycle but was sometimes disturbed by the pumping action of water exchange, which re-suspended the organic particles in the water column. When particles in the water column increased and augmented the biological oxygen demand, visibility and pH decreased. Temperature did not significantly correlate with any of the other *in situ* water quality variables. Final measures of 3-day BOD exceeded Thai government standards for 5-day BOD (<10 mg/L) in all of the ponds. All of the measured variables for water quality and sludge were equal between the control and probiotic treatment ponds over the entire crop cycle. There was no interaction between the treatment and the week of the measurements.

5. DISCUSSION

This chapter discusses the results for each water quality parameter and sludge characteristic that was measured and statistically analyzed in Chapter 4. It then compares these findings with previous aquaculture studies. A discussion of the effectiveness of EM-1 as a probiotic for inland, low-salinity shrimp aquaculture ponds is then provided, followed by an overview of the implications that the findings of this study may have for managers of biophysical and socio-economic resources.

5.1: Interpretation of data analysis

5.1.1: Dissolved Oxygen

Naturally, the oxygen requirements of the shrimp increase as they grow larger. The increased demand though, should be offset by increases in photosynthesis brought about by the increased nutrients available (that result from the production of larger amounts of waste which ultimately can lead to phytoplankton growth) and by the greater amount of mechanical aeration that takes place. However, these natural and non-natural offsets are not enough to stabilize the Dissolved Oxygen concentrations at a level suitable for the growth of the shrimp, and this necessitates water exchange. The practice of water exchange in combination with heavy rains can explain the pattern in the Dissolved Oxygen concentrations (peaks and troughs) over time.

The fact that the treatment ponds (particularly treatment pond 1) displayed a trend of lower mean Dissolved Oxygen concentrations than the control pond--both throughout the crop season and for the final measurement--indicates that the probiotic treatment did not improve Dissolved Oxygen concentrations, and may in fact have lowered them (although not significantly). One explanation for the slightly lower concentrations in the treatment ponds is the fact that the treatment ponds appeared to have had a higher number of shrimp, and the larger population size in these ponds would naturally consume greater amounts of oxygen overall. However, the lower concentrations may also be due to enhanced microbial activity in the probiotic treatment ponds, which causes a greater increase in oxygen consumption. McMillan *et al.* (2003) described the decomposition of organic particles as a problem in aquaculture, as the consumption of oxygen by microbes during the process of breaking down organic compounds decreases the amount of oxygen available for the cultured organisms. As long as mechanical aeration provides ample oxygen for shrimp survival, the lower *in situ* Dissolved Oxygen concentrations are a short-term trade-off since an important part of the water treatment process involves reducing the organic load.

The lack of significant improvement in the mean Dissolved Oxygen concentrations in the ponds treated with EM-1 is similar to the findings of other microbial additive research. The laboratory study by Rengpipat *et al.* (1998) and the outdoor experiments of Shariff *et al.* (2001) and Yusoff *et al.* (2002) found that Dissolved Oxygen concentrations were not significantly different between probiotic treatment ponds and control ponds. As Shariff *et al.* (2001) explains, water exchange and continuous aeration play a crucial role in maintaining acceptable water quality. In this study, the Dissolved Oxygen was clearly more influenced by these factors than by the probiotic treatment. Rengpipat *et al.* (1998) found that Dissolved Oxygen concentrations were fairly stable (between 5-6 mg/L) throughout their experiment, while the evidence presented in this study shows a much larger and varied range (3.00-13.77 mg/L). The variation observed in this study though, is likely more representative of natural pond systems, whereas Rengpipat *et al.* (1998) performed the experiment under controlled, laboratory conditions.

5.1.2: Temperature

As explained in Chapter 4, temperature may have potentially influenced the Dissolved Oxygen concentrations within the ponds for the first eight weeks of the crop season, either due to the adjusted saturation points, or the metabolic activities, or both. However, in the pond systems observed in this study, temperature had an insignificant influence on all of the measured water quality variables based on the correlation statistics and was not significantly different between probiotic treatment and control ponds. Therefore, any differences that were observed in water quality parameters in any of the treatment or control ponds cannot be attributed to variation in temperature.

The manufacturer does not make any claims regarding the effect of the EM-1 probiotic on water temperature, and enhanced microbial activity would not necessarily raise or lower water temperature. Thus, the findings that both the final measurements and the entire crop season do not significantly differ between the probiotic treated and control ponds are reasonable. The results of this study are supported by the findings of Prabhu *et al.* (1999), which recognized that probiotics do not influence pond temperature. Similarly, Rengpipat *et al.* (1998) and Shariff *et al.* (2001) found stable water temperatures throughout their periods of observation between treatment and control ponds. However, a study by Yusoff *et al.* (2002) presented opposing data that showed that temperature was significantly lower in the treatment ponds. Unfortunately, the discussion provided by Yusoff *et al.* (2002) does not provide an explanation for this observation.

Since microorganisms experience optimal growth at temperatures of 25-35°C and the majority of shrimp ponds are located in tropical regions, decomposition of organic matter by microbes in Thailand's shrimp ponds is rarely limited by temperature (Boyd, 1992). Therefore, the temperatures observed during this study would not have diminished the effectiveness of the probiotic.

5.1.3: pH

The pH values for all of the ponds were consistently within the recommended range of 7-9 for optimal shrimp growth (from Boyd and Fast, 1992). The significant positive correlation between Dissolved Oxygen and pH could be attributed to the fact that during the day, phytoplankton will remove carbon dioxide from the water to use for photosynthesis to produce oxygen and pH will increase (becomes more basic) (Boyd and Tucker, 1998). At night, however, this process ceases while respiration (i.e. consumption of Dissolved Oxygen and production of carbon dioxide) by the shrimp, phytoplankton and other organisms continues, which creates an increase in carbon dioxide, causing pH to decline (Boyd and Tucker, 1998). Since the process of respiration affects pH, Dissolved Oxygen and pH could be expected to follow similar patterns within aquatic systems. Supporting this hypothesis, the mean pH measurements for all of the ponds were positively correlated with mean Dissolved Oxygen concentrations. Moreover, pH was also significantly negatively correlated with Secchi disk visibility, which can be explained by the fact that an increase in Dissolved Oxygen may have occurred with an increase in the production of oxygen, as result of a greater presence of phytoplankton. The appearance of phytoplankton within the water column would simultaneously decrease the clarity or the depth of visibility.

With augmented microbial activity and nutrient cycling through the use of EM-1, an increase in photosynthesis might be expected to take place in the treatment ponds compared to the control, if the phytoplankton are nutrient limited. The increased photosynthesis activity would cause an increase in pH, a result observed in previous experiments by Prabhu *et al.* (1999). Yet the final pH measurements of this study do not support this hypothesis or the research of Prabhu *et al.* (1999), since trends indicate that pH was slightly lower (more acidic) for the treatment ponds. More than likely, the reduced pH was observed due to the enhanced microbial activity, which would lead to greater levels of respiration. The consumption of oxygen may have occurred at a quicker rate than its production by phytoplankton, subsequently creating an increase in carbon dioxide and a decline in pH. Most importantly, however, the values for the entire crop season do not significantly differ between the probiotic treatment ponds and the control, indicating that EM-1 does not significantly affect the pH of pond water, and ultimately, will not affect the pH of the effluent and effluent-receiving waters. Laboratory data presented by Rengpipat *et al.* (1998) and the field studies conducted by Shariff *et al.* (2001) and Yusoff *et al.* (2002) support these findings.

The instruction manual for EM-1 states that the solution is ready to use when the pH is below 4.0. Unfortunately, the farmer in this study generally monitored the pH of the ponds only, and not the pH of the EM-1 solution added to the pond. When the pH of the ponds became too high (i.e. very basic), the farmer concluded that the sugar in the molasses of the EM-1 solution had increased the organic carbon concentrations in the ponds. In reality, the small amount of molasses added (relative to pond size) would have had a minimal effect on the pH levels. By providing a substrate for the microorganisms, the addition of sugars would have (if anything) increased respiration and thereby decreased pH, making the water more acidic. Although the grower's motivations for changing the application protocols of the product were sincere, the recommended methods for applying the EM-1 product were not properly followed, thus complicating the findings of this study.

5.1.4: Salinity

The reservoir used to fill the pond water at the study site often stores brackish water that is collected during the dry season from the Bang Pakong River. Additionally, the reservoir may be filled in between crop cycles, allowing the salt to become concentrated over time due to evaporation. Once the crop cycle has begun, water is exchanged too frequently to allow salts to accumulate, so freshwater is used for the remainder of the crop. Hence, the salinity of the ponds consistently decreased for the entire 13 weeks.

Natural and non-natural adjustments (e.g. rainfall, water exchange, mechanical aeration) to the ponds may have interfered with a more typical pattern or relationship between Dissolved Oxygen and salinity, and may provide an explanation for the lack of a significant correlation between the two *in situ* water quality parameters. The correlation between Secchi disk visibility and salinity can be attributed to the high water clarity that existed when saline values were high. This relationship exists for the simple reason that the water was most saline at the beginning of the shrimp crop cycle and had not yet been exposed to the organic inputs of the shrimp aquaculture farm and the associated phytoplankton, and thus was least turbid. Salinity declined throughout the season with additional freshwater inputs, which corresponded with the pattern of organic inputs and phytoplankton that consistently increased with feed and feces production. Worth noting is the fact that a large number of ions (e.g. calcium, magnesium) and organic matter will readily coagulate with small clay particles (Boyd, 1995a), and will remain suspended even in still water if the water is fresh (Boyd, 1992). However, in brackish water, clay will settle out much faster since the saline concentration interferes with the attraction of particulate matter (Boyd, 1995a). Therefore, the behaviour of soil particles and coagulates may also provide an explanation for the inverse relationship between turbidity and salinity.

Since microbial activity itself does not raise or lower salinity levels, it stands to reason that a significant difference did not exist between the probiotic treatment ponds and the control for the final measurements and for the entire crop season. The findings of Prabhu *et al.* (1999) provide further support for this claim. Likewise, Rengpipat *et al.* (1998), Shariff *et al.* (2001), and Yusoff *et al.* (2002) found no significant difference between the salinity values of treatment and control ponds. The instructions manual of EM-1 never states that the microbial solution performs better in different salinity concentrations, and since the product has been promoted for shrimp aquaculture (a marine species), it can be assumed that salinity would not have destabilized the efficacy of the probiotic.

5.1.5: Secchi disk visibility

Overall trends for Secchi disk visibility showed that the treatment ponds contained less turbid conditions on average for the entire crop cycle, especially in treatment pond 1. These results are inconsistent with the idea that EM-1 would increase nutrient cycling and phytoplankton growth, since these activities would increase turbidity and Dissolved Oxygen levels. However, with the enhanced microbial activity provided by the EM-1 solution, it is possible that decomposition of organic particles in the water column was more efficient than in the control pond, leading to clearer conditions. These findings support the concept presented in the *in situ* Dissolved Oxygen concentration and *in situ* pH sections that proposed the idea that the enhanced microbial activity in the treatment ponds leads to the consumption of oxygen, which in turn lowers *in situ* concentrations of Dissolved Oxygen and pH. The greater Secchi depth values for the treatment ponds could be the result of the microorganisms' activity, which would decrease the Dissolved Oxygen concentrations while simultaneously reducing the concentration of organic particles in the water column.

The relationship between mean pH with mean Dissolved Oxygen concentrations and mean Secchi disk visibility was highlighted in the preceding sections. Essentially the argument stated that pH would increase with Dissolved Oxygen concentrations and lower Secchi disk visibility due to increased presence and activity of phytoplankton. Further support for this theory could be provided if mean Secchi disk visibility and mean Dissolved Oxygen concentrations were correlated. However, a significant relationship between mean Secchi depth and mean Dissolved Oxygen did not exist. The lack of correlation corresponds to earlier claims by this study and by Shariff *et al.* (2001) that indicated Dissolved Oxygen concentrations were influenced largely by mechanical aeration (which as discussed in Chapter 4 may increase turbidity and decrease Secchi disk visibility) and the freshwater inputs from the reservoir and monsoon rains rather than the biological functioning of the pond systems.

The same activities that influence Dissolved Oxygen concentrations may also disturb the clay-based pond sediments, creating greater amounts of particles to be suspended, which invariably would lead to lower Secchi depth values. Chiayvareesajja and Boyd (1993) also commented on the dense turbidity that excessive aeration could create by suspending bottom soil particles. As discussed previously, the clay particles in the ponds would not easily settle, especially as the water became less saline throughout the duration of the crop cycle. The turbidity caused by the clay particles raises an important issue. Many recommendations for aquaculturists have included building additional reservoir ponds to act as sedimentation tanks, where particles can settle out before the water is pumped into ponds for crop production. The results observed in this study, and the observations by Boyd (1992) explain why sedimentation is not an effective technique for the inland ponds in

Thailand where soils are predominantly clay. Hence, finding alternative strategies such as probiotics is further justified.

A significant difference did not exist between the probiotic treatment ponds and control pond for mean Secchi disk visibility in the final week prior to harvest discharge or throughout the entire crop cycle. The lack of difference indicates that the microbial activity of the probiotic treatment could not maintain a visibility depth any greater than the untreated pond and that on average the visibility in the treatment ponds still fell below the recommended levels of 30-45 cm (from Boyd and Tucker, 1998). Similarly, Yusoff *et al.* (2002) found a significant difference did not exist in pond water transparency between treatment and control ponds.

One problem that arises when measuring Secchi disk visibility is the fact that both phytoplankton and suspended particles may influence water clarity in the same manner, but may have different impacts within the pond. Thus, interpreting the measurements becomes difficult because a lack of difference between two or more ponds is not necessarily indicative of similar conditions. One may have low visibility due to greater phytoplankton growth which would lead to higher Dissolved Oxygen concentrations within the pond, whereas another may have low visibility due to extremely high levels of suspended organic particles (such as feed) which invariably would lead to lower *in situ* Dissolved Oxygen concentrations.

5.1.6: Biological oxygen demand (BOD)

The biological oxygen demand measurements may be the most meaningful for aquaculture managers and policy-makers as the legal standard already set is clearly not being enforced. Policy-makers need to acknowledge that meeting legal requirements is difficult for farmers when the starting conditions of the ponds already exceed the water quality standards. Obviously, source water in the area has deteriorated due to both shrimp aquaculture and possibly other agricultural and industrial activities to a level that would require treatment before the shrimp crops could even begin. Nonetheless, the results of this study also clearly indicate that regardless of the reason for this initial poor water quality, the inputs from shrimp aquaculture contribute to further degradation of the aquatic resources. The 3-day BOD measurements calculated for this study are similar to, or slightly higher than, the 5-day BOD values reported in previous probiotic research in shrimp ponds (see for example: Yusoff *et al.*, 2002; Shariff *et al.*, 2001). The fact that these values were matched or surpassed in only a 3-day test indicates that the BOD may in fact be much higher in the ponds of this study. The high BOD values observed throughout the crop cycle in this study are not only indicative of the effect of aquaculture activities on water quality, but may also illustrate that the mechanical aeration and pumping action that occurs during water exchange result in a high concentration of organic materials being suspended and re-suspended in the water column. With the low salinity levels typical of inland ponds, particles will not readily sediment out to the pond bottom. The high concentration of organic particles in the water column may then reduce light availability for photosynthesis, which in turn, leads to even poorer water quality conditions.

The overall trend for the mean BOD values showed a lower demand for the treatment ponds, which indicates a lower (although not significantly) level of oxidizable materials in the water column for these ponds. These findings may lend further support to the hypothesis presented in the *in situ* Dissolved Oxygen, pH, and Secchi disk visibility sections that the enhanced microbial activity within the treatment ponds that reduced Dissolved Oxygen levels and pH were the result of organic decomposition within the pond water.

The correlation between mean Secchi disk visibility and mean BOD illustrates that pond conditions that have high organic content of the pond water tend to exhibit reduced pond water clarity. Ultimately, the effluent released will also contain this combination of poor water quality characteristics. The relationship between the two variables, and the fact that the BOD values are high throughout the crop cycle, also indicates that not only will the final harvest discharge stress the overall waterway system, but the effluent released for water exchange purposes will also be detrimental to neighbouring waterways. This type of effluent release would be much more frequent during all shrimp grow-out periods than the final discharge and therefore, may be equal or more significant in terms of impacts than the harvest output. However, based on the statistical findings, the impact of the effluent would not have significantly differed for the mean biological oxygen demand measurements between the control and probiotic treatment ponds for the final week or for the duration of the crop cycle. Shariff *et al.* (2001) and Yusoff *et al.* (2002) also found that biological oxygen demand was not significantly affected by probiotic treatments.

Since a significant difference did not exist between the treatment and control ponds, the final measurements can be averaged to calculate a single mean biological demand value for the harvest discharge, which equals 28.80 mg/L (or 0.00002880 kg/L). Based on wastewater outflow calculations by Braaten and Flaherty (2000), one report has estimated that an inland shrimp farm releases 16 300 000 L/ha/crop of effluent (Szuster and Flaherty, 2002). Using this value, an average biological oxygen demand of 469.46 kg/ha/crop will be produced, or 938.92 kg/ha/year (assuming two crops per year) by a single small-scale, inland farm. Further research reported by Szuster and Flaherty (2002) established that the Bang Pakong east bank produces 146 500 000 m^3 (or 146 500 000 000 L) of effluent each year. If the week 13 BOD measurements of this study are assumed to be an accurate representation of the effluent released at harvest by other farms within the east bank region (an assumption supported by observations of farm management by Braaten, 2000), then the biological oxygen demand of the effluent released each year in this area would equal approximately 4.2 million kg (Table 6). The total effluent each year produced by shrimp farms in the entire Bang Pakong basin (pond area equals 18 530 ha) has been estimated to be 604 100 000 m^3 (604 100 000 000 L) (Szuster and Flaherty, 2002). Again, extrapolating the data from this study to provide an average of the small-scale farms in the entire basin, the biological oxygen demand of the effluent released each year would equal approximately 17.4 million kg (Table 6). If these calculations closely reflect reality, then the oxygen demand created by shrimp farm effluent measures between the best and worst case scenario values estimated by Szuster and Flaherty (2002). The demand of the effluent is slightly lower, but contributes a level of the same magnitude, as the BOD production estimates for chicken and pig farming in the Bang Pakong region, which number 28.8 million kg/year and 18.7 million kg/year respectively (Table 6)(Land Development Department, 1998; Pollution Control Department, 1998).

Difficulties may arise when attempting to quantitatively compare the environmental degradation caused by different activities (e.g. shrimp farms versus other industries), particularly if comparing different areas of the world. Consequently, it is imperative that these values are understood in the context of this region of Thailand. However, the impacts may also be relevant to other tropical areas where the shrimp industry is much more prevalent than other forms of agriculture, and truthfully, any solution for controlling this problem would be useful to any aquaculture-producing region worldwide.

Table 5.1 Total BOD (kg/year) production estimates for selected activities in the Bang Pakong River region (Modified from Szuster and Flaherty, 2002)

Area of Bang Pakong River region	Low-salinity shrimp (BOD kg/year)	Chicken (BOD kg/year)	Pig (BOD kg/year)
East bank sub-basin	4 200 000	N/A	N/A
Total Basin	17 400 000	28 800 000	18 700 000

5.1.7: Total percent organic content

The mean total percent organic content of the pond bottoms measured between 2-4% throughout the crop cycle. These values are similar to data presented by Boyd (1992), in which the average total percent organic matter for the sediment of two shrimp ponds in Thailand was 3.1% over a 4-month period. Despite the accumulation of sludge on the pond bottom from the previous shrimp crop and the organic inputs for the crop being reared during this study, mean total percent organic content of the sludge was low and fairly constant throughout the season. The organic particles that were present in the sludge may have been naturally occurring, and not the result of aquaculture activities. However, the slight increase between week 1 and week 2 indicates that the presence of shrimp and the organic inputs that begin to enter the pond system due to aquaculture practices do alter the natural organic content of the sediments.

The slight reduction in total percent organic content observed in the control pond for week 8 and for all ponds in week 12 was described in Chapter 4 as corresponding with the timing of water exchange. One possible explanation may be that on occasion water exchange may disturb the flocculent layer of the sediments, thereby resuspending organic materials in the water column. Support for the proffered explanation can be found in the relationships between total percent organic content, Secchi disk visibility and BOD. The positive correlation between Secchi disk visibility and total percent organic content indicates that as the visibility in the water column becomes more clear, the organic content of the sludge increases— a reasonable relationship that illustrates that as organics settle out of the water column onto the pond bottom, water clarity improves. BOD and Secchi disk visibility have a significant relationship, with BOD increasing as visibility within the water column decreases. Although a statistically significant correlation did not exist between organic content and BOD, the high BOD measurements during weeks 8 and 12 do support the theory that a large amount of organic material could be re-suspended in the water column by the pumping action involved in water exchange.

A study by Funge-Smith and Briggs (1998) that determined the nutrient budgets of shrimp ponds found that 58-70% of the organic matter within a shrimp aquaculture pond accumulated in the sediment. But this study also found that the organic content of solids discharged in routine water exchange was 13% (Funge-Smith and Briggs, 1998). Based on this information, it can be assumed that the accumulation in the pond bottom is much greater than the concentration in the water column, and consequently, the total percent organic content of the sludge must be extremely high (much greater than 13%). Therefore, the results of Funge-Smith and Briggs (1998) oppose the findings of this study, where the organic content of the pond sludge was fairly low but was much higher in the water column based on BOD calculations. The difference may exist because of differing farm practices, or may be due to the location of the shrimp ponds, which were coastal farms, unlike the inland ponds used in this study. As described previously, clay particles will not settle out as easily in low-salinity ponds such as those used in inland regions, when compared to coastal marine farms. It should be noted, however, that Funge-Smith and Briggs (1998) also recognize that certain particle types will have a higher tendency to remain suspended in the water column.

They describe how frequent aeration may allow heavier soil particles to settle and form accumulated sediment, but phytoplankton, bacteria and organic particles do not easily amass in a flocculent layer on the pond bottom due to natural buoyancy (Funge-Smith and Briggs, 1998). This argument may also be supported by the idea that the organic matter (adsorbed to the clay particles) was remaining suspended in the water column due to the mechanical aeration and the low saline environment.

Although the enhanced microbial activity of the probiotic treatment ponds was expected to break down the accumulating organic materials on the pond bottom, a significant difference did not exist between the total percent organic content of these ponds and the control. The lack of difference between the treatment and control opposes the findings of Ehrlich *et al.* (1989) who found organic content of sediments decreased when treated with a bacterial additive. The difference between these studies, however, is that Ehrlich *et al.* (1989) used lake sediments that received multiple sources of pollution rather than actual aquaculture pond sediments, and may not provide accurate expectations for aquaculture pond sludge that has ben treated with a probiotic. Ehrlich *et al.* (1989) also avowed that the organic content of aquaculture wastes might exceed 80%--much higher than the results found in this study. The values were much lower than previous claims (e.g. Ehrlich *et al.*, 1989), providing evidence that farm management practices for inland, low-salinity ponds have controlled the excessive organic accumulation in recent years, unlike the former coastal ponds that would have been the subject documented in the 1989 study by Ehrlich *et al.* Nevertheless, as Boyd and Sonnenholzner (2000) point out, certain conditions may still exist in other regions practicing aquaculture where organic sediment may be high, or where populations of microorganisms are inadequate to efficiently decompose organic matter. In this case, the potential of EM-1 to reduce the organic sludge should still be tested.

However, in inland, low-salinity ponds it appears that EM-1 may be both unnecessary and ineffective at reducing the small amount of organic sludge that may be created within the aquaculture ponds, although this conclusion is difficult to ascertain given the modified application procedures of EM-1 by the farmer. Similar to the results of this study, Sonnenholzner and Boyd (2000) found that the application of a bacterial suspension did not enhance the decomposition of organic matter in shrimp pond bottoms.

5.2: Effectiveness of EM-1 in an inland, low-salinity shrimp pond

One of the observations regarding the growth and health of the shrimp recognized that although the control pond shrimp grew to a greater size than the shrimp of the treatment ponds, the larger growth likely occurred because there were fewer shrimp in the control pond and the shrimp had more space to grow. This assumption is supported by previous research that has discussed the density-dependent characteristics of *P. monodon* (e.g. Abdussamad and Thumpy, 1994). Although the same amount of seed was placed in each pond at the beginning of the crop cycle, fewer must have survived the initial few weeks within the control pond, leaving the two treatment ponds to contain higher densities. In this respect, EM-1 may have improved the survival of the shrimp seed upon being placed within the treatment ponds, through improved water quality and sediment conditions or by providing an extra food source.

The biggest difficulty that arises when attempting to assess the effectiveness of EM-1 in this study is that the product was not applied in accordance with the manufacturer's instructions. The instructions manual requires that a minimum of 5 L of stock EM-1 solution be added to the pond each week after being mixed with appropriate amounts of water and molasses. If the majority of small-scale farmers operate 3 ponds each crop for a

110 day cycle, with the cost of EM-1 at 60 baht/L a farmer would need to purchase approximately 195 L, costing a total of 11 700 baht (~CAN$394.04)(Universal Currency Converter, 2003). The total amount is a large investment if the product cannot be guaranteed to work effectively. Realistically, some shrimp farmers would be able to afford the initial costs. Yet, the primary goal of shrimp aquaculture is similar to agricultural operations, which is to rear a high quality crop for profit. For many farmers, conserving the environment is secondary to more immediate financial security priorities. Thus, farmers have adapted their own techniques to propagate the microbes in the additives to create more solution for themselves at home, thereby minimizing costs. Problems with this technique may include reduced effectiveness if not all of the microbes within the solution are propagating to create a balanced solution (which cannot be observed by the naked eye), or if the initial EM-1 stock solution is a poor quality "batch". For probiotic manufacturers to be truly successful in implementing their product as a farm management tool, they need a better understanding of the amount that a farmer will be able to afford and be willing to invest, and create a technique that incorporates that information. Otherwise, farmers will adopt entirely new and possibly improper application techniques or will look elsewhere for solutions.

During this study, the farmer followed the manufacturer's instructions for the first seven weeks of the crop cycle and then modified the application technique to no longer include the addition of molasses to the EM-1 solution. Reality does dictate that modifications may be necessary when applying the additive to outdoor earthen ponds, particularly if the crop and the corresponding profits are in jeopardy. However, this creates a problem requiring a two-part solution. Firstly, manufacturers need to understand how the application of the probiotic product may need to be adjusted to account for certain environmental conditions that will commonly occur in small-scale shrimp ponds, and secondly need to train farmers about the application protocols for probiotics. In terms of allowing for local conditions, developing a solution that is practical for the schedule and financial capabilities of farmers will be crucial for the successful adoption of this type of bioremediation technique. The success of probiotics in improving water quality will depend on the ability of the selected bacteria to meet specific functions for bioremediation, and to survive at appropriate population densities (Moriarty, 1999). Future research by EM-1 manufacturers then, should consider different combinations of microbial strains, and should also recognize that probiotics may perform differently in areas with different water and soil conditions. To maximize their success as bioremediation tools, microbial strains will undoubtedly have to be applied in different combinations in different regions of the world. Different species of shrimp may also yield different results with the probiotic products, and this variable should be investigated as well. As other researchers have discussed, continuing to conduct this type of research is critical for advancing the science of microbial ecology, and for providing well-informed recommendations to the global aquaculture industry (e.g. Jory, 1998; Moriarty, 1997).

In terms of training for farmers, it became apparent by documenting the application techniques of a small-scale farmer that awareness regarding the importance of following the manufacturer's instructions is not emphasized in promotional materials. As a result, the probiotics in this case were not utilized as intended. EM-1 is supposed to be combined with molasses for the microorganisms to propagate to a high concentration before the entire solution is added to the pond. The high concentration of microbes must be established so that the organisms are present in sufficient type and number in order to outcompete pathogenic organisms and other microbes already present in the pond, to survive predation by other aquatic organisms, and to create a "balanced" system. By applying a lower concentration of microorganisms than was prescribed, the new microbes were greatly outnumbered at the time they entered the system, and therefore the impact of the probiotic was possibly less than it would have been if the product had been applied as suggested by the manufacturer. Consequently, the lack of significant results between the control and treatment ponds is not surprising, since EM-1 did not recommend or guarantee the application method that the farmer employed. One important point to note is the fact that the farmer studied in this work has been involved in shrimp farming for more than a decade and is well educated in aquatic sciences. Therefore, with this depth of knowledge, the grower may be representative of a "best-case" scenario in terms of the management and application practices of the probiotic. Interestingly, improper application techniques were still practiced. The findings of this study suggest that adequate training and education may not be readily available for small-scale shrimp farmers in Thailand, especially regarding the use of additive products such as probiotics. Increased information sharing between local growers and probiotic manufacturers will enhance the success of probiotic implementation at the farm level.

While the results of the statistical analysis indicate that a significant difference for the *in situ* water quality parameters, the biological oxygen demand, and the total percent organic content of sludge did not exist between the control and probiotic treatment ponds, the use of probiotics in aquaculture ponds as a bioremediation technique should not be entirely dismissed. Some of the trends observed in this study indicate slight differences in the behaviour of the dynamics of the ponds treated with the probiotics. Although not all of the effects of EM-1 appear to be immediately beneficial (e.g. lower Dissolved Oxygen, lower pH), even the variables that indicated poorer *in situ* water quality conditions could be explained by enhanced microbial activity that acted to reduce the concentration of organic material in the water column. The findings do suggest that (although not statistically significant) a potential may exist for EM-1 to be an effective tool in improving water quality. Possibly, longer-term use of the probiotic product may yield more positive results, and could provide a more accurate assessment of probiotics in outdoor aquaculture ponds. Also, the success of microbial additives in other areas of research, as described in Chapter 2, provides evidence of their potential to improve environmental conditions, which could reduce the negative impact that shrimp farm effluent has on surrounding water bodies. More than likely, probiotics will not be an effective solution when used in isolation of other wise management strategies, but could help to improve pond and effluent water quality if applied as one element of a more comprehensive and well-conceived water management plan.

5.3: Management Implications

Several different levels of management exist within the aquaculture industry in Thailand. For the purposes of this discussion, however, managers are divided into two main categories. The first division involves those who are concerned with biophysical resources, and are focused upon site-specific details, local/regional ecosystems, and local markets (e.g. local farmers or distributors). The second category involves those dealing with the implementation and enforcement of policies that govern the first level of managers, such as the Thai Department of Fisheries.

5.3.1: Implications for managers of biophysical resources

One of the most remarkable findings of this study was that the organic load of shrimp aquaculture ponds is a more extensive problem in the actual water column than in the sludge sediment removed from the pond bottoms at harvest. The high biological oxygen demand of the water in all of the weeks tested indicates that water outflows for exchange throughout the crop cycle place great demands on the receiving waters, in addition to the final harvest discharge. Boyd and Musig (1992) note that although the effluent will typically contain adequate Dissolved Oxygen concentrations to support the life of aquatic organisms (particularly shrimp), the water does contain elevated concentrations of organic matter that will create an oxygen demand and can accelerate phytoplankton growth and eutrophication of receiving water bodies.

While the findings regarding the high biological oxygen demand confirms the hypotheses proposed by previous aquaculture research (Szuster and Flaherty, 2002; Dierberg and Kiattisimkul, 1996), the results differ from other research that has emphasized the impact that the organic content of sludge would create (see for example Funge-Smith and Briggs, 1998; Primavera, 1998). Small-scale shrimp farmers recognized that the creation of organic sludge needed to be minimized and have made efforts to reduce the amount of fertilizers and organic inputs into the pond systems. Farm managers also need to be aware that the "broadcast" feed method may add excessive amounts of organics to the ponds and due to the low salinity levels within inland ponds, particles within the water column may not sediment out as readily as similar types of particles in coastal-based ponds. Additionally, water exchange practices appear to occasionally disturb the flocculent layer of sludge, thereby increasing the concentration of organic particles in the water column, which decreases water transparency. Thus, the very practice that aims to improve pond water conditions may temporarily degrade the quality even further.

Despite the fact that management practices have been modified to decrease the amount of organic sludge accumulating on the pond bottoms, the sludge removed at harvests will continue to cause problems of siltation and accumulation of inorganic sediment in neighbouring waterways. The increased sediment load in the neighbouring water supplies will increase the turbidity, which as explained previously, can reduce the level of light that may penetrate the waters, causing a decline in photosynthesis and oxygen production. The load of sediment released at harvest could also alter benthic community structure and productivity (Phillips *et al.*, 1993) and fill in benthic habitats as accumulation occurs, which ultimately decreases the holding capacity of that waterbody. As such, the impacts of the sludge will remain the same in that the quantity and quality of accessible freshwater will be reduced. Since the organic content of the pond bottom does not reach extremely high levels that would be detrimental to the pond system and the load of sediment released at harvest causes damage to surrounding ecosystem communities, the removal of the sludge may potentially be more harmful than helpful, and may be a futile exercise.

The health of all organisms (humans included) that consume the water and/or shrimp resources, and the degraded environmental conditions that result from the impacts of aquaculture's intensive activities were discussed in Chapter 2. The research presented here does not specifically address consumer health impacts. However, the demands placed on the seafood industry by advocates for human health is a large part of the reason that concerns about the impacts of aquaculture are being raised today. The results of this study indicate that more research is required to improve the efficacy of probiotics for inland, low-salinity shrimp aquaculture ponds before local and international policy-makers and resource managers can create reliable standards to implement and enforce within the industry. Although the probiotic treatment did not clearly benefit the aquatic environment or the health of the shrimp, it is possible that long-term effects may exist for consumers that ingest water or shrimp that have been treated with probiotics. In order to determine this, a comprehensive research program needs to be developed to address this gap in knowledge, and to investigate the positive and negative aspects that arise when employing this type of bioremediation technique in aquaculture operations.

5.3.2: Implications for managers of socio-economic resources

One of the most disconcerting aspects of the shrimp aquaculture industry in Thailand and many other countries is the limited regulatory capacity for ensuring that shrimp farmers maintain environmentally-friendly management practices. Criticisms have been made of countries involved in shrimp aquaculture for their lack of long-term vision in conserving the environment and sustaining the industry (e.g. Primavera, 1998). The lack of conservation efforts possibly stems from the fact that many of the countries producing cultured shrimp (Thailand in particular) do not have the same institutional controls in place for business entrepreneurs (e.g. business licenses, property taxes for sales arenas) as other countries. Therefore, people are able to build businesses with few restrictions--leading to an "anything, anywhere, anytime" philosophy. With this mentality, operators do not need to concern themselves with questions of sustainability when options for another business can easily arise. The lack of socio-economic regulations, then, perpetuates the lack of concern for the biophysical resources.

The fact that farmers are able to purchase various additives for their ponds, despite the lack of evidence that these products are truly effective, is also a concern. The products need to be screened and labelled with appropriate instructions and information before promotions and sales are permitted. Whilst some might argue that capabilities will be limited by economic constraints, the fact that shrimp aquaculture is a multi-billion dollar industry in Thailand tends to negate this argument. Despite soaring profits, it seems that Thailand (along with most other shrimp producing nations) has invested very little back into the industry, especially at the rural, small-scale level. Furthermore, few environmental regulations are enforced. Yet, if continued profits and healthy consumers and environments are desired, an onus does exist on the forces governing the industry to begin to manage aquaculture more responsibly.

Research regarding possible improvements for aquaculture practices should not be coordinated by randomly-involved scientists on public land. Although in the case of this study, minimal environmental manipulation took place, all experiments do carry a certain risk. If research facilities were developed to test and certify aquaculture additive products in a safe manner before the products were permitted to enter local markets for use, farmers could make more informed decisions regarding management practices. Such a certification process would also prevent farmers from performing their own experiments that may otherwise only contribute further to the deterioration of the environment and confusion, since the choice to select a legitimately guaranteed product would then be available. The experimental research would also allow for adaptive management to take place, as new discoveries about alternative methods for management are made.

The success of bioremediation may vary greatly depending on the type of products used and the technical information available to the end user (Moriarty, 1999). Therefore, local small-scale farmers need to be trained in the use of probiotics to ensure their effectiveness. In addition to research facilities, a training program needs to be developed by either manufacturers or local aquaculture-governing bodies to enhance the understanding of how to utilize probiotics to maximize beneficial results. The outcome will build local capacity to rear shrimp using bioremediation techniques, while mitigating the environmental impacts of shrimp aquaculture.

Terstad (1999) describes areas where traditional conservation efforts (e.g. environmental legislation, protection of land) have not controlled environmental deterioration but newer and more market-oriented incentives have been more successful. The aquaculture industry is an excellent example of this trend, since many of the countries receiving shrimp imports have begun to demand certain standards for the products in recent years, driven by humans' own desire to protect their health (Table 5.2). For example, following reports by the Netherlands that shrimp imported from Thailand were contaminated with chemical antibiotics, the European Union adopted a zero-tolerance food safety policy that required 100% of imported shrimp to be inspected (Thapanachai, 2003). Many countries are also beginning to develop eco-labelling programs that promote products that reduce environmental impacts compared to other similar products on the market in response to consumers' demands for more information about their purchases (e.g. Official Journal of the European Commission, 2000; Australian Environmental Standards Association, 2001). Currently, importers rely on an index for food labelling known as the standards of Codex as approved by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) (Hastein *et al.*, 2001). The requirements for food packages by Codex include: name of the food (e.g. shrimp), quantitative list of ingredients, net contents and drained weight in metric units (for shrimp this includes the "count size, i.e. the number of whole shrimp per 100 g), the name and address of the manufacturer, packer, distributor, importer, exporter, and/or vendor of the product, country of origin, lot identification (for packaging factory), and the date of processing or storage instructions (Hastein *et al.*, 2001).

In response to increasing pressures from shrimp receiving nations, the Thai Department of Fisheries took an important step in February 2002 by creating a Code of Conduct for the marine shrimp industry (Department of Fisheries Marine Shrimp Culture Research Institute, 2003). The Code of Conduct outlines an operational guideline and manual for the shrimp industry, the certification process for all operators, and market incentives for shrimp produced under the Code of Conduct guidelines (Department of Fisheries Marine Shrimp Culture Research Institute, 2003). The goal is to provide a high quality product for consumers that can be traced from the final destination back to the original source of seed, ensuring responsible practices at each step. However, concerns remain about compliance to enforcement.

While the export requirements of other countries have necessitated that certain standards be met, a similar model does not exist for shrimp products being traded in Thailand's domestic markets. Cultured shrimp may be directly transferred from farms to local markets for consumers within hours of being harvested, with no formal inspection. The health of the natural environment and the consumers of shrimp are equally important within the producing nations as the health of consumers in export countries. Accordingly, suitable governance of the industry is required at local levels. More information needs to be made available both locally, and internationally, regarding the rearing conditions of the shrimp. As awareness increases regarding the quality of the final product and how that target is achieved at the farm level, an understanding of alternatives will be raised. The international market demands have already begun to reflect societal preferences for the least chemically altered product. Demanding that this type of choice exists for consumers in producing countries needs to be encouraged.

Table 5.2 The certificate requirements of selected black-tiger shrimp importing countries (modified from: Food Market Exchange, 2003).

Country	Certificate requirements
USA	Certificate of Analysis or Analysis and Health Certificate Sanitary Certificate
Canada	Certificate of Analysis or Analysis and Health Certificate Sanitary Certificate
European Union	Certificate of Analysis or Analysis and Health Certificate Sanitary Certificate
Italy	Certificate of Analysis or Analysis and Health Certificate Sanitary Certificate Mercury Certificate (if products are not cooked)
Benelux	Certificate of Analysis or Analysis and Health Certificate
Japan	Certificate of Analysis or Analysis and Health Certificate Sanitary Certificate

5.4: Summary

Many of the patterns and interrelationships found in this study illustrate that non-natural alterations to pond systems, such as water exchange and mechanical aeration, play a larger role in influencing the measured water quality and sludge variables than natural biological functions. One of the most interesting findings involved the organic load, which was much lower in the pond sludge than expected by previous studies. However, oxidizable materials were still extremely high within the water column, indicating that organic particles remain suspended in the water, possibly due to mechanical aeration, rather than accumulating on the pond bottom. Therefore, effluent released throughout the crop season for water exchange will place repeated negative demands on receiving waterways. The sludge discharged at harvest will contain less of an organic load, but will still create sedimentation problems that will reduce the quantity and quality of accessible water.

Overall, the EM-1 probiotic did not significantly affect any of the parameters, although certain trends showed evidence of benefits. The lack of significant findings was most likely due to the improper application techniques adopted by the farmer— a result of the lack of available education and training for shrimp growers in this region.

The results from this study indicate that aquaculture does indeed contribute to the deterioration of water resources, but also demonstrates that pond water quality conditions are extremely poor at the beginning of the crop cycle. Essentially, water must not only be treated before being discharged from the ponds, but also before ponds are filled from the source.

Farmers are making efforts to minimize the creation of sludge, but in doing so, are experimenting with various additives on the market despite a lack of concrete evidence regarding their effectiveness, and with very little training in their use. Since little information is available about the purpose and the application methods of the bioremediation products, farmers often adopt their own treatment techniques. Stricter regulations need to be created in order to prevent this type of haphazard management, and for this industry to remain competitive on the international market where human health concerns are driving major changes to environmental and food safety policies. Thailand's Department of Fisheries has begun to address this need by creating the Code of Conduct for marine shrimp export products. However, similar controls are not in place to protect the health of local consumers and their environment and the truth is that much more research is required before probiotics can be deemed an effective management tool for rearing *P. monodon* in inland ponds.

6. CONCLUSIONS

This chapter summarizes the expansion of the black-tiger shrimp industry in recent decades and the environmental management issues that have arisen as a result of this rapidly growing international trade. Next, an explanation of the goals of this study and the main findings are offered, followed by a discussion of the recommendations for future research and the implications of this work for resource managers in the aquaculture industry.

6.1: Background summary

With the impending global fisheries crisis and the human population continuing to grow, the need for increased food production has never been more apparent. Many nations have turned to aquaculture as a way of resolving food security concerns. Aquaculture has been embraced as an alternative source of income and food supply by many development agencies that have promoted and encouraged the growth in various aspects of the industry through financial investments (e.g. World Development Bank). Due to this support, the industry experienced explosive growth throughout the world, but particularly in the developing nations of Asia, with little infrastructure in place to guide its establishment and enforce effective management practices. Although complaints regarding rearing practices and the impacts of this industry have been voiced by many researchers and NGO conservation groups, the reality is that aquaculture provides not only a source of food to many nations, but also acts as a huge economic force in many developing countries, placing them at the forefront of a very competitive international market.

Mirroring the "Green Revolution" that agriculture has undergone in the last 50 years, the intensification of farming methods that has occurred in aquaculture has created a "Blue Revolution" in the culturing of aquatic organisms, especially for one of the international market's most sought after species— *Penaeus monodon*, black-tiger shrimp. Thailand has become the world's leading producer of black-tiger shrimp, but unfortunately is now facing the socio-economic and environmental impacts of an industry that experienced unparalleled growth in only two decades. New methods for supplying seed, artificial and nutritionally enriched feed pellets, and mechanical aeration have all become common farm management practices that enhance the growth and yield of shrimp crops. Unfortunately, the addition of non-natural feed and chemicals that may be used to fertilize or "clean" aquaculture pond water has created a problem regarding the removal of wastewater. The wastewater being released from these ponds contains high concentrations of organic particles and is frequently followed by the release of the pond bottom sediments, or sludge. Research has begun to recognize the negative impacts of shrimp pond effluent, such as eutrophication and siltation (e.g. Graslund, 2001; Funge-Smith and Briggs, 1998; Tookwinas, 1996; Phillips *et al.*, 1993). Yet, the problem of sludge management has typically been ignored on aquaculture farms, in spite of frequent disease outbreaks among shrimp crops. Such epidemics indicate the conditions have deteriorated to a state where pathogenic organisms can easily proliferate. With the movement of shrimp farms into the inland regions of Thailand, waste is now released into local streams, rivers and canals where the assimilative capacity is much lower than coastal areas where farming typically occurred in the past, and therefore can be exceeded much more quickly than in a larger body of water such as the Gulf of Thailand. Unfortunately, research to date has yet to find a technique for minimizing the creation and impact of the organic effluent removed from shrimp ponds, and the quantity and quality of inland freshwater supplies is being continually diminished.

The world's human population will continue to require food for sustenance. But the world also needs clean water; without it, the health of humans and all other organisms relying on that resource will be in peril. While aquaculture may provide hope for the provisions of one resource (food), the destructive practices that are commonplace on many farms today will invariably threaten that heightened security if other resources (such as water) continue to be degraded. A potentially dangerous element of the black-tiger shrimp industry is that the main producers of the cultured seafood are tropical, developing countries while the consumers are typically developed countries. The distance between the producer and consumer creates a critical issue since the consumers in developed nations are often unaware of how the product was created. Developed nations are beginning to demand more informative labels on products and trends that place value on "environmentally-friendly" products have emerged in recent decades. In response, governing bodies such as FAO and WHO are enforcing standards and regulations on imported products. However, regardless of consumer preference (i.e. captured fish, cultured seafood, or agricultural products), the demand for sustenance will undoubtedly continue to grow. Ultimately then, food needs to be produced with minimal impact on the health of all organisms and the natural environment everywhere. Therefore, research needs to focus on improving the waste handling practices of shrimp aquaculture and in doing so, may provide information that can be transferred to other types of aquaculture or agriculture throughout the world.

One possible solution for managing the sludge created in aquaculture ponds involves probiotics— a commercially prepared solution of microorganisms selected for their beneficial impacts on pond systems. Probiotics have been used previously in human health studies and are already common within foodstuffs, such as dairy products containing live *Lactobacillus* cultures. However, evidence regarding their effectiveness as a management tool in aquaculture is limited and inconsistent (e.g. Shariff *et al.*, 2001; Sonnenholzner and Boyd, 2000; Prabhu *et al.*, 1999). Previous studies, however, have not yet documented the application techniques that typical small-scale farmers adopt, or the impacts of probiotics on water quality and sludge through a crop cycle.

6.2: Goals and results of the study

The main research objective of this study focused on the wastewater management of an inland shrimp aquaculture farm in rural Thailand and investigated the efficacy of a commercial probiotic (already marketed in shrimp producing regions of Thailand) in reducing the organic wastes and biological oxygen demand of shrimp farm effluent. To do this, the potential impacts of intensive, inland, low-salinity shrimp aquaculture on the health of the natural environment and the possible implications for human health were reviewed. *In situ* water quality parameters, biological oxygen demand, and the total organic content of the sludge of a typical inland, low-salinity shrimp pond were then documented and compared to ponds treated with EM-1 on a weekly basis for an entire crop cycle.

The main findings of this study include the following:

- The true effectiveness of EM-1 cannot be properly assessed by the findings of this study since the probiotic was not applied according to the manufacturer's instructions. Clearly, the importance of following the application instructions has not been emphasized enough to growers purchasing the products in either the manuals or on the labels. As a result, the small-scale farmer modified the application method and the adopted treatment did not significantly improve any of the *in situ* water quality variables. Consequently, mean Dissolved Oxygen, temperature, pH, salinity, and Secchi disk visibility were equal in the effluent released for exchange throughout the crop cycle from all of the ponds, and for the final discharge released at harvest.

- From week 5 onwards, the water released for exchange and for the final harvest exceeded the 10 mg/L legal standard for 5-day BOD as set by the Thai government, despite only being measured as a 3-day test. Although results were not significant, general trends indicated a lower demand for the treatment ponds. Extrapolations from the data collected during this study period provide evidence that low-salinity shrimp farms in the east bank sub-basin of the Bang Pakong River region may release effluent with a total BOD of 4.2 million kilograms each year, and that the farms in the entire Bang Pakong River basin may discharge effluent with a total BOD of 17.4 million kilograms each year.
- With the improperly applied EM-1, the total percent organic content of the pond sludge was not significantly reduced in the probiotic treatment ponds, although all ponds contained low levels of organics in the flocculent layer of the pond sediments, indicating that farm management practices have already adapted to minimize the accumulation of sludge.

6.3: Recommendations for future research

The scope of this project was designed with the purpose of studying a single small-scale aquaculture farm. If logistical constraints (e.g. time, laboratory space, finances) were not a concern in future studies, the inconsistencies within the data set collected during this experiment may be avoided or more easily interpreted with alterations to the design and methodology. Enlarging the sample size could increase confidence that the analysis provides an accurate representation of all possible results that could be found in the study area. However, since the focus of this study involved small-scale farms— that is, 2-3 ponds each-- increasing the number of ponds within the study would require increasing the number of actual aquaculture farms sampled. When different farms become included in the study, variation will be introduced that may create confounding effects on the data. For example, slight changes in management practices (e.g. feeding rates, aeration time) may differ among farms that could create a discrepancy in pond conditions. For example, some farmers may have larger reservoirs that could contain more water to concentrate salt. The larger holding capacity would result in greater amounts of saline water being available for water exchange, and ultimately, would affect the Dissolved Oxygen concentrations throughout the crop cycle. Depending on the location of the farm, different climatic conditions could also become a factor in analyzing collected samples. Although the analysis of the results of a study with a larger sample size may be more complex with greater variability, a more realistic conclusion could be drawn since every farm that employs probiotics will not be the same. Repeating the experiment throughout numerous seasons (wet and dry) would also be beneficial for researchers to address the differences that seasonal changes may have upon the efficiency of the microbial additive.

In order to have a broader understanding of the effect of probiotics upon the pond dynamics, standard instructions must be followed. Also, the number of variables measured in this model should be increased. In addition to a full range of water quality parameters and sludge characteristics, information regarding the microbiological aspects of bioremediation techniques needs to be collected. As Moriarty (1997) explains, developments in molecular biology techniques have allowed scientists to determine the growth, presence, and survival of particular species of microbes in their environment. Determining which microbes from the probiotic solution excel in the environment of a given area, which microbes are outcompeted by other organisms already present in the aquatic system of that area, and understanding the impacts that specific environmental conditions have upon the processes of organic matter decomposition could provide valuable insight for farmers as they attempt to effectively incorporate probiotics into shrimp aquaculture.

Finally, one problem that exists within the shrimp aquaculture industry that could overpower any attempts to improve the environment is the extent of degradation that has already occurred in aquatic systems. Based on the data collected at the start of the crop cycle, it appears that water quality is extremely poor even before shrimp crops begin. Thus, bioremediation is expected to not only minimize the impacts of the industry's practices, but also to reverse previous damage in order to restore or improve the state of the resources. Although testing probiotics in controlled laboratory conditions will not provide results that accurately reflect how the product will work in outdoor ponds, it may be worth attempting to replicate the laboratory conditions outdoors. That is, if the water that was used in the laboratory had a minimal biological oxygen demand, starting shrimp crops with similar water may prove to be useful. The BOD measurements provide evidence that water, before entering the pond system, needs to be treated to improve the quality. Although expensive biofiltration units could be purchased, many cheap alternatives are also on the market that can filter mass quantities and still improve water quality. The combination of improved starting conditions and the use of probiotics throughout a crop cycle may yield vastly improved results.

6.4: Management implications of the research

The outcomes of this study provide insight on the use of probiotics in aquaculture for managers within Thailand's shrimp producing regions. However, the findings also have broader implications for resource managers worldwide that are faced with minimizing the impacts of wastewater containing high concentrations of organics, whether the effluent is created from aquaculture, agriculture, or other industries. The recommendations for managers are summarized below.

- Low-salinity shrimp farms are contributing to the increased degradation of aquatic resources with poor quality effluent being released throughout the grow-out period, in addition to the final harvest discharge. In spite of farmers in the Bang Khla area eliminating the use of chemo-therapeutants and fertilizers, the water contains a high concentration of organic material which can increase the biological oxygen demand and reduce the turbidity of receiving waters, leading to eutrophication and algal blooms, and subsequently to decreased levels of photosynthesis and Dissolved Oxygen concentrations.
- The sludge released once every third crop cycle will increase the turbidity and cause sedimentation in receiving canals and rivers, thereby reducing the quantity and quality of accessible water. The low level of organics in the sludge samples may provide evidence that removing the sludge from ponds is an unnecessary task that does not actually improve pond conditions.
- Due to the novelty of probiotic research in aquaculture, sufficient evidence does not exist to support the use of microbial solutions as a management tool in inland low-salinity ponds. The findings of this study illustrate that farmers have not been

properly trained in the use of probiotics. With the modified techniques that small-scale farmers adopt, EM-1 will not be completely effective in reducing the environmental impacts of shrimp farms in the inland regions of Thailand. Therefore, investments into probiotic products by farmers are currently an unnecessary expense if application procedures are not followed in accordance with the manufacturer's instructions.

- Despite the lack of statistically significant improvement in the state of the ponds, the variables measured in this study did indicate that the enhanced microbial activity has the potential to improve pond conditions. More research will be needed to ascertain whether probiotics may be a useful tool for managing the resources of shrimp farms.
- Probiotic manufacturers need to invest in outdoor, experimental ponds that more accurately reflect local conditions in order to better understand how the probiotic will work in that specific area. However, if a company were to exploit such an opportunity, monetary capital would be required to build the research facilities. Thus, funding agencies may find allocating support for research facilities more valuable than subsidizing shrimp farmers' initial investments. A teaching facility would also prove to be useful to communicate with local stakeholders about proper application techniques and farm management when utilizing probiotics.
- As with all agriculture and aquaculture farmers throughout the world who are attempting to maximize their profits, shrimp aquaculturists in Thailand would easily embrace any opportunity to improve their crop yields. Therefore, if application techniques and environmental impacts are thoroughly researched and the results are easily available for farmers and consumers, probiotics may still have potential to be

properly incorporated as a tool into the management regime of those culturing *P. monodon*.

- Farmers may also consider filtering the incoming water prior to stocking the ponds with shrimp seed to ensure optimal starting conditions for the crop.
- Based on the measured variables and general observations regarding the health of the shrimp, probiotic treatment did not appear to cause any negative effects during this experiment. However, the health impacts for those consuming either the water or shrimp resources have not yet been documented. Consequently, research needs to be expanded to focus on these issues.
- Future experiments could produce a more comprehensive data set by including a larger sample size, measurements from numerous crop cycles in different season, and more information on the microbiological activities of the probiotic with respect to pond dynamics.
- Although developing standards within the shrimp industry has thus far been motivated more by international demand for high quality export products than by concern for domestic health and safety, enforcing strict policies at the local level may be impractical, based on previous experience with low levels of compliance. Education of the farmers and the consumers may be a more effective means of communicating the goals for maintaining a sustainable shrimp aquaculture industry and for promoting health safety.
- Shrimp farmers aim to produce profitable crops, but their success is determined by societal response to the product. If people begin to demand a product that is exposed to predominantly "natural" conditions with the least environmental impact possible, farmers will be forced to adapt to provide an adequate supply.

Aquaculture is becoming a true necessity in the world today, both for the food it provides for the ever-increasing human population, and for the economic benefits it supplies to rural areas. Although this study has mainly focused on the farming of *P. monodon*, and the resultant environmental degradation within Thailand, the problem of organic effluent cannot be isolated to one nation. Worldwide, the treatment of wastewater and its impacts on the health of surrounding habitats and the organisms inhabiting those areas is a grave concern. Thus, the results and insights provided by research in shrimp aquaculture could be applicable for managers of biophysical and socio-economic resources in other industries, worldwide.

Although controlling further deterioration of the environment may be constrained by socio-economic circumstances within developing countries such as Thailand, the truth is that shrimp farming is a profitable industry and not merely a subsistence activity for most farmers. With the high economic returns that seafood exports are reaping on international markets today, a certain amount of accountability guaranteeing the safety of the product and environmentally responsible management practices should be expected.

One step towards improving the standards of this industry includes the use of bioremediation techniques for improving water quality and for minimizing the impacts of the effluent released from shrimp ponds. Although the use of a commercial probiotic in this study yielded insignificant results, general trends indicated that organic decomposition was enhanced in the pond water by the addition of the microbial treatment. Future efforts need to focus on educating and training aquaculturists about using probiotics to ensure their effectiveness. Further research also needs to examine other bioremediation alternatives for water quality control that may be used in conjunction with probiotic treatments. In order for the shrimp aquaculture industry to maintain its current production levels or to continue expanding, more research will be crucial to ensure that resource managers involved are able to adopt the most sustainable management models.

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