FISH WELFARE INITIATIVE

INVESTIGATING DISSOLVED OXYGEN CHALLENGES IN AQUACULTURE SYSTEMS

By Ravi Teja Sangeetha, Thomas Billington, and Marco Cerqueira

Data analysis by Dr. Sai Kumar Sangeetha and Kevin Patrick Lim

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Executive Summary

From August 18th to October 13th, Fish Welfare Initiative conducted an important exploratory study on six ponds known for historically low dissolved oxygen (DO) levels (AJU1, AJU2, CRP, GRA, NRO, and VMS). The study's primary aim was to assess DO and its potential causes in each selected pond (the study was not designed to compare data or assess correlations across ponds). This initiative aimed to enhance our understanding of the extent of DO issues and identify specific causes in each targeted pond. Quantitative measurements were recorded in each pond once every two days, complemented by a qualitative analysis of farmer behaviors, achieved through regular interactions and close observation of their daily practices. The results were deliberated in reference to FWI-established water quality ranges deemed necessary for the well-being of the species, as supported by existing literature. A high-level overview of the quantitative data is shown in the table below.

***Table** with the Frequency DO, pH, and ammonia were **inside** of optimal and required ranges*

Parameter	No. Measurements	% of measurements within range	
		Optimal (The ideal range for the species)	**Required** (The acceptable range for the species)
DO (Morning)	100	11%	23%
DO (Evening)	104	16%	32%
pH	205	9%	63%
Ammonia	101	0%	42%
Chl-a	195	25%	37%

The main findings were:

- In five out of six ponds, DO was a significant welfare concern. On average, **morning DO was within our required range only 23% of the time**, with one pond recording 0% of morning DO measurements within range.

- In three out of six ponds, a notable **correspondence was observed between fish biomass and DO levels**. High biomass in AJU1 before harvesting (an industry term for removing fish from the pond to be sold) was connected with low DO levels. As fish biomass steadily increased throughout the study in both VMS and NRO, a consistent decline in DO levels was observed. A lower biomass in GRA was related to balanced DO levels. This suggests that fish biomass may play an important factor in managing DO levels, a factor previously undervalued by FWI.

- **The relationship between phytoplankton and DO was complex.** In some ponds like AJU1 and CRP, a clear link was evident, while in others, it was less apparent. In general, phytoplankton's impact on DO seemed dependent on fish biomass, with high fish biomass exerting a stronger impact on DO than phytoplankton biomass.

- **The Chlorophyll-a (Chl-a) range defined by FWI might be set too low.** In CRP and NRO, despite Chl-a levels being within the prescribed range, DO levels remained suboptimal. In GRA, Chl-a levels above our optimal range were linked with good DO levels.

- **Fish gasping events** (where fish will rise to the water's surface to "gasp" for air) tended to **coincide with DO levels below 1 mg/L and ammonia levels above 0.5 mg/L**. These stressors likely act to magnify each other, as ammonia toxicity impairs respiratory function, making fish more susceptible to low oxygen conditions. This is likely one of the most stressful experiences fish commonly experience within the pond.

- **Ammonia levels posed a significant welfare issue**, staying within the required range in only 42% of the measurements. Various factors, including heavy rainfall, fertilization, unbalanced feeding ratio, and pond bed disturbances, were identified as potential causes of increased ammonia levels.

- **All farmers in the study reported larger pond sizes than actual,** with discrepancies ranging from approximately 1 to 11 acres. This raises concerns about the accuracy of reported stocking densities and suggests that fish may be more densely stocked than previously thought.

Implications for Our Work

1. Fish biomass should be increasingly investigated as a potential cause of DO problems. Based on the findings from this study, FWI could consider conducting a study of biomass and its relationship with DO.
2. Consequently, a more nuanced view of phytoplankton should be taken forward as not the sole actor causing DO, but as needing to be understood in the context of fish biomass.
3. Collection of daily farmer practices regarding information related to fertilization, medicines, water addition from source, feed stoppages, etc., is necessary to understand the root causes of water quality issues at ponds.

4. The combination of low DO and high ammonia should be considered as one of the worst welfare infringements fish experience within ponds. Ammonia should be reviewed as a potential focus for future interventions and research.
5. The current Chl-a range should be re-evaluated, potentially changing the optimal range from 100–150 mg/L to 150–220 mg/L.
6. The review of true pond size should continue, as should the reduced reliance on farmers' self-reported acreage.
7. Research into the quality of inflow water should be added to the potential future studies list, as current evidence implies water exchange's efficacy may be blocked by low-quality inflow water
8. FWI resources, as part of its Alliance for Responsible Aquaculture (ARA) program should be focused on ponds with historically poor DO levels, and processes for giving recommendations for corrective actions should integrate more information about the pond and daily management activities.
9. A replication study taking place in a different season should be considered. This would enable further understanding of the impact of weather conditions on the parameters reviewed below.

Acknowledgment

We express our sincere gratitude to those whose invaluable contributions made our intensive study a success. Special thanks to the five cooperative farmers and their staff, particularly Vijay, David, and Jaanu, for allowing our team access to their ponds and going above their regular duties to assist with additional boat measurements, adding considerable time to their daily workload. Their cooperation and effort were remarkable and greatly appreciated.

Our data collection team, Manikanta, and Gandhi, along with Durga Prasad, who occasionally filled in, displayed outstanding dedication in challenging conditions. Their commitment, enduring heavy rains and extended hours, was crucial in maintaining the quality and integrity of our data throughout the eight-week study. We also extend gratitude to Sid, whose significant role in formulating the study protocol and aiding the data collection team was indispensable. His meticulous attention to designing the survey tool and ensuring accuracy in data entry was fundamental to the project's success.

Additionally, we acknowledge Vivek for his expert guidance and enthusiasm, particularly in meter usage and sample collection. His contributions to weekly data discussions and corrective action prescriptions were invaluable. We are also thankful to external data analysts Dr. Kumar and Kevin Patrick Lim for their detailed and insightful data analysis.

To all involved, your dedication and cooperation have not only enabled this study's successful execution but have also deepened our understanding of these ecosystems. Thank you.

Introduction

Over the past three years, the Fish Welfare Initiative has managed the Alliance for Responsible Aquaculture (ARA), an alliance primarily comprising farmers who commit to higher welfare standards in their aquaculture facilities. The ARA, focusing on Indian major carp farmers in Andhra Pradesh, now works with 92 fish farms, offering water quality monitoring and guidance to maintain key water quality parameters within required and ideal ranges.

A major issue identified through the ARA's monitoring is the instability of dissolved oxygen (DO) levels, suspected to be linked to unbalanced phytoplankton concentrations in the ponds. DO refers to the level of oxygen available for fish to breathe in the system, and is critical for the welfare of the animals. Phytoplankton, which can be measured through chlorophyll-a (Chl-a) levels, regulates DO via photosynthesis, which generates oxygen, and respiration, which consumes it.

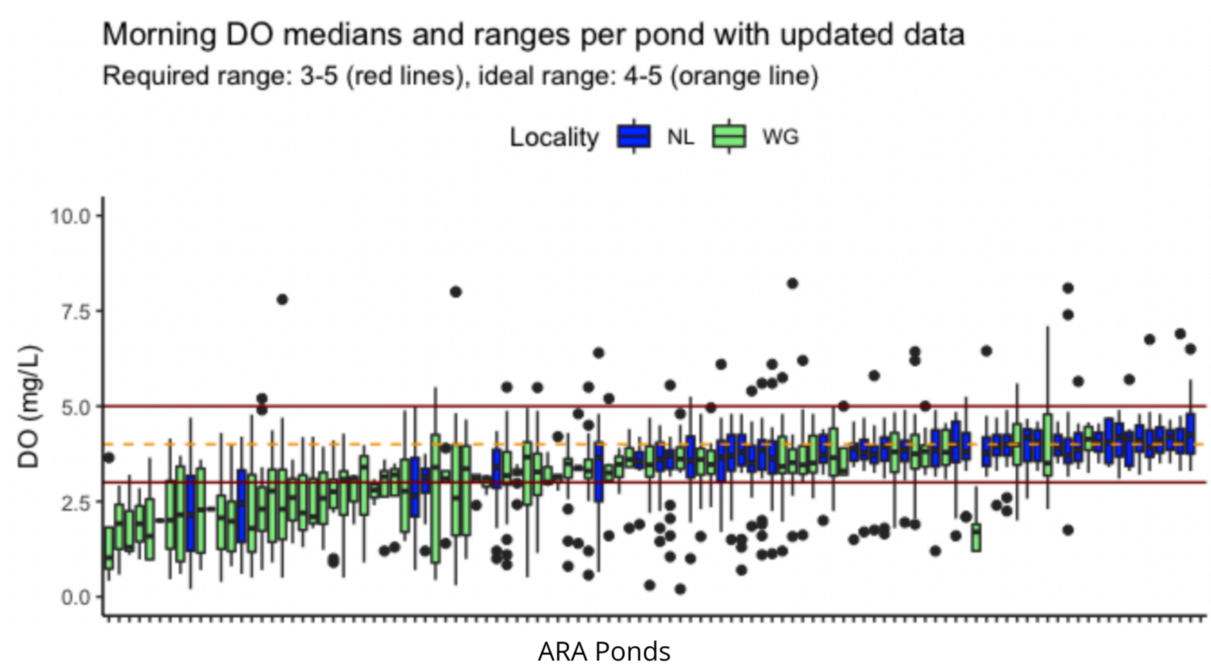


***Figure 1**: Box plot of all morning DO measurements for all ARA ponds. From this, we can see that a large minority of ponds are consistently below the required range.*

The ARA's data reveals that DO issues vary significantly among ponds. To better understand these issues, the Fish Welfare Initiative conducted a two-month observational study on six ponds in the West Godavari region. These ponds were specifically chosen for their historically low DO levels. We define historically low DO levels as any pond where less than 60% of morning measurements taken by the ARA had DO in our required range (3-5 mg/L).

The primary goal of this study was to evaluate DO levels and explore potential causes in each pond. While the limited sample size restricts our ability to compare ponds, the study was structured to identify trends and practices within each pond individually, offering insights into potential causes of DO problems.

The study included both quantitative and qualitative aspects. The quantitative aspect involved measuring key water quality parameters (DO, pH, ammonia, and Chl-a) along with factors like feed quantities and weather conditions. The qualitative component aimed to gather information from farmers and farm workers about practices at these ponds, to identify potential behavioral factors impacting water quality.

The findings from this exploratory research will guide strategies and interventions to improve the ARA and future programs.

Data collectors Gandhi and Mani collect water quality measurements from the boat

Methodology

Selection and Recruitment of Ponds

In West Godavari, 13 ponds (owned/leased by 10 farmers) were identified that met the criteria of having historically poor DO (less than 60% of morning measurements taken by the ARA had DO in our required range (3–5 mg/L)).

All 10 farmers were informed about the study's purpose and benefits. Alternative day water quality measurements were highlighted as an incentive. Brief individual interviews were then conducted with all 10 farmers to assess their interest. These interviews involved:

a. Gauging their enthusiasm and willingness to participate in the research.
b. Evaluating their openness to sharing information and experiences, mainly considering ARA staff's previous interactions.
c. Confirming their understanding of the study's objectives and their potential role.

Based on these interactions, farmers meeting the selection criteria and showing higher interest and willingness were chosen, ensuring a mix of farm sizes, water quality histories, and socioeconomic backgrounds for representation. This left us with five farmers and six ponds within the study. The selected farmers were approached, informed of their inclusion, and briefed on the study. Upon confirmation of their participation, the farmers' information was documented, finalizing the sample for the study (Table 1). The remaining farmers continued to be part of the ARA protocol with ongoing water quality monitoring.

The six ponds were divided into two clusters based on geographical proximity. Clusters were visited on alternate days.

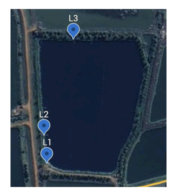
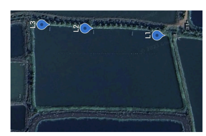
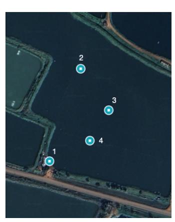
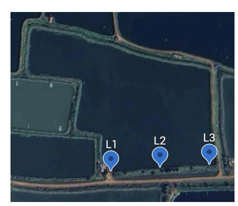
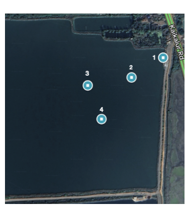
Table 1*: Key background data for each pond selected*

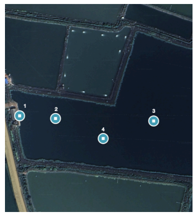
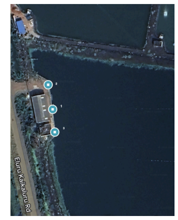
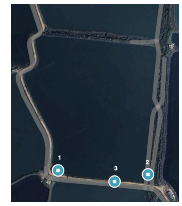
Cluster no.	Pond number	Lifestage	Reported Pond Size (acres)	Depth (feet)	Reported Fish Per Acre	Species
1	AJU1	Grow-out	13	4-6	3400	Catla, Rohu
	AJU2	Breed-out	7	4-6	7850	Catla, Rohu
	VMS	Grow-out	16	4-6	2100	Catla, Rohu
2	GRA	Breed-out	50	4-6	1250	Catla, Rohu
	NRO	Grow-out	36	4-6	2500	Catla, Rohu
	CRP	Grow-out	15	4-6	2100	Catla, Rohu

Water Quality Measurements

Quantitative water quality data was collected every other weekday, twice a day (once in the morning and once in the evening). The collection window for the morning measurements was 6 to 9 AM and for the evening 4 to 6:30 PM. Measurements were either taken from inside a boat or from the side of the pond, depending on attributes like weather and farmer willingness to allow use of their boat. Both pond-side measurements and boat measurements are used in the analysis (Table 2). Smaller ponds would have three data collection points, whereas larger ponds would have four. Data from these collection points was averaged into a final value for each water quality parameter.

***Table 2**: water quality measurement points for each pond, both within the boat and from the pond side.*

Farmer	Boat	Pond side
AJU1	NO BOAT MEASUREMENTS	
AJU2	NO BOAT MEASUREMENTS	
VMS		
GRA		

NRO		
CRP	NO BOAT MEASUREMENTS	

Measurements were taken using a ProDSS water quality meter for DO, Temperature, and Chl-a, and a separate Taiwan meter for pH due to equipment limitations. The calibration of these meters was scheduled weekly. A spectrophotometer was employed to measure ammonia levels in the samples collected.

Non-Water Quality Measurements

Measurements such as weather (sunny, rainy, foggy, percentage cloud cover, and wind levels), number of dead fish, and water color were directly observed by the data collectors at the pond location. The number of individual fish gasping (where fish come to the surface of the water to breathe) was recorded by data collectors observing the pond for one minute and counting the number of instances they could see. Data collectors also ensured that any data pertaining to feed/fertilizer/chemicals/medicines that took place between the measurements were recorded in the subsequent measurements notes sections.

For collecting data on the acreage of ponds, we asked the farmer for their acreage assessment and used Google Earth satellite imagery to verify. Stocking densities were determined using our Google Earth acreage assessments and the farmer's self-reported quantity of fish in the pond. At multiple points during the test period, farmers partially harvested (an industry term for removing fish from the pond to be sold) their ponds. These instances were noted by our data collection team and are factored into stocking density numbers. Pond biomass was assessed predominantly using farmer's self-reported size of their fish, multiplied by the estimated number of fish present in the pond (as assessed by farmers' self-reporting). On a few occasions, however, we were able to verify fish size by observing farmers weighing their fish.

Data was entered into a designated JotForm within one day of collection. Standard operating procedures were established for various aspects of the study, including water quality probe handling, calibration, maintenance, equipment error code resolution, disinfection, storage, and stock maintenance for equipment and solutions.

Qualitative Measurements

Qualitative observations were done by shadowing and observing the workers/farmers as they carried out their day-to-day operations such as feeding, water and disease management, harvest and sale of fish, etc. The main objective of this process was to engage more closely with the farmer/worker and understand their daily contributions that might affect the water quality parameters—be it negatively or positively. Farms where there were any processes of interest for the qualitative observation—be it feeding, disease management, stocking of fish, harvest, etc.—were prioritized for observation for the particular day.

Corrective Actions

Although this test was only observational, and thus did not cause negative welfare effects for fish, we still decided to ensure that fish received a minimum standard of care. We did this by suggesting corrective actions (CAs); interventions to farmers when water quality parameters went outside of range). CAs were determined weekly based on the water quality measurements taken over the prior week. These actions were communicated to farmers either in person, over phone calls, or through WhatsApp, depending on the farmers' availability and preference. Predominantly, we believe that farmers did not follow these CAs, but we decided to not formally monitor farmer’s adherence, mostly due to the risk of this damaging the farmer relationship and because CAs were not the focus of our study.

Results

The raw data can be found here.
For detailed results and additional information from each farm individually, see appendices (1–6).

Quantitative

The study planned to make 20 morning and 20 evening measurements for each pond. The final numbers are shown in Table 3. The discrepancy between planned and actual visits was mainly due to the data collectors taking breaks during public holidays, festivals such as Dussehra, and pausing the data collection on rainy days when the ponds were inaccessible

***Table 3**: Number of morning and evening measurements per pond*

Pond ID	Morning Visits	Evening Visits
AJU1	19	18
AJU2	17	18
VMS	18	19
GRA	16	18
NRO	18	17
CRP	13	13

Across the duration of the study, DO was the most common parameter outside of the required range, followed by Chl-a, ammonia, and pH (Tables 4 and 5).

***Table 4**: Frequency DO, pH, and ammonia were **inside** of optimal and required ranges, compared to [other ARA ponds]*

Parameter	*No. Study Measurements*	Required Range		Optimal Range	
		% Study Measurements	% ARA Measurements	% Study Measurements	% ARA Measurements
DO (Morning)	*100*	23%	79%	11%	29%
DO (Evening)	*104*	32%	83%	16%	63%
pH	*205*	63%	92%	9%	91%
Ammonia	*101*	42%	--	0%	--
Chl-a	*195*	37%	--	25%	--

Morning DO levels were frequently below our required range in five of the six ponds studied (Figure 2 and Table 5). DO reduces during the night, as no photosynthesis is taking place, and as such the morning DO levels likely represent the lowest DO level present in the ponds across the testing period. One pond (GRA) was frequently above the required range, though this is typically less detrimental to fish welfare.

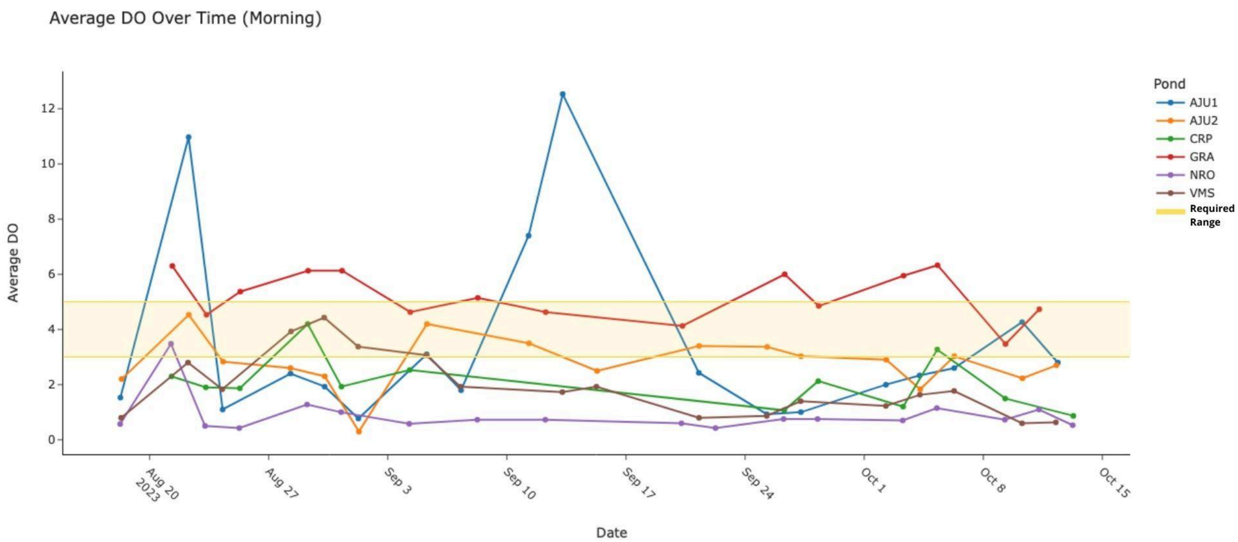


Figure 2: *Morning DO across all ponds for the entire testing period*

Evening DO levels were very erratic during the testing period (Figure 3). During the day, photosynthesis increases DO levels. Typically the amount of DO increase depends on factors such as fish biomass, phytoplankton biomass, and the presence of other organisms in the pond such as zooplankton or invasive species. As such, Evening DO is often much more varied than morning DO.

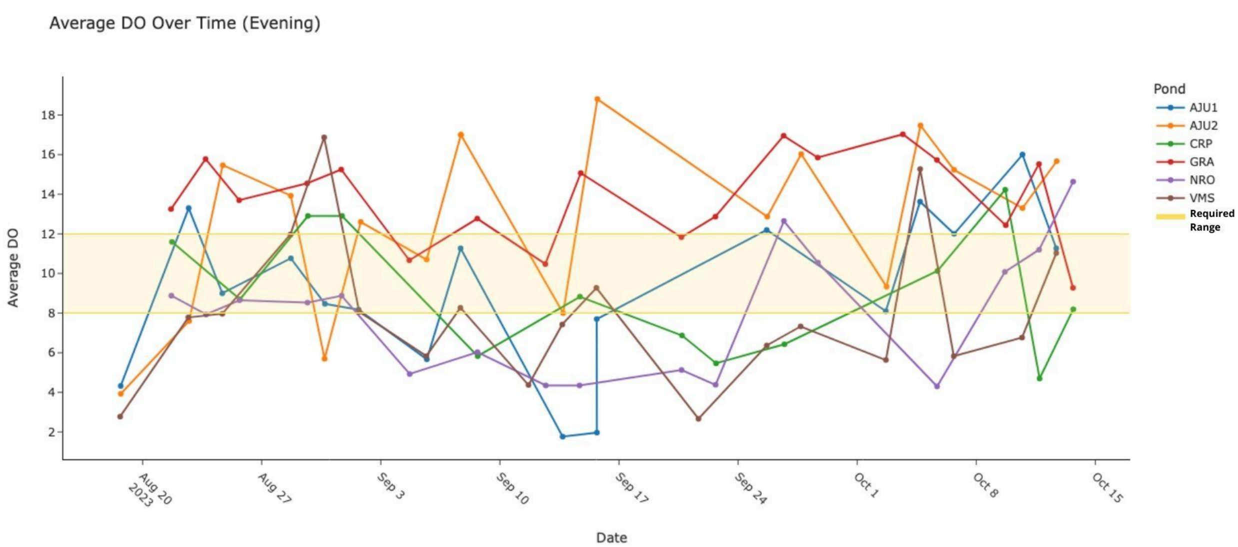


Figure 3*: Evening DO across all ponds for the entire testing period*

The pH levels were consistently within the appropriate range the majority of the time for five out of the six ponds (Table 4 and Figure 2). This suggests that neither alkalinity nor hardness were the likely sources of stress in the system. Balanced alkalinity and hardness play a crucial role in maintaining water stability and preventing abrupt fluctuations in pH.

During the week of 24th September to 1st October, there was a marked decrease in pH levels across all ponds. This was likely due to a calibration issue with the pH probe rather than actual changes in the pond environment.

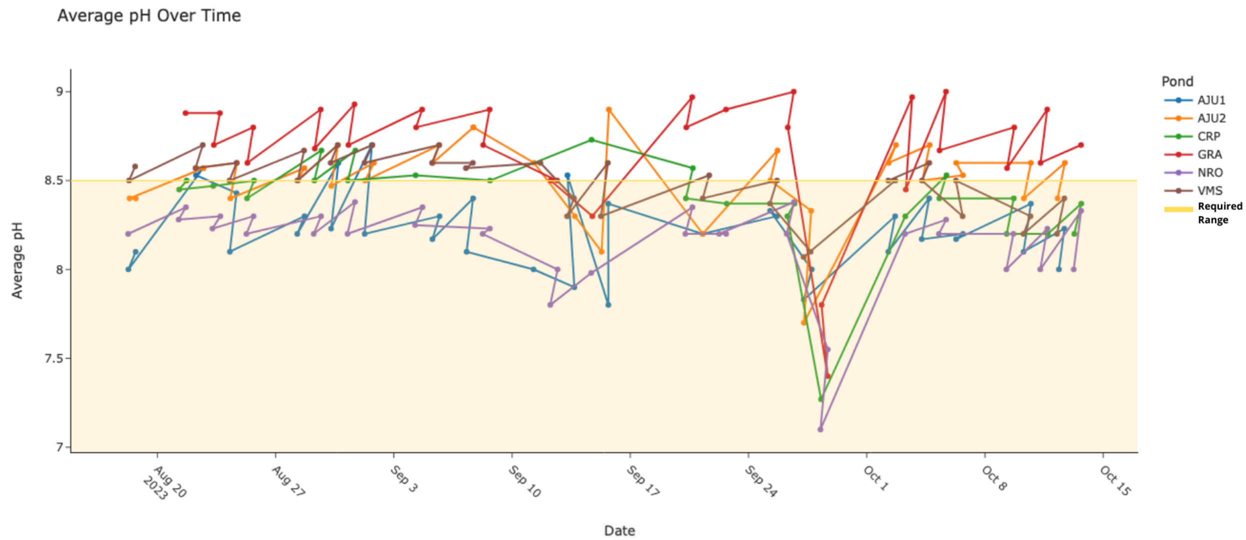


Figure 4: *pH across all ponds for the entire testing period*

Ammonia was a consistent issue for the ponds in the study, with some ponds experiencing significantly more issues than others (Figure 3 and Table 5). High ammonia concentrations are toxic to fish. As fish experience stress or health issues due to elevated ammonia, their oxygen consumption may increase, further intensifying the oxygen demand. High ammonia levels often coincide with elevated feeding ratios and warmer temperatures.

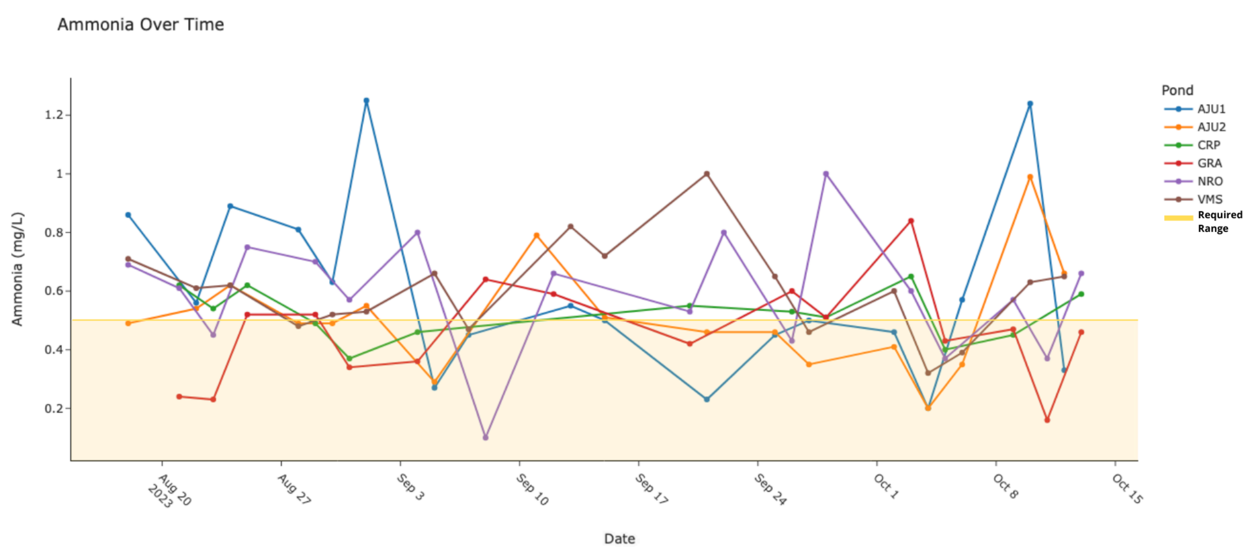


Figure 5: *Ammonia across all ponds for the entire testing period*

Within the study, Chl-a levels were extremely varied among ponds, spanning from ponds with 90% of Chl-a measurements within range to only 15% (Figure 4 and Table 5). Well-balanced Chl-a levels indicate a thriving phytoplankton community, contributing to increased photosynthesis. This process results in elevated oxygen production, especially during daylight (i.e. phytoplankton undergo photosynthesis during daylight, leading to higher DO levels, while at night, they consume oxygen through respiration. This can cause diurnal fluctuations in DO levels).

High Chl-a levels indicate high phytoplankton levels, which can lead to a destabilized system where risks such as eutrophication, where phytoplankton populations crash and oxygen is consumed during their decomposition of accumulated organic matter, potentially leading to oxygen depletion. Lower Chl-a levels indicate reduced phytoplankton levels and, consequently, lower photosynthetic activity. With a lower presence of phytoplankton, the oxygen dynamics tend to be more stable, with less pronounced diurnal variations and a reduced risk of sudden drops in DO levels. Nevertheless, if phytoplankton levels are not enough to ensure the biochemical oxygen demand of the pond actors, this will mean low DO in the water and ultimately affect the welfare of the fish.

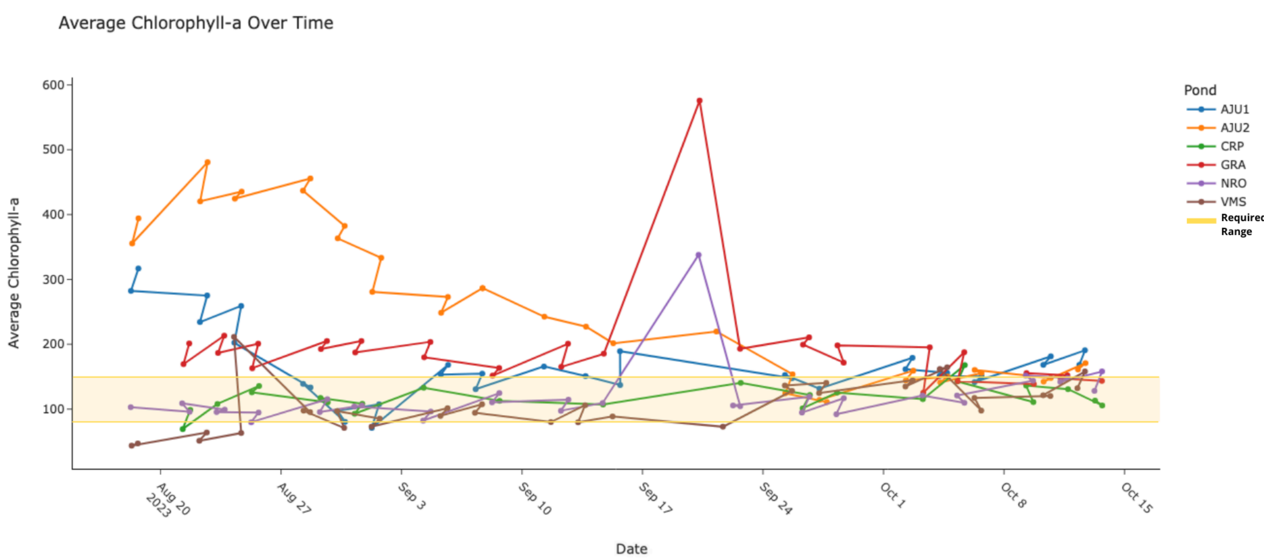


Figure 6: *Chl-a across all ponds for the entire testing period*

Table 5: *Percentage of measurements within the required range per pond for key parameters*

Pond ID	% measurements *within* required range					Biomass /acre
	DO (Morning)	DO (Evening)	Chl-a	pH	Ammonia	
AJU1	11%	44%	35%	86%	49%	1100-5130
AJU2	39%	21%	20%	43%	61%	800-2625
VMS	22%	26%	89%	54%	28%	711-1659
GRA	44%	22%	15%	21%	56%	114-396
NRO	0%	41%	91%	100%	28%	2979-3646
CRP	15%	38%	96%	77%	31%	726-940

Inflow
Only seven measurements across three inflow points were taken. This small sample size was due to challenges accessing some of the inflow points as well as the infrequency of farmers adding water into their ponds (as inflow measurements were only taken when farmers were inflowing water, to make sure the measurements represent the quality of the water entering ponds).

Five measurements were taken of the inflow for AJU1 and AJU2, and one measurement each was taken of VMS's and GRA's inflow (Table 6). All measurements showed at least one water quality issue, and most showed multiple.

Table 6: *Frequency of pond inflow water quality being out of range on key parameters*

Water Quality Issue	No. Measurements out of required range
DO	7/7
Ammonia	3/7
pH	2/7
Chl-a	2/7

All farmers were found to be misrepresenting the size of their ponds (Table 7). This was always overreported (farmers would claim more acres than their pond was). This has important implications for how stocking densities are calculated.

Table 7: *Farmer's self-reported pond size and the pond size as measured using Google Earth*

	Reported pond size	Actual pond size	Difference
AJU1	13	10	-3
AJU2	7	6.1	-0.9
VMS	15	12.4	-2.6
GRA	50	39.4	-10.6
NRO	36	29.3	-6.7
CRP	16	15.1	-0.9

Gasping

Fish gasping was observed 7 times across all ponds (Table 8). Fish gasping is typically a sign that fish are unable to access enough oxygen. This can be caused both by a low level of DO and too high ammonia levels (which reduce fishes' ability to uptake oxygen).

Table 8: *Frequency of gasping events across all studied ponds*

Pond ID	Number of gasping events
AJU1	1
AJU2	0
CRP	1
GRA	1
NRO	4
VMS	0

Qualitative

All data below was collected either through surveys, interviews, or informal conversations with the selected farmers. Analyzing the data, several trends emerge, particularly in the context of feeding, fertilization, and disease management practices. These trends provide insights into why these farms may consistently experience poor water quality:

Experience-Based Feeding and Fertilization Practices:

- All farmers, except AJU1 and AJU2's farmer (one of the five farmers in the study, and the only farmer to have two ponds included in the study), primarily relied on personal experience and satiation feeding methods (where farmers provide as much as fish will consume). **This often led to feeding quantities we believe to be excessive and thus potentially feed wastage**.
- Only AJU1 and AJU2's farmer strictly followed a weight-based feeding system. However, most farmers did consider some elements such as weather when making decisions on how much to feed.
- VMS's farmer, while being absent from the pond, collaborates with peers for decision-making, indicating a more community-based approach to feed decisions.
- **Fertilization and treatments are typically carried out without halting feeding or adhering to a strict plan**.

Regular Fish Weight Monitoring:

- All farms demonstrate regular weighing practices, indicating an awareness of the importance of weight-based feeding regimes. However, the frequency and methodology vary.
- Farmer's reasoning is mostly to keep track of the monthly growth rates, and not to inform future feeding decisions.

Adjustments for External Factors:

- Ceasing feeding during heavy rainfall or post-medicine application is a common practice across farms, showing a responsive approach to environmental changes. Most farmers were tenable to corrective actions around feed stoppage during heavy rains.
- One step that has been identified as a common practice during the rains is that the uneaten feed bags are emptied into the pond before they are left to dry. The worker believes that this excess feed is not consumed, and thus likely contributes to nutrient loads within the pond.

Trends in Disease Management Protocols:

- All farms employ dedicated workers for daily pond maintenance and disease observation. Most farmers also routinely visit the ponds themselves.
- Responses to disease outbreaks, like lice infestations, are prompt and systematic. However, levels of success in treatment efficacy are varied.

- Regular water sanitization routines, which involve the usage of BKC to ensure that the pond does not accumulate too many parasites, are common across farms, indicating a standard practice in disease prevention.
- Dependence on external sources for medicine and advice, notably Coastal Aqua Ranga Rao, points to a reliance on local expertise and networks.
- AJU is the only farmer who currently uses organic fertilizers every month to ensure that the pond gets higher-quality inputs. He accesses these through a Biotech company that provides zooplankton checks every 10 days through their field technicians.

Discussion

As part of the ARA, farms are visited once a month by our data collectors who collect key data for water quality parameters. We defined ponds with poor DO as any pond that has DO inside of FWI's required range (3–5 mg/L) at 60% or more of the ARA's recorded morning visits. There are 12 such poor DO ponds currently in the ARA. As FWI considers DO to be a key water quality parameter linked to fish welfare, we wanted to collect more frequent data at these selected ponds to develop a more thorough understanding of the DO issue, and to attempt to understand the reasons why DO is such a concern at these ponds. This study, combining qualitative and quantitative methodologies, was conducted at six ponds in the West Godavari region.

This study was designed to help us understand if DO is a consistent concern at these selected ponds and if so, to identify practices at individual farms that may contribute to the problem. A discussion for each of the individual ponds is presented in Appendices 1 through 6. Discussions around key findings across all ponds are presented below.

***Table 9**: Summarized thoughts for each pond*

Pond	Comments
Pond 1 (AJU1)	Extremely high biomass led to Chl-a and DO crashes. "Harvesting" fish increased ammonia, and the combined effect of these stressors led to fish gasping. Post-harvest, DO improved as biomass decreased and regular fertilization stabilized Chl-a.
Pond 2 (AJU2)	AJU2 experienced similar issues to AJU1, with high biomass causing Chl-a and DO crashes. Post-harvest, DO levels improved with the reduction of biomass and phytoplankton stabilization. Ammonia levels spiked, possibly due to heavy rainfall. The lack of supplemental feed for 26 days would have stunted fish growth.
Pond 3 (VMS)	DO levels in VMS consistently declined during the study, with no clear indicators identified for the progressive decrease in DO (though it is

	possibly connected to increasing biomass).
Pond 4 (GRA)	GRA was unique as DO levels did not pose a significant welfare issue, primarily due to the pond's very low biomass (about 20% of CRP's biomass). This allowed phytoplankton to generate more DO than the biochemical oxygen demand, despite not having high Chl-a levels. However, the pond experienced a severe gill fluke outbreak likely due to high ammonia/organic matter in the pond possibly caused by excess fish feed
Pond 5 (NRO)	NRO had the study's highest consistent biomass, exacerbated by delayed harvesting, leading to the worst DO levels and the most gasping events. Ammonia levels were also exceptionally high. Chl-a levels were low, indicating that there was not enough phytoplankton to meet oxygen demand.
Pond 6 (CRP)	In CRP, Chl-a and DO levels were closely related, fluctuating in tandem. The typical low DO levels suggest that Chl-a might also have been too low, even though it was often within the required range. The pond's low biomass may explain the clear relationship between Chl-a and DO (as it is one less variable affecting DO).

Dissolved Oxygen (DO)

DO was a persistent welfare issue across 5/6 of the ponds studied, with 77% of total morning and 68% of total evening DO measurements falling outside the required ranges. This supports the hypothesis that these ponds consistently struggle with DO challenges. Given the frequency of suboptimal DO levels, it's plausible that this contributes to stress in the aquaculture systems under study.

An analysis of each pond (see appendix) revealed a relationship between critical DO levels, increased biomass, and unbalanced phytoplankton biomass. Fish biomass was particularly influential in certain ponds. For instance, AJU1 experienced severe oxygen depletion until harvesting, which then led to a rapid increase in DO levels. In contrast, NRO, with high fish biomass and no harvest during the study, faced continuous DO issues. Conversely, GRA, with significantly lower fish biomass, presented balanced DO levels with Chl-a being within the required range. These all suggest that fish biomass has a pronounced influence on DO.

CRP, another low-biomass pond, displayed a stronger relationship between DO and Chl-a, indicating that in environments with lower fish biomass, phytoplankton exerts a greater influence on DO levels. Consequently, Chl-a's influence on DO levels was highly contingent on fish biomass within the pond. Low Chl-a levels (below 150 mg/L) seem insufficient to counterbalance the biochemical oxygen demand from fish and other organisms, such as zooplankton.

Considering DO, it is clear that it is a persistent welfare issue within all of these ponds. 77% of morning and 68% of evening DO measurements were outside of the required ranges. Considering the frequency with which fish were subjected to these poor levels of DO, we believe this is likely a contributing factor to stress in the selected aquaculture systems.

Fish gasping events became more evident when DO levels dropped below 1 mg/L, suggesting that such low levels are intolerable for the fish. It's important to note that these observable behavioral responses typically occur at the final stage of welfare compromise, indicating that the fish would have already experienced stress before reaching this point.

Fish gasping events were notably frequent when DO fell below 1 mg/L and ammonia levels exceeded 0.5 mg/L. This may be due to high ammonia toxicity affecting phytoplankton, leading to their die-off and subsequent low DO levels. However, this pattern wasn't consistent, as not all ammonia increases led to reductions in phytoplankton. Another explanation is that high ammonia directly impairs fishes' respiratory function, hindering their ability to use available oxygen. Therefore, the simultaneous presence of low DO and elevated ammonia levels is likely to be especially stressful for the fish.

It's noteworthy that despite these persistently poor DO levels, no significant mortality events were observed in any of the ponds. This aligns with our prior findings that suggest DO alone is unlikely to cause major die-offs.

Regarding water management, most farmers reported only one instance of pumping water into the pond during the four-month monsoon season. In our two-month study period, only three ponds added water, with a total of seven instances of water inflow. There's some evidence suggesting that DO issues could be caused or exacerbated by the lack of quality inflow water, as DO challenges were present in all instances of inflow. This can be particularly problematic during periods of high biomass (as in AJU1 farm) and increased metabolic activity (feeding time), leading to stress or even oxygen depletion in extreme cases.

This test was run with the ponds that most frequently exhibit water quality issues as part of the ARA, and at the worst time of year for DO, so results likely represent close to the worst DO levels typically found in the Kolleru region.

Mass Gasping event in the morning following a cloudy day (although it is hard to see, the small disruptions in the pond surface are each a fish struggling to breathe).

Other Water Quality Parameters

Chl-a was another parameter frequently outside the required range, alongside DO. It was typically too high, but variations were observed depending on the pond, with some exhibiting too low Chl-a levels. The relationship between feed quantities and Chl-a was not consistently clear, though notable reductions in Chl-a were observed during extended periods of non-feeding in AJU1 (14 days) and AJU2 (26 days).

37% of pH and 58% of ammonia measurements were outside the required range. This suggests that ponds with poor DO levels are also prone to additional water quality issues. Ammonia levels were of particular concern, with high spikes (1 mg/L or above) in four out of six ponds, exacerbating welfare issues, especially when coupled with low DO levels. Identified potential causes for these spikes included heavy rainfall, fertilization, overfeeding, and disturbance of the pond bed.

Likely Causes of Poor Water Quality

- High biomass
- Uncontrolled phytoplankton
- Poor inflow water quality
- Heavy rainfall
- Ammonia-rich fertilizers
- Lack of pond preparation between cycles (leading to toxic gas build-up)
- Farmer resistance to altering feeding practices (as in CRP's farm)

Farm Practices

All farmers in the study overreported the acreage of their ponds, indicating a systemic issue in reporting practices. This discrepancy may stem from farmers including the total land area rather than just the pond area in their reports. Considering this trend of overestimation, it's likely that farmers adhering to our stocking density recommendations may inadvertently be exceeding these guidelines.

The farming practices observed highlight a mix of traditional knowledge and adaptive methods. However, prevalent trends such as overfeeding, maintaining high biomass, and, in some cases, reluctance to adopt new practices, are contributing to the consistently poor water quality. To address these challenges, a multifaceted approach involving additional research, educational initiatives (such as proper fertilization regimes), advanced technology, and community engagement could lead to improved outcomes.

Corrective Actions

Overview of Corrective Actions

Throughout the study, a range of CAs were suggested to the farmers to manage and improve water quality parameters within their ponds. Initially, CAs were recommended every two days, but this approach was soon modified to a weekly basis after observing workers' discomfort with frequent oversight and some farmers' defensiveness. The most common CAs involved reducing feed quantities, with variations ranging from slight reductions (10–15%) to complete feed stoppage, depending on the severity of the water quality issues, followed by suggestions to turn on aerators wherever they were available.

Based on the analysis of the six weeks' corrective actions suggestions, the ponds with the most number of CAs suggested throughout the study period are:

- AJU1, CRP, and NRO: Each of these ponds had four CAs suggested.
- GRA and VMS: Both of these ponds had three CAs suggested.
- AJU2: This pond had the least number of CAs suggested, with only one CA.

This indicates that AJU1, CRP, and NRO required the most intervention in terms of corrective actions, possibly due to more significant or persistent water quality issues compared to the other ponds.

Farmers' Compliance and Outcomes

Compliance with the recommended CAs varied significantly among the farmers, impacting the effectiveness of these interventions. In Week 2, AJU2 adhered to the CA of stopping feed, resulting in a noticeable decrease in phytoplankton levels, as evidenced by lower Chl-a levels. This contrasted with the outcomes in other ponds where farmers did not follow the CAs.

As the study progressed, non-compliance was a recurring theme. For instance, in Week 5, despite multiple CAs suggesting feed reductions (AJU1, CRP, NRO, VMS), adherence was minimal, with only NRO partially following the advice. Similarly, in Week 6, despite high ammonia levels and low DO, CRP and VMS did not reduce feeding as advised. However, NRO responded to gasping events by using aerators, showing partial compliance. In Week 7, some improvement in compliance was observed, with CRP and GRA reducing feed as suggested, but AJU1, NRO, and VMS still showed resistance to following the CAs.

The study provided CAs on a weekly basis, whereas farmers were accustomed to monthly CA recommendations as per ARA's protocol. The overall low adherence to the suggested CAs presented a challenge in conducting further analysis of their effectiveness on water quality parameters.

Limitations

Some limitations affected the study’s ability to draw meaningful conclusions.

- Variations in baseline conditions across different ponds preclude conducting a meaningful comparative analysis, which constricted our evaluation to only review each facility individually.
- The temporal gaps between measurements at times complicate data analysis, increasing the likelihood of misinterpretation.
- Accompanying notes to the water quality data were not of consistently high quality, meaning that some insights were likely lost that could have informed the evaluation.
- Pre-study information was not collected (such as the farmer’s actions in the preceding weeks), which affected our ability to evaluate initial measurements.
- The study took place during the monsoon season and is not representative of an entire yearly cycle for the selected ponds.
- We were unable to access inflow water sources and the number of inflow events was sparse, meaning that we were not able to obtain a significant amount of data as to inflow water quality.

Concluding Remarks

1. This study shed light on the critical issue of DO levels in fish ponds, emphasizing also the importance of proactive management strategies. The observed patterns of DO fluctuations underscore the need for targeted interventions to ensure optimal fish welfare and sustainable aquaculture practices. Moving forward, implementing better management practices and addressing the identified challenges will play a crucial role in enhancing the overall health and productivity of aquaculture systems in Andhra Pradesh.
2. These results hold many implications for Fish Welfare Initiative's work moving forward. Below, we highlight some of the key implications we have identified:
3. Fish biomass should be increasingly investigated as a potential cause of DO problems. Based on the findings from this study, FWI could consider conducting a study of biomass and its relationship with DO.
4. Consequently, a more nuanced view of phytoplankton should be taken forward as not the sole actor causing DO, but as needing to be understood in the context of fish biomass.
5. Collection of daily farmer practices regarding information related to fertilization, medicines, water inflow, feed stoppages, etc. is necessary to understand the root causes of water quality issues at ponds.
6. The combination of low DO and high ammonia should be considered as one of the worst welfare infringements fish experience within ponds. Ammonia should be reviewed as a potential focus for future interventions and research.
7. The current Chl-a range should be re-evaluated, potentially changing the optimal range from 100–150 mg/L to 150–220 mg/L.
8. The review of true pond size should continue, as should the reduced reliance on farmers' self-reported acreage.
9. Research into the quality of inflow water should be added to the potential future studies list, as current evidence implies water exchange's efficacy may be blocked by low-quality inflow water
10. FWI resources, as part of its Alliance for Responsible Aquaculture (ARA) program should be focused on ponds with historically poor DO levels, and processes for giving recommendations for corrective actions should integrate more information about the pond and daily management activities.
11. A replication study taking place in a different season should be considered. This would enable further understanding of the impact of weather conditions on the parameters reviewed below.

Appendix

Appendix 1: AJU1 Fish Farm

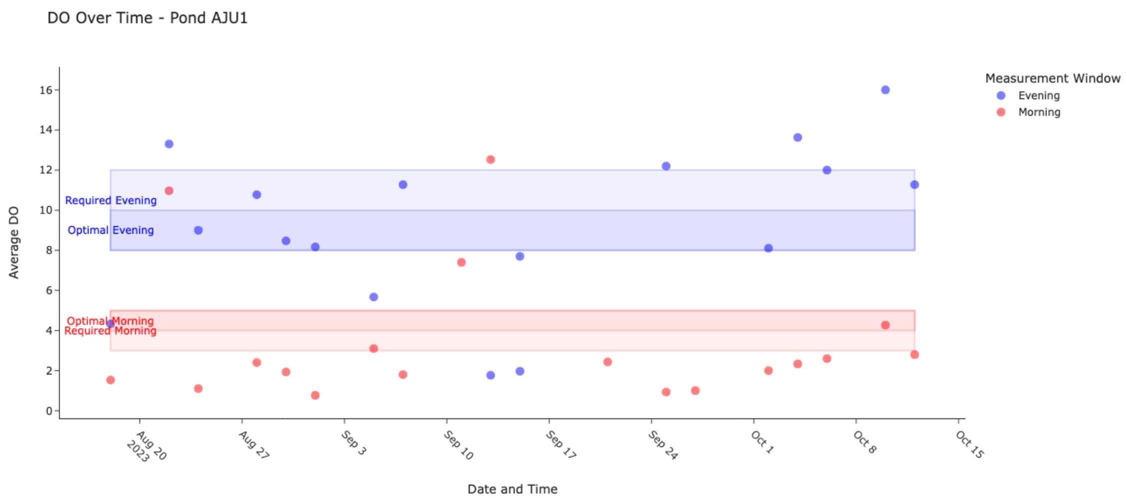


Fig 7: *DO measurements at AJU1*

Fig 8: *Line graphs showing key parameters for AJU1*

Fig

Table 10: Percentage of measurements within the required range for AJU1

AJU1					
Parameter	DO (Morning)	DO (Evening)	Chloro phyll-A	pH	Ammonia
% of measurements in required range	11%	44%	35%	86%	49%

AJU1's Farmer employs one worker from the village to be at the pond during the day to carry out all the pond operations such as feeding, pond maintenance, etc. He owns the pond and has aerators. Stocking densities were high (5400–4400/acre).

- Before "harvest", AJU1's farmer **"conditioned" fish**, a practice where fish are intentionally stressed, primarily to prevent fish from eating phytoplankton. It is believed that feed in the gut of fish will spoil their flesh more quickly in transit. Fish were stressed by being caught and released, as well as by running boats across the water's surface.

- Fish at AJU1 were **caught twice using drag nets** (large nets that are pulled across the pond bottom), once during conditioning and once during "harvest". This likely caused an ammonia spike by disturbing toxic gasses that build up on the uncleaned pond bed.

- **Chl-a levels**, initially high, dropped significantly in August. They stabilized at a healthy level after the farmer harvested, reintroduced feed, and began fertilizing regularly. However, this didn't correspond to a significant improvement in DO.

- AJU1, the **most frequently fertilized pond** (4 times over roughly 40 days), likely benefited from the farmer's use of biotech advice. These companies conduct bi-monthly zooplankton checks and suggest a fertilization plan. This likely contributed to stabilizing phytoplankton levels, indicating the positive role of biotech companies in influencing farmer practices.

- AJU1 was **drastically overstocked** pre-harvest, with 5500 fish per acre. This was reduced to 4400/acre due to slow growth in the previous cycle. The reduction in biomass post-harvest aligns with an improvement in oxygen and phytoplankton levels.

- Two of the four largest **ammonia spikes** coincided with **heavy rainfall**, suggesting rainfall could have caused some of the ammonia issues potentially due to runoffs from surrounding areas, decomposition of organic matter, or dilution effects of the ammonia present which causes a temporary increase in ammonia concentration.

- AJU maintained conscious, consistent feed ratios, feeding less than most, which likely helped avoid overfeeding-related water quality issues. However, feed was added after medicating the pond, which is not advised.

- The **farmer weighs 100 Rohu and 30-40 Catla monthly**, exceeding typical ARA numbers. This allows for a more representative understanding of fish's feeding requirements, though does cause fish stress during weighing.

- A **lice outbreak** in early August was quickly managed, followed by weekly netting for lice checks and fish being sold more quickly to prevent further lice infestations. Monthly sanitization was also practiced as a preventive measure.

- Considering **medical advice**, the farmer consults Coastal Aqua from Eluru market for medicine and diagnostics.

Final thoughts on DO:
DO was a significant issue within AJU. It appears the main driver was fish biomass within the pond, as DO improves after harvest. It is considered likely that the extremely high biomass meant that biological oxygen demand was exceeding oxygen production within the pond, as well as straining phytoplankton populations as fish consumed them.

The link between phytoplankton and DO was not very clear, though DO was at its best toward the end of the study when phytoplankton had stabilized.

Appendix 2: AJU2 Fish Farm

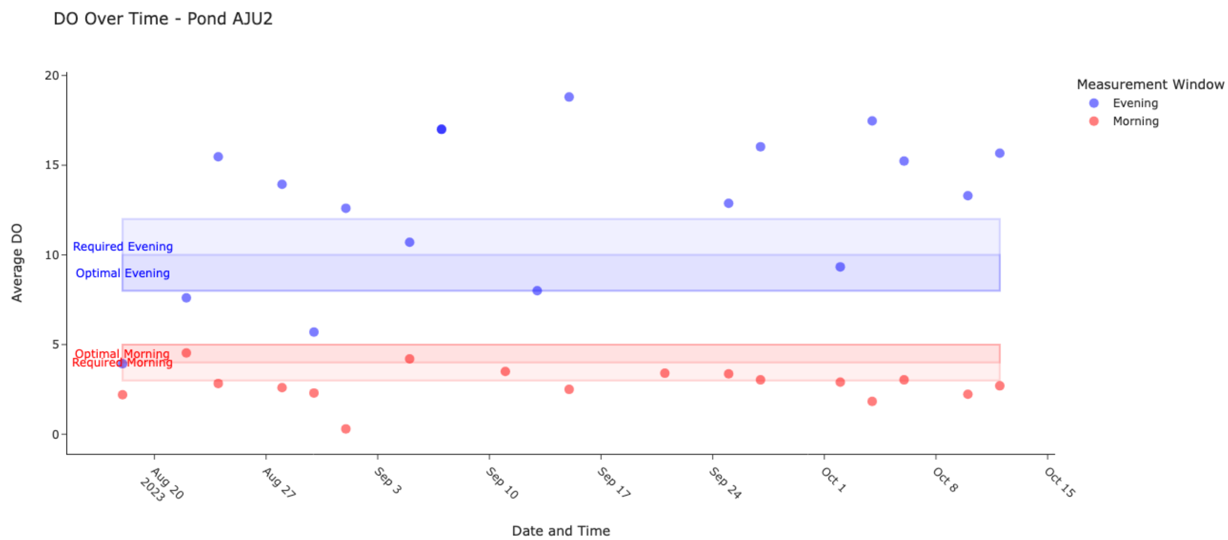


Fig 9*: DO measurements at AJU2*

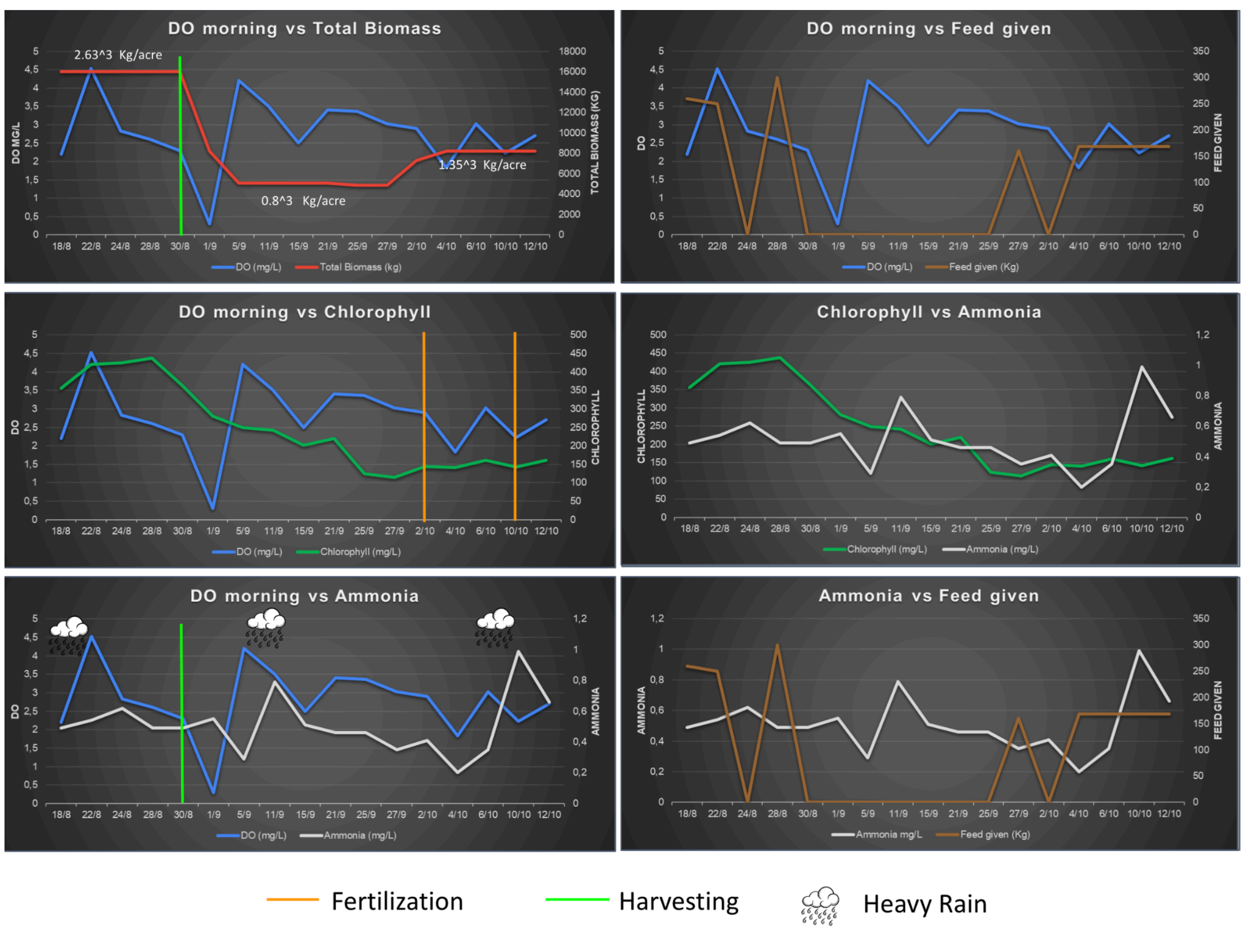


Fig 10*: Line graphs showing key parameters for AJU1*

***Table 11**: Percentage of measurements within the required range for AJU2*

AJU2					
Parameter	DO (Morning)	DO (Evening)	Chloro phyll-A	pH	Ammonia
% of measurements in required range	39%	21%	20%	43%	61%

For information on the farmer's practices, see AJU1. AJU2 is a "Breed-out" pond, where younger fish are held before moving to the "Grow-out" pond. This means that fish are smaller, and thus the pond can hold more fish per acre.

- **Chl-a levels** were dangerously high at the beginning of the study period. However, after harvest they dropped significantly, only restabilizing after the pond was restocked, and feed and fertilizer were reintroduced. This likely shows an unstable pond going through "boom and bust" cycles of phytoplankton.

- The largest **DO crash corresponded with a significant decrease in Chl-a**. This could imply that the DO crash was caused by a phytoplankton mass mortality (potentially related to harvesting activity). Potentially, this was because dead phytoplankton and zooplankton (who feed on phytoplankton) created "scum" at the pond surface that blocked sunlight and photosynthesis. It could also be that the dead phytoplankton and zooplankton decomposed, a process that uses oxygen.

- The largest **ammonia spike** proceeded **heavy rainfall** and was measured on the same day as **fertilizer** was added. Both of these may have caused the ammonia spike. The second largest ammonia spike also proceeded heavy rainfall.

- After partial harvest (where some but not all fish are removed from the pond), fish remaining in the pond were **not fed for roughly one month**. This would have **stunted the fishes' growth**. This common practice in breed-out ponds aims to boost profit through faster "compensatory growth" in the grow-out stage but can increase stress and affect immune systems. It may also reduce phytoplankton levels.

Final thoughts on DO:
DO consistently struggled to stay consistently within the required range. The largest DO crash coincided with a drop in phytoplankton, likely pointing to phytoplankton being a more significant actor in AJU2 than could be evidenced in AJU1.

After the crash, DO continued to be volatile but was perhaps helped by the reduction of biomass. This link is less clear, however, than in AJU1.

Appendix 3: VMS Fish Farm

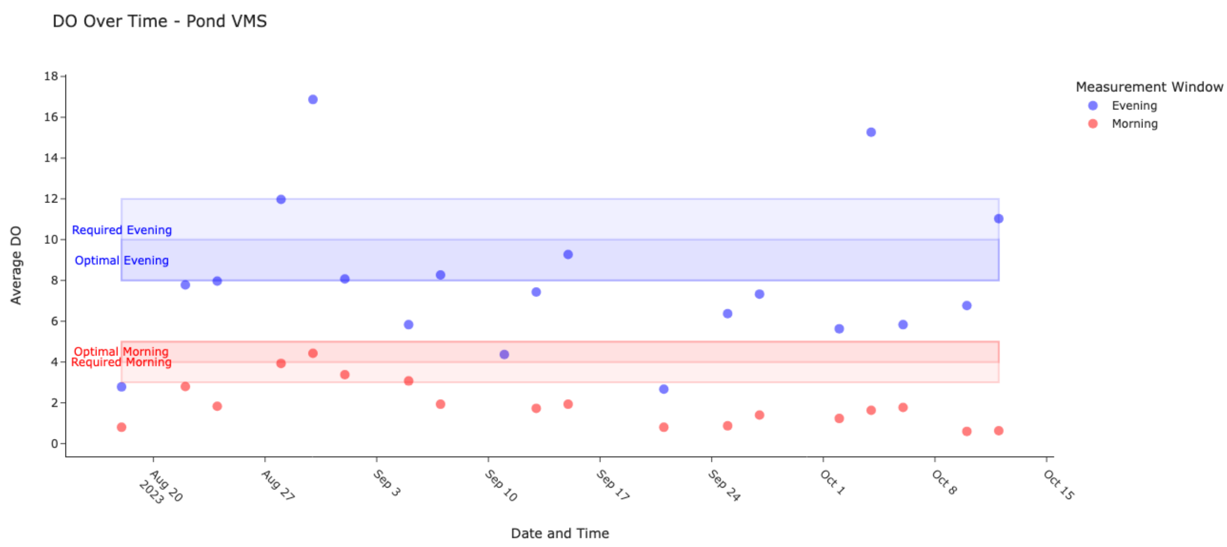


***Fig 11**: DO measurements at VMS*

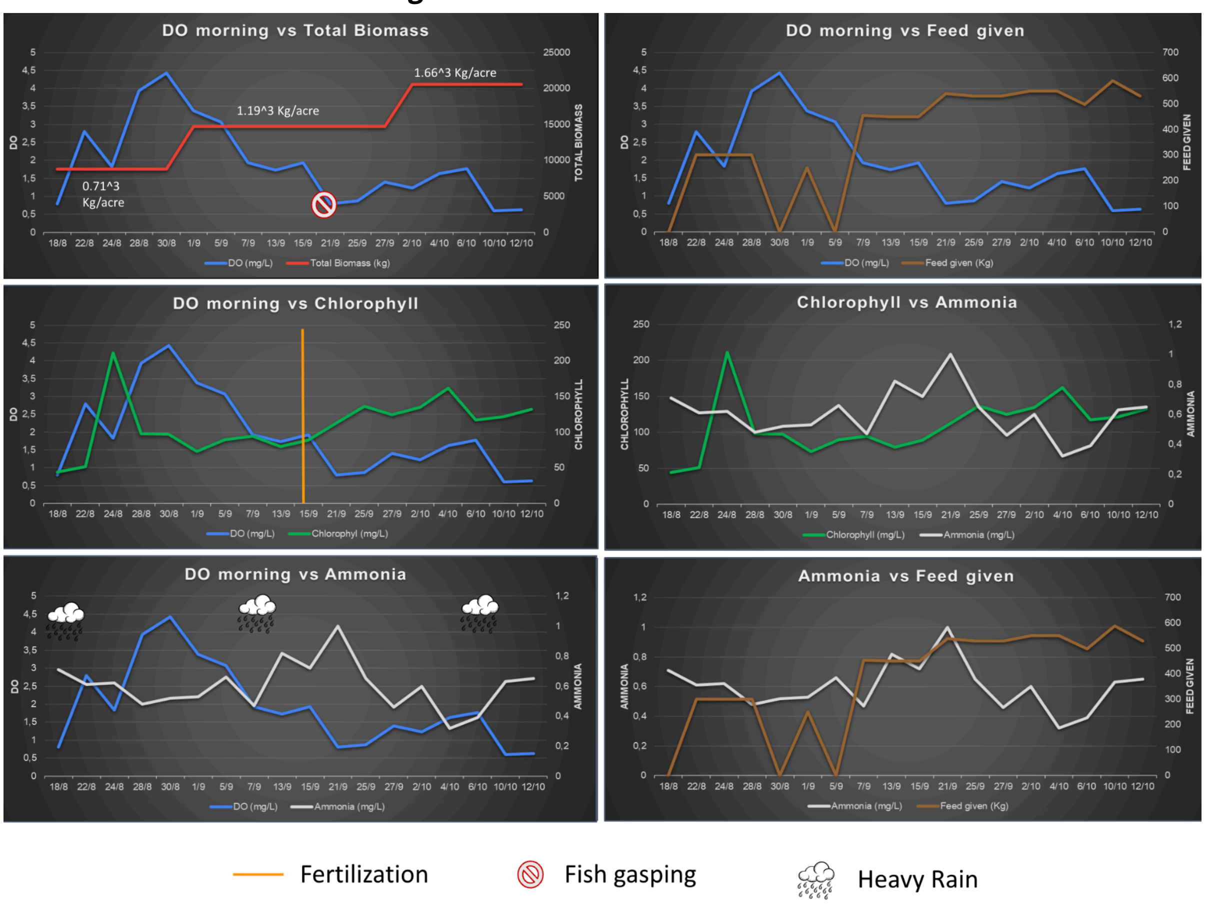


***Fig 12**: Line graphs showing key parameters for VMS*

***Table 12**: Percentage of measurements within the required range for VMS*

VMS					
Parameter	DO (Morning)	DO (Evening)	Chloro phyll-A	pH	Ammonia
% of measurements in required range	22%	26%	89%	54%	28%

VMS is an absentee farmer. He manages the operations through his worker who lives full time by the pond. The worker is very diligent in observing and mitigating any emergencies that come up. Stocking densities are 2370/acre, and VMS owns the pond.

- **DO was fairly poor** and decreased over time. This could potentially correlate with fish biomass increasing, however, it is unclear as this was due to fish growth and not increased numbers of fish.

- **Ammonia levels were high**. Heavy rain and fertilization tended to be followed by ammonia spikes in the proceeding measurement, but considering the multiple days between measurements it appears unclear as to whether these caused the ammonia issues. The farmer responded to high ammonia levels with the use of probiotics, which do not appear to have had a lasting impact.

- There was a single **fish gasping** event which happened as DO dropped to 0.8mg/L and ammonia increased above 1. These two things in tandem act to severely stress fish, as DO is low and respiration is simultaneously impaired by ammonia.

- **Chl-a increased after fertilization**. However, there appears to be a lack of a clear fertilization strategy, with fertilization only being applied once. Thus, fertilization is unlikely to cause long-term benefits. There is some possibility that Chl-a affected DO, though with a delay.

- **VMS's farmer does not live near the pond** and rarely visits. To run his operation, he relies on a dedicated full-time worker and collaboration with a peer group of neighboring farmers (which he seems to rely on more than other farmers within the study).

- **Feeding rates were not excessively high**. This could potentially explain the lower Chl-a levels than other ponds within the study. The removal of feed correlated also with the lowest point for Chl-a, potentially suggesting that feed was a driver of Chl-a levels. VMS's farmer reduces or stops feeding during stress periods or for medicine application.

- The **farmer weighs 100 Rohu and 30 Catla monthly**, exceeding typical ARA numbers.

- There was a lice infestation during the study period, which did not appear to have a large effect and mostly subsided. The farmer relies on Coastal Aqua from Eluru market for medicine and diagnostic advice.

Final thoughts on DO:
DO was an issue at VMS and worsened over the test period. There is not as clear a relationship between DO and other factors, however, it is possible that unsuitable levels of phytoplankton and the high ammonia levels were responsible for the DO issues found. The fertilization strategy was likely too poor to re-establish good levels of phytoplankton.

It appears that the combination of low DO and high ammonia caused fish to gasp, indicating that this combination is likely one of the most stressful experiences fish are subjected to within ponds.

Fish harvest

Appendix 4: GRA Fish Farm

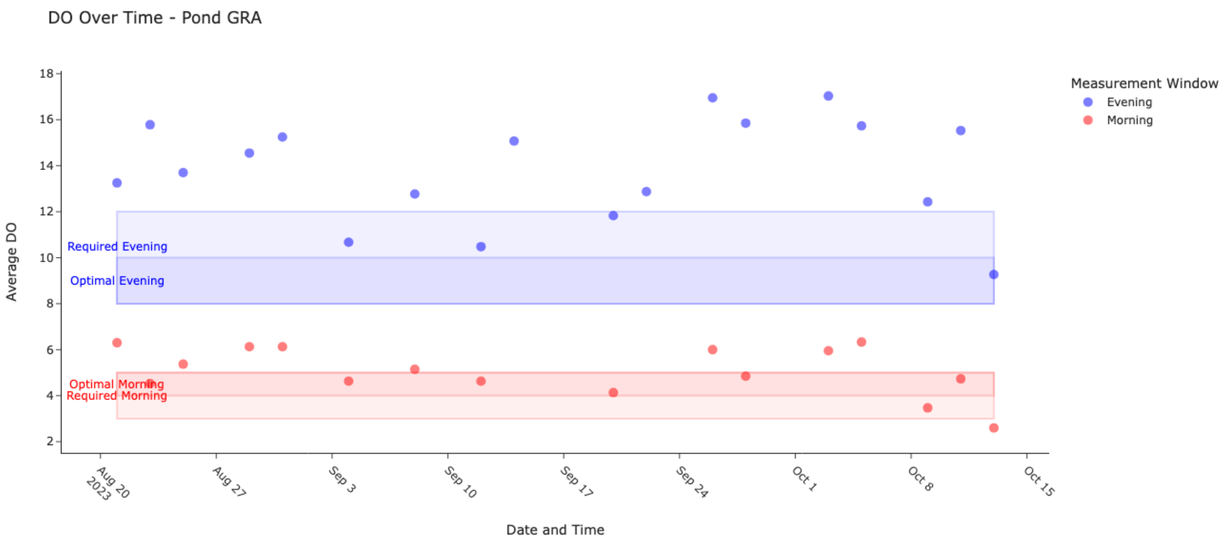


Fig 13: *DO measurements at GRA*

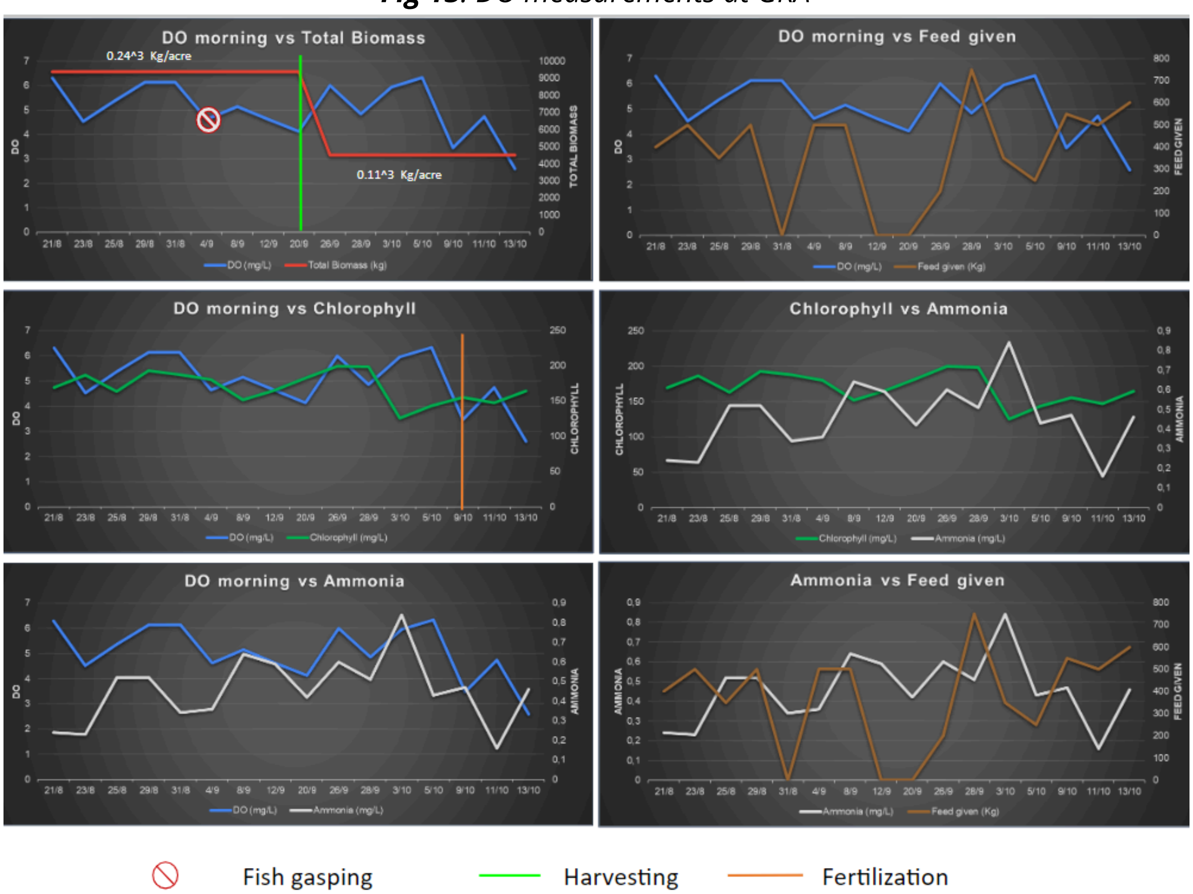


Fig 14: *Line graphs showing key parameters for GRA*

***Table 13**: Percentage of measurements within the required range for GRA*

GRA					
Parameter	DO (Morning)	DO (Evening)	Chloro phyll-A	pH	Ammonia
% of measurements in required range	44%	22%	15%	21%	56%

GRA's farmer employs two full-time workers at the pond site. The pond is leased to the farmer, which restricts the level of investment the farmer is willing to make into the pond, as well as meaning there is an additional expenditure. The stocking density was 800 to 1600 fish/acre. It was an anomalous period, as the farmer had sold most of their fish and was trying to sell the rest before beginning a new cycle. GRA is a "Breed-out" pond, where younger fish are held before moving to the "Grow-out" pond. This means that fish are smaller, and accounts somewhat for the low biomass present.

- This pond was severely **infected with gill fluke**, a common parasitic flatworm that latch themselves inside the fish gills. The farmer tried to solve the issue through multiple natural treatments, such as bitter gourd juice. At the end of the study, the gill fluke infection had subsided. This could show that these natural remedies worked, although there are very few evidenced interventions for the effective removal of gill fluke. It could also be that good water quality coupled with low SD ceased the spread of disease.

- There was one **fish gasping event**, though this is difficult to understand as DO levels were significantly above the required range (roughly 4.5 mg/L). It is possible that this was measured in error, as sometimes fish consuming phytoplankton at the surface at the water can appear to be gasping. Assuming that gasping was not erroneously documented, it could be due to compounding from other stressors (fish were, at the time, infected with gill fluke) or that DO had dropped significantly during the night, and thus had stressed fish despite still being within an adequate range.

- **DO was not an issue**. DO was consistently above 4 mg/L with a few periods between 5 and 7 mg/L. These levels, though optimal for the fish, caused hyperoxia (too high DO levels) in the afternoon. This was likely due to the low fish biomass in the pond and the oxygen production through photosynthesis exceeding the rate of oxygen consumption by the fish and other organisms. However, these levels of hyperoxia in aquaculture are common, and unlikely to be a serious welfare issue.

- **Fish biomass was extremely low** in this pond. This is likely because this was a breed-out pond that the farmer had already partially harvested before the study period began. This could connect to the good DO levels, as there was little biological oxygen demand.

- The **farmer weighs 100 Rohu and 30–40 Catla monthly**, exceeding typical ARA numbers. The farmer conducted this three times within two months, which is an atypical frequency that was caused by the farmer trying to sell fish multiple times without success (mostly due to bad weather on harvest days). This can lead to excessive stress on those fish being weighed.

- GRA's farmer did not use the common process of providing **supplemental feed** by tying permeable bags filled with feed to lines along the pond. Instead, he directly inputted feed into the water, and the feeding ratio looked above what is recommended for fish of 150 g. This would plausibly lead to high levels of phytoplankton, but this is not supported by the Chl-a measurements likely because the levels of high ammonia caused by both the feeding method and ratio. GRA's farmer relied largely on personal experience and fish appetite to decide feed quantities.

- **Diseases** are prevented through monthly sanitation and daily pond monitoring. GRA's farmer has 2 primary sources in the Eluru market where he purchases medicine, but has stated that medicine effectiveness is varied, often requiring trials of multiple medicines.

Final thoughts on DO:
GRA was the only pond in our study where DO levels did not present a significant welfare issue. This appears largely attributable to the extremely low fish biomass in the pond, the lowest among all studied ponds and approximately 20% of that in CRP, which had the second-lowest biomass. The reduced biomass resulted in lower biochemical oxygen demand, allowing the modest levels of phytoplankton present to easily produce sufficient (and excess) DO.

Although DO measurements were above the ARA ranges (especially in the afternoon, which can be dangerous, we do not believe that this ever led to welfare issues and so is only a minor concern.

Appendix 5: NRO Fish Farm

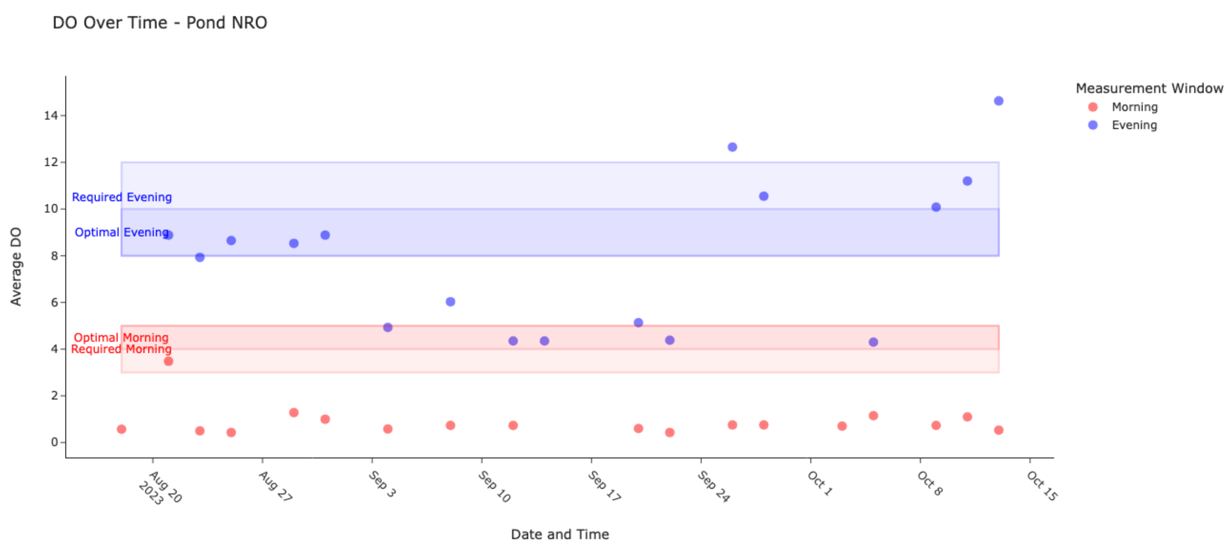


Fig 15: *DO measurements at NRO*

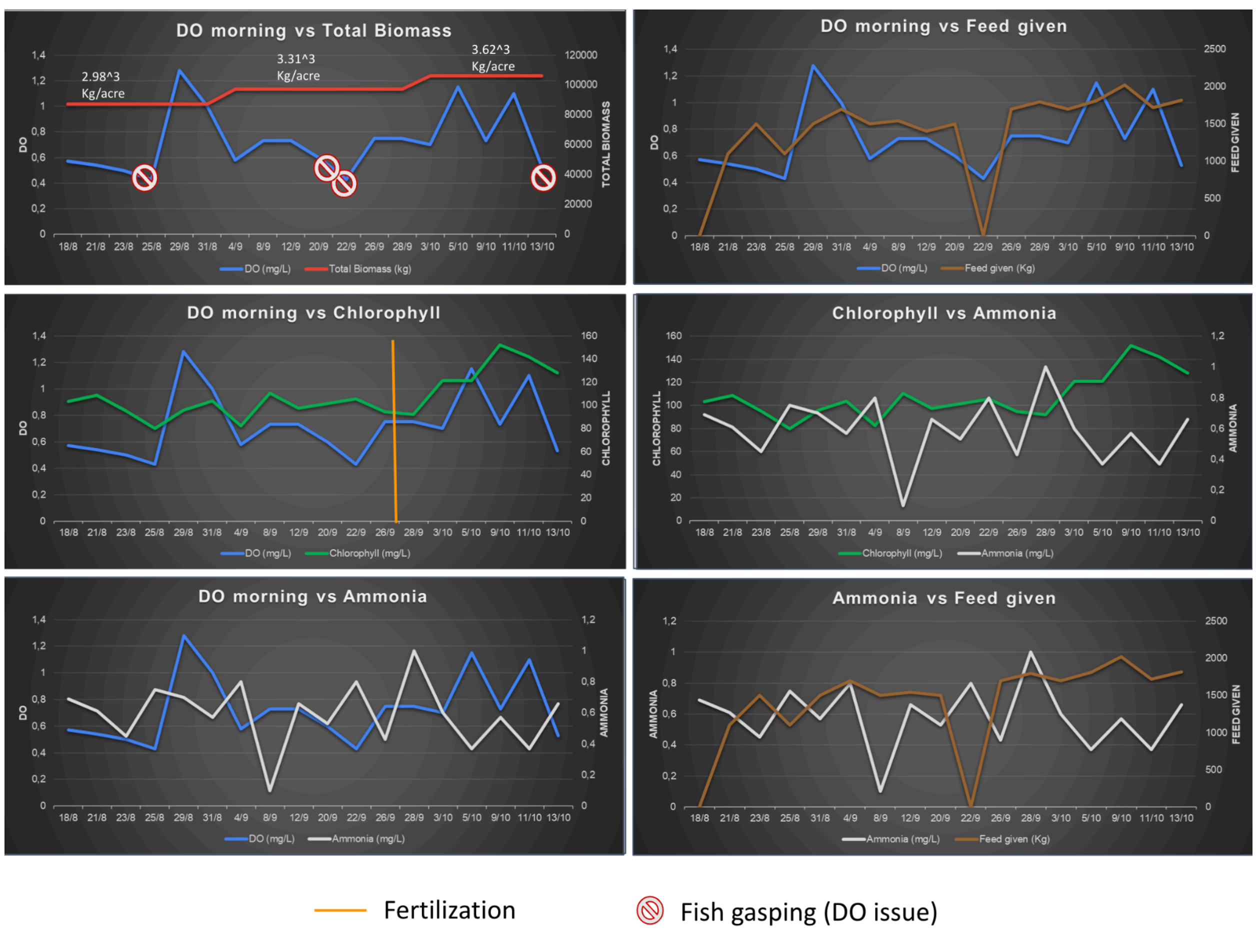


Fig 16: *Line graphs showing key parameters for NRO*

***Table 14**: Percentage of measurements within the required range for NRO*

NRO					
Parameter	DO (Morning)	DO (Evening)	Chloro phyll-A	pH	Ammonia
% of measurements in required range	0%	41%	91%	100%	28%

NRO's farmer employs two workers who are present full-time at the pond site. The farmer owns the pond and stocks it with 3100 fish/acre.

- **DO was critically low** in the pond, with the lowest average of any pond (0.9 mg/L).
- **Chl-a** readings were mostly within range, though on the lower side. Considering how poor DO was, this could indicate that our current Chl-a requirements are too low.
- **Fish gasping** events were far more common in this pond compared to the other study ponds and corresponded with all major DO crashes. This appears to indicate that DO levels below 1 mg/L are particularly harmful to Indian Major Carp. However, ammonia was also out of range for each of the measurements taken, and so likely fish were stressed by multiple factors. Ammonia toxicity can stress fish by impairing their respiratory function, which exacerbates already critically low DO levels.
- The **farmer responded to fish gasping** with aerators and DO granules (an aquaculture product that claims to increase DO by being added to pond water).
- **Ammonia was exceedingly high** within this pond. This is despite a lack of heavy rainfall or consistent fertilization (although the one fertilization conducted did precede an ammonia spike). While phytoplankton utilize ammonia as a nutrient, high concentrations of ammonia are toxic to phytoplankton and fish. Ammonia toxicity occurs when the production or input of ammonia exceeds the capacity of the system to assimilate or remove it. The relative consistency of the ammonia levels could indicate that extreme levels of toxic gasses have built up on the pond bottom, in which case the pond may not be able to resolve the ammonia issue without pond preparation. Low DO levels and elevated ammonia concentrations in the pond may have resulted in a situation where the feed added to the pond consistently contributed to the already high ammonia levels.
- **Fish biomass was very high**. This was because the farmer chose to delay harvest beyond normal lengths in order to wait for a better market price and a higher fish weight. This likely contributed to poor DO levels.

- **The farmer overfed.** Fish were fed even on days when fish were gasping, where it is very unlikely that any feed would be consumed. The farmer consistently fed exceedingly very large amounts, far above the 1% of body weight that is recommendable for fish above 1 kg in size, potentially wasting up to 9 tonnes in excess feed (before considering the feed not consumed due to high stress levels). Despite this, Chl-a levels stayed within range. This was likely a cause of high ammonia, as excess feed can decompose and trigger increased ammonia. NRO's farmer claims to use a weight-based feeding table, augmented with experience and satiation methods. Feeding is halted during heavy rainfall or medicine application.

- **Ammonia reduced significantly after the addition of jaggery** (cane sugar high in carbohydrates). The addition of carbohydrates can lower the level of inorganic nitrogen, which is a key ingredient in ammonia. However, its impact is momentary and not enduring, which implies the need for parallel strategies to enhance ammonia.

- The **farmer weighs 100 Rohu and 30-40 Catla monthly**, exceeding typical ARA numbers.

Farm worker mixes groundnut cake with DORB before transferring it to the boat for feeding.

Final thoughts on DO:
NRO's pond was the worst in terms of DO. This is both in terms of the absolute levels (as seen in the percentage of measurements within range) and the lived experience of fish (as seen through frequent gasping events).

One likely cause is the excessively high fish biomass within the pond. This means that the oxygen demand was outstripping the production. It is also possible that phytoplankton levels were too low, though this would imply that our Chl-a ranges are currently inaccurate. Low phytoplankton levels may have been caused by high ammonia, which is toxic to phytoplankton. Ammonia levels likely also contributed to the experienced DO issues of fish, through inhibiting their respiration.

The farmer's interventions of aeration and DO granules appear to have not made a significant difference to the overall pond. This is not particularly surprising, as such a large pond (29 acres) is unlikely to be affected by DO granules or low numbers of aeration devices. However, these likely did cause localized and temporary respite from critical DO levels (but our methodology meant that we did not detect these smaller changes). This helps to reinforce that proactive action is necessary to prevent DO issues, and it is hard to resolve DO issues when they are already present.

Appendix 6: CRP Fish Farm

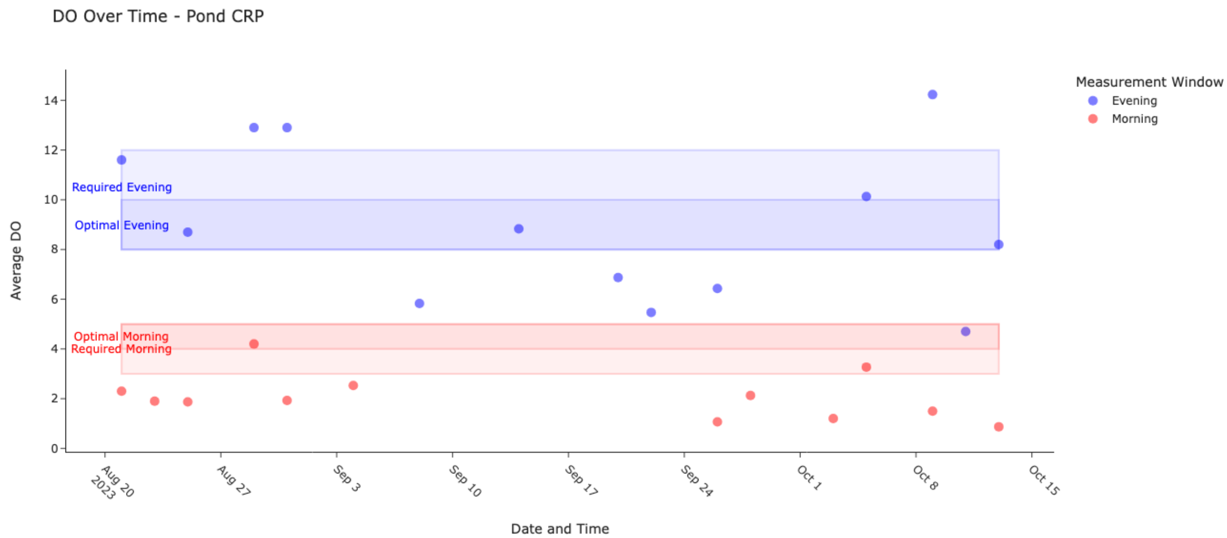


***Fig 17**: DO measurements at CRP*

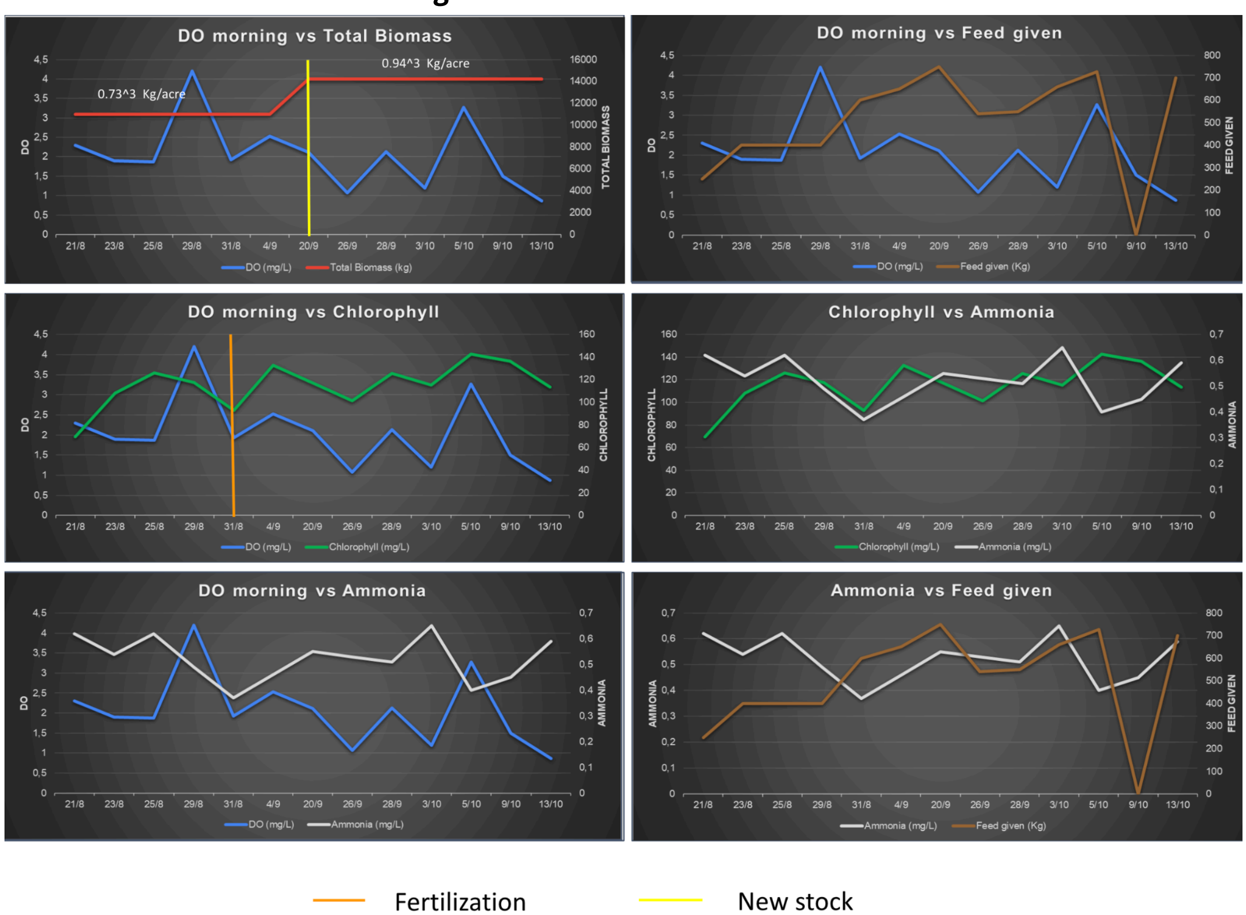


***Fig 18**: Line graphs showing key parameters for CRP*

***Table 15**: Percentage of measurements within the required range for CRP*

CRP					
Parameter	DO (Morning)	DO (Evening)	Chloro phyll-A	pH	Ammonia
% of measurements in required range	15%	38%	96%	77%	31%

CRP's farmer employs a worker from the village. As a leased pond, CRP's farmer faces limitations in implementing higher welfare regimes. Lease rates are high where he farms (65,000 INR/acre/annum). CRP is stocked with 2100 fish/acre. Owing to the location of the pond within Manuru, this pond had the least number of measurements in the entire pool as the roads were inaccessible after rains.

- **Chl-a and DO were closely linked** within this pond, the two mirroring each other's rises and falls for the majority of the study.
- **Chl-a** was within our required range, but DO performed particularly poorly. This could imply that our current Chl-a range of 100 to 150 mg/L is too low.
- **Fish biomass was very low**, so it is not particularly surprising that it did not appear to have much effect on DO, even after stocking. This could also help explain why Chl-a had such a clear connection to DO, as biomass had minimal impact.
- **Ammonia was too high** the majority of the time. It is unclear what the cause of this may have been. However, there was a systematic bias where this pond was not accessible during rainfall, meaning that all heavy rain data is missing (a potential cause of ammonia).
- **Feed quantities were somewhat high**. This didn't, however, seem to translate into phytoplankton issues, perhaps because feeding was halted at key points such as heavy rain or medicine application. CRP's farmer relied largely on personal experience and fish appetite to decide feed quantities. CRP's farmer was broadly resistant to reducing feed quantities, which is common as farmers feel it will reduce fish growth.
- The **farmer weighs 100 Rohu and 30–40 Catla monthly**, exceeding typical ARA numbers. This allows for a more representative understanding of fish's feeding requirements, though does cause fish stress during weighing.
- **Diseases** are prevented through monthly sanitation and daily pond monitoring. CRP's farmer has 2–3 sources in the Eluru market where he purchases medicine, but

has stated that medicine effectiveness is varied, often requiring trials of multiple medicines.

Final thoughts on DO:
DO was particularly poor in this pond, barely going into the required range. The most likely cause here appears to be too low phytoplankton, considering how linked the two variables appear. However, phytoplankton was within our required range, implying we may need to reassess our ranges. It is not impossible that a confounding variable, such as sunlight, caused the relationship between these DO and phytoplankton. However, this would be irregular.

Biomass appears to have been too low to affect DO. This implies that a stocking density of 2100/acre is not harmful in a 15 acre pond.

Data Collectors Gandhi and Manikanta taking measurements from a pond boat.