Department of Agriculture, Trade and Consumer Protection

Division of Agricultural Development

Agricultural Development & Diversification Program (ADD)

Grant Project Final Report

Contract Number: 16058

Grant Project Title: Establishment of Aquaculture Effluents Profile, Years 1&2

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FINAL REPORT

Establishment of Aquaculture Effluents Profile, Years 1&2

Agricultural Development and Diversification Grant Program

Contract # 16058

Program Summary

The original intent of the project was to collect data and draw basic scientific research conclusions regarding quantifiable changes in water chemistry resulting from intensive aquaculture on typical private Wisconsin flow-through fish farms. The benefits to Wisconsin aquaculture include giving concrete, useable information in a regulatory climate which is both restrictive and lacking data, and applying such data and conclusions in deriving regulatory policies that are fair to farmers and protective of the highest standards of water quality in Wisconsin. The US-EPA is currently involved in a major revamping of federal standards for aquaculture effluents, in which the paucity of data is acknowledged. This makes the project particularly timely and appropriate.

The project is not directly involved creating jobs, expanding or attracting investment to the industry, diversification of production or processing, market expansion, commercial application or competitive positioning. Rather, it hopes to promote fair, science based, and correspondingly appropriate regulatory and permitting policies, which could benefit all of the above. Encouraging new farms employing appropriate practices would expand production, increase employment, provide exceptional quality healthy food in an area where trade imbalances are huge, foster healthy rural development, etc. On the basis of environmental impact misconceptions, due in part to the lack of real data, aquaculture suffers excessive regulation. Hopefully, correcting this lack will relieve us of these misconceptions and allow the numerous benefits of aquaculture to develop. Among the benefits is the farmers' commitment to sustainable practice, as fairly determined.

Evaluation of Results

Data has been submitted the Science Team, as described in the Plan of Work. In the initial stage, data will be tabulated by the Biology Dept, University of Wisconsin - Stevens Point, supervised by Dr. Chris Hartleb. Results will then be reviewed and any appropriate conclusions and comments submitted by the fall team, consisting also of Jeff Malison UW-Madison, and Steve Yeo, UW-Milwaukee.

Final conclusions, as well as complete descriptions of farms and practices, will be submitted separately, and held in custodianship of the project head veterinarian, Dr. Robert Smith.

These results will be the final results of the project. The ultimate application of the results will determine the project's impact on development, revenues and jobs generated. With positive results, aquaculture could grow and develop markets. Until then, in our current regulatory climate, aquaculture in Wisconsin will not grow.

The project has largely lived up to expectation. Data-collection required great time and patience, but has proven positive as far as the basic intents of the project were defined. We have established a substantial database that quantifies effluents and water chemistry changes typical of Wisconsin flow-through aquaculture systems, measuring major waste-water contaminants at four locations (source, discharge, up&downstream receiving waterways) at each of seven farms (including an unused 'control' facility), four times per year for two years. Background information for each farm (physical description, discussion of sampling sites, description of effluent treatment if any, wt. of feed used & stocking volumes) along with maps and observations of data collectors (veterinarians) is included for each sampling at each farm.

Future Projections

The effects of this project on the development of private aquaculture are soon to be seen depending on the conclusions drawn from the data. As the facts dictate, we hope for a best case scenario of relaxed standards for aquaculture, commensurate with evidence of non-degradation and subsequent new farm startups, increased production, supply and markets. Worst case, existing restrictive policies will be justified.

Fiscal Summary

The project operated within budget throughout the two plus years of data-collection and beginning analysis. Expenses in some budget categories exceeded budgeted amounts, but were balanced by budget exceeding expenses in other categories. See chart below.

	Year 2 Budget		
Budget Items:	Funds Requested	Invoiced to Date	Funds Remaining
Consultant Services	4,000.00	3,750.00	250.00
Laboratory Testing	2,665.00	4,087.60	-1,422.60
Bookkeeping/Clerical	4,335.00	2,612.18	1,722.82
TOTAL	11,000.00	10,499.78	550.22

The final payment of remaining funds will follow the final report, to cover continued bookkeeping and clerical costs involved in reporting, evaluating and dissemination of results.

FARM DESCRIPTIONS

Farm I:

Two wells, large indoor hatchery, concrete raceways and earth ponds
Sample 1: Source: Well, constant 45"
Sample 2: Discharge from final raceway
Sample 3: Discharge to take from earth settling pond
No upstream receiving waterway; no 4th sample
Discharge description: On-line settling basin; discharge to lake (no outflow)
Feed: 45% protein, 22% fat; peak summer -2500#, avg.- 1800# per week
Stocking volume: varies from 350,000 fish @ 20,000# to 800,000 fish @ 50,000#

Farm II:

Creek diversion feeds 4 banks of raceways and one large earth pond
Sample 1: Creek at influent to raceways; temps vary seasonally from low 30's to low 60's
Sample 2: Outfall from final pond
Sample 3: Downstream in creek, below farm discharge
Discharge description: direct discharge from intensive culture area to creek.
Feed: 45% protein, 12% fat. Usage varies seasonally from 0# (below 36º water temp) to 4000# midsummer peak of water temps & growth
Stocking volume: also varies seasonally, 22,000# to 60,000# (comb. Brown, Brook & Rainbow)

Farm III:

Consists primarily of two buildings, each with numerous hatching trays, fry tanks and raceways. Water is supplied by either of two 750 gpm wells, only one of which is normally in use. Source water runs through a degassing head box in each building prior to use.
Sampling: early in the project, several locations were experimented with in order to determine the best, most characteristic profile. Typically these locations include source water, discharge water from one of the buildings, and discharge water from the wetland filtration area, into a creek. Caution: sampling sites are not consistently numbered.
Discharge description: water from intensive culture discharges into wetland settling area prior to entering small creek.
Feed: 48% protein, II% fat
Stocking volume: varies seasonally from ~2000# to ~6000#, typically consisting of large numbers of eggs and fry.

Farm IV:

Well and artesian sources feed indoor hatchery and fry area and numerous outdoor earthen raceways and ponds.
Samplings: vary somewhat in exact location, in effort to profile all sources and discharge characteristics. Typically each sampling date covers at least one source and discharge into and out of settling pond. Discharge combines with seasonal overflow from impounded lake, but is usually considered a 'headwater discharge' Caution: numbering of samples doesn't follow a consistent pattern.
Discharge description: Farm employs a settling pond prior to final discharge to public waters.

Feed: 48% protein, 18% fat; usually ~5-6000# per week
Stocking volume: ~700,000fish,~150,000#total,allsizes

Farm V:

Three spring sources feed indoor hatchery and four outdoor earthen ponds. First use pond water is pumped to hatchery and returned after use
Sample 1: Spring source
Sample 2: Final use pond discharge to river
Sample 3: 50' upstream in river from farm discharge point
Sample4: -40'downstreaminriverfromfarmdischargepoint
NOTE: Samplings sometimes include test of municipal storm-sewer runoff, which combines with farm discharge prior to water entering river (when runoff is present)
Discharge description: Top-draw from final farm pond through culvert direct to river
Feed: 45% protein, 15% fat; varies 10-20# per week
Stocking volume: - I 000# mixed Brook and Rainbow Trout

Farm VI:

Spring sources feed indoor hatchery and numerous outdoor earthen ponds
Sample 1: Spring source
Sample 2: Discharge from final farm use pond
Sample 3: -50'upstream from farm discharge point
Sample 4: -300'downstreatn from farm discharge point
Discharge description: Top-draw through culvert direct to river
Feed: 40-45% protein, 12-16% fat; 1000# per week
Stocking volume: 300,000 fish @ ~ 15,000#

Farm VII:

Springs originating in ~30 acre wetland form a creek which feeds several earthen ponds before discharging into a continuation of the creek that flows into river.
Sample 1: creek source above ponds
Sample 2: below large concrete darn that impounds farm ponds
Sample 3: creek below farm, prior to river
Sample 4: upstream from mouth of creek, in river (approx. 1/4 mile upstream from Farm VI) Discharge description: direct overflow from lowest farm pond into creek
Feed: none, no fish culture at present
Stocking volume: none, no fish culture at present

Scientific Review

Title of Project: Establishment of Aquaculture Effluents Profile

Objective of Study: Understand aquaculture water use impacts, in order to fairly allow or disallow new fish farms in the state of Wisconsin. This is to be achieved by creating a database for on-farm effluent characteristics and by demonstrating the impact of aquaculture effluents from farms and their receiving waters.

Data Analysis: Samples of water temperature, dissolved oxygen (DO), pH, ammonia-nitrogen (NH_3-N), phosphorus (P), total suspended solids (TSS), and biological oxygen demand (BOD) were collected quarterly in 2001 and 2002 from water source, discharge, upstream and downstream receiving waters. Records were also kept for weather conditions, species of fish raised, production quantity, feed type & quantity, and on-site observations by a veterinarian.

Farm codes and original water analysis results are kept on file by the principal investigator, Mac Graham. Water analysis was performed at Commercial Testing Laboratories, Inc., Colfax, WI. [**Note**: some water analysis data were reported by Commercial Testing Laboratories as < #. These data were modified to the # reported for statistical analysis, since < # cannot be used in calculations. Therefore, the values reported may overestimate the actual values at the farm in some instances.]

Since only one water sample, from each of the four sampling locations, was collected at each farm and analyzed quarterly, statistical analyses were limited to linear regression analysis for trends (where R^2 = coefficient of determination) and Analysis of Variance (ANOVA) test of the significance of R^2 when $R^2 > 0.6$.

[**NOTE**: R^2 is an indicator of the percentage of the Y-variable explained by the linear regression model. $R^2 > 0.6$ is commonly used to denote a significant percentage of the observed trend to be explained by the X and Y variables plotted in the graph. An ANOVA is only calculated when $R^2 > 0.6$ and, when $p \leq 0.05$ from the ANOVA, this indicates that predicting the Y-variable based on the X-variable is reasonable.]

Since data analyses were limited to linear regression analysis of parameters, because of the small sample sizes, comparison of effluent levels among farms could not be performed. Also, occasionally, water samples were either not collected at an individual farm or the sampling location at an individual farm was moved from the previous sampling location. These occurrences are noted in each farm report and data analysis was modified or omitted when this occurred.

Water Temperature:
Water temperature values were reported and graphed when available. Water temperature did not vary significantly among the seven farms sampled and only occasionally did quarterly sampling date affect water temperature. Overall, water temperature did not appear to be impacted by passage through the facility and most farms showed little variation in water temperature between 2001 and 2002.

Dissolved Oxygen (DO):
Dissolved oxygen levels varied by farm and the resulting impact that DO levels had on effluents depended on the facility. Three farms showed no significant changes in DO levels from source to discharge water. The remaining farms showed significant depletion of DO in water as it traveled through the culture facility, but DO levels returned to original levels once the water entered the downstream receiving water. Seasonal effects on DO appeared at two farms, with lower DO levels in late summer and fall, but in both instances, DO returned to higher levels in winter.

pH:
pH values showed an inconsistent pattern among fish farms and within individual facilities. Occasionally, source water showed declining pH during one year, while the overall trend among fish farms was that pH would decline each year and return to original values during winter. It appears that the pH of water declines as it passes through the fish farm, but the range that it declines was rarely more than one pH unit.

Ammonia-Nitrogen (NH3-N):
Ammonia-nitrogen values consistently increased in water as it passed through the facilities. NH_3-N values were usually higher in culture and discharge water than in source water. Sampling date could not account for changes in NH_3-N and the rate of increase in NH_3-N was not consistent among fish farms. Overall, ammonia-nitrogen levels were elevated when discharge water re-entered the receiving stream in both 2001 and 2002, but the cause of the increase was not identified in this study.

Phosphorus (P):
There were no significant trends in phosphorus levels among sampling locations or fish farms. Variations in phosphorus included some instances of lower phosphorus levels entering the receiving water, while other farms reported slightly increased phosphorus levels leaving the facility. Overall, there were no consistent or predictable trends and water leaving the farms rarely had levels of phosphorus greater than the source water.

Total Suspended Solids (TSS):
Total suspended solids showed no strong trends in discharge from most aquaculture facilities. At most facilities the TSS in effluents did not differ significantly from the source water. In one instance, the TSS released into the receiving water was lower than the source water, while at another facility the TSS were significantly higher than the source water. Overall, no general conclusion can be made and TSS did not appear to be elevated when discharged from the facilities.

Biological Oxygen Demand (BOD):
Biological oxygen demand did not show any clear trends among fish farms. Most BOD levels did not change significantly from the source to outflow water. In one case, the BOD levels were significantly higher in discharge water, while at another facility the BOD decreased in discharge water from the source. Overall, BOD levels varied only slightly between 2001 and 2002 and did not appear to be affected by sampling date.

Conclusions:
The original project description indicated that a database of on-farm effluent characteristics were to be established. The collection of quarterly data on water temperature, dissolved oxygen, pH, ammonia-nitrogen, phosphorus, total suspended solids, and biological oxygen demand for 2001 and 2002 at seven fish farms (one used as a control) has established initial characteristics for flow-through culture facilities. Data from one facility could not be used in statistical analyses due to fragmented data collection and inconsistencies in sampling locations. Also, data from three other facilities do not represent a complete set for either 2001 or 2002 (noted on individual farm evaluation) and should be considered when forming conclusions. Statistical analyses were performed on the remaining data, since missing values were often at the beginning or end of one year.

Individual fish farm evaluations are included and are subdivided into an analysis of each parameter. Comparisons among fish farms was not included, since from the data collected, it became apparent that each farm was significantly different and comparisons could not include differences in design, flow rate, fish biomass, feeding regiment, feed quantity, and geographic location. Brief conclusions for each characteristic were included in the summary report that indicated sampling date was rarely the determining factor when parameters changed at each farm, but that other factors were most likely influencing the variation observed.

Recommendations:
The following recommendations are made when referencing the data analysis and using the database as guidelines for future aquaculture enterprises:

1) During data analysis, it became apparent that each farm had a unique passage of water through the facility and that variation in each characteristic may have been due to factors present only at that farm. Therefore, when comparing or reviewing parameters for assessment, individual fish farms should be evaluated for likeness to the new facility and only similar farms should be compared.

2) Only a few parameters consistently showed variation at many facilities (ammonia-N and DO). Further

investigation, including a more detailed analysis of controlling factors such as fish biomass, feed quantity & type, and facility design should be conducted to determine the most influential factors on effluent composition.

3) Data analyses were limited to linear regression analysis for seasonal trends in parameters due to the fact that only one sample was collected on each date and at each location. This greatly minimized the types and power of statistical analyses that could be performed.

   When designing effluent profile studies, multiple samples of the same variable must be collected, during each sampling interval, for replication and data analysis. Statistical analysis of data, that can be used to support the project, is greatly improved when multiple samples (data points) are collected. For example, five or more water samples should have been collected at each sampling date (quarterly) and location (water source, discharge, upstream and downstream receiving waters) for each parameter. This will increase the number of samples and cost, but will improve the data analysis and determination of the impact that fish farming has on water quality.

Farm I Evaluation

Temperature

Well input for 2001 showed consist water temperature of approximately 7°C throughout the year. Raceway discharge and downstream receiving water showed significant ($R^2 > 0.6$) decreasing trends in water temperature throughout 2001. An Analysis of Variance (ANOVA) showed that the trend was not significant ($p > 0.05$) and, therefore, date alone was not a good predictor of water temperature. Water temperature was consistent at all sampling locations in 2002 at approximately 7°C.

Dissolved Oxygen (DO)

Well input for 2001 showed a significant increasing trend ($R^2 = 0.80$) in DO, but remained relatively constant in 2002. ANOVA showed that the trend in 2001 was not significant ($p > 0.05$) and could not be explained by sampling date. Raceway discharge DO increased throughout 2001 but was not significant ($R^2 = 0.55$). Raceway discharge DO in 2002 remained relatively constant. Output decreased slightly in both 2001 and 2002, but neither showed a significant trend. Overall, dissolved oxygen patterns showed a slight depletion following passage through the culture system.

pH

Well input pH increased slightly throughout 2001 and 2002 but neither showed a significant trend ($R^2 < 0.6$). Raceway discharge pH decreased significantly ($R^2 = 0.90$) in 2001 and ANOVA showed that sampling date was a reasonable predictor of pH from raceway discharge ($p = 0.05$). Raceway discharge in 2002 decreased slightly. Output levels increased slightly in 2001 and decreased slightly in 2002. Overall, the culture facility did not significantly affect pH levels of the effluent water based on quarterly trend patterns.

Ammonia-Nitrogen (NH_3-N)

Well input ammonia-nitrogen remained unchanged in both 2001 and 2002 at 0.1 mg/L. Raceway discharge in 2001 increased only slightly, however, 2002 exhibited a significant increase ($R^2 = 0.81$), but sampling date could not explain the NH_3-N patterns in 2002 (ANOVA, $p = 0.10$). Output NH_3-N showed a significant increasing trend ($R^2 = 0.70$) in 2001, but this was not attributed to sampling date (ANOVA, $p = 0.17$). Output in 2002 also showed a significantly increasing trend ($R^2 = 0.99$) that could not be explained by sampling date ($p > 0.05$). Overall, higher ammonia-nitrogen levels were found in the discharge water from the farm when compared to water source values, but these increases could not be explained by sampling date alone. In 2002, outflow exhibited an increase of 0.3 mg/L as compared to the well input. In 2001, outflow ammonia-nitrogen also increased from well input to discharge but only by 0.1mg/L.

Phosphorus (P)

Well input phosphorus remained relatively constant throughout 2001 and 2002 and showed no significant changes between sampling dates ($R^2 < 0.6$). Raceway discharge and output levels increased in 2001 as compared to 2002, but were statistically not significant ($R^2 < 0.6$) among sampling dates.

Total Suspended Solids (TSS)

Well input total suspended solids were constantly low for 2001 at 1 mg/L, but in 2002 showed a dramatic increase due to one data point (sample collected on 11/18/02 measured 11 mg/L). Conclusions were not based on this increase, since this one data point was most likely due to an error in the collection process or laboratory analysis. Raceway discharge and output remained constant throughout 2001 and 2002 between 1-2 mg/L.

Biological Oxygen Demand (BOD)

Well input and output biological oxygen demand remained between 2-2.5 mg/L in 2001 and 2002 and showed no significant trend ($R^2 < 0.6$) among sampling dates. Raceway discharge remained relatively constant in 2001, same range of well input, but decreased in 2002. The decrease was not significant ($R^2 = 0.51$). Overall, biological oxygen demand was not affected by this facility's use of the water among the sampling dates.

Farm II Evaluation

Temperature

Temperature increased slightly among sampling dates in both 2001 and 2002, but the trend was not significant ($R^2 < 0.60$). Overall, water temperature remained between 5 and 7°C at all three sites: input, outlet discharge, and downstream for both years and the culture facility did not appear to influence downstream water temperatures.

Dissolved Oxygen (DO)

Input, outlet discharge, and downstream dissolved oxygen decreased slightly throughout 2001 and 2002, especially during the summer months, but the following year, returned to pre-winter levels. For both, 2001 and 2002, dissolved oxygen was higher in input water, depleted upon use at the facility, but increased upon reentry into the stream. However, overall dissolved oxygen concentrations did not show significant trends among sampling dates ($R^2 < 0.60$).

pH

Input pH increased slightly throughout 2001 and decreased slightly throughout 2002 but showed no significant pattern among sampling dates ($R^2 < 0.60$). Outlet discharge slightly decreased in 2001 and slightly increased in 2002 were showed no statistically significant pattern ($R^2 < 0.60$). Downstream pH in 2001 remained relatively constant at 7.5-7.6. Downstream pH in 2002 showed a significant decrease ($R^2 = 0.81$) in pH throughout the year, but an ANOVA showed that sampling date was not a reasonable predictor of pH changes ($p = 0.10$). Overall, water pH was slightly decreased upon its reentry into the stream for both 2001 and 2002.

Ammonia-Nitrogen (NH_3-N)

Input ammonia-nitrogen concentrations remained unchanged throughout 2001 at 0.1 mg/L. Input NH_3-N for 2002 decreased slightly but was not statistically significant ($R^2 = 0.11$). Outlet discharge NH_3-N levels in 2001 stayed constant at 0.1 mg/L. In 2002, ammonia-nitrogen increased significantly ($R^2 = 0.67$), but could not be predicted by sampling date alone (ANOVA, $p = 0.18$). Downstream NH_3-N increased significantly for both 2001 (R2 = 0.69) and 2002 ($R^2 = 0.81$). An ANOVA did not find the trend to be significantly related to sampling date for either year (2001, $p = 0.17$; 2002 $p = 0.10$).

Overall, ammonia-nitrogen levels were elevated when they water re-entered the stream in both 2001 and 2002 when compared to source water values.

Phospohorus (P)

Input phosphorus in 2001 showed a significant increasing trend ($R^2 = 0.78$) throughout the year, but an ANOVA did not indicate that sampling date was a good predictor of phosphorus levels ($p = 0.11$). Input P for 2002 decreased slightly throughout the year but was not statistically significant ($R^2 < 0.60$). Outlet discharge increased slightly in 2001 and remained relatively constant in 2002. Downstream phosphorus remained relatively constant throughout 2001 and 2002. Overall, in 2001 phosphorus levels were higher in discharge water; however, in 2002 phosphorus levels were lower in discharge water throughout the year.

Total Suspended Solids (TSS)

Input total suspended solids increased slightly in 2001 and decreased slightly in 2002, but neither showed a statistically significant pattern ($R^2 < 0.60$). Outlet discharge in 2001 decreased slightly, however, in 2002 TSS levels increased significantly ($R^2 = 0.81$), but could not be predicted by sampling date (ANOVA, $p = 0.10$). Downstream increased slightly in 2001 but showed a significant increasing trend in 2002 ($R^2 = 0.61$) that could not be explained by sampling date (ANOVA, $p = 0.22$). Overall, total suspended solids were not elevated in discharge water in 2001, but showed a significant increase in 2002.

Biological Oxygen Demand (BOD)

Input biological oxygen demand remained relatively constant throughout 2001 and 2002 between 2-3 mg/L. Outlet discharge in 2001 remained constant and in 2002 showed a slight decrease, but was not statistically significant ($R^2 = 0.51$). Downstream BOD also remained constant. Overall, biological oxygen demand was not affected by fish culturing in 2001 and 2002 for this facility.

Farm III Evaluation

Due to large inconsistencies with data collection, as shown in Table 1, analysis could not be performed on data collected from this farm. Samples were often not collected from the same sampling location, sampling dates were missed for some parameters and sampling locations were changed from 2001 to 2002. With these sampling inconsistencies it was not possible or prudent to compare parameters within this farm or with other farms. Scientific recommendations based on Farm II effluent profiles are not recommended and, therefore, statistical analysis was not performed.

Table 1. Farm III Sampling

	4/26/2001	7/23/2001	10/23/2001	2/4/2002	4/30/2002	7/17/2002	10/30/2002
Head Box	X					X	X
Building #1 Drain Pipe	X					X	X
Building #2 Drain Pipe		X		X	X		
North Wetland Outlet	X						
West Wetland Outlet		X	X	X	X	X	X
East Wetland Outlet		X	X	X	X	X	
Settling Pond #1			X				

Farm IV Evaluation

Throughout this study, the sampling locations at the farm were not consistent. The effluent water, labeled as outflow, and the head tank, labeled as inflow, were the only consistent sampling sites in both 2001 and 2002. The downstream location was sampled 3 times in 2001, allowing analysis. This same location was only sampled once in 2002, so it was not included in 2002 analysis. In 2001, the artesian pond was only sampled 2 times, so data analysis could not be completed. However, in 2002, it was sampled consistently and was included in analysis. A spring source was sampled once in 2001, so data for this location were not analyzed. A pre-settling pond was sampled in 2002, but not in 2001. The data from this location were analyzed in 2002. Data for lake effluent was taken in 2001, but was not analyzed because it had no visible relationship to the farm outflow.

Dissolved Oxygen (DO)

Downstream dissolved oxygen, in 2001, showed the only significant trend among sampling dates ($R^2 = 0.61$). An ANOVA determined that sampling date was not a reasonable predictor of DO levels downstream ($p = 0.43$). There were no significant trends in DO for 2002 indicating that sampling date did not influence the DO levels in effluent water. Overall, dissolved oxygen showed no consistent patterns and only outflow in 2002 showed changes in DO levels. It does not appear that DO is influenced by the culturing environment and shows no significant difference from source water.

pH

There were significant decreases in pH among sampling dates in 2001 at the inflow ($R^2 = 0.77$) and outflow ($R^2 = 0.77$) sites. An ANOVA showed that sampling date was not a significant predictor of pH for either location (inflow, $p = 0.12$; outflow, $p = 0.12$). In 2002, there were significant increasing trends in pH for the inflow ($R^2 = 0.80$) and presettling pond ($R^2 = 0.75$). ANOVA results showed no significant patterns in pH among sampling dates at each location (inflow, $p = 0.29$; presettling pond, $p = 0.14$). The pH did not change in 2002 in the artesian pond. There were no consistent patterns in pH among the sampling locations during the study.

Ammonia-Nitrogen (NH_3-N)

There were no significant trends in NH_3-N values among sampling locations in 2001 ($R^2 < 0.60$). Both inflow and downstream locations showed no change in ammonia-N levels throughout 2001. In 2002, samples taken at the inflow (slightly increasing) and at the artesian pond (slightly decreasing) showed significant trends in NH_3-N among the sampling dates ($R^2 = 0.80$ and $R^2 = 0.84$, respectively). An ANOVA showed that sampling date was not a reasonable predictor of ammonia-N levels (inflow, $p = 0.29$; artesian pond, $p = 0.08$). Overall, the changes in ammonia-N could not be predicted by sampling date and the slight changes that occurred were the result of some other factor.

Phosphorus (P)

There were no significant trends in phosphorus values among the sampling dates in both 2001 and 2002 ($R^2 < 0.60$) at all locations. Overall, phosphorus levels increased slightly throughout the year, but remained within a narrow range (0.1 – 0.35 mg/L).

Total Suspended Solids (TSS)

Total suspended solids showed a significant increasing trend among sampling dates at the inflow location ($R^2 = 0.72$), but an ANOVA showed that sampling date could not be used as a reasonable predictor ($p = 0.15$). There were no other significant trends in TSS for 2001 or 2002. Inflow and outflow locations showed practically no change in TSS in 2002.

Biological Oxygen Demand (BOD)

Biological oxygen demand showed a significantly increasing trend at the outflow in 2001 ($R^2 = 0.71$). An ANOVA showed that sampling date could not be used as a predictor of BOD for this location ($p = 0.16$). Inflow and downstream showed little or no change in BOD for 2001. There were no significant trends in BOD at all locations in 2002, with values ranging from 2-5 mg/L. Overall, only BOD levels in outflow water showed any pattern among sampling dates and locations.

Farm V Evaluation

Data collection for 2001 was not consistent for ditch outflow. Data analysis was performed on three data points only (5/1/01, 7/23/01, 10/23/01).

Temperature

Upstream and downstream locations had higher water temperatures in 2001 than in 2002, however, the change was not significant to infer any correlation between years. Hatchery discharge water temperature remained constant in 2001 and 2002, ranging from 6 - 11˚C. Ditch outflow, in 2001, showed a significant decrease in water temperature among sampling dates ($R^2 = 1.00$) and an ANOVA showed that sampling date was a reasonable predictor of water temperature for this location ($p = 0.02$). The ditch outflow water temperature (2002) remained constant throughout the year, ranging from 7 - 9˚C.
Overall, from the graphs it can be seen that water temperature decreased as it passed through the farm and was lower than the source upon reentry into the stream.

Dissolved Oxygen (DO)

Upstream and downstream dissolved oxygen decreased in 2001 and remained constant in 2002. Hatchery discharge DO (2001) showed a significant decreasing trend ($R^2 = 0.84$), but an ANOVA determined that sampling date was not a reasonable predictor of DO ($p = 0.08$). Hatchery discharge, in 2002, showed a slight decrease in DO but the trend was not significant ($R^2 < 0.60$). Ditch outflow, in 2001, also showed a slight decrease in DO but the trend was not significant ($R^2 = 0.52$). Ditch outflow, in 2002, showed a significant decreasing trend in DO ($R^2 = 0.76$), but it was not predictable by sampling date (ANOVA, $p = 0.09$). Overall, dissolved oxygen is depleted as water travels through the fish farm, but sampling date and year cannot be used as predictors of the rate of decline in dissolved oxygen.

pH

Upstream and downstream pH increased in 2001 and 2002, but neither showed a statistically significant trend among sampling dates ($R^2 < 0.60$). Hatchery discharge pH, in 2001, increased significantly ($R^2 = 0.68$), but could not be predicted by sampling date (ANOVA, $p = 0.17$). Hatchery discharge pH increased slightly in 2002 among sampling dates. Ditch outflow pH, in 2001 and 2002, decrease throughout the year but the trend among sampling dates was not significant ($R^2 < 0.60$). Overall, pH changed only slightly upon entering and leaving the facility.

Ammonia-Nitrogen (NH_3-N)

Upstream ammonia-nitrogen concentration remained unchanged throughout 2001 and 2002 at 0.1 mg/L. Hatchery discharge NH_3-N, in 2002, showed a slight increase throughout the year when compared to 2001, however, the change did not produce a significant trend ($R^2 = 0.34$). Downstream NH_3-N, in 2001, show a significant increasing trend ($R^2 = 0.65$), but an ANOVA showed that sampling date could not be used as a reasonable predictor ($p = 0.19$). Overall, ammonia-nitrogen concentrations did not vary greatly among sampling date and locations and there was little difference between source and discharge values.

Phosphorus (P)

Upstream phosphorus concentrations remained constant throughout 2001 and 2002 at 0.1 mg/L. Hatchery discharge P also remained constant in 2001 and 2001 at 0.17-0.19 mg/L, slightly elevated from upstream concentrations. Ditch outflow P in 2001, showed a significant decreasing trend ($R^2 = 0.63$), which could not be attributed to sampling date (ANOVA, $p = 0.42$). Ditch outflow P, in 2002, fluctuated slightly ($\pm$0.1 mg/L), but the pattern was not significant ($R^2 < 0.60$). Downstream concentrations of phosphorus, in 2001 and 2002, remained constant at 0.1 mg/L. Overall, phosphorus concentrations did not change upon discharge into the stream and varied slightly at sampling locations within the facility.

Total Suspended Solids (TSS)

Upstream total suspended solids remained constant in 2001 and 2002. A slight decreasing trend was observed in 2002 ($R^2 = 0.77$), but was not related to sampling date (ANOVA, $p = 0.12$). Hatchery discharge TSS increased

slightly in 2001 and a little more in 2002, however, neither trend was significant ($R^2 < 0.60$). Ditch outflow TSS, in 2001, showed an increase in 2002 displayed a decrease; neither were significant patterns. Downstream TSS remained relatively constant throughout 2001 and 2002 at 1.5 mg/L.
Overall, total suspended solids showed a slight increase downstream from the farm, but the data shows the increase to be minimal.

Biological Oxygen Demand (BOD)
Upstream biological oxygen demand was constant in 2001 and 2002, however, 2002 exhibited a lower BOD at 2 mg/L (2001 average 2.75 mg/L for the year). Hatchery discharge BOD, in 2001, showed a significantly decreasing trend ($R^2 = 0.77$), but an ANOVA showed it not be significantly related to sampling date ($p = 0.12$). Hatchery discharge BOD in 2002 was constant at 2 mg/L. Ditch outflow BOD, in 2001, significantly decreased ($R^2 = 0.72$), but could not be explained by sampling date (ANOVA, $p = 0.35$). Ditch outflow BOD, in 2002, was constant at 2 mg/L. Downstream BOD concentration, in 2001, also showed a significant decreasing trend ($R^2 = 0.77$), but was not predictable by sampling date (ANOVA, $p = 0.12$). Overall, biological oxygen demand decreased downstream in 2001; however, BOD was relatively constant at all sampling locations in 2002.

Farm VI Evaluation

Dissolved Oxygen (DO)
Dissolved oxygen showed no significant trends for either 2001 or 2002. Overall, DO remained constant among all sampling dates for each location in 2001 and 2002. DO levels for outflow and downstream sampling sites were consistently higher than upstream and inflow in both 2001 and 2002, therefore, discharge water contained higher DO than source water.

pH
pH showed no significant trends for either 2001 or 2002. All sampling locations showed increased pH levels among sampling dates in both 2001 and 2002, but increases were minimal and ranged from 7.5 to 7.8.

Ammonia-Nitrogen (NH_3-N)
In 2001, the outflow sampling location showed a significant decreasing trend in ammonia-nitrogen among sampling dates ($R^2 = 0.62$), but an ANOVA test produced inconclusive results. All other sampling locations did not have significant trends in NH_3-N in 2001. In 2002, NH_3-N levels showed a significant increase for the outflow (R^2=0.60), inflow (R^2=0.80), and downstream (R^2=0.60) sampling locations. ANOVA results did not show sampling dates to be significant predictors of NH_3-N levels (outflow, $p = 0.34$; upstream, $p = 0.33$; downstream, $p = 0.33$). In 2002, the NH_3-N level at the upstream location stayed constant. Overall, while ammonia-nitrogen levels remained constant in the source water (upstream), slight increases in NH_3-N occurred in inflow, outflow and downstream water. These increases could not be predicted by sampling date, but were influenced by factors within the facility.

Phosphorus (P)
Phosphorous levels, in 2001, showed no significant trends among sampling dates or locations ($R^2 < 0.60$). Linear regression analysis of the outflow P, in 2002, showed a significant increasing trend ($R^2 = 0.60$), but ANOVA results showed that sampling date was not a reasonable predictor of phosphorus levels ($p = 0.33$). Overall, phosphorus levels were nearly identical at all sampling locations in 2001, and showed little variation in 2002 (range 0.1 to 0.25 mg/L) at all sampling locations.

Total Suspended Solids (TSS)
In 2001, TSS at the inflow showed a significant decreasing trend ($R^2 = 0.62$), but sampling date could not be used as a predictor of TSS ($p > 0.05$). In 2002, outflow showed a significant decreasing trend in TSS ($R^2 = 0.89$), but changes in TSS could not be explained by sampling date (ANOVA, $p = 0.34$). Overall, TSS declined in 2001 but there was little difference in TSS among sampling locations. TSS were nearly identical at all sampling locations in 2002, except at the downstream sampling location where TSS values were generally higher.

Biological Oxygen Demand (BOD)
In 2001, only the inflow showed a significant declining trend in BOD ($R^2 = 0.81$), but sampling date could not be used as a predictor of BOD levels (ANOVA, $p = 0.31$). BOD levels at all sampling locations in 2002 were not significantly different and ranged from 2.5 to 4.5 mg/L. Overall, BOD levels changed slightly at all sampling locations and dates and no clear pattern was observed.

Farm VII Evaluation

Downstream data for 2001 was not used in statistical analyses because the sampling location changed during mid-year, resulting in only 2 data points per sampling location. Also, the first inflow data point for all parameters in 2002 was not included in statistical analyses because the first sampling location was different from the remaining three.

Dissolved Oxygen (DO)
Dissolved oxygen showed a significant decreasing trend in 2001 for the inflow location ($R^2 = 0.75$). An ANOVA showed that sampling date could not be used as a predictor of DO levels at this location ($p = 0.60$). All other sampling locations in 2001 and 2002 showed no significant trends in DO for each sampling date. Overall, DO levels were consistent among sampling locations and showed only a slight decline in concentration during 2001.

pH
pH showed no significant trends at all sampling locations in 2001. In 2002, outflow pH showed a significant increasing trend ($R^2 = 0.60$), but an ANOVA showed that the increase in pH was not attributed to sampling date ($p = 0.33$). Overall, pH increased at all sampling locations in 2001 and 2002, but values among sampling locations varied only slightly, ranging from 7.4 to 8.0.

Ammonia-Nitrogen (NH_3-N)
Ammonia-nitrogen values showed no significant trends in 2001. In 2002, outflow and downstream NH_3-N both had significant increasing trends ($R^2 = 0.6$ for both), but an ANOVA showed that sampling date could not be used as a reasonable predictor of ammonia levels ($p = 0.22$ for both). Overall, ammonia-nitrogen levels were higher in inflow water than any other sampling location and there was little variation in ammonia levels at all sampling sites.

Phosphorus (P)
There were no significant trends in phosphorous in 2001. In 2002, inflow phosphorus showed a significant increasing trend over the sampling dates ($R^2 = 0.76$), but an ANOVA showed that sampling date could not be used as a predictor of phosphorus levels ($p = 0.33$). Overall, phosphorous levels were higher in the inflow water, with other sampling sites having similar phosphorus levels.

Total Suspended Solids (TSS)
There were no significant trends in TSS in 2001. In 2002, inflow, downstream, and upstream locations showed significantly decreasing trends in TSS (inflow, $R^2 = 0.96$; downstream, $R^2 = 0.63$; upstream, $R^2 = 0.80$) among sampling dates. ANOVA results showed that none of the changes in TSS could be predicted by sampling date (inflow, $p = 0.13$; downstream, $p = 0.21$; upstream $p = 0.11$). Overall, TSS was similar at all sampling locations, but declined throughout the year in both 2001 and 2002.

Biological Oxygen Demand (BOD)
In 2001, there were no significant trends in BOD among sampling locations. In 2002, both the outflow and upstream sampling locations showed significant declining trends in BOD among sampling dates (outflow, $R^2 = 0.80$; upstream, $R^2 = 0.80$). ANOVA results showed that sampling date could not be used as a predictor of BOD level at either location ($p = 0.11$ for both locations). Overall, BOD levels declined throughout 2001 and 2002 for all sampling locations and varied only slightly at each sampling location.