Effects on Water Quality of Additional Mechanical Aeration in the Waste-Treatment Cells in Split-Pond Aquaculture Systems for Hybrid Catfish Production

by

Lauren Nicole Jescovitch

A dissertation submitted to the Graduate Faculty of
Auburn University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Auburn, Alabama
May 7, 2017

Keywords: split-ponds, aeration, water quality, pond engineering

Approved by

Claude E. Boyd, Chair, Professor Emeritus, School of Fisheries, Aquaculture and Aquatic Sciences
Yolanda Brady, Associate Professor Emerita, School of Fisheries, Aquaculture and Aquatic Sciences
Donald Allen Davis, Alumni Professor, School of Fisheries, Aquaculture and Aquatic Sciences
Philip Chaney, Associate Professor, Department of Geosciences
George W. Crandell, Associate Dean, Graduate School

Abstract

Split-pond aquaculture is a new, innovative system for intensification of pond aquaculture in the southeastern USA. Split ponds have a fish cell and a waste cell, approximately 20% water surface area and 80% water surface area, respectively, in which water recirculates to improve water quality and allow more intensive production than possible in traditional ponds. This three-year study focuses on the possible benefits of using mechanical aeration in the waste-treatment section of the split-pond culture system.

The present study was conducted on a commercial catfish farm in west Alabama that has eight split-ponds, each with a fish-holding section of approximately 8,000 m^2. Water quality was assessed through a variety of parameters that had the potential to be affected by oxygen using standard analytical chemical procedures in the field and laboratory. Further investigation also determined poor circulation rates and aeration in split-ponds because of poor management.

This dissertation discusses water quality and intensification of pond aquaculture, water quality and aeration in split-pond waste cells, and best practices of the split-pond design.

Acknowledgments

The author would like to offer her love and sincere gratitude to her family for their continuous support throughout this dissertation. She also wants to thank Dr. Claude E. Boyd for giving her the opportunity to learn and study aquaculture, and gain teaching experience for the past 5-years under his guidance and wisdom.

The author would like to express appreciation to June Burns, committee members, colleagues and lab mates - especially Piyajit Pratipasen and Hisham Abdelrahman – for assistance in this study and support for various professional opportunities that she completed while attending Auburn University.

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Chapter 1 – Introduction & Review of Literature

1.1 Water Quality in Aquaculture

There are many aspects of aquaculture management, and one of the most important is water quality. Water quality is dependent on various physical factors (climate, light, temperature, etc), water composition (phosphorus, nitrogen, metals, etc.), aquatic plants, soil, and aquaculture species, and type of production system. Water quality can be managed, but because of the complex nature of the factors mentioned above, water quality variables cannot be predicted accurately and must be measured at frequent intervals. Water quality variable concentrations measured at a particular time provide managers with real-time data, but such data often cannot be used to accurately project the concentrations that these parameters will be 24 hours later. Farmers need to monitor water quality so they can observe trends in changes of concentrations and adapt their management practices accordingly. Many water qualities are interrelated and interact with each other (Xu and Boyd, 2016), and changes in one variable gives insight about changes in a related variable. Water quality can have severe effects on living organisms if not managed properly. Rapid changes in concentration or high levels of some variables are thought to compromise immunocompetence of animals and make them more susceptible to pathogenic organisms (Hargraves and Tucker, 2003).

1.1.1 Mechanical Aeration and Dissolved Oxygen

Dissolved oxygen (DO) in water is an extremely important water quality parameter to living organisms, and especially to fish. Chronically low DO concentration is associated with poor appetite, and low feed consumption (Boyd, 2015; Boyd and Tucker, 2014; Green and Rawles, 2011; Torrans, 2005).

Dissolved oxygen concentrations in aquaculture systems should be maintained at resonable levels at all times in order to meet the oxygen demand for biota and production species. While DO can be supplied through reaeration (diffusion, wind, etc.) and photosynthesis, mechanical aeration is necessary in ponds with feeding rates above 30 to 40 kg/ha/day. Photosynthesis is the largest oxygen producer in a pond (Hargraves and Tucker, 2003). Net DO production fluctuates daily as a result of the balance of photosynthesis and respiration as well as to the rate of organic matter decomposition and other oxidative processes. Photosynthesis (Equation 1) consumes carbon dioxide and produces energy for the plankton and releases oxygen. Thus, DO increases in sunlight, but at night, respiration, or the reverse reaction of photosynthesis, occurs and oxygen is consumed. This causes lower DO concentrations during the night. The lowest DO concentration usually is observed just before dawn and that is the most critical time to add additional aeration, because DO levels often fall below acceptable levels at this time. For warmwater fish, early morning DO should remain above 3-4 mg/L, and for coldwater fish above 5-6 mg/L. Warmwater and coldwater fish can survive with concentrations as low as 1.0-1.5 mg/L and 2.5-3.5 mg/L, respectively, but these concentrations will increase stress, diminish appetite or aggressiveness to eat, and – if low enough for a long period of time – they can be lethal (Boyd, 2015).

$$6CO_2 + 6H_2O \xrightarrow{\text{sunlight}} C_6H_{12}O_6 + 6O_2$$

Equation 1. Chemical reaction of photosynthesis.

During feeding, DO decreases because of the increased metabolic rates of the fish feeding in the area. Metabolic rate increases because fish are using more energy to competitively eat. Uneaten feed and fecal matter also create a DO demand. This waste is a source of plant nutrients that stimulate phytoplankton growth. At a greater abundance, phytoplankton can demand more DO for respiration at night increasing DO demand. Phytoplankton also are continually dying and decomposing to increase DO demand. The addition of fertilizer can stimulate algae growth that can produce more oxygen and the increased algal growth removes potentially toxic ammonia. Algicides can be used to thin phytoplankton blooms, but they are not recommended because of the potential for a large die-off of algae and oxygen depletions following algicide application. The balance of phytoplankton and bacterial abundance are very important factors in DO dynamics in ponds (Boyd, 2015; Boyd and Tucker, 2014; Zhou and Boyd, 2015).

If DO levels drop below 3-4 mg/L, mechanical aeration should be provided. Mechanical aeration supplements DO supply, and to raise low DO and maintain DO at satisfactory levels in aquaculture systems. Several types of mechanical aerators are used in aquaculture: paddlewheels, aspirators, fountains, etc. Mechanical aeration is one of the most important management inventions in feed-based, pond aquaculture. Paddlewheel aerators dominate around the world as the most effective mechanical aerator for earthen ponds (Hargreaves and Tucker 2003).

1.1.2 Organic Matter

Organic matter is present in ponds in the form of fish, phytoplankton, bacteria, plants, and even feed. Living organic matter consumes oxygen in respiration, dead organic matter can cause great oxygen depletion when decomposed by bacteria. Organic matter requires a specific amount of oxygen, or a specific oxygen demand, when decomposed and used as energy. The rate of organic matter decomposition is greatly affected by temperature and oxygen availability.

The oxygen demand is expressed as either biological oxygen demand (BOD) or chemical oxygen demand (COD). The BOD is the amount of DO required by the respiration of microorganism in a water sample held in the dark at 20°C for a specific time (commonly 5 days). The COD refers to the oxygen equivalent of the dichromatic ion required to completely oxidize the organic matter in a water sample. These are generally used as indicators for pollution, because a greater BOD or COD is indicative of a greater oxygen demand in effluents.

The concentration of bottom soil organic matter increases drastically in catfish ponds during the first 6-12 months after a new pond is put into production and then reaches equilibrium after 3-5 years (Steeby, 2002). Organic matter can be decomposed by either aerobic or anaerobic processes. If oxygen is unavailable, other agents such as nitrate, sulfate, carbon dioxide, etc. will act as the terminal election acceptors in respiration.

1.1.3 Nitrification

Nitrogen (N) occurs in several forms in pond water: gaseous N (N_2), nitrate (NO_3^--N), nitrite (NO_2^--N), ammonia (NH_3-N), ammonium (NH_4^+-N), and dissolved and particulate N. The most critical forms in aquaculture are ammonia nitrogen and nitrite that are potentially toxic to fish. Feed, uneaten or eaten, and fish feces will decompose releasing ammonia into the system.

Ammonia is either in one of two forms: ionized ammonium or unionized ammonia. The amount of nitrogen present in each form is dependent on pH and temperature: the greater the pH and temperature, the more ammonium ion that is present, which is the toxic form of ammonia. This response is described by the following equation:

$$NH_3 + H_2O = NH_4^+ + OH^- \qquad K_b = 10^{-4.74}$$

The methods for measuring ammonia nitrogen do not distinguish between ammonia and ammonium. The forms must be fractionated based on the pH and temperature. Tables of the percentage of un-ionized ammonia at different temperatures and pH values are available, and on-line ammonia calculators are helpful. Together, the ionized and unionized forms are called total ammonia nitrogen (TAN). The TAN concentration can build up and, if enough of the un-ionized form is present, can stress the fish; symptoms usually include lesions on the gills. However, few cases of direct mortality result from ammonia in aquaculture ponds. More often, ammonia stresses fish and opens the opportunity for other health issues (Boyd, 2015; Boyd and Tucker, 2014; Zhou and Boyd, 2015).

The LC_{50}, or lethal concentration of 50% survival of an organism, for warmwater fish in respect to NH_3-N ranges from 0.3- 3.0 mg/L. First signs of toxicity will appear around 0.01-0.05 mg/L (Boyd 2015). The US EPA (2013) acute and chronic criterion for NH_3-N is 0.067 mg/L and 0.008 mg/L, respectively; however, there is no “safe” ammonia concentration established by law – these are only recommended concentration limits to protect freshwater organisms. According to Zhou and Boyd (2015), the no-observed-effect level (NOEL) for channel catfish is estimated to be 1.0 mg/L NH_3-N in ponds with pH of 7.5 or greater. Adequate aeration and efficient feed management should be used to prevent excessively high TAN concentrations, especially because the alternatives of immediate, emergency practices (i.e. algicides, exchange water, adding an acid, etc.) are expensive and have negative environmental impacts. Unfortunately, an ammonia standard for hybrid catfish still needs to be determined through research.

Ammonia in aquaculture has been a growing concern because of the increased feeding rates in intensive systems. In feed-based aquaculture, 60-80% of nitrogen contained in the protein of feed enters the pond as uneaten feed and feces or is excreted by fish as ammonia nitrogen. Intensification and high production increases the nitrogen input and leads to greater TAN concentrations. With photosynthesis causing higher pH during the day, NH_3-N levels increase during the day (Boyd and Tucker, 2014). The concentration of TAN increases in late fall and early winter despite the reduced feeding rates. This results from decomposition of organic matter that has accumulated during the summer (Hargreaves and Tucker, 20003).

Total ammonia N can be removed through uptake by phytoplankton and by the nitrification process. Ammonia N is used by *Nitrosomas* bacteria and converted into nitrite (NO_2) and nitrite is used by *Nitrobacter* bacteria and converted to nitrate (NO_3). Nitrite is potentially toxic, but fortunately nitrification usually continues to nitrate, which is not considered toxic. Both genera of nitrifying bacteria are autotrophic and require aerobic conditions in order for nitrogen oxidation to occur. However, nitrate will remain in the water until absorbed by plants, denitrified, or lost in outflows. Denitrification, or nitrogen reduction, is conducted by heterotrophic bacteria (many species) that under anoxic conditions convert nitrate into nitrogen gas (N_2). These heterotrophic take oxygen from NO_3 as an alternative to molecular oxygen. In the process, nitrogen gas is formed and released into the water. Nitrogen gas diffuse from water into the atmosphere.

A rapid oxidation rate of ammonia nitrogen and nitrite minimizes their concentration in ponds. Higher concentrations of ammonia nitrogen in the water will block ammonia that is in the fish gills from diffusion into the water thus remaining in the fish's blood – becoming toxic. Toxic ammonia in the blood will adversely affect the fish's health, diminish feeding rates, increase feed conversion ratio (FCR), and thus even more feed will be wasted as a response. Phytoplankton will compete with nitrifying bacteria for ammonia which could manipulate the microbial community present leading to production of odorous compounds that when absorbed render fish off-flavor (Hargreaves and Tucker, 2003).

Unfortunately, very quick nitrification of ammonia can lead to high concentrations of nitrite in the water, which can lead to methemoglobinemina or brown blood disease in fish (a condition causing brown blood, gills, and internal organs). Bowser et al. (1983) showed that in the presence of high nitrite, DO of 5 mg/L is not sufficient for channel catfish. Increasing aeration drives nitrification to the nitrate (not as toxic) form thus reducing nitrite. Also, by elevating concentrations of chloride or bromide in the water, the uptake of nitrite by fish is blocked (Kroupova et al., 2005).

1.2 Traditional Pond Design in Southeastern USA

Traditional ponds are either excavated, levees formed around the area in which to impound water, or watershed catchments dammed to capture and hold water for fish production. Catfish ponds may reach up to 16 ha in size (Hargraves and Tucker, 2003). These ponds typically exhibit various levels of intensification as described above and can produce a variety of production species.

In the southeastern USA, the most common species grown in ponds is the catfish. In the past, channel catfish was the most common species, but, in recent years, farmers have decided to produce hybrid catfish. Current estimation of hybrid catfish production is 30-40% of total catfish production in the US (Li et al., 2014). Hybrid catfish are created when a channel catfish female (*Ictalurus punctatus*) and a blue catfish male (*Ictalurus furcatus*) mate. These hybrids are more disease resistant, grow faster and bigger, and are more tolerant to poor water quality conditions than their channel catfish parents (Dunham and Masser, 2012; Green and Rawles, 2011).

Farmers have found that management practices are more effective in smaller ponds, and thus the average size of ponds decreased from 8-16 ha in the 1940s-1990s to 2-6 ha today. Traditional pond production typically ranges from 5,600-6,700 kg/ha (Heikes, 1996). Typically, ponds are subject to semi-intensive, or intensive, management. These farms also use multiple-batch approach to stocking and harvesting. Thus, fish are being stocked every year and harvested any time of the year when the farmer can get the best price and/or needs the money. Multiple-batch production also reduces the economic risks associated with off-flavor because another pond can be chosen for harvest rather than the one with the presence of off-flavors. This management style is also advantageous for reducing effluents. These ponds can be operated continuously for many years without draining unlike many single-batch cropping systems (Hargreaves and Tucker, 2003).

Off-flavors and blue-green algae communities may dominate because of the high degree of eutrophication and high waste loading rates that are associated with intensification. Waste treatment and assimilation capacity of aquaculture ponds is a limiting factor for intensification as a result of deterioration in water quality from over feeding (Hargreaves and Tucker, 2003). Many designs to improve production and increase the water quality limit on production. One method is based on transfer of water from an intensive fish confinement area to less extensive culture pond where water is treated by natural processes for reuse. The improved mixing practice increases algal production and settling to stabilize algal populations. The advantages of a smaller confinement area for fish reduce labor in the form of water quality management, animal and bird predation, feeding, harvesting, and sorting. The major disadvantage to these systems is the increased use of energy intensive pumping systems that are necessary to move high volumes of water between the two ponds. In addition, algal production produces diurnal oxygen and ammonia cycles that can lead to algal population crashes (Brune et al., 2003).The partitioned aquaculture system, in-pond race way, and the split-pond are some of more popular systems using this technique.

1.2.1 Split-Pond Design

Partitioned aquaculture system, or PAS, developed by David Brune at Clemson University were modified and implemented by Craig Tucker at Mississippi State University into what are now called split-ponds. Split-ponds are created by dividing a traditional pond into two sections: fish section and waste-treatment section. The fish section, or cell, is approximately 20% of the total area, while the waste cell is approximately 80%. This system is an intensification of the traditional pond system in order to yield higher production, and up to five times the density of traditional ponds. This system provides reduced labor for harvest, reduced cost in chemical treatments, and lower feed conversion ratio (FCR). This new system is becoming popular within the catfish industry in Mississippi and Arkansas, and it is now starting to develop in Alabama (Tucker, 2009).

Traditional, semi-intensive ponds can yield 6,000 kg fish/ha, but intensification from a split-pond system can produce yields of over 12,000 kg fish/ha (Tucker, 2009). The high production requires a higher cost than what most farmers are used to; thus, farmers may try to modify the design to create their own mixed practices and designs of a traditional pond and split-pond. These un-researched modifications may result in failure of production at higher stocking densities.

Implemented commercially in 2009, split-ponds are a new system of ponds for which little data exists on water quality of these systems. The present research will expand on this knowledge by exploring water quality in a large commercial farm, in which some ponds have aerators in the waste cell and others do not. The present research also has the potential to determine if additional aeration results in increased ammonia oxidation, through nitrification, leads to more production than with un-aerated waste cells. The present research will provide an assessment of best management practices used to manage split-pond systems. For instance, transferring research findings to the commercial industry has always been a challenge. The present on-farm research will be able to depict a more accurate result or application of split-ponds than does a highly, controlled approach. Farmers and researchers will be able to apply these results for future research in split pond management and water quality.

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Chapter 2 – Split-Pond Water Quality

2.1 Abstract

Split ponds have a fish cell and a waste cell accounting for approximately 20% and 80% of total water surface area, respectively. Water passes from the fish cell to the waste cell for water quality improvement and flows back to the fish cell. The present study was conducted on a commercial catfish farm in west Alabama that has eight split-ponds, each with a fish-holding section of about 8,000 m^2. Two, 10-hp floating, electric paddlewheel aerators were placed in the waste treatment section of each of four ponds; while four ponds – the controls – had un-aerated waste treatment cells. Water samples were collected biweekly at the inflow and outflow of the waste-treatment cells; once the water became cooler in the fall and winter, the samples were collected monthly. Analyses were made for pH, dissolved oxygen (DO), temperature, secchi disk visibility, Chlorophyll *a*, total ammonia nitrogen, nitrite-nitrogen, nitrate-nitrogen, total nitrogen, total phosphorus, soluble reactive phosphorus, chemical oxygen demand (total and soluble), biological oxygen demand, and acidification potential. Water circulation rates and aeration hours were determined as well as sediment samples analyzed. The study period was too short in Year 1 (2014) to obtain meaningful results. In Year 2 (2015), differences between control and ponds with aerated waste cells were found for Secchi disk visibility, total ammonia nitrogen, total nitrogen, chemical oxygen demand (soluble and total) and DO. In Year 3 (2016), differences

were analyzed between control ponds and ponds with aerated waste cells for total ammonia nitrogen, total phosphorus, and soluble chemical oxygen demand. Nevertheless, no differences were found between treatments and control ponds for production, yield, and FCR. The effects of fish mortality in several ponds probably had a great influence on production and FCR than did aeration in the waste cells. Best management practices that could help the farmer minimize fish mortality and improve production are discussed.

2.2 Introduction

Alabama and Mississippi are the two leading catfish-producing states; the production area in Alabama was 30,000 acres while Mississippi had 78,000 acres in production in 2014. Both states have experienced losses in catfish production since 2009 (USDA, 2016). These losses can be attributed to the competition of imported catfish from Asia (Bosworth et al., 2015; Hanson and Sites, 2013). Some farmers who have had troubles with maintaining profitable production during the last decade converted their farms to agricultural land or dedicated the land to other purposes.

In order to prevent more loss to the catfish industry, new, innovative production systems such as the partitioned aquaculture system (PAS) and split-ponds have been promoted. Split-pond aquaculture is a version of the PAS that has similar characteristics such as confinement of fish in a smaller area, controlling dissolved oxygen in a smaller portion of the water area, and aggressively treating for diseases and cyanobacteria (Brune et al., 2004). Split-ponds can be created using existing, traditional catfish ponds through renovation rather having to build new production facilities thereby lessening the cost of adoption of a new production method. Split-ponds are formed when a levee is added inside an existing pond to divide the pond into a 1:4

relationship: 20% water surface area designated to fish production and 80% designated to waste-treatment. The water should be able to move freely between these two cells, and screens must be installed to isolate fish within the smaller cell (Tucker, 2009).

Many advantages come from using an intensive system such as the split-pond. Fish may be stocked at a higher stocking density, fish are easier to feed and harvest, medicated treatments can be isolated to only the fish cells thereby reducing cost, and greater yields may be achieved. In 2009, a commercial-sized, split-pond with a stocking rate of 1,334 kg/ha produced a yield of 17,880 kg/ha at a feed conversion ratio (FCR) of 1.83. This commercial-sized, split-pond consisted of a 0.4-ha fish cell and 1.42-ha waste-treatment cell. The 2009 study provided a promising alternative production method for farmers struggling to make ends-meet (Tucker, 2009).

Farrelly et al. (2015) conducted a study comparing water quality conditions between different pond production systems that including split-ponds and traditional ponds. Net production for traditional ponds was 4,962 kg/ha and for split-ponds it was 13,390 kg/ha. Of course, split-pond net production was slightly lower than the harvest weight reported in the study above. This study found that the feeding rate was significantly greater in split-ponds than traditional ponds (which is to be expected with intensification), but there also were greater concentrations of total phosphorus, alkalinity, and hardness in the split-ponds. Both Farrelly et al. (2015) and Tucker (2009) reported that total ammonia nitrogen (TAN) concentrations rarely exceeded 2.0 mg/L.

Presently, there is limited information on commercial split pond systems and the need for aeration within the waste treatment cell. Hence, the objective of this study was to determine if additional paddle-wheel aerators in the waste-treatment cells of split ponds affected water quality within split-ponds from June 2014-September 2016.

2.3 Materials and Methods

2.3.1 Design

This experiment was conducted from June 2014 through September 2016. A commercial catfish farm in west-central Alabama was selected for the study because it had six, split-ponds constructed with the intention of creating more in the near future. Ponds 3, 4, 5, 7, 8, and 9 were already active as split-ponds in May 2014, pond 10 became operational in August 2014, and pond 13 was operational in June 2015. All ponds had two or three 10-hp paddlewheel aerators for maintaining DO in the fish cells. Ponds 4, 8, 9, and 10 (the treatment ponds) were designed to include two additional 10-hp paddlewheel aerators at the inlet of the waste cells as indicated by the red and white indicators (Figure 2.1). These ponds were operational by August 2014; the other ponds were considered the control group. Ponds were randomly assigned to each group.

A custom-made, axial pump consisting of a propeller of 50-cm in diameter, shaft and 12.5 kW electric motor was placed between the fish and waste cells. The propeller was inserted in the end of the 90-cm diameter corrugated pipe extending between the two cells of a split-pond. Between the pipe and the screen, a dam was installed to maintain division and circulation between the cells. Screens were placed at the corner with the propeller pump to protect fish from the propeller and to prevent fish from moving into the waste cell. Water then returned without additional pumping back into the fish cell through a 1.1 m x 6 m screen. There was no baffle in the waste treatment cells for all ponds. A typical split-pond with additional aerators in the waste cell is show in Figure 2.2.

Control ponds and ponds with aerated waste had an average of 6:1 waste cell: fish cell water volume ratio (Table 2.1) as determined by Google Earth Pro for surface area and average depth as determined from measurement made intermittently along an S-shaped pattern (Boyd and Tucker, 1998). Ponds were stocked with hybrid catfish (*I. punctatus* ♀ x *I. furcatus* ♂). A multiple-batch culture was practiced and most ponds were stocked and harvested at least two times during the duration of the present study. Fish were provided a 32% crude protein, floating, pelleted feed (Alabama Catfish Feed Mill, Uniontown, AL, USA) that was distributed by truck-mounted feeders that propelled the feed into the fish cells only. The daily feed input per pond was recorded by the farm manager. The Feed Conversion Ratio (FCR) was determined using annual production and annual feed inputs.

The electrical system for the farm was managed through the farmer's own company, AirCon Technologies, LLC. Electrical meters that could control aerator operation and record DO concentration, water temperature, and time of aeration operation was installed in the farm. Fish cell aerators were turned on when a DO concentration reached a limit set by the farm manager, and the waste cell aerators were programed to turn on if the DO in the waste cell fell below 2.0 mg/L and then off once the DO exceeded this threshold.

2.3.2 Water quality analyses

Pond water were sampled at the inflow (in) and outflow (out) of the waste-treatment cells for the control ponds and ponds with aerated waste cells. These sampling locations are thus referred as control-in, control-out, aerated-in, and aerated-out. Secchi disk visibility and DO data were collected on site. Water samples were collected using a 3-m plastic rod attached to a dipper that collected water below the surface. Samples were transferred into 1-L dark, plastic bottles that were held on ice in insulated chests for transport from the farm to the laboratory at Auburn University's E.W. Shell Fisheries Center in Auburn, Al. Background samples were taken weekly between June and July 2014. Aerators in waste cells were wired and operational at the beginning of August 2014. Samples were collected biweekly during summer months and monthly during cooler months until the end of September 2016.

Water samples were filtered using glass fiber filters and analyzed using standard protocols as follows: pH (Orion 3 Star Probe, Thermo Scientific City Co.), chlorophyll *a* by membrane filtration, acetone-methanol extraction of phytoplankton, and spectroscopy; total ammonia nitrogen (TAN) by the salicylate method (Bower and Holm-Hansen, 1980; Le and Boyd, 2012); nitrite nitrogen by the diazotization method (Boyd and Tucker, 1998); nitrate nitrogen was measured by the Szechrome NAS reagent method (Van Rijin, 1993). Total nitrogen (TN) and total phosphorus (TP) were analyzed by the ultraviolet spectrophotometric screening method and ascorbic acid methods, respectively, following digestion in potassium persulfate solution (Gross et al., 1999; Eaton et al., 2005). Total and soluble chemical oxygen demands were analyzed by the heat of dilution technique (Boyd and Tucker, 1992). Ammonia-nitrogen concentrations were calculated using an online calculator (http://www.hbuehrer.ch/Rechner/Ammonia.html) with corresponding pH and temperature.

2.3.3 Non-routine Analyses

Some other water quality parameters were measured three to four times during of 2015 and 2016. These variables were soluble reactive phosphorus; total, carbonaceous, and nitrogenous biological oxygen demand; calcium, and magnesium hardness; total alkalinity; total suspended solids and total suspended volatile solids (Clesceri et al, 1998).

The acidification potential of the pond water was determined by finding the calcium carbonate equivalent of the potential acidity resulting from nitrification of ammonia produced by microbial decomposition. One nitrogen molecule consumes two oxygen molecules; thus, 1 mg/L N, 4.57 mg/L of DO is consumed. This process produces two hydrogen ions – or acidity – that reacts with one calcium carbonate; thus, neutralizing total alkalinity (Boyd, 2015). Overall, total alkalinity, or acidification potential, can be used to determine the nitrification potential of the water. So, water samples were measured for pH, total alkalinity, and TAN every other day from the same container that was constantly open to the atmosphere. Analyses stopped when TAN measurements reached 0 mg/L; thus, no more nitrification of ammonia was possible. Acidification potentials were determined four times.

A 24-hour pH study was conducted to determine daily fluctuations in pH. A portable pH meter (HACH Pocket Pro Tester; Loveland, CO. USA) was used to measure pHs at the inflow and outflow locations for all eight ponds. The pH of samples was measured every 3 hours for 24 hours in Year 3. Samples were taken in the same order for each time period to assure 3 hour separation between measurements because 1 hour was necessary to complete measurements at all locations.

Soil was collected from the bottom of ponds by using an Ekman dredge dropped from a boat (Boyd and Tucker, 1998) at multiple places in each pond and compositing the dredge grabs to form a single composite sample. The samples were dried and pulverized to pass a 20-mesh screen and sent to the Soil, Forage, & Water Testing Laboratory in Auburn, Alabama for analysis of pH, 18 elements, nitrate-nitrogen, nitrogen, carbon, and organic matter concentrations.

2.3.4 Statistical Analyses

Data were analyzed for means and standard deviation, repeated measures analysis of variance (ANOVA) on ranks followed by Tukey for multiple comparison procedure, and t-tests by means of SigmaPlot version 11.0 statistical software (Aspire Software International, Ashburn, VA, USA).

2.4 Results

2.4.1 Production

Fish production only occurred in the smaller, fish cells of the split-ponds, however, to accurately portray production values, the entire area of both the fish and waste-treatment cells were used. No differences were found for stocking rate, feed input, total production, yield, and FCR between control and aerated waste-cell ponds (Table 2.2). Yields for the control and aerated waste-cell ponds were 9,003 kg/ha and 7,936 kg/ha, respectively. Both groups had a FCR of 4.4. While survival or mortality were not measured, there were reports of fish kills that had a negative effect on the FCR. Feed was used to produce the dead fish, but only the weight of live fish harvested was used in calculating FCR.

2.4.2 Background water quality

Eight paddlewheel aerators were used for this study. Problems with delivery and installation of the aeration system delayed the beginning of the study. During June and July 2014, routine water quality parameters were measured to determine background levels before the study began (Table 2.3). Only six ponds were in production as split-ponds in 2014. Nitrite-nitrogen was found to be at greater concentrations in the ponds that were assigned to the treatment group with 0.131 mg/L in inflow to the waste cell compared to 0.045 mg/L in outflow of the control waste cells. All other water quality variables were analyzed to ascertain if there were differences between treatment and control ponds.

2.4.3 Water quality

The waste-cell aerators were operational at the beginning of August 2014. Thus, during Year 1 data were collected from August through December 2014. The only differences in Year 1 were in the ammonia nitrogen concentrations which had a higher level in aerated-in ponds with 0.21 mg/L than control-in with 0.14 mg/L. However, there were no differences between the ammonia nitrogen in the control and ponds with aerated waste cells with the outflow water from the waste cells (Table 2.4).

In Year 2, data were collected from January through December. During this time, differences were found between total ammonia nitrogen, ammonia nitrogen, total nitrogen, and total and soluble COD (Table 2.5). Total Ammonia N, total chemical oxygen demand, and soluble chemical oxygen demand followed the same trends of having no differences between in and out locations within the control and aerated pond groups, but the ponds with aerated waste cells had lower concentrations than the control ponds. Average concentrations of TAN were 2.73

mg/L and 3.13 mg/L for the control-in and control-out locations, respectively, and 1.67 mg/L and 1.74 mg/L aerated-in and aerated-out locations, respectively. There were no differences between control-in and aerated-in for ammonia nitrogen; however, aerated-out locations were significantly lower ($P<0.05$), 0.06 mg/L than control-out concentrations at 0.12 mg/L. Total COD had greater values for control-in and control-out than aerated-in and aerated-out: 38.72 mg/L, 40.31 mg/L, 33.06 mg/L, and 34.12 mg/L, respectively. Soluble COD had slightly less averages in the same manner with 32.25 mg/L, 35.95 mg/L, 27.73 mg/L and 29.04 mg/L, respectively.

In Year 3, data were collected from January through the end of September 2016. Differences were found between TAN, ammonia nitrogen, total phosphorus, and soluble COD (Table 2. 6). Concentrations of TAN were less in the ponds with aerated waste cells. Averages for TAN in control-in ponds were 1.889mg/L and control-out ponds were 2.09, while aerated-in ponds were 0.79 mg/L and aerated-out ponds were 0.87 mg/L. There were no differences between control-in, aerated-in, and aerated-out for ammonia nitrogen; however, control-out locations were higher with a concentration of 0.05 mg/L. Total phosphorus had averages of 0.459 mg/L for control-in ponds and 0.481 mg/L for control-out ponds, with significantly lower concentrations in aerated-in ponds with 0.284 mg/L, but not with aerated-out ponds with a concentration of 0.332 mg/L. Soluble COD only had differences between control-in and aerated-in ponds with concentrations of 35.60 mg/L and 30.65 mg/L, respectively.

There were differences between background data and data collected during the rest of the study with Years 1, 2, and 3 within the same treatments for the following parameters: pH, Secchi disk visibility, TAN, nitrate, total nitrogen, total COD, and soluble COD (Figure 2.3, Figure 2.4, Figure 2.5). Chlorophyll *a*, nitrite, and total phosphorus had no differences within the same treatment and control over the course of the background collection and study. Overall, pH decreased from background data into the rest of Year 1 and 2 for control-in, control-out, and aerated-in; aerated-out did not differ over this time. Secchi disk visibility decreased from Year 1 to Year 3 for only the aerated-in and aerated-out treatments. The TAN concentration drastically increased from background data into year one, but then decreased until year three for all treatments. Nitrate was only different for aerated-in and aerated-out treatments with higher concentrations for year 2 than the rest of the study period. Total nitrogen only changed with the control-out treatment with a higher concentration in Year 2 than for the background data. Total COD had very low concentrations during the background months, but it drastically increased within each treatment at by Year 1. Control-in, control-out, and aerated-out continued to increase through Year 3; aerated-in remained greater than the background data, but did not increase over time. Soluble COD followed the same patterns in background and the study for control-in and control-out, but both aerated-in and aerated-out treatments did not increase after Year 1.

2.4.4 Non-Routine analyses

Additional water quality parameters were also collected during Years 2 and 3 of the study. These parameters include soluble reactive phosphorus, total biological oxygen demand (BOD_5), carbonaceous biological oxygen demand (CBOD), nitrogenous biological oxygen demand (NOD), total hardness, calcium hardness, magnesium hardness, total alkalinity, total suspended solids (TSS), and totals suspended volatiles solids (TSVS). These parameters exhibited no differences ($P>0.05$) between treatments, but their averages and standard deviations are shown in Table 2.7.

The acidification potential of control and additional aerated waste cell waters were different based on the average regressions of four, separate trials. There were no differences between the control ponds and ponds with aerated waste cells for acidification potential. Control ponds had a potential of 1.14 mg/L $CaCO_3$/day and ponds with aerated waste cells of 1.32 mg/L $CaCO_3$/day. Regression equations for these potentials are shown in Figure 2.7.

The 24-hour pH study revealed that pHs of all treatments fluctuated, on average, between 7.38 and 9.31 (Figure 2.4). No differences (P>0.05) occurred between treatments.

Average values for typical soil parameters for control and treatment ponds for Year 2 and Year 3 are shown in Table 2.8. Only difference (P>0.05) occurred between treatment ponds for barium (Ba) with 4.5 mg/L present in Year 2 that was reduced to 1.5 mg/L in Year 3.

2.5 Discussion

Fish are stocked and harvested at various intervals in a multiple-batch culture system. Thus, the longer the period in which the data are collected, the more accurate is the prediction of average, annual production. Net yield estimations included a wide range in yields, 9,003 ± 4,764 kg/ha/yr for control ponds (n=3) and 7,936 ± 4,737 kg/ha/yr ponds with aerated waste cells (n=4). The net yield data from this production were more than that from traditional ponds (Heikes, 1996) and up to the lower end of yields for split-ponds (Farrelly et al, 2015). The FCRs for treatment and control ponds of 4.4 must be considered an extremely low result – especially for hybrid catfish. Despite Dunam and Masser (2012) finding that the FCR of hybrid catfish being 10-20% better than channel catfish being for younger fish, channel catfish typically have a FCR of 1.6-1.8 in research (Boyd and Tucker, 1998), and farmers usually obtain a FCR less than

2.5. Poor feed management by the farmer combined with mortality from diseases likely resulted in the extremely high FCR.

During this study, no differences in water quality occurred between control-in and control-out or between aerated-in and aerated-out were observed. The lack of differences shows that water quality entering the fish cell is of the same quality as the water exiting the fish cell. Thus, no conclusion can be made about whether split-ponds waste-treatment cells improve water quality. This could be because of the large size of the ponds or other contributing factors. Further studies should determine pond sizes and water circulation design affecting water quality. In the present study, Secchi disk visibilities were similar to those reported by Brune et al. (2001) in the algal cell of a PSA system.

There were, however, differences between treatments and controls seasonally. The most significant water quality finding related to TAN concentrations. The greatest averages for TAN in all treatments were during September and October in Year 1 – ranging from 6.1-7.6 mg/L. By this same time in Year 2, ponds with aerated waste cells had significantly lower concentrations of TAN than did control ponds. Peak TAN concentrations were above 6 mg/L for both inflow and outflow control locations, while ponds with aerated waste cells had concentrations between 1.8-2.1 mg/L (Figure 2.5). The higher concentrations of TAN were the results of intensification of production that lead to high inputs of nitrogenous waste from high feed inputs and high stocking rates. Concentrations above 5.0 mg/L are common among similar farms in this area of west-central Alabama (Zhou and Boyd, 2015). Brune et al. (2004) also had comparable TAN results throughout the year in the PAS system with greater fluctuations in August.

Zhou and Boyd (2015) found that TAN concentrations were not correlated with aeration, total feed input, and weight of harvest fish. However, they stress that low DO concentrations inhibit ammonia oxidation by nitrification, thus increasing the TAN concentration and favoring NH_3 toxicity. Ammonia nitrogen often exceeded the EPA acute and chronic limits (Figure 2.6), but no values exceeded the NOEL of 1.0 mg/L determined by Zhou and Boyd (2015). Ponds with aerated waste cells had significantly lower proportions of ammonia in the water that was coming out of the fish cell; this suggests a reduction in TAN concentrations and also indicates that aeration of the waste cell improves ammonia management.

High TAN concentrations were reduced through the nitrification process. The split-ponds had low nitrite concentrations compared to the 96-hr LC50 for nitrite (Figure 2.5). However, this nitrite standard can fluctuate based on ammonia, pH, oxygen, temperature, and fish size and age (Kroupova, Machova, and Svobodova, 2005). Channel catfish can typically tolerate oxygen concentrations that fall below 5 mg/L, but according to Bowser et al. (1983), this concentration is not sufficient for channel catfish in the presence of elevated nitrite. The 96-hr LC50 for nitrite in hybrid catfish has not been determined. However, as a general rule-of-thumb, larger fish of several species have 96-hr LC50 values around 8 mg/L for N-NO_2 (Kroupova et al., 2005). Only 3.5% of nitrite-nitrogen measurements this this study were ≥1.0 mg/L – the greatest concentration was 3.2 mg/L. However, there was one occurrence where the nitrite-nitrogen concentration was above 1.0 mg/L on two consecutive sample dates. The Alabama Fish Farming Center often attributes fish kills to sharp increases of nitrites (such as these observed in the present study). These high nitrite episodes do not allow the fish to appropriately acclimate to poor water quality conditions. No differences were found between control ponds and ponds with aerated waste cell for nitrite during the present study.

Total phosphorus concentrations were lower in ponds with aerated waste cell inflow compared to the control ponds in Year 3. Soluble reactive phosphorus values (Table 2.7) showed no differences between treatments. Thus, the control ponds accumulated more particulate phosphorus and nitrogen than the ponds with aerated waste cells.

Total and soluble COD concentrations remained less than 15 mg/L and 9.0 mg/L, respectively, before September in Year 1. By the beginning of September, six initial ponds had already been in full operation for three months, but aeration in these ponds with aerated waste cells had only been provided for 1 month. By the beginning of September in Year 1, total and soluble COD increased as high as 44 mg/L and 34 mg/L, respectively. Total and soluble COD concentrations continued to increase and there was a greater difference in control pond concentrations than ponds with aerated waste cells in Year 2. By Year 3, all values increased but the only differences between control and treated ponds were soluble COD. This contributes to the concern of organic matter accumulation in split-pond systems.

Despite there being no differences between control and treatment for Year 1, differences in water quality data were found starting between Year 1 and the background data when treatments were compared across years. This should be interpreted cautiously, as most of the parameters that showed differences (TAN, nitrate, and total nitrogen) followed the trend of increasing drastically at this time of year. However, COD increased three-fold after the split-ponds were operational. The ponds that were constructed during and integrated into the study were outliers during the first year they were in operation. This allowed for only n=4 for both control and additional aerated waste-treatment cell pond groups to only occur during Year 3.

All other non-routine water quality variables measured were within acceptable ranges for fish culture with no differences between control and treatment. The variable BOD, Carbonaceous BOD (CBOD), and Nitrogenous Oxygen Demand (NOD) were analyzed for the purpose of determining how much oxygen is required for the nitrogenous bacteria in response to the additional aeration in the waste cell, but no differences were observed. Soil samples, TSS and TSVS do not show differences between control and treatment either. This does not support the observation that ponds with aerated waste cells had more organic and particulate matter. Of course, these parameters were not analyzed as frequently as those in Table 2.3, Table 2.4, and Table 2.5, and if more samples had been analyzed, possibility of a difference could have been shown in BOD, CBOD and NOD.

Acidification potentials were not different in ponds with aerated waste cells than in control ponds. However, there were only four trials completely randomly throughout the study. More trials during peak seasons of TAN and NH_3 concentrations could provide further insight to the treatments acidification potential. Thus, it is important to have amble supply of dissolved oxygen in order to increase nitrification rates since the aerated waste cell ponds have a greater potential to nitrify more of the TAN than the control ponds. This statement is supported by the evidence that TAN concentrations are reduced in the ponds that have additional aeration.

The 24-hour pH study showed that the daily low and high pH value follow the typical pattern for aquaculture ponds (Boyd and Tucker, 1990). However, it should be noted that the routine sampling was done between 1000hr and 1100hr. Thus, the routine pH sampling was taken 2 or 3 hours before maximum daily pH usually occurs.

2.5.1 Complications

Design and construction of the split-ponds, aerator/pump placements, wiring, and aeration rates were preset by the farmer and followed convenience of installation of operation and cost reduction. For instance, to reduce the length of wire from the aerator to the electrical box, the aerator in the fish cell was placed such that water impinged on the embankment between the cells. These aerators ideally would have been at 90°with the inflow from the waste-treatment cells to direct the water along the long axis of the fish cell. Placing the aerators this way would have increased circulation and proper mixing in the fish cell, as well as reducing erosion on the dividing levee. Moreover, the propeller pumps were not operated constantly during summer months, reducing mixing and circulation from fish cells to waste cells.

The multiple-batch system made analyzing actual fish production, feed conversion ratio (FCR) or survival difficult on an annual basis. The FCR was also further skewed because of reoccurring fish kills due to nitrite stress and *Microcystis* poisoning (personal communication, fish health specialist at the Alabama Fish Farming Center). The weights of the dead fish were not included in the production data contributing to a higher FCR.

The motors of the paddlewheels that were placed in the waste-treatment cells of the split-ponds sporadically failed during year 2 and 3 of the study and had to be replaced. Thus, the waste cell aerators were not operational for 4-6 weeks while waiting for motor replacement. These motor failures were thought to have affected the water quality results.

2.6 Conclusions

Overall, water quality was improved over the 3-year study in ponds with paddlewheel aerators positioned at the inflow of the waste-treatment cells of split-ponds. TAN and COD were constantly lower in the ponds with aerated waste cells compared to the control ponds. Ammonia nitrogen proportions were the same concentrations in the water that was leaving the fish cell; however, ammonia nitrogen concentrations were lower in water entering the fish cell in the ponds with aerated waste cells rather than the control ponds. Production was not affected by the observed difference between control and treatment, but lower un-ionized ammonia concentration should have reduced stress to fish.

There were no differences between quality of water going into the waste-treatment cells and that of water leaving the waste-treatment cells in either control or treatment ponds during the present study. This could have been the result of lack of circulation in these large ponds. Split-ponds should be designed and managed to facilitate complete mixing of water within each cell and good circulation between cells. It is likely that if the waste cell is too large, short circuiting of flow between the fish cell and waste cell will result in deterioration of water quality.

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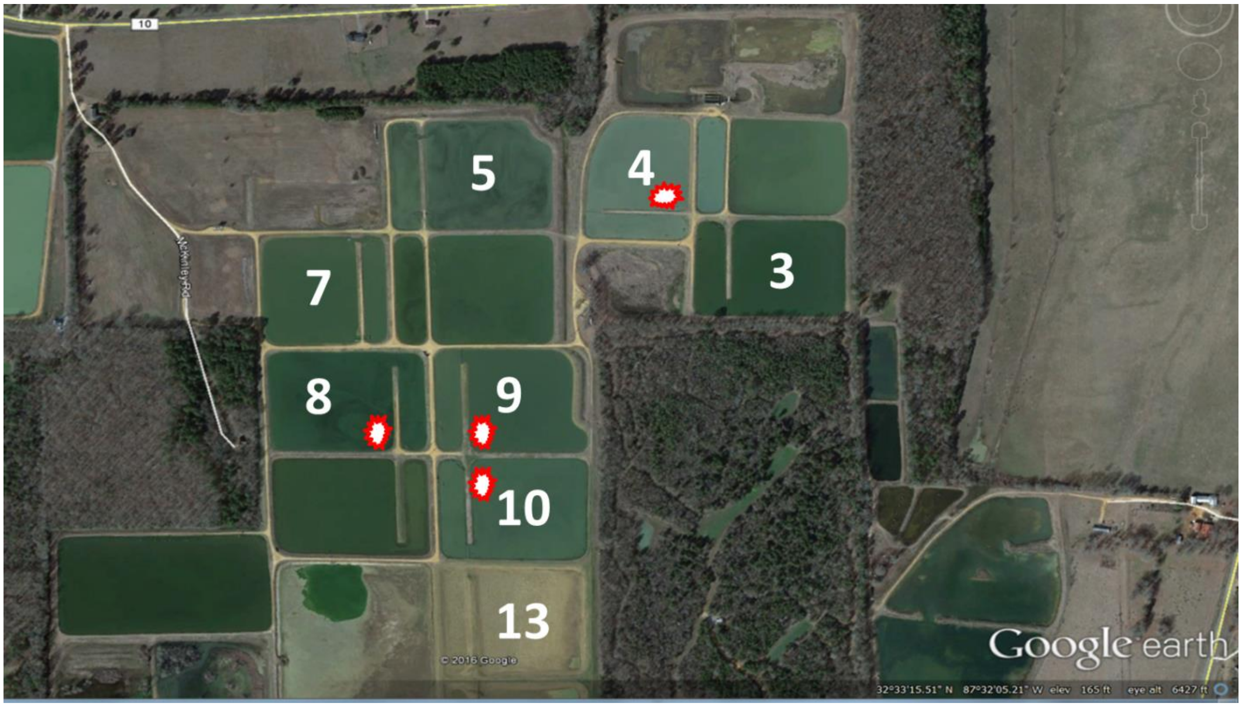

Figure 2.1. Study site in Hale County, Alabama. Control Ponds: 3, 5, 7, 13; Aerated waste-treatment cell ponds: 4, 8, 9, 10 as noted by symbols. Picture taken using Google Earth Pro.

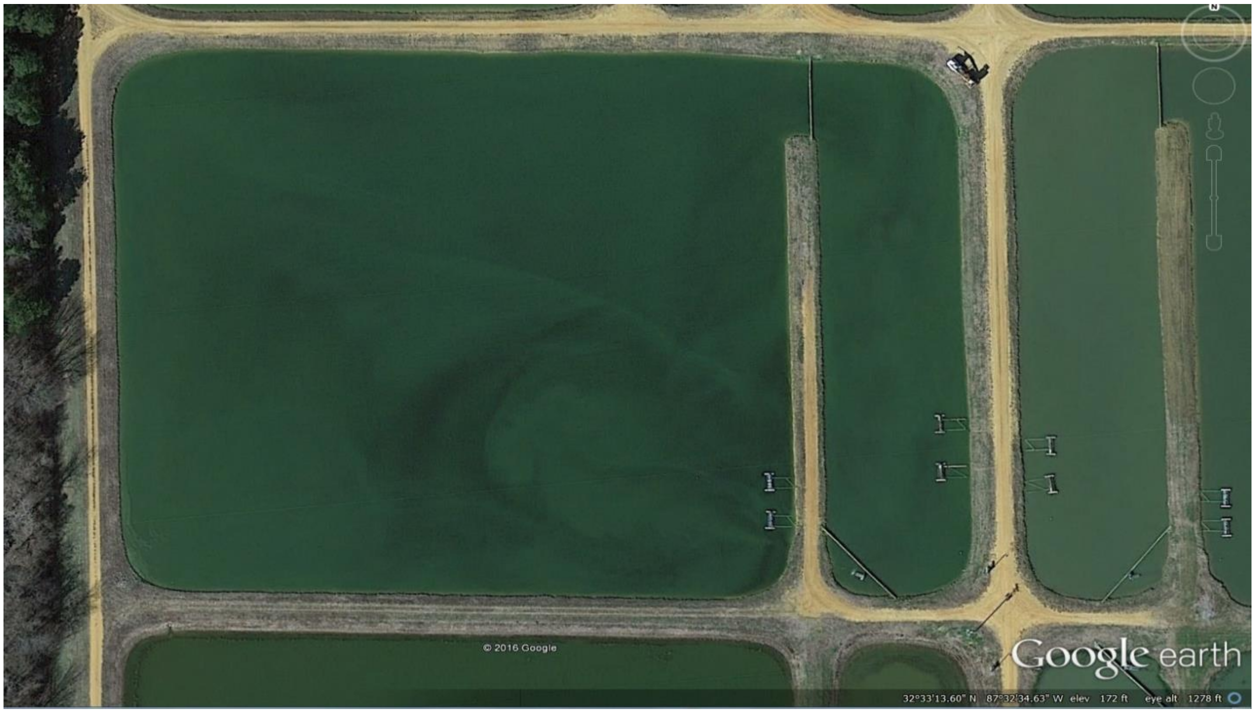

Figure 2.2. Typical split pond used in this study. This pond has waste cell aerators placed where water is traveling through a pipe between the fish cell and waste cell. Picture taken using Google Earth Pro.

Table 2.1. Average pond measurements for fish and waste cells for control and ponds with aerated waste cells using Google Earth Pro for surface area and a calibrated rod for depth.

	Surface Area (m^2)		Depth (m)		Volume (m^3)	
	Fish	Waste	Fish	Waste	Fish	Waste
Control Ponds (n=4)	6,593	28,681	1.66	2.10	10,970	60,775
Ponds with Aerated Waste Cells (n=4)	6,138	26,517	1.57	2.29	10,946	60,468

Table 2.2. Average stocking rates, feed inputs, production, net yields, and feed conversion ratio (FCR) for control and ponds with aerated waste cells for multiple-batch management system over three years (2014-2016). Area includes both fish and waste cell assimilation. Significant differences are noted by letters (P<0.05)

	Stocking Rate (kg/ha/yr)	Feed Input (kg/ha/yr)	Total Production (kg/ha/yr)	Net Yield (kg/ha/yr)	FCR
Control Ponds (n=3)	1,746 ± 132	33,170 ± 5537	10,749 ± 4868	9,003 ± 4764	4.4 ± 2.0
Ponds with Aerated Waste Cells (n=4)	1,827 ± 671	28,900 ± 3363	9,763 ± 4403	7,936 ± 4737	4.4 ± 2.0

Table 2.3. Average pH, Secchi disk visibility, and concentrations of other water quality variables in control and treatment ponds with aerated waste cells for six sampling data as background data (June-July, 2014). Significant differences are noted by letters (P<0.05)

	Control (n = 3)		Treatment (n = 3)	
Variable	In	Out	In	Out
pH	8.33	8.36	8.47	8.15
Secchi disk visibility (cm)	4.17	3.96	5.12	4.83
Chlorophyll *a* (µg/L)	196.93	193.56	157.05	169.61
Total ammonia nitrogen (mg/L)	1.340	1.313	1.142	1.287
Ammonia-nitrogen (mg/L)	0.133	0.083	0.174	0.116
Nitrite (mg/L)	0.062**ab**	0.045**a**	0.131**b**	0.112**ab**
Nitrate (mg/L)	0.194	0.161	0.333	0.222
Total nitrogen (mg/L)	4.314	4.122	3.705	3.731
Total phosphorus (mg/L)	0.395	0.289	0.266	0.258
Chemical oxygen demand, total (mg/L)	9.43	9.54	8.55	8.23
Chemical oxygen demand, soluble (mg/L)	7.02	7.41	6.46	6.69

Table 2.4. Average pH, Secchi disk visibility, and concentrations of other water quality variables in control and treatment ponds for seven sampling data in year one (August-December, 2014). Significant differences are noted by letters ($P<0.05$)

	Control (n = 3)		Treatment (n = 4)	
Variable	In	Out	In	Out
pH	7.85	7.94	8.05	8.02
Secchi disk visibility (cm)	3.81	3.98	4.82	5.39
Chlorophyll *a* (μg/L)	171.68	183.34	187.60	203.14
Total ammonia nitrogen (mg/L)	4.357	4.684	3.406	3.475
Ammonia-nitrogen (mg/L)	0.144**a**	0.197**ab**	0.205**b**	0.146**ab**
Nitrite (mg/L)	0.209	0.187	0.347	0.325
Nitrate (mg/L)	0.279	0.277	0.340	0.335
Total nitrogen (mg/L)	5.843	5.730	5.303	5.048
Total phosphorus (mg/L)	0.344	0.273	0.362	0.361
Chemical oxygen demand, total (mg/L)	31.04	32.07	31.15	31.49
Chemical oxygen demand, soluble (mg/L)	25.88	25.78	27.39	27.70

Table 2.5. Average pH, Secchi disk visibility, and concentrations of other water quality variables in control and treatment ponds for seven sampling data in year two (January-December, 2015). Significant differences are noted by letters (P<0.05)

	Control (n = 3)		Treatment (n = 4)	
Variable	In	Out	In	Out
pH	7.90	7.88	7.93	7.87
Secchi disk visibility (cm)	4.08	4.41	4.03	4.81
Chlorophyll *a* (µg/L)	212.92	262.63	202.37	208.11
Total ammonia nitrogen (mg/L)	2.734**a**	3.132**a**	1.671**b**	1.738**b**
Ammonia-nitrogen (mg/L)	0.119**ac**	0.146**a**	0.067**bc**	0.059**b**
Nitrite (mg/L)	0.211	0.208	0.203	0.193
Nitrate (mg/L)	0.268	0.227	0.425	0.433
Total nitrogen (mg/L)	5.378**ab**	5.979**a**	4.320**b**	4.657**ab**
Total phosphorus (mg/L)	0.519	0.681	0.471	0.672
Chemical oxygen demand, total (mg/L)	38.72**a**	40.31**a**	33.06**b**	34.12**b**
Chemical oxygen demand, soluble (mg/L)	32.25**a**	35.95**a**	27.73**b**	29.04**b**

Table 2.6. Average pH, Secchi disk visibility, and concentrations of other water quality variables in control and treatment ponds for eight sampling data for year three (January-September, 2016). Significant differences are noted by letters ($P<0.05$)

	Control (n = 4)		Treatment (n = 4)	
Variable	In	Out	In	Out
pH	7.86	7.84	7.94	7.92
Secchi disk visibility (cm)	3.71	4.20	3.01	3.60
Chlorophyll *a* (µg/L)	251.29	253.96	245.92	292.23
Total ammonia nitrogen (mg/L)	1.886**a**	2.088**a**	0.787**b**	0.866**b**
Ammonia-nitrogen (mg/L)	0.042**ab**	0.048**a**	0.024**b**	0.025**b**
Nitrite (mg/L)	0.217	0.225	0.202	0.195
Nitrate (mg/L)	0.013	0.014	0.031	0.031
Total nitrogen (mg/L)	4.540	4.550	3.893	3.985
Total phosphorus (mg/L)	0.459**a**	0.481**a**	0.284**b**	0.332**ab**
Chemical oxygen demand, total (mg/L)	43.98	42.97	39.71	40.75
Chemical oxygen demand, soluble (mg/L)	35.60**a**	34.38**ab**	30.65**b**	31.89**ab**

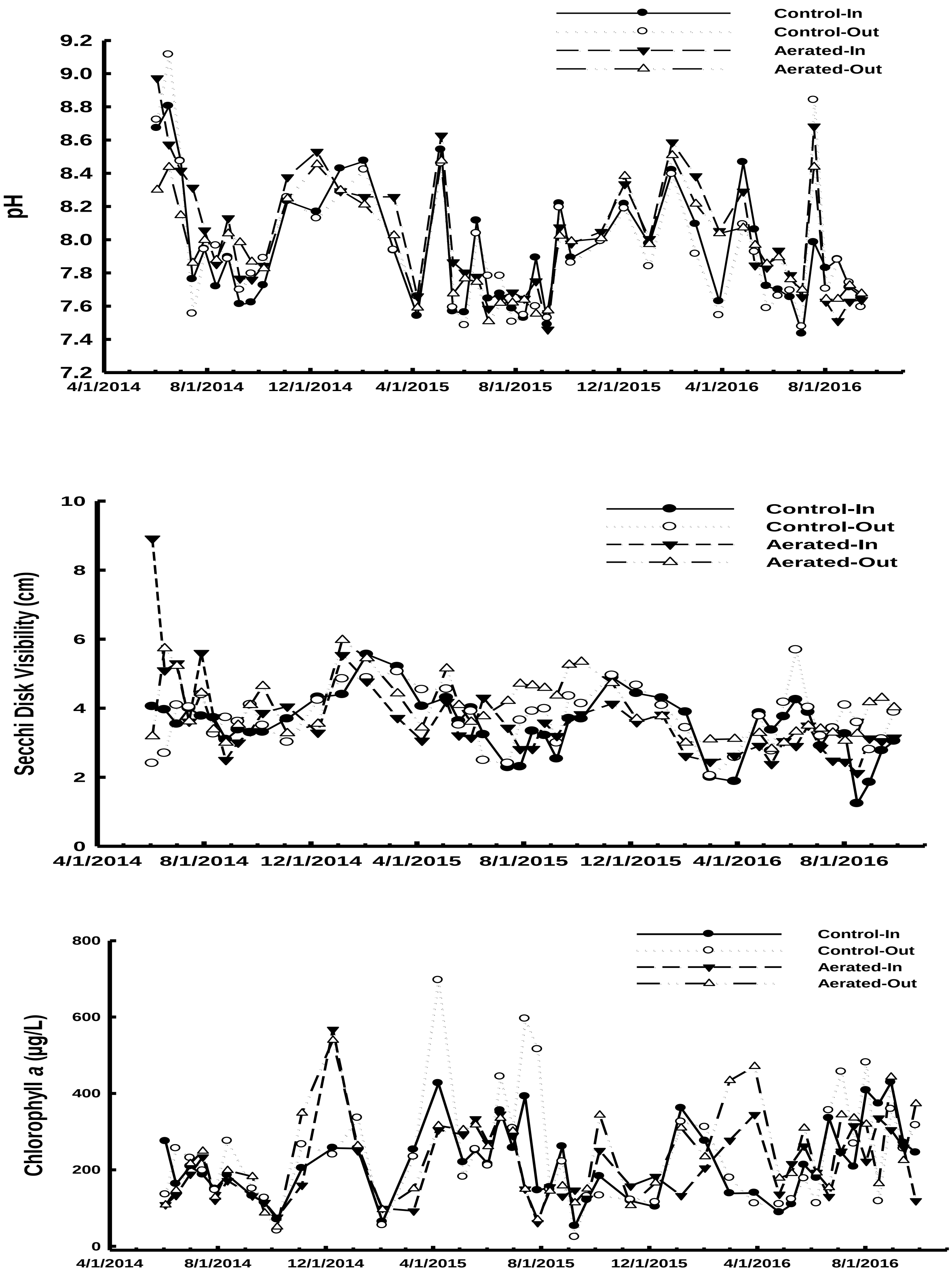

Figure 2.3. Water quality averages (pH, secchi disk visibility, and chlorophyll *a*) for background, and years 1-3 of study for control-in, control-out, aerated-in, and aerated-out sample locations.

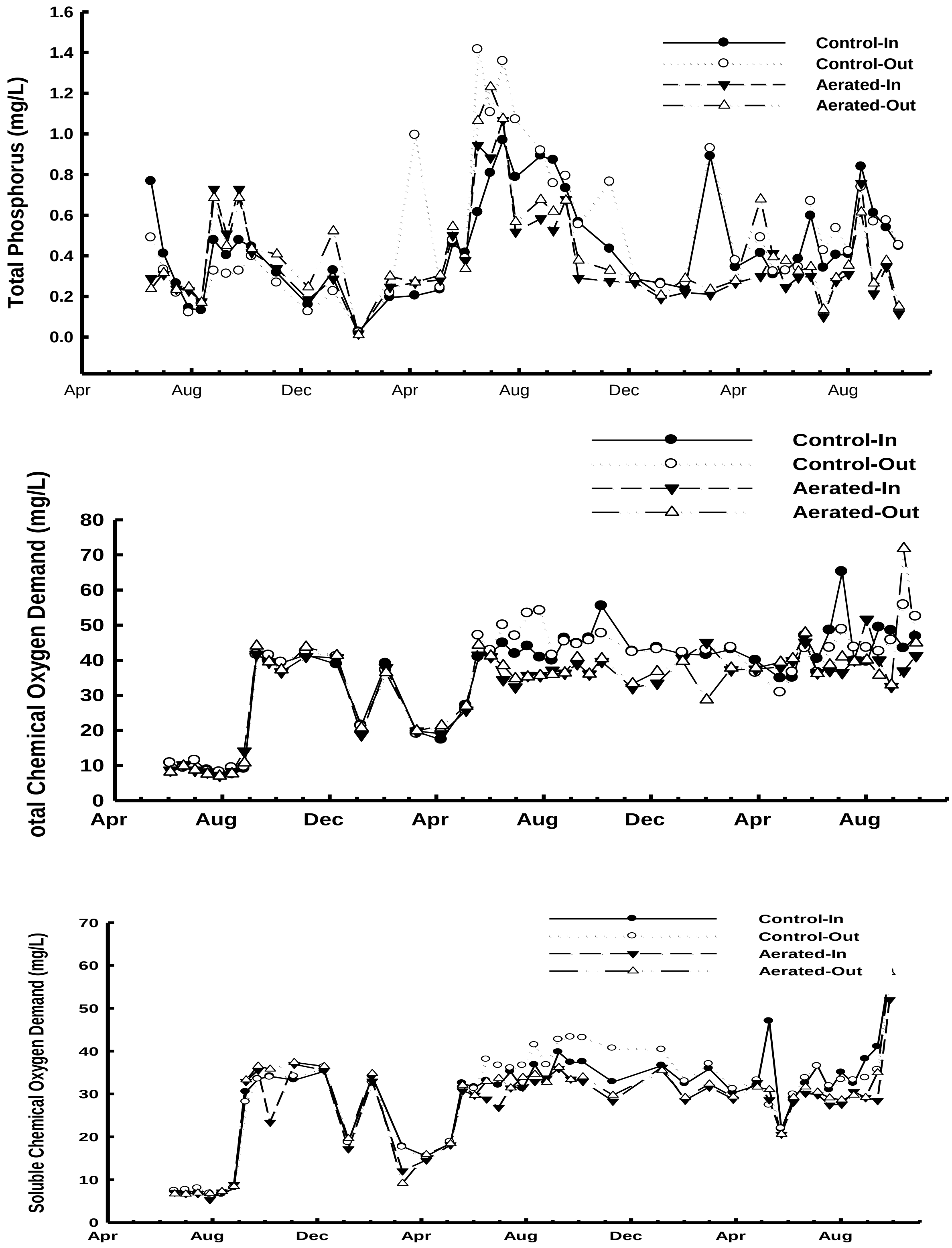

Figure 2.4. Water quality averages (total phosphorus, total and soluble COD) for background, and years 1-3 of study for control-in, control-out, aerated-in, and aerated-out sample locations

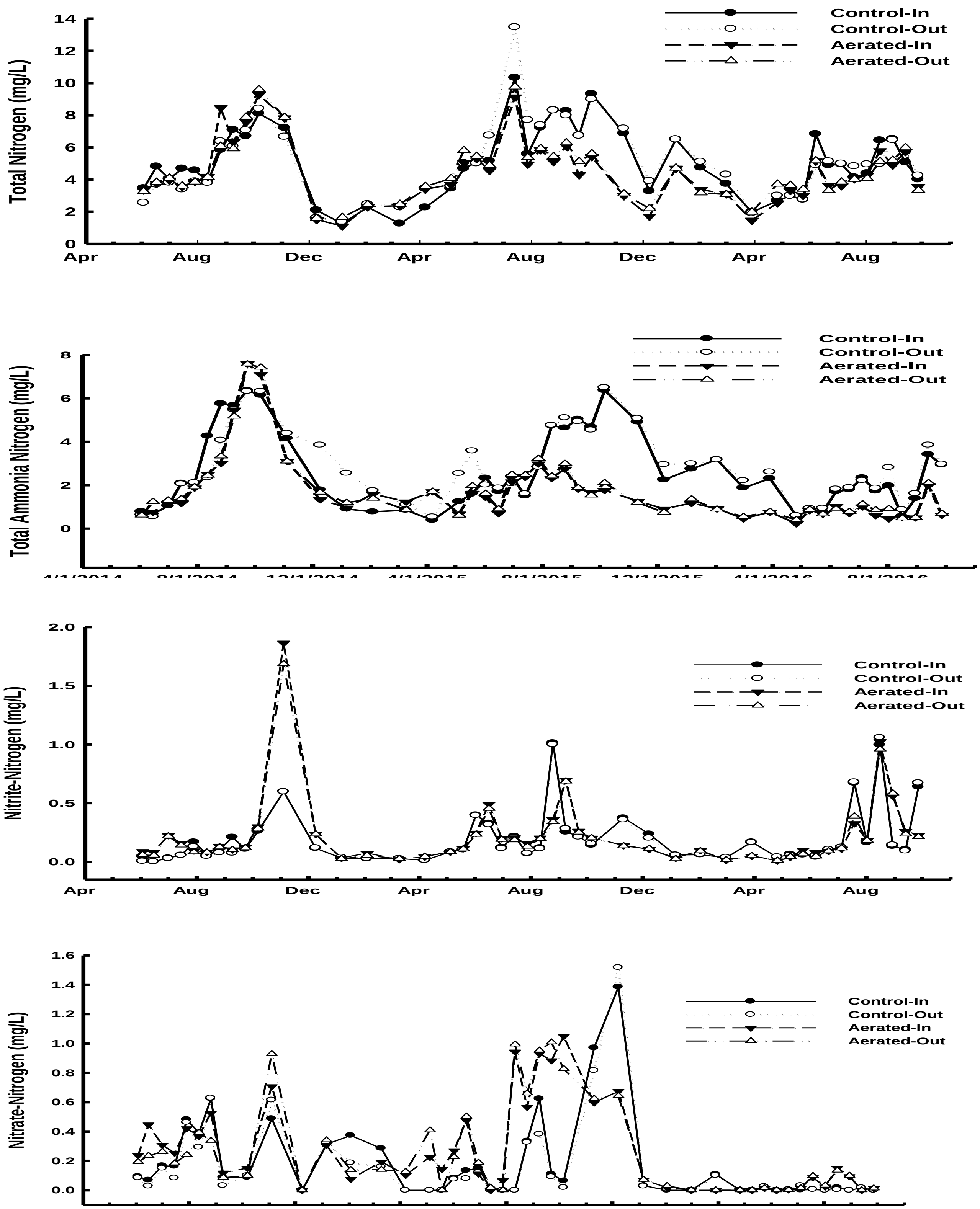

Figure 2.5. Water quality averages (total nitrogen, TAN, nitrite nitrogen, nitrate nitrogen) for background, and years 1-3 of study for control-in, control-out, aerated-in, and aerated-out sample locations

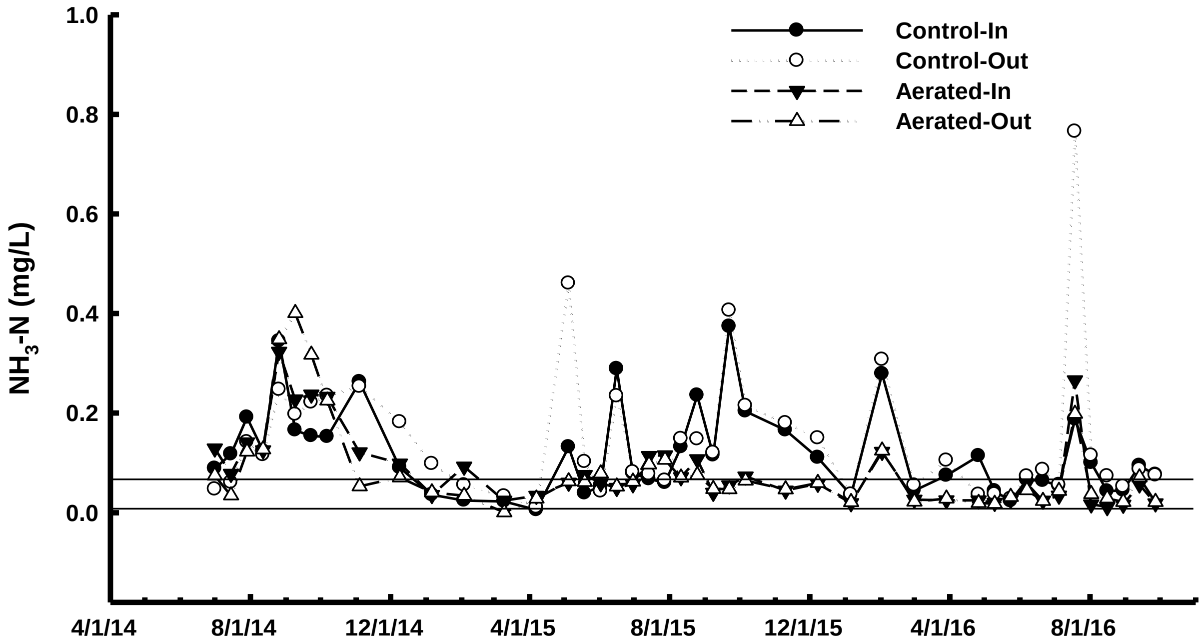

Figure 2.6. Ammonia nitrogen averages for background, and years 1-3 of study for control-in, control-out, aerated-in, and aerated-out sample locations. US EPA (2013) limits for acute and chronic ammonia nitrogen concentrations are illustrated.

Table 2.7. Average values for non-routine variables (2015-2016). Significant differences are noted by letters (P<0.05)

	Control (n = 4)		Treatment (n = 4)	
Variable	In	Out	In	Out
Soluble Reactive Phosphorus (mg/L)	0.154	0.175	0.185	0.189
Total Biological Oxygen Demand (mg/L)	9.39 ± 4.03		11.10 ± 6.48	
Carbonaceous Biological Oxygen Demand (mg/L)	7.71 ± 3.51		7.58 ± 3.81	
Nitrogenous Biological Oxygen Demand (mg/L)	2.95 ± 2.60		3.36 ± 4.65	
Total Hardness (mg/L)	83.43 ± 37.30		80.54 ± 26.99	
Calcium Hardness (mg/L)	70.27 ± 34.47		68.21 ± 25.41	
Magnesium Hardness (mg/L)	13.16 ± 6.22		12.33 ± 3.81	
Total Alkalinity (mg/L)	116.54 ± 31.06		125.20 ± 28.61	
Total Suspended Solids (µg/L)	46.7 ± 18.5		51.3 ± 21.7	
Total Suspended Volatile Solids (µg/L)	46.1 ± 17.3		51.4 ± 18.7	

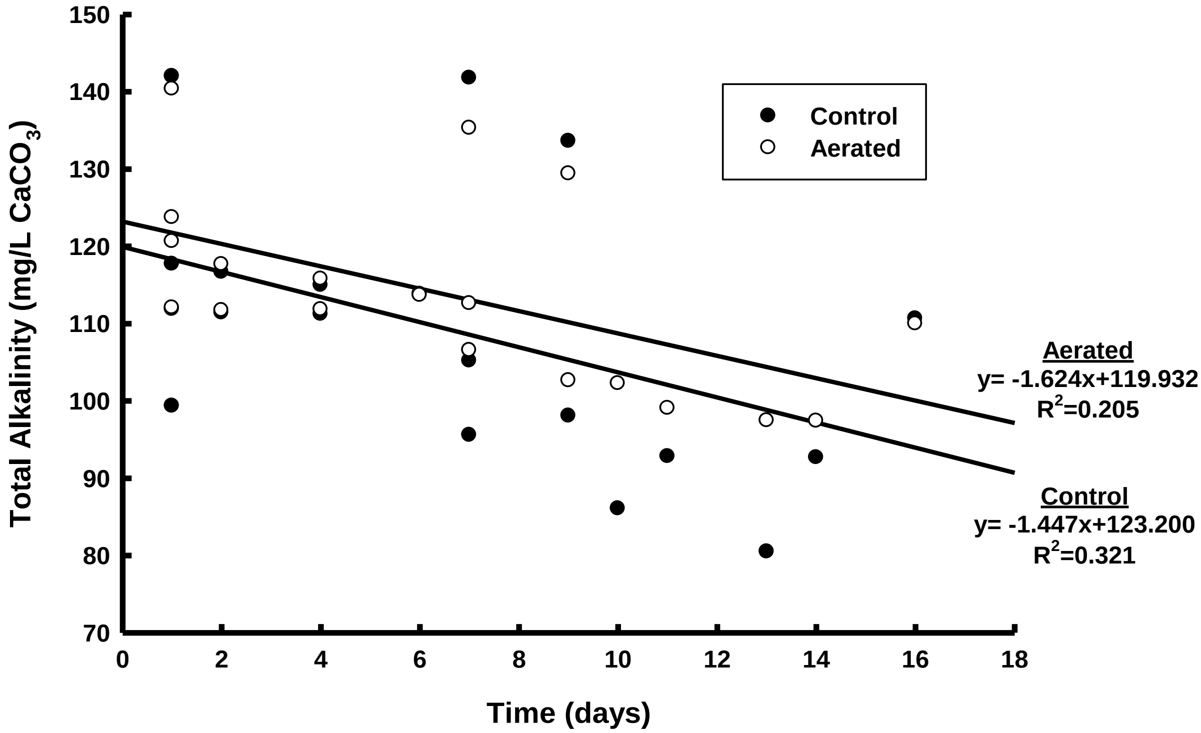

Figure 2.7. Total alkalinity concentrations for water samples from control and treatment ponds during acidification trials.

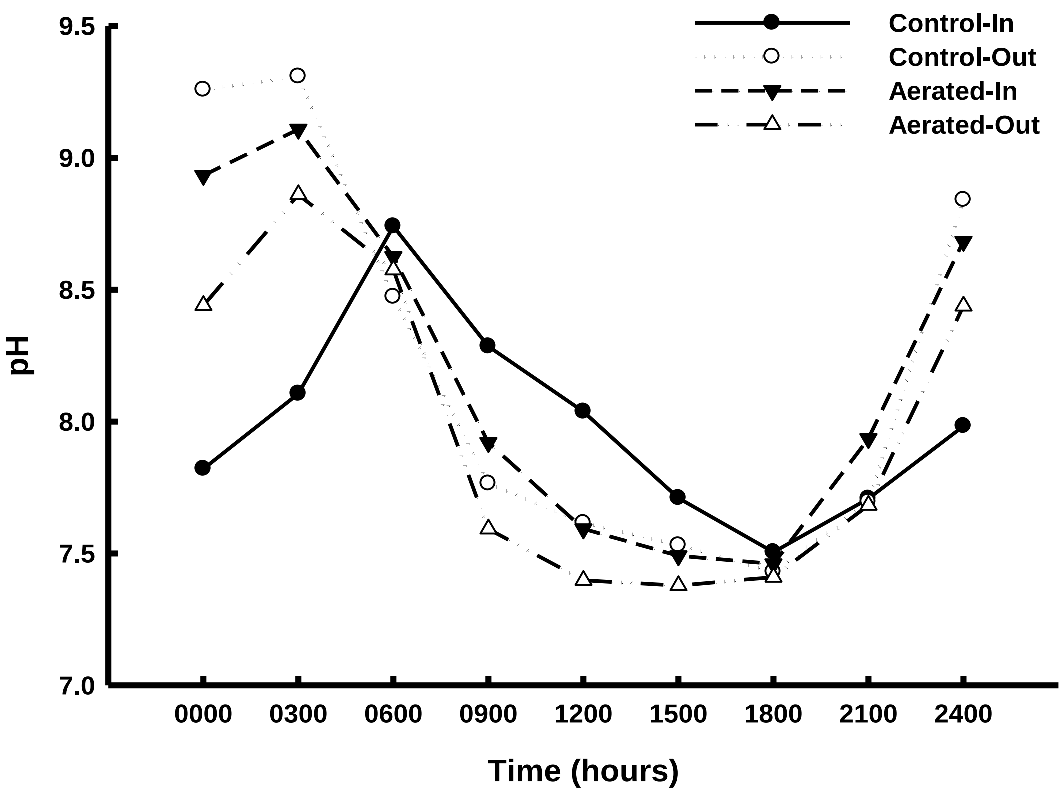

Figure 2.8. Average pH measurements. Measurements were taken every 3 hours for 24 hours for control-in, control-out, aerated-in, and aerated-out sample locations.

Table 2.8. Average values of soil parameters for eight sampling data in Year 2 and Year 3 (2015-2016). Significant differences are noted by letters ($P<0.05$)

	Year 2 (2015)		Year 3 (2016)	
	Control	Treatment	Control	Treatment
(ppm)				
Ca	5911.25	7855.75	12133.75	23935
K	166.5	171.75	312.25	449
Mg	167.75	173	397	497.25
P	14.8	8.825	87.5	272.75
Al	80.25	29.75	104.5	43.5
As	0.1	0.1	0.1	0.1
B	0.425	0.3	0.275	0.25
Ba	3.5**ab**	4.5**a**	1.5**b**	1.5**b**
Cd	0.1	0.1	0.1	0.1
Cr	0.1	0.1	0.1	0.1
Cu	15.75	10.25	1.75	1.0
Fe	43.5	15.5	67.25	8.275
Mn	104.25	106.5	81.75	53.75
Mo	0.1	0.1	0.1	0.1
Na	446.5	527.25	294.25	387.5
Ni	0.1	0.1	0.325	0.225
Pb	0.1	0.1	0.1	0.1
Zn	4.75	3.75	1.25	1.025
NO3-N	3.65	3.35	5.23	5.13
(%)				
N	0.255	0.345	0.085	0.171
C	1.575	2.475	0.765	1.43
OM	2.725	4.25	1.325	2.45
pH	7.08	7.21	6.32	7.19

Chapter 3 – Split-Pond Aquaculture System Design and Dissolved Oxygen Management

3.1 Abstract

Split-pond aquaculture is a new, innovative system for intensification of pond aquaculture in the southeastern USA. Split ponds have a fish cell and a waste cell, approximately 20% water surface area and 80% water surface area, respectively, in which water recirculates to improve water quality and allow more intensive production than possible in traditional ponds. This is a continuation of a study that focuses on the possible benefits of using mechanical aeration in the waste-treatment section of the split-pond culture system.

Work was conducted on a commercial catfish farm in west Alabama. The farm currently has eight split-ponds, each with a fish-holding section of about 8,000 m^2. Two, 10-hp floating, electric paddlewheel aerators were placed in the waste treatment cell of each of four ponds; while four ponds – the controls – had un-aerated waste treatment cells. Water samples were collected biweekly at the inflow and outflow of the waste-treatment cells; once the water became cooler in the fall and winter, the samples were collected monthly. Water analyses included general water quality parameters, dissolved oxygen (DO), and water temperature. Water circulation rate was assessed and the hours of aeration recorded. No differences were found between control and treatment ponds for production, yield, and FCR. Examination of DO

concentration records determined that at least one fish cell and one waste cell in both control and additional aerated ponds fell to 2.5 mg/L or lower at least for one hour during the present study. Aeration hours were greater than reported for traditional ponds but without much benefit in DO concentration as a result of carrying capacity and increased feeding rates in the split-ponds. Circulation and mixing were virtually non-existent even when all pumps/aerators were operating – mainly because of the amount of aeration and the positioning of aerators.

Additional aeration in waste-treatment cells of split-ponds did improve water quality, and especially for total ammonia nitrogen. This should have reduced stress on fish, but no beneficial effects on fish production resulted. The poor performance of the study ponds is thought to be the results of poor pond design and management.

3.2 Introduction

Usually, one thinks of recirculating aquaculture systems (RAS), as indoor, fully controlled, recirculating tanks such as those used in salmonid hatcheries. Such systems do not contribute much to global aquaculture production. About 70% of global aquaculture production is from ponds (Boyd and McNevin, 2015). Nevertheless, there is much interest in expanding the application of RAS systems, and the technology is being applied in pond aquaculture.

Intensification of production was achieved in catfish ponds using a variation of the partitioned aquaculture system (PAS) known as the split-pond system (Brune et al., 2004; Tucker, 2009). Split-ponds are created by taking a traditional pond and splitting the water surface area into two, unequal sections: 20% dedicated for fish grow-out and 80% for waste treatment. The water is then recirculated – using aspirators or slow rotating paddlewheel aerators – between these two sections to improve water quality for a greater carrying capacity (Tucker, 2009). These types of designs allow a systematic approach to engineering aquaculture processes in series. Fish carrying capacities of 11,000-28,000 kg/ha are possible with on-site waste treatment and consequently reduces its' environmental impact (Brune et al., 2003).

Intensification of pond aquaculture requires greater aeration to meet the higher respiratory demand. The increase in feeding rates leads to increased phytoplankton and greater potential for low nighttime DO concentrations. Great phytoplankton blooms reduce underwater light penetration resulting in self-imposed limits on photosynthesis. The majorities of farmers have sized and position aerators to meet the respiratory demands of the fish, but not the oxygen demand of chemical and microbial processes. Additional aeration for waste treatment cell in split-pond systems might increase waste assimilation capacity and thus increase production in the fish cell. However, there are no data upon which economic benefits of waste cell aeration were analyzed (Hargreaves and Tucker, 2003).

Split-pond aquaculture is growing rapidly in the United States (US). US farmers have been struggling to sell their hybrid catfish in the face of the competition with imported catfish from other countries, so they were looking for a simple, yet effective solution. Researchers at Mississippi State University have proved that the split-pond system for catfish production significantly improves production; however, it does require a greater initial investment (Tucker, 2009). If done correctly, farmers only have to renovate their current ponds to create split-ponds rather than constructing new ones. This is certainly favorable aspect of split-ponds as compared to PAS ponds.

Because split-ponds are a new practice, the purpose of this research was to illustrate engineering design and dissolved oxygen management in split-pond aquaculture. The current study focused on adapting to larger ponds used in the commercial industry – rather than using small, highly-controlled research ponds.

3.3 Materials and Methods

3.3.1 Design

This experiment was conducted from June 2014 through September 2016. A commercial catfish farm in west-central Alabama was selected for the study because it already had six, split-ponds constructed with the intention of creating more in the near future. Ponds 3, 4, 5, 7, 8, and 9 were already active as split-ponds in May 2014, pond 10 became operational in August 2014, and pond 13 was operational in June 2015. All ponds had two or three 10-hp paddlewheel aerators for maintaining DO in the fish cells. Ponds 4, 8, 9, and 10 (the treatment ponds) were designed to include two additional 10-hp paddlewheel aerators at the inlet of the waste cells as indicated by the red and white indicators (Figure 3.1). These ponds were operational by August 2014; the other ponds were considered the control group. Ponds were randomly assigned to each group.

A custom-made, axial pump consisting of a propeller of 50-cm in diameter, shaft and 12.5 kW electric motor was placed between the fish and waste cells. The propeller was inserted in the end of the 90-cm diameter corrugated pipe extending between the two cells of a split-pond. Between the pipe and the screen, a dam was installed to maintain division and circulation between the cells. Screens were placed at the corner with the propeller pump to protect fish from the propeller and to prevent fish from moving into the waste cell. Water then returned without additional pumping back into the fish cell through a 1.1 m x 6 m screen. There was no baffle to separate inflowing and outflowing water in the waste treatment cells for all ponds. A typical split-pond with additional aerators in the waste cell is show in Figure 3.2.

Ponds for the control and additional, waste-treatment cell groups had an average of a 6:1 ratio of water dedicated to waste treatment to fish grow-out (Table 3.1). Ponds were stocked with hybrid catfish (*I. punctatus* ♀ x *I. furcatus* ♂). A multiple-batch system was followed; however, most ponds were stocked and harvested at least two times each during the time of this study. Fish were provided with a 32% crude protein, floating, pelleted feed (Alabama Catfish Feed Mill, Uniontown, AL, USA) that was distributed by truck-mounted feeders that propelled the feed into the fish cells only. The daily usage per pond was recorded by the farm manager. The Feed Conversion Ratio (FCR) was determined using annual production and annual feed inputs.

3.3.2 Circulation and mixing

A circulation study was conducted on one pond that had two paddle-wheel aerators in the fish cell, a pump, and two paddle-wheel aerators in the waste-cell, to determine the flow rate between fish and waste-treatment cells. This study was conducted using trial-and-error techniques to record readings. First, a current velocity and stream discharger indicator Model 3000 (Swoffer Instruments, Inc.; Seattle, WA, USA) was used to determine flow from the pipes and screens between the fish cell and waste cell. The velocity meter was used from a boat to record readings at different places and depths throughout the waste-treatment cell.

Next, water jugs were filled half way and placed right at the water flow location in the waste treatment cell for disbursement. At 2-minute intervals, the locations of the jugs were recorded.

The above procedures were completed over a series of trials consisting of: no aerators turned on, only aspirator pump turned on, only fish cell-aerators turned on, and all fish cell and waste cell aerators turned on including the pump. The aerators and/or pump were turned on for one hour before trials were conducted in order to ensure maximum flows and mixing.

3.3.3 Dissolved oxygen

There were two DO probes: one in front of the fish cell aerators, and another in front of the waste cell aerators (or pump if control pond). These probes also turned aerators on and off in response to the DO concentration The system recorded fish and waste cell DO and temperatures, aeration time, and kilowatts of electricity used. Data were downloaded and stored every hour as Microsoft Office Excel files on USBs. Fish cell aerators were turned on when a DO reached a limit set by the farm manager (usually between 3-4 mg/L), and the waste cell aerators were programed to turn on if DO in the waste cell reached below 2.0 mg/L and then off once the DO exceeded this threshold. USBs were switched and collected monthly.

3.3.4 Statistical Analysis

Data were analyzed using repeated measures one-way analysis of variance (ANOVA) and t-tests by means of SigmaPlot version 11.0 statistical software (Aspire Software International, Ashburn, VA, USA).

3.4 Results

3.4.1 Production

Fish were produced only in the smaller, fish cells of the split-ponds; however, to accurately portray production, the entire area of both the fish and waste-treatment cells were used. No differences were found for stocking, feed input, total production, yield, and FCR between control ponds and ponds with aerated waste cell (Table 3.1). Yields for the control and aerated waste-cell ponds were 9,003 kg/ha and 7,936 kg/ha, respectively. Both groups had a FCR of 4.4. While survival and mortality were not measured, there were several reports of fish kills.

3.4.2 Circulation and mixing

Velocities through the corrugated pipes had an average of 0.82 m/sec when the propeller pumps were turned on. With all fish aerators, waste aerators and pump turned on, screen velocity returning into the fish cells had an average of 0.002 m/sec.

Water velocities in one pond were measured with two fish cell aerators operating, two waste cell aerators operating, and the propeller pump running. Multiple measurements were taken at the surface, midway (0.9 m) and bottom (1.8 m) in the waste treatment cell (Figure 3.3). Velocity of flow could not be measured except directly in front of the waste cell aerators. The greatest velocity of 0.748 m/sec was measured close to the aerators. This value may not be the greatest velocity, because closer measurements could not be taken safely. Velocities were greatest at midway points. Flows were nonexistent on the opposite bank of the waste cell aerators.

Bottles were released in front of the aerators and their movement timed until all of them reached the bank. In one trial, after 5 minutes all 20 bottles reached the pond bank nearest the aerators and near the end of the waste cell. However, observations indicated that the wind affected this result more than did the aerators. In another trial with less wind, it took 15 minutes before all the bottles to reach the side- and opposite-banks of the waste cell aerators.

3.4.3 Dissolved oxygen

Dissolved oxygen concentrations and water temperature data were difficult to analyze because of the large number of measurements available from the continuous recordings. However, DO and water data were analyzed for means and deviation (Table 3.3) associated water temperatures. All control and treatments were different except for the DO in the fish cells during the background data collection and the temperatures in the fish cells in year three. Rather than DO averages, limits and frequencies become important to where the fish grow efficiently, stressed, or die over time. DO diurnal fluctuations and frequencies for fish and waste cells for both control and ponds with aerated waste cells are shown in Figures 3.4 – 3.11. Table 3.4 shows the frequency of how many hours were recorded when the DO dropped between 0-0.5 mg/L, 0.6-1.0 mg/L, 1.1-1.5 mg/L, 1.6-2.0 mg/L, 2.1-2.5 mg/L, 2.6-3.0 mg/L for control and additional aerated waste cell ponds. There are clear differences between control ponds and ponds with additional aeration for DO hours that were recorded for 1.0 mg/L or less. Total hours recorded for that time period are also present since the DO probes did not record the same amount of hours because of malfunctions.

Because DO concentration was supplemented by aeration in all ponds, Table 3.5 shows how many of each pump and aerators were divided between treatments. This was important when determining the number of hours each aerator worked, on average, for each treatment (Figure 3.8). Propeller pumps were operational for an average of 12.2 kw/hr, fish cell aerators at 24.6 kw/hr, and waste cells aerators at 25.1 kw/hr. Additional aerated waste cell ponds' pumps had significantly more hours/aerators during the length of the study than the control ponds with median values of 157.6 hours/pump and 79.5 hours/pump, respectively. Fish cell aerators were not different between treatments with medians of 121.5 hours/aerator in control ponds and 126.7 hours/aerator in waste cell aerator ponds. The median for waste cell aerators was 5.0 hours/aerator with the maximum value of 79 hours/aerator in August 2016.

3.5 Discussion

3.5.1 Design

Original design features of the split-pond system were described in Tucker (2009) as well as in the introduction of this dissertation. Mississippi State University constructed a pilot system for expanding to commercial settings. In 2013, there were more than 1,300 acres of ponds in Mississippi, Arkansas, and Alabama that are dedicated as split-ponds (Brown and Tucker, 2013).

Split-ponds have two critical design features: 1. water flow rate between the two basins and 2. amount of aeration required in the fish-holding area (Brune et al., 2012). Feature 1 is managed by directing pump discharge from the fish cell to the waste cell during daylight and early evening hours, but the pump is turned off during night when oxygen levels drop in the fish cell. At this time, fish cell aerators are turned on to provide the requirements for feature 2. In previous studies, there has been no attempt to manage the DO in the waste-treatment area.

The required flow rate between cells should be calculated and corrected as water temperature changes and fish grow (Brown and Tucker, 2013). Tucker (2009) initiated one paddlewheel aerator in the fish cell with DO concentration fell below 5 mg/L and a second paddlewheel when the DO fell below 3 mg/L. The pump in the channel sluiceway only pumped water when the DO was above 5 mg/L.

The DO collected in the present study confirms that the farmer only supplied enough aeration to meet the fish demand, rather than the bacterial and algal processes that are also involved. The lack of differences between fish cell DO and waste cell DO for both control and ponds with aerated waste cells could also be attributed to seasonal variation and mechanical malfunctions.

Screens are used to prevent fish from travelling between cells at each sluiceway channel. These screens can reduce flow depending on the opening size and type of screen (Brown and Tucker, 2013). Flow rates with a slow rotating paddlewheel of 4 rpm was reduced from 19,330 to 17,320 gpm, and 14,847 gpm, with a polymer-coated steel-mesh wire and expanded metal barriers, respectively. Like the study above and study performed by Park et al. (2014), this study also found that these screens collect mats of algae, plants, sticks, and dead fish. If not properly cleaned, these objects impede flow rates. Biofouling was also common as a result with algal growth on the screens. Biofouling also blocked the screen openings and restricted water movement. Screen cleaning requires more labor, but is necessary for maximum growth of fish. Brown and Tucker (2013) recommends using maximum mesh size possible and possibly even the use of bar screens that are used in wastewater treatment plants to pre-filter the waste stream; thus, reducing maintenance and labor associated with the screens. This study did not evaluate the flow loss of the screen and channels. This study, however, indicated that flow rates were not high enough to allow full use of the waste cell. The short-circuiting of flow in the waste cell is illustrated in the aerial view in (Figure 3.2) and in the circulation velocity results. This study also observed that flows could have been influenced by fish that congregated around the screens, and wind effects.

Farrelly et al. (2016) discusses the placement of aerators near the sluiceways as possible elevated DO concentrated water loss away from the fish cell. DO concentrations measured several meters distant from the data loggers in the split-pond system. This reduced aeration zone in the fish cell increases can be eliminated a much faster rate. This diffusion zone can be increased by aerator placement and creating proper circulation through water flows. Future studies should include measuring DO at different depths and positions in the waste treatment cells and fish cells. The aerators and DO probes in the fish cell and waste cell for this study were positioned with respect to convenience of electrical installation rather than function.

Aerator placement is a critical design feature which cannot be stressed enough. Aerators can be used as point sources of turbulent water that can be efficient at distributing uniform flows of elevated DO throughout the fish cell or inefficient by creating a small zone of irregular DO concentrations that only diffuse throughout the fish cell (Hargreaves and Tucker, 2003). For instance, concentrations in a mass of water directed at the cell will be greater than if the aerator flow is spread over a greater area before reaching the fish cell. Vigorous turbulence can also force fish to swim in a stronger water current if DO concentrations are low, thus using more energy for swimming rather than growth. Erosion and turbidity also can result if aerator as placed so that a strong water current impinges on the pond embankment.

3.5.2 Production and water quality management

There were no differences in fish production (Table 3.2) in this study. That apparently resulted from low DO concentration, algal toxins, and nitrite toxicity. There were multiple fish kills in treatment and control pond alike. The faulty design of the split-ponds and resulting impaired circulation of water with the waste cells and low flow of water through the fish cells were a major factor leading to the fish kills.

The farmer did not promptly provide harvesting data over the course of all three years. Harvests are still ongoing at the conclusion of the study; thus, the estimates of production given below must be considered as approximate. Net yield estimations included a wide range in yields, 9,003 ± 4,764 kg/ha/yr for control ponds (n=3) and 7,936 ± 4,737 kg/ha/yr ponds with aerated waste cells (n=4). The averages FCR for both groups were 4.4. The lower production experienced with the present study were not supportive of the literature due to the modifications made by the farmer with the design and management practices (Craig Tucker, personal communication).

Tucker (2009) reported that a commercial-sized split-pond stocked at 1,334 kg/ha produced a yield of 17,880 kg/ha with a feed conversion ratio (FCR) of 1.83. This commercial-sized split-pond consisted of a 0.4 ha fish cell and a 1.42 ha waste-treatment cell. Another study by Farrelly et al. (2015), had a net production of 13,390 kg/ha. Although somewhat lower than reported in the study of Tucker mentioned above, the production in the work by Farrelly et al. (2015) was greater than reported in the present study.

Water quality conditions during the present study were described in detail in Chapter 2. The main feature was reduced total ammonia nitrogen levels in ponds with aerated waste cells.

Farrelly et al. (2015) also studied water quality by comparing split-ponds, traditional ponds, and in-pond raceways. The split-ponds showed greater total phosphorus (2.1±1.0 mg/L) than the traditional and in-pond raceways, greater alkalinity (309 ± 51 mg/L $CaCO_3$) than traditional ponds, and greater hardness (463 ± 64 mg/L $CaCO_3$) than in-pond raceways. Hybrid catfish grow best between 32-36 °C (Stewart et al, 2013); thus, temperatures in the present study were only in this range during mid- and late-summer.

3.5.3 Paddlewheels/Pumps

In conventional catfish ponds, aeration usually is applied for about 1,000 hr. Aeration rates in traditional ponds are around 7.5 hp/ha, and the annual electrical use is about 2,238 kW-hr/ha. In the present study, ponds were aerated between 1,000-1,600 hrs annually, and energy use for aeration in the fish cells (pump and paddlewheels) averaged 1,873 kW-hr/ha/aerator and 242 kW-hr/ha for waste cell aeration in the four ponds that had aerated waste cells. With two-three paddle wheel aerators in the fish cells with the inclusion of one pump, the energy use in the waste cells would be within the range of 5,619-7,492 kW-hr/ha. Thus, the amounts of aeration used in the split-ponds of the present study were greater than for conventional ponds. More aeration should be expected in a more intensified design in accordance with the increased stocking and feeding rates in order to meet all the required oxygen demands.

The study by Farrelly et al. (2016) compared DO concentration in split-ponds (2.8 ha) and conventional earthen ponds (3.24 ha). The split-ponds had 35 kW/ha of aerators while the conventional ponds had only 6.9 kW/ha of aerators. Farrelly et al. (2016) had all split-ponds oxygenated above 2.5 mg/L in fish cells for all 24 hours in July except for one pond at 0500 hr.

that was most likely due to a large algal bloom. Farrelly's study was limited due to availability of pond access, data loggers, and personnel. The present study had at least once fish cell and one aerated cell in both control and treatment groups drop below 2.5 mg/L. This shows that the amount of aeration used in split-ponds for the present study should be increased since the oxygen requirements of the fish alone are not being met.

Brown and Tucker (2013) advise that if a split-pond has a slow rotating paddlewheel in the channel sluiceway, the paddlewheel should have a rotational speed between 1.0-2.0 rpm. The paddlewheel must operate for long periods, 12-18 h/d during mid and late summer and the slow speed paddlewheel is recommended because it is less likely to surge and cavitate, and has a longer service life than paddlewheels that operate at greater rotational speed. This relationship of speed and efficiency was also supported by Park et al. (2014). In the present study, propeller pumps moved water from fish cells into waste cells. The efficiency of such pumps has not been compared to the efficiency of slow moving paddlewheels. But, at 1.0 rpm, the slow-rotating paddlewheel will move about 4,500 gpm, which at US$0.12/kW-h, has an operating expense of $30.24/yr (Brown and Tucker, 2013). Economic analyses were not done due to the over-all (lack-of) results of this experiment. However, it can be noted that the propeller, fish cell aerators, and waste cell aerators used an average of 12.2 kW/pump/hr, 24.6 kW/pump/hr, and 25.1 kW/pump/hr, respectively.

3.5.4 Complications

The DO probes that were used in all fish and waste cells were in a position that could only be reached by using a boat. The probes malfunctioned and required frequent maintenance by the farmer. When probes malfunctioned, they recorded 0.0 mg/L DO. Thus, recorded values of 0.0 mg/L were assumed to be the result of a malfunctioning probe and removed from the data set. It is not known how well the DO and aerator readings at this point represented the average DO and water temperature within a cell.

The lack of circulation and mixing in the split-ponds was unanticipated when the farm was selected for as the study site. The farmer claimed to have constructed the split-ponds according to the design recommendations given by Mississippi State University. However, he chose to deviate from the design of the split-ponds. These deviations included: the use of and a propeller pump instead of a slow-moving paddlewheel, they did not include a baffle in the waste cells, and did not properly position aerators and DO sensing probes. These modifications resulted in poor mixing and circulation that, in turn, most likely resulted in poor survival and production.

3.6 Conclusion

The poor results obtained for water circulation in the split-pond is likely the reason that aeration of the waste cells caused little improvement in water quality on fish production. Comparatively poor production performance was observed at this farm as compared to results obtained in experimental split-ponds. This may be related to the inability to properly scale pond design in terms of pump, and aeration capacity as well as placement relative to experimental split-ponds. Moreover, management of the split-ponds in the present study may not have been as efficient as in the experimental ponds at Mississippi State University.

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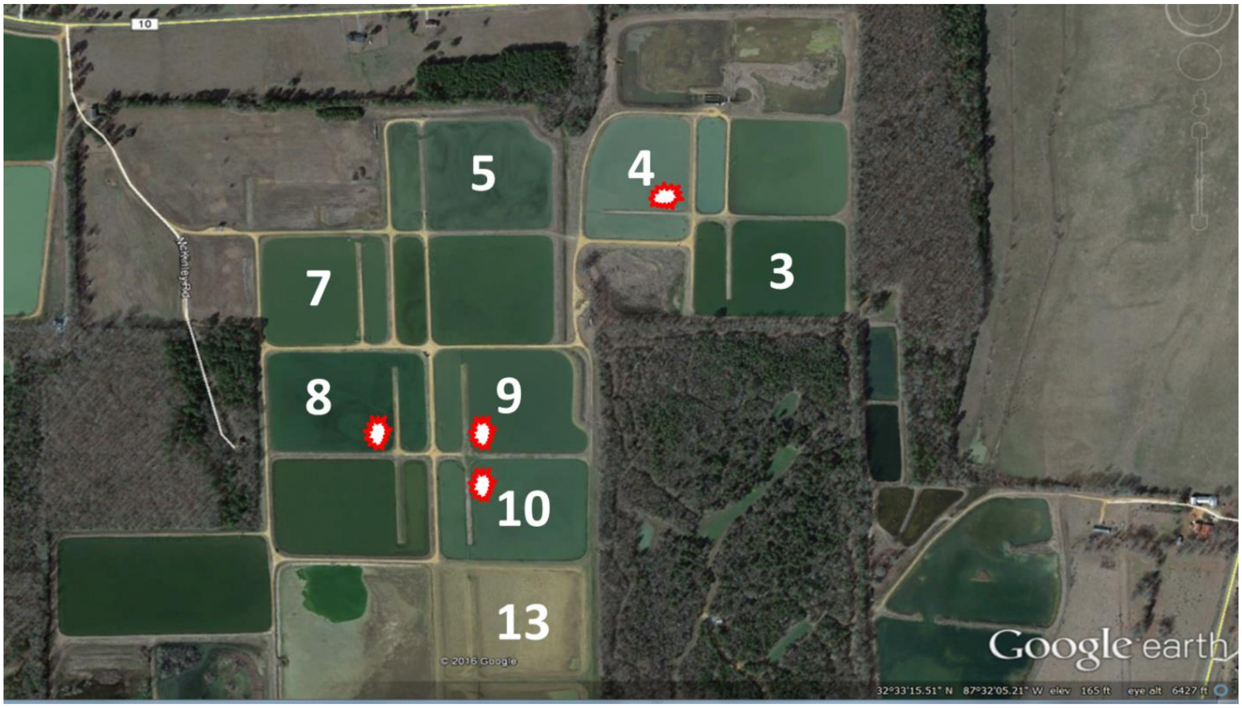


Figure 3.1. Study site in Hale County, Alabama. Control Ponds: 3, 5, 7, 13; Aerated waste-treatment cell ponds: 4, 8, 9, 10 as noted by symbols. Picture taken using Google Earth Pro

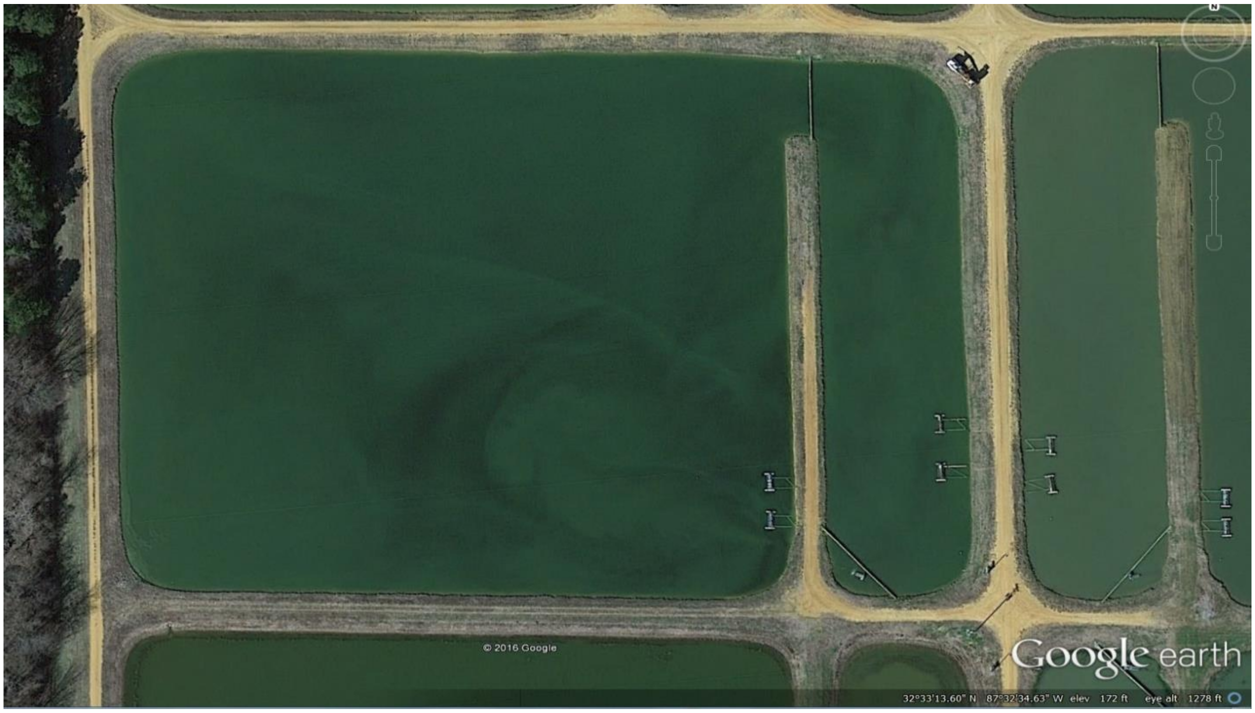


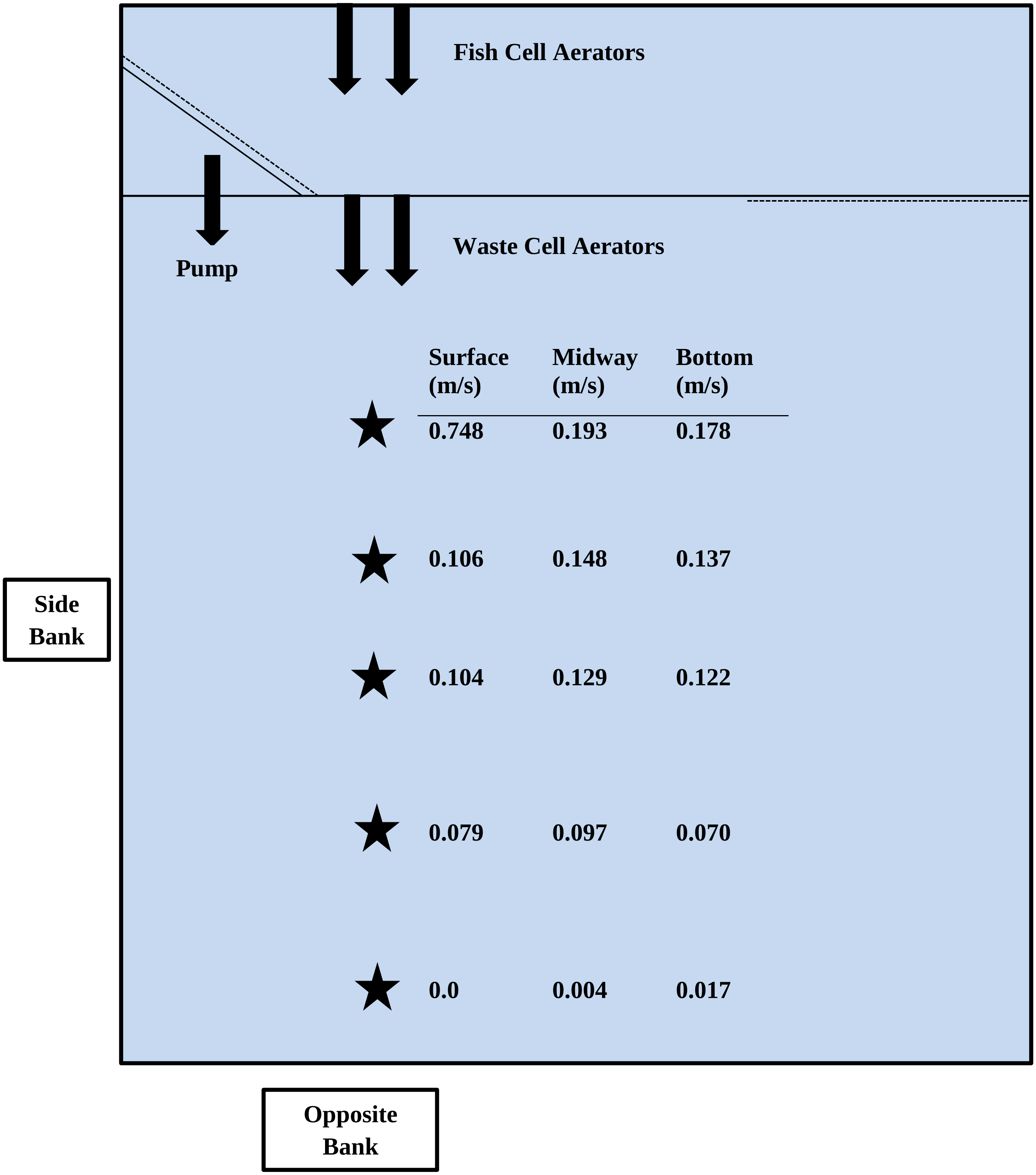
Figure 3.2. Typical split pond used in this study. This pond has waste cell aerators placed where water is traveling through a pipe between the fish cell and waste cell. Picture taken using Google Earth Pro.

Table 3.1. Average pond measurements for fish and waste cells for control and aerated-waste cell ponds.

	Surface Area (m^2)		Depth (m)		Volume (m^3)	
	Fish	Waste	Fish	Waste	Fish	Waste
Control Ponds (n=4)	6,593	28,681	1.66	2.10	10,970	60,775
Aerated -Waste Cell Ponds (n=4)	6,138	26,517	1.57	2.29	10,946	60,468

Table 3.2. Average stocking rates, feed inputs, production, net yields, and feed conversion ratio (FCR) for control and aerated-waste cell ponds for multiple-batch management system over three years (2014-2016). Area includes both fish and waste cell assimilation. Significant differences are noted by letters ($P<0.05$)

	Stocking Rate (kg/ha/yr)	Feed Input (kg/ha/yr)	Total Production (kg/ha/yr)	Net Yield (kg/ha/yr)	FCR
Control Ponds (n=3)	1,746 ± 132	33,170 ± 5537	10,749 ± 4868	9,003 ± 4764	4.4 ± 2.0
Aerated Waste-Cell Ponds (n=4)	1,827 ± 671	28,900 ± 3363	9,763 ± 4403	7,936 ± 4737	4.4 ± 2.0



Surface (m/s)	Midway (m/s)	Bottom (m/s)
0.748	0.193	0.178
0.106	0.148	0.137
0.104	0.129	0.122
0.079	0.097	0.070
0.0	0.004	0.017

Figure 3.3. Average velocities measured during circulation study at surface, midway, and bottom of waste cell. Stars indicate where measurements were collected.

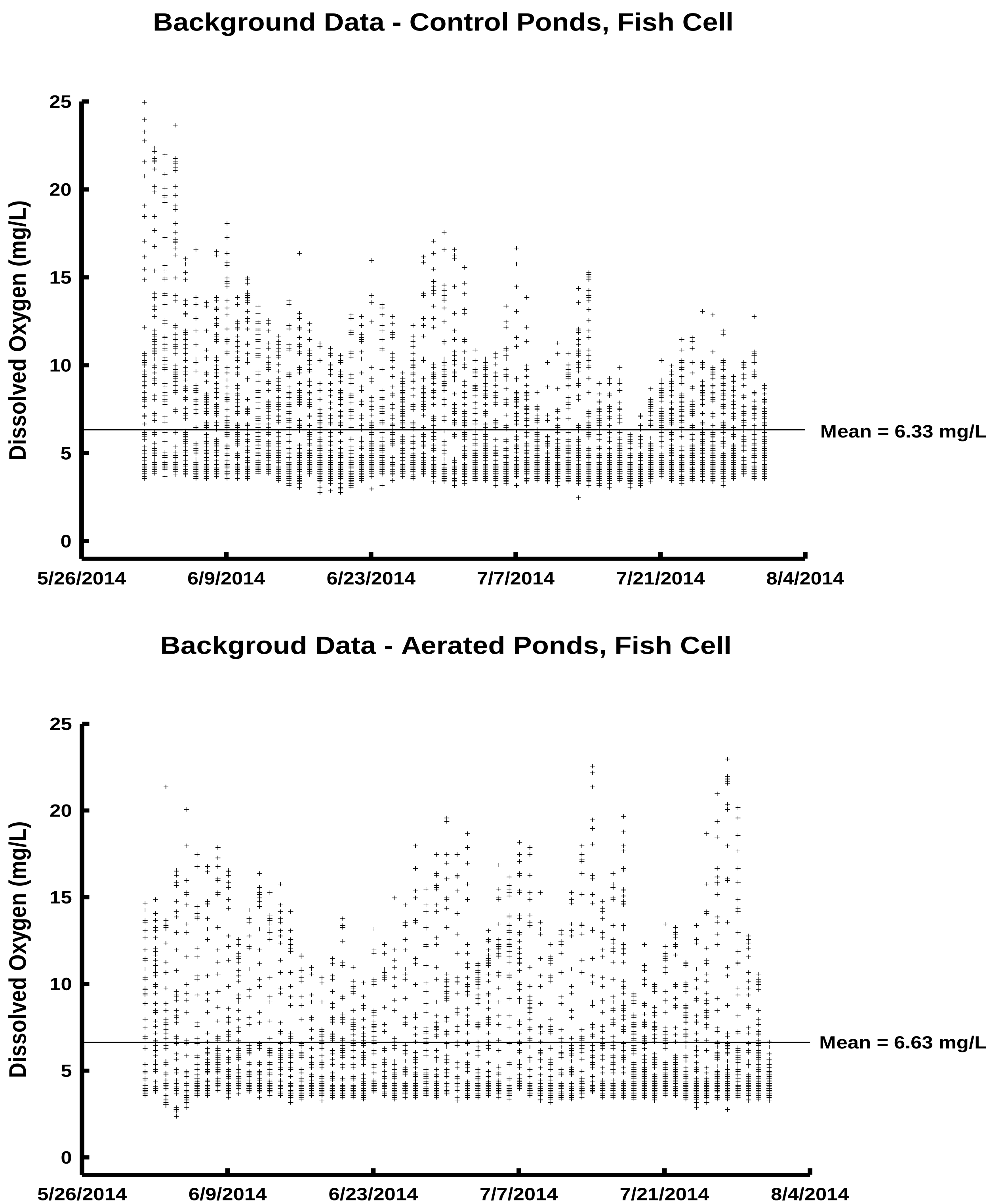


Figure 3.4. Background dissolved oxygen data in the fish cells of the control and aerated waste cell ponds. Dots indicate daily fluctuations on a given day. Darker areas have higher occurrences.

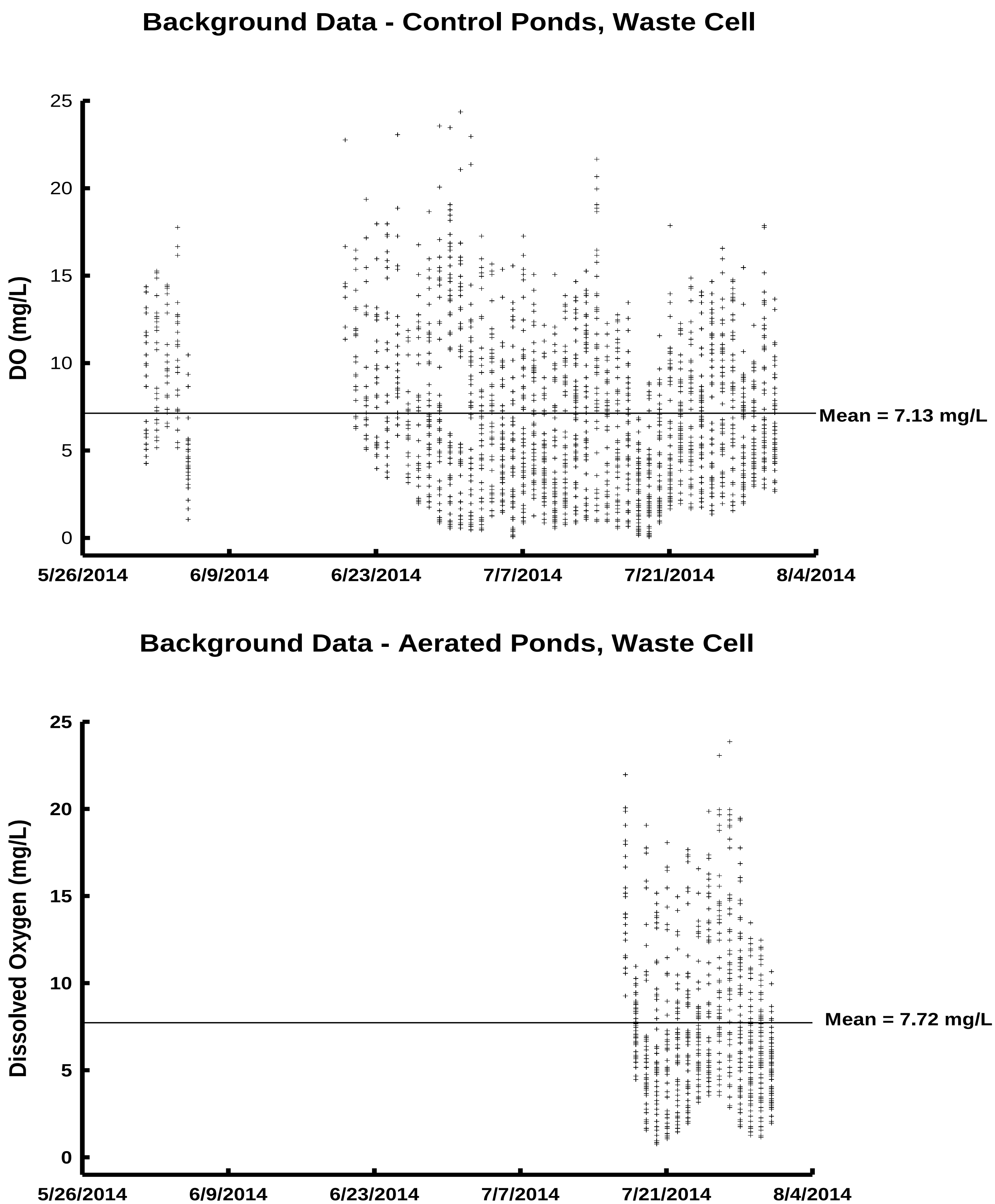


Figure 3.5. Year 1 (Aug- Dec 2014) oxygen data in the fish cells of the control and aerated waste cell ponds. Dots indicate daily fluctuations on a given day. Darker areas have higher occurrences.

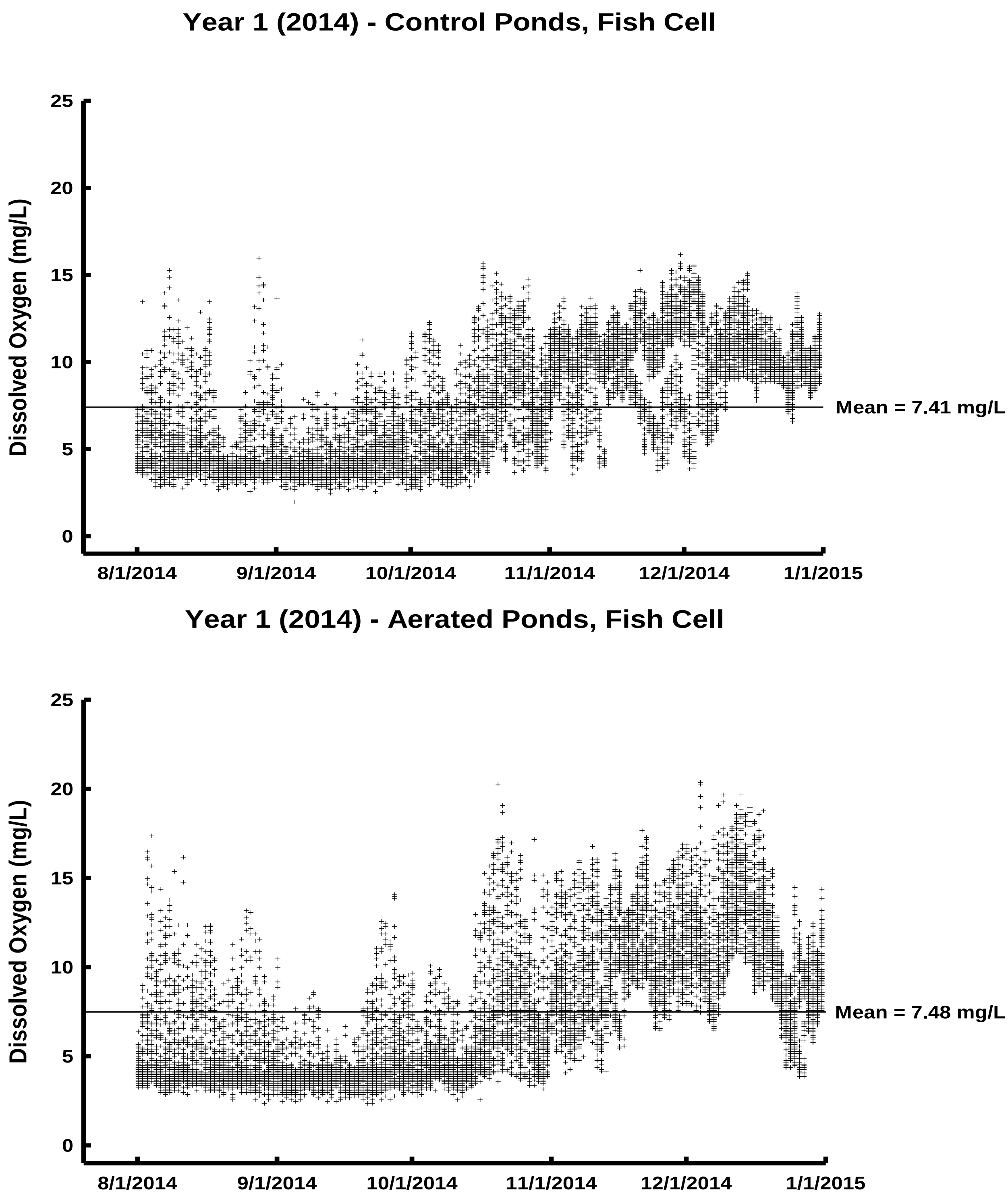
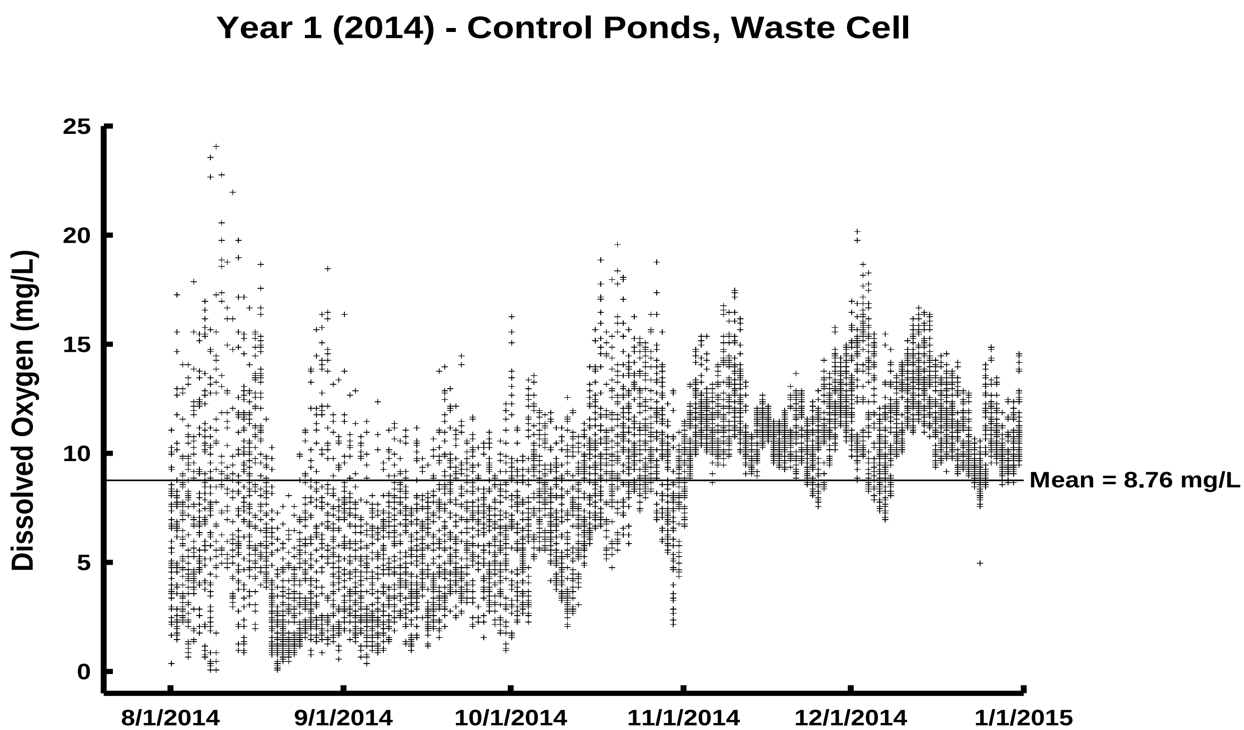


Figure 3.6. Year 1 (Aug- Dec 2014) oxygen data in the fish cells of the control and aerated waste cell ponds. Dots indicate daily fluctuations on a given day. Darker areas have higher occurrences.



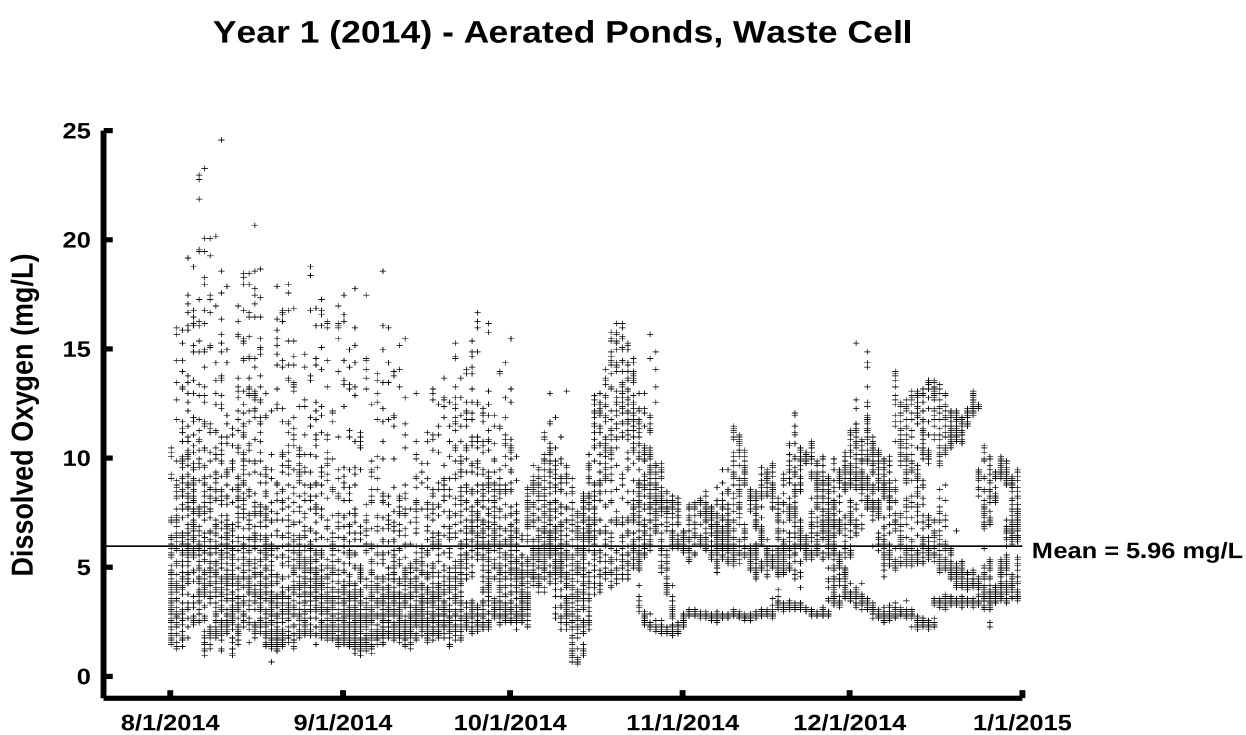
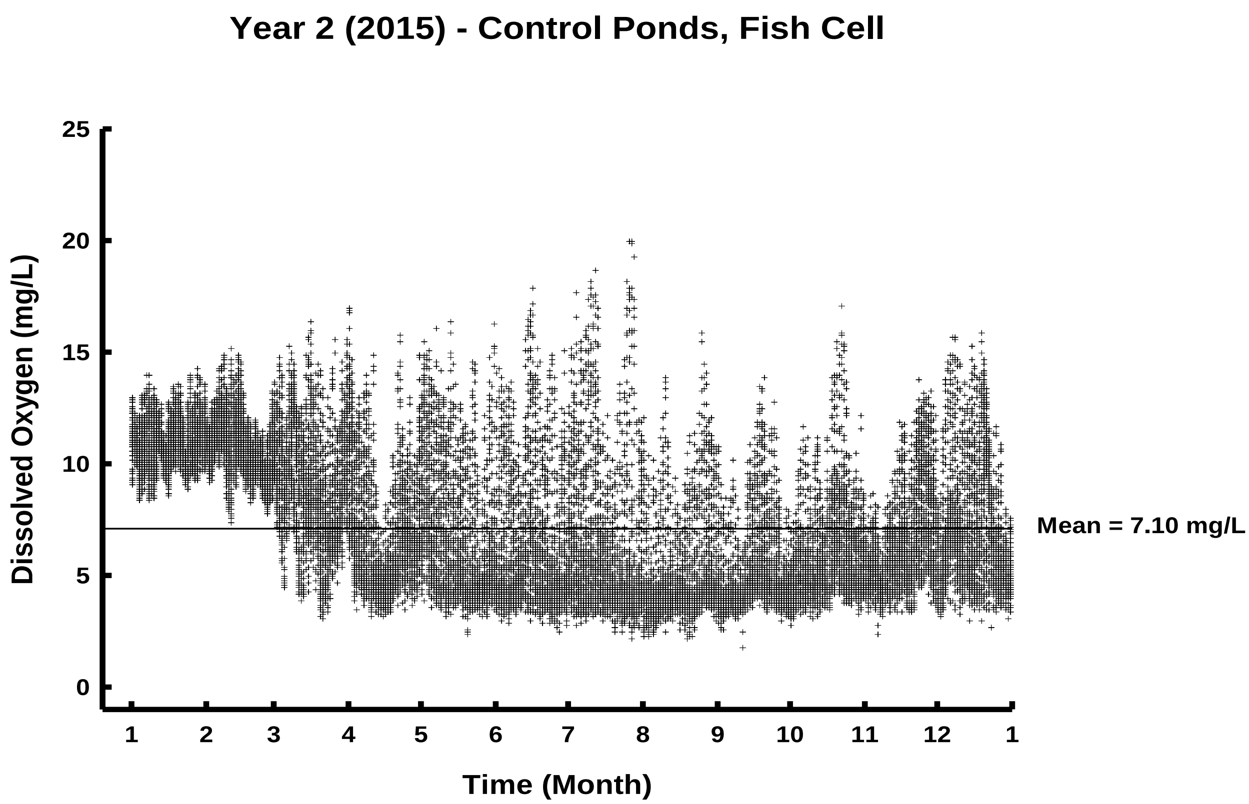


Figure 3.7. Year 1 (Aug- Dec 2014) oxygen data in the waste cells of the control and aerated waste cell ponds. Dots indicate daily fluctuations on a given day. Darker areas have higher occurrences.



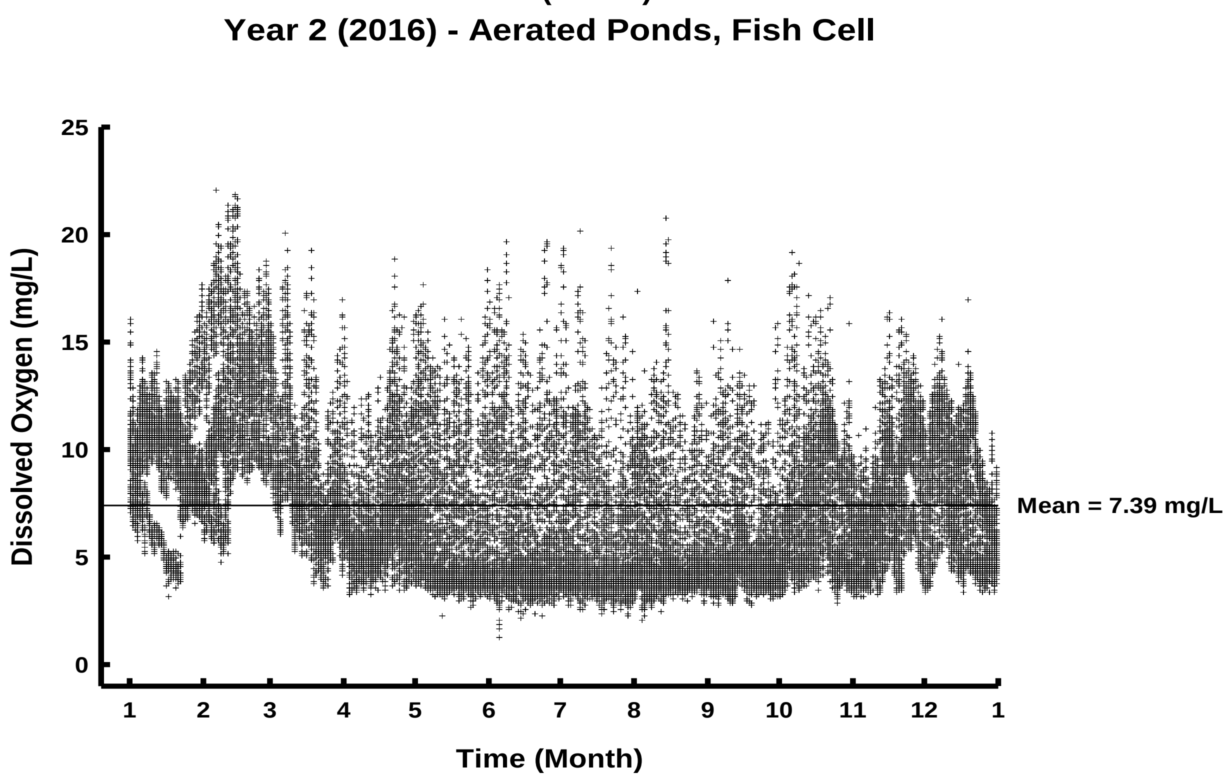
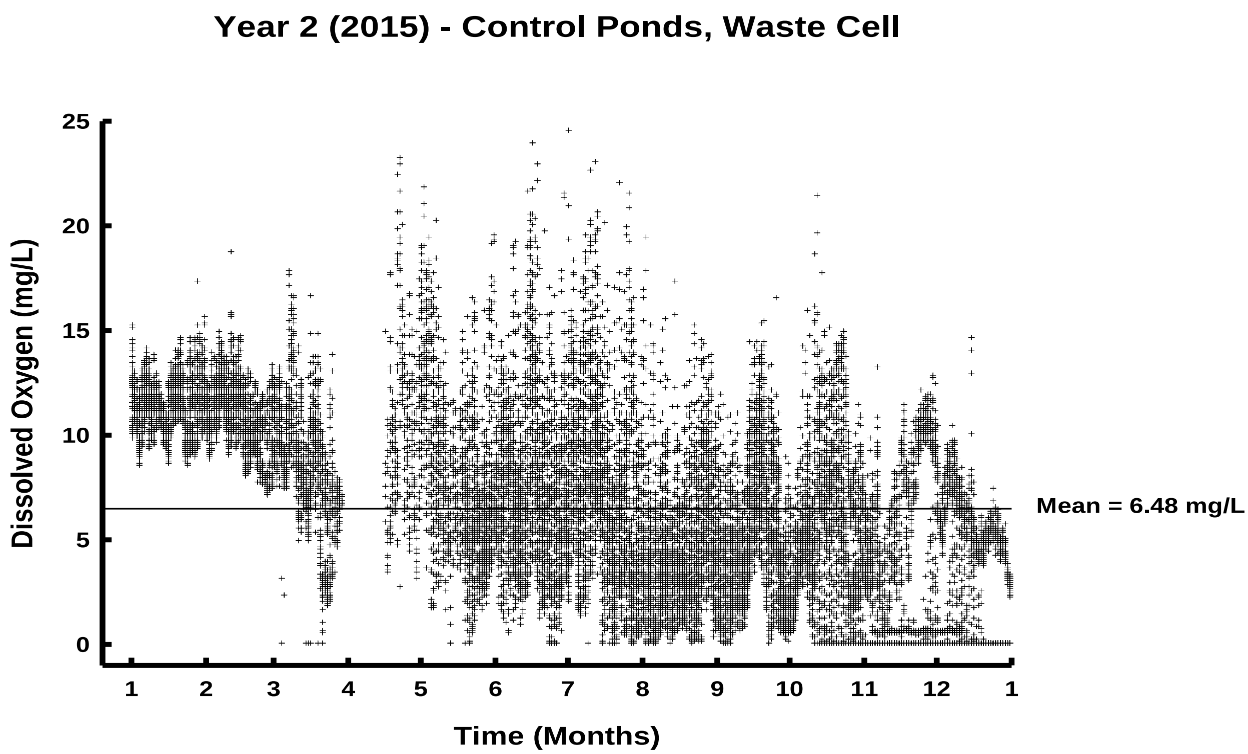


Figure 3.8. Year 2 (Jan-Dec 2015) dissolved oxygen data in the fish cells of the control and aerated waste cell ponds. Dots indicate daily fluctuations on a given day. Darker areas have higher occurrences.



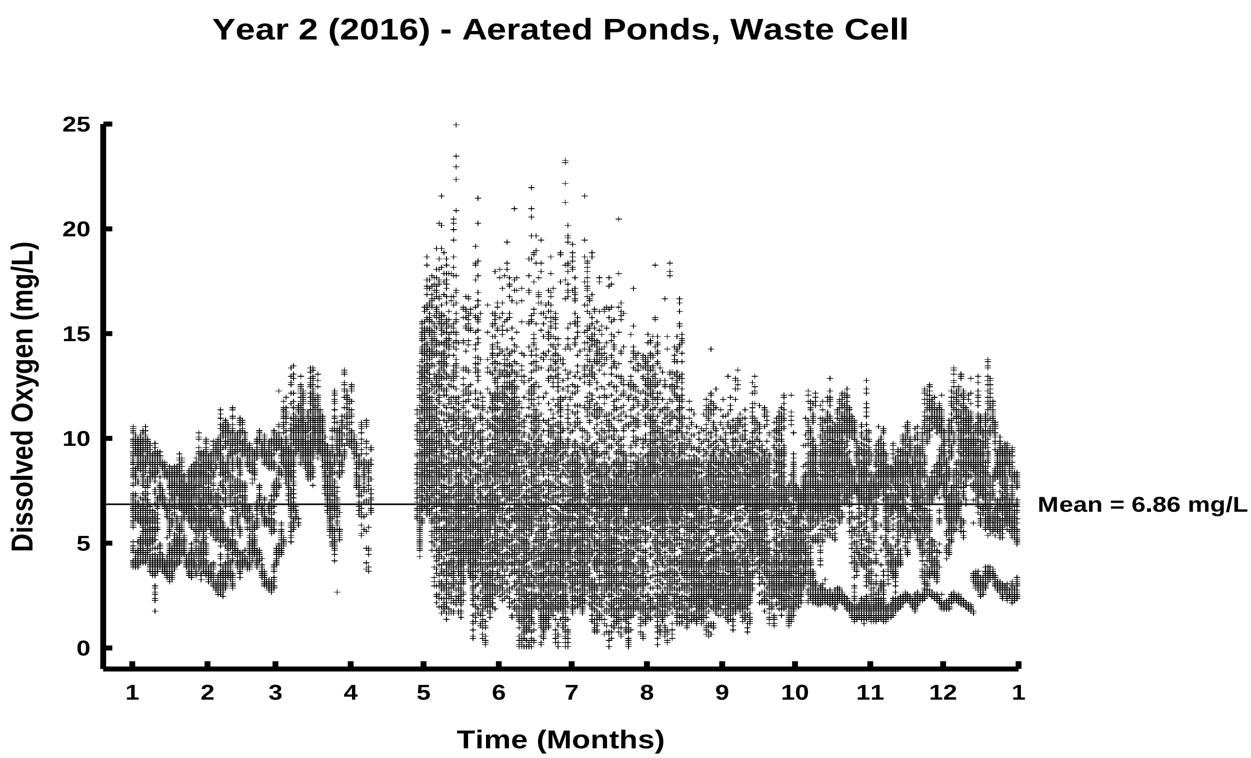
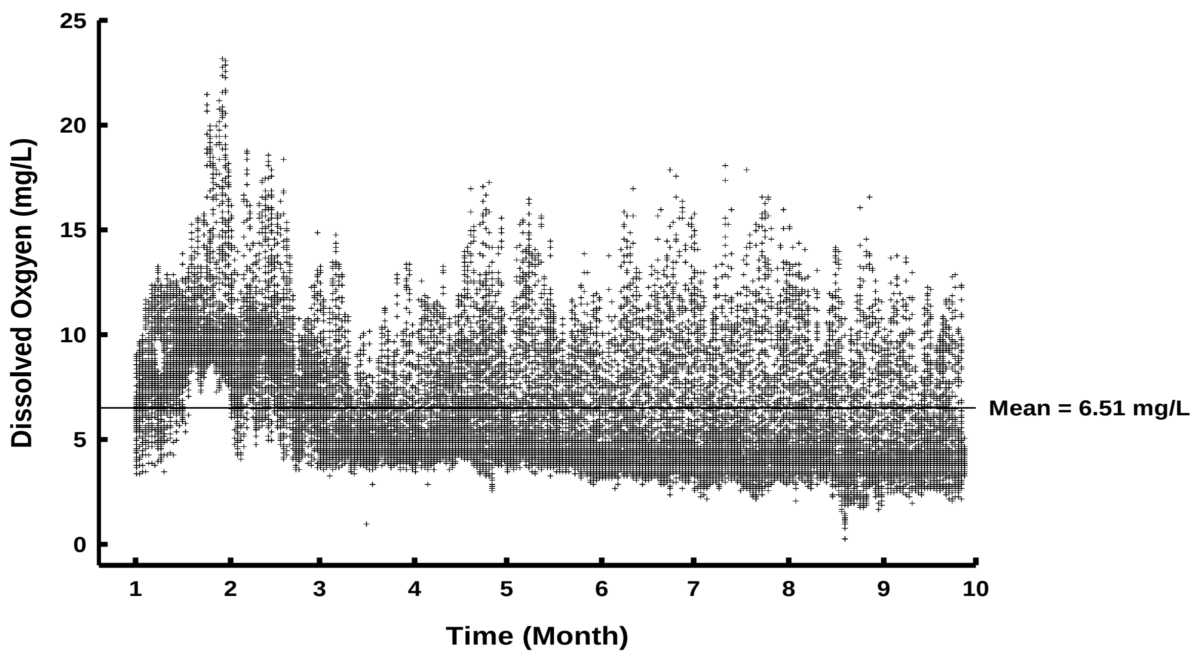


Figure 3.9. Year 2 (Jan - Dec 2015) oxygen data in the waste cells of the control and aerated waste cell ponds. Dots indicate daily fluctuations on a given day. Darker areas have higher occurrences.

Year 3 (2016) - Control Ponds, Fish Cell



Year 3 (2016) - Aerated Ponds, Fish Cell

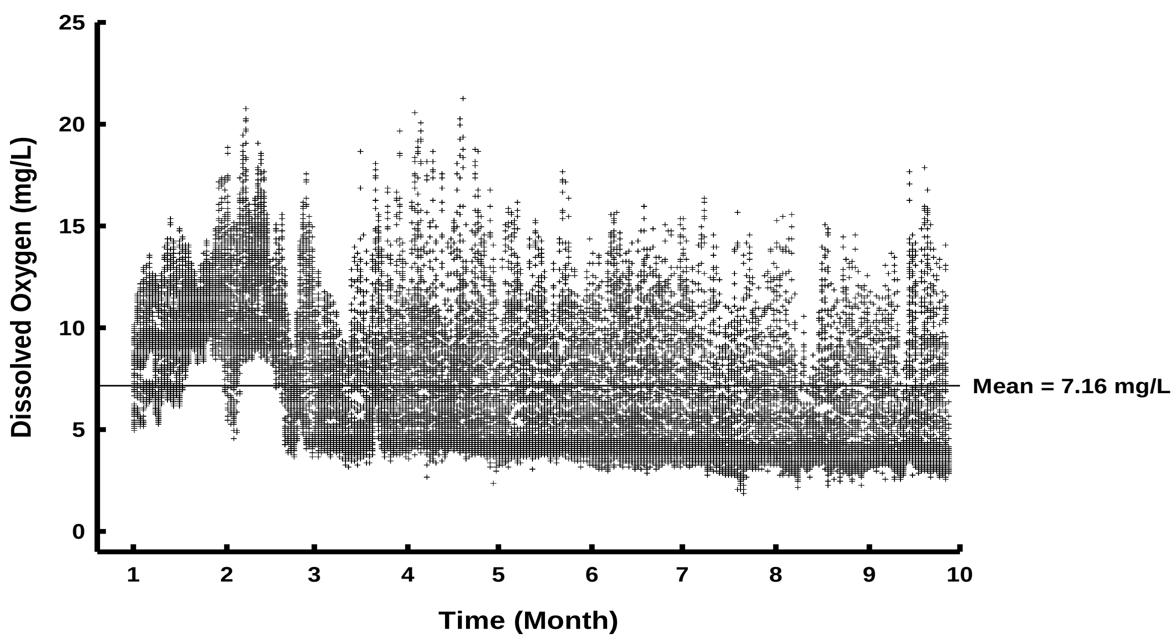
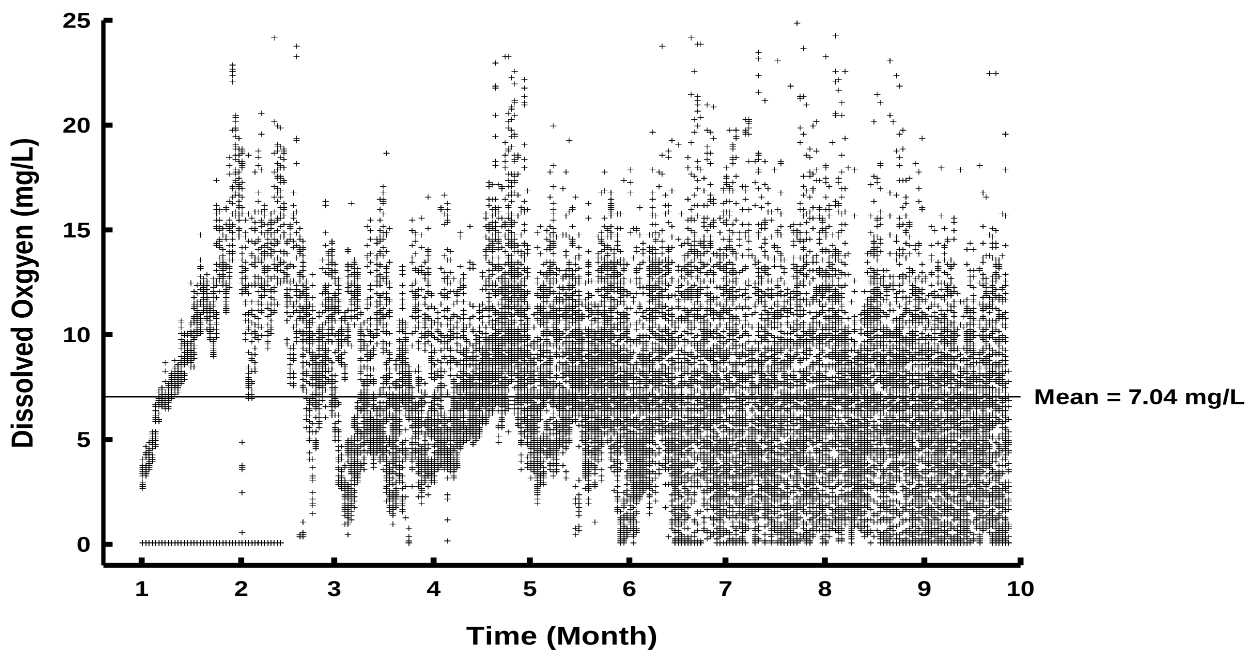


Figure 3.10. Year 3 (Jan-Oct 2016) dissolved oxygen data in the fish cells of the control and aerated waste cell ponds. Dots indicate daily fluctuations on a given day. Darker areas have higher occurrences.

Year 3 (2016) - Control Ponds, Waste Cell



Year 3 (2016) - Aerated Ponds, Waste Cell

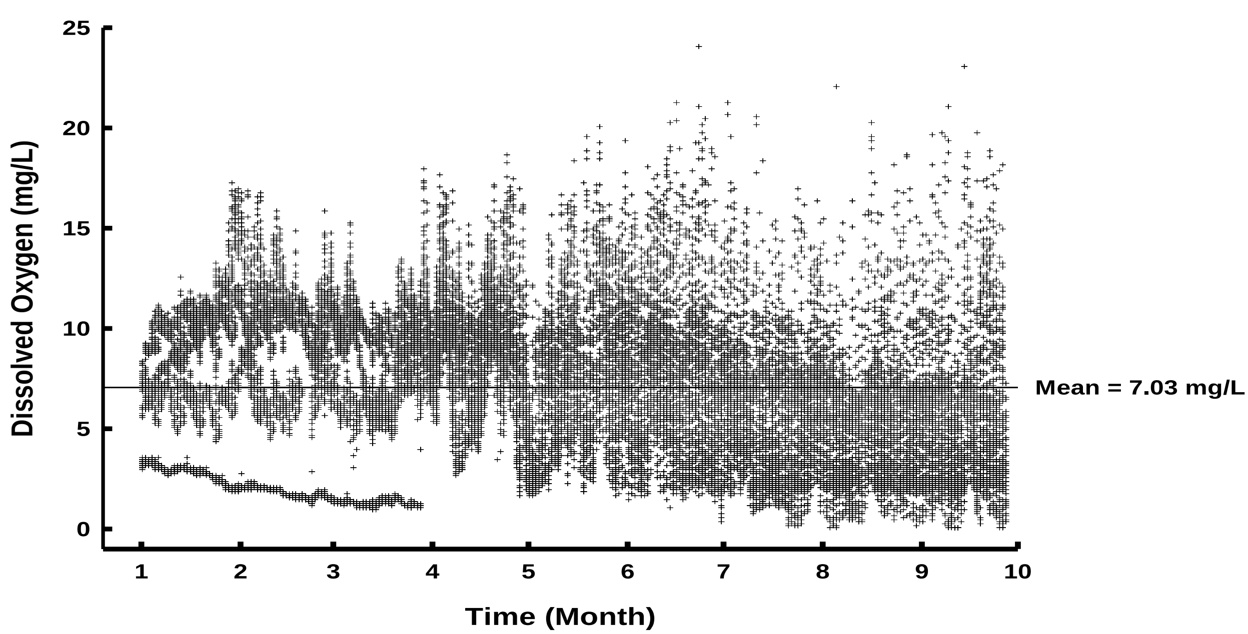


Figure 3.11. Year 3 (Jan-Oct 2016) oxygen data in the waste cells of the control and aerated waste cell ponds. Dots indicate daily fluctuations on a given day. Darker areas have higher occurrences.

Table 3.3. Averages of DO and temperature for background and year 1-3 (2014-2016) in control and additional aerated waste cell ponds in the fish and waste cells.

	Background		Year 1		Year 2		Year 3	
	Control	Aerated	Control	Aerated	Control	Aerated	Control	Aerated
Fish Cell DO (mg/L)	**6.3±3.1a**	**6.6±3.7a**	7.4±3.2	7.5±3.7	7.1±3.0	7.4±3.3	6.5±2.9	7.2±3.3
Waste Cell DO (mg/L)	7.1±4.4	7.7±4.4	8.8±3.8	6.0±3.3	6.5±4.3	6.9±3.2	7.0±4.4	7.1±3.6
Fish Cell Temperature	30.3±1.5	30.1±1.6	22.1±6.7	21.9±7.2	21.9±6.7	22.1±7.2	**23.6±7.2a**	**23.4±7.8a**
Waste Cell Temperature	30.3±1.6	30.1±1.8	21.9±7.2	21.6±7.2	23.1±7.2	21.9±7.2	25.0±7.2	23.4±7.8

Table 3.4. Number of hours recorded when the DO dropped between 0-0.5 mg/L, 0.6-1.0 mg/L, 1.1-1.5 mg/L, 1.6-2.0 mg/L, 2.1-2.5 mg/L, 2.6-3.0 mg/L, and total hours of DO collected for control and additional aerated waste cell ponds.

			Dissolved Oxygen (mg/L)						Total Hours Collected
			0.1-0.5	0.6-1.0	1.1-1.5	1.6-2.0	2.1-2.5	2.6-3.0	
Background	Fish Cell	Control	0	0	0	0	1	6	4,350
		Aerated	0	0	0	0	1	17	3,241
	Waste Cell	Control	25	59	54	80	79	71	1,923
		Aerated	0	4	13	22	24	23	771
Year 1	Fish Cell	Control	0	0	0	1	1	90	10,943
		Aerated	0	0	0	0	7	121	10,961
	Waste Cell	Control	25	64	94	141	186	173	7,215
		Aerated	0	21	103	393	707	1,101	10,671
Year 2	Fish Cell	Control	0	0	0	1	28	132	26,203
		Aerated	0	0	1	2	15	185	33,450
	Waste Cell	Control	1,757	1,311	439	485	639	682	20,853
		Aerated	79	120	348	966	1,638	1,361	30,921
Year 3	Fish Cell	Control	3	3	8	30	144	381	25,838
		Aerated	0	0	0	1	21	235	25,515
	Waste Cell	Control	1870	359	387	393	446	509	21,180
		Aerated	100	151	906	1,052	1,278	1,057	25,375

Table 3.5. Number of pumps and aerators for each treatment group throughout all three years of the study.

	Control Ponds			Aerated Waste Cell Ponds		
	Pump	Fish Aerators	Waste Aerators	Pump	Fish Aerators	Waste Aerators
Background	3	9	---	3	6	---
Year 1	3	9	---	4	8	8
Year 2	3	8	---	4	8	8
Year 3	4	11	---	4	8	8

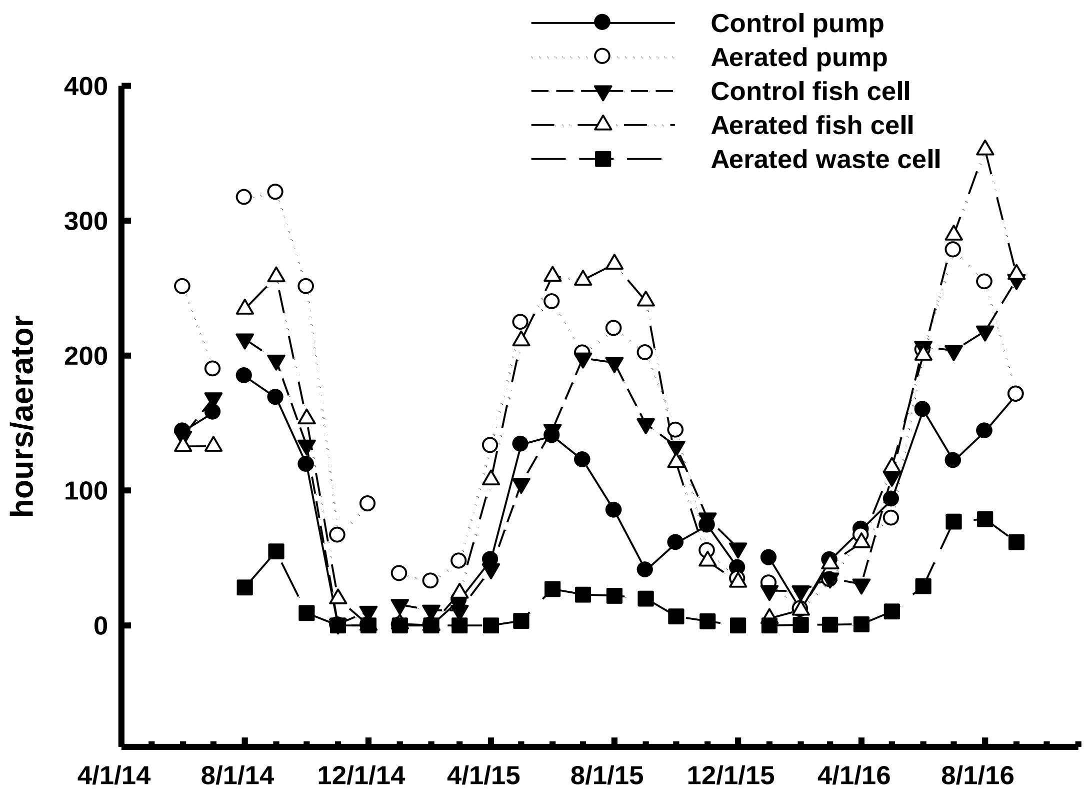


Figure 3.12. Average hours/aerator there were operational during background and year 1-3 (2014-2016) of this study for pumps, fish, and waste cells in control and additional aerated ponds.