Project Title: Assessment of Carbon Dioxide (CO_2) and Inorganic Nitrogen Compounds to Enhance Winter Kill in Natural Rearing Ponds Used for Fish Production in The North Central Region [Termination Report]
Key Word(s): Other
Total Funds Committed: $175,000
Initial Project Schedule: September 1, 2011 to August 31, 2013
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Extension Liaison: Jeffrey Gunderson, Minnesota Sea Grant, Minnesota
Industry Liaison: Gregory Oswald, Ellendale, Minnesota
Reason for termination: Project objectives completed and funds have been terminated.

Project Objectives

1. Conduct a literature review to summarize the toxic effects of carbon dioxide (CO2) and inorganic nitrogen compounds (e.g. N2, NO2–, NH3, etc.) on fish with an emphasis on common carp *Cyprinus carpio*, black bullhead *Ameiurus melas* and walleye *Sander vitreum*.
2. Estimate the cost per acre of pond treatment using either CO2 or inorganic nitrogen compounds to enhance winter kill conditions during late winter periods in the North Central Region (NCR).
3. Consult with EPA to determine the registration eligibility and requirements for the use of CO2 or inorganic nitrogen compounds to enhance winter kill conditions.
4. Determine, through laboratory study, application rates required of CO2 or inorganic nitrogen compounds to enhance winter kill conditions to remove unwanted fish from natural rearing ponds. Studies required for the registration of CO2 or inorganic nitrogen compounds to enhance winter kill conditions will be conducted according to GLP regulations (40CFR160).
5. Evaluate, through laboratory pond experiments, the efficacy of laboratory-derived application rate data for CO2 or inorganic nitrogen compounds to enhance winter kill conditions.
6. Collect late winter water chemistry condition data in representative NCR natural rearing ponds.
7. Obtain an experimental use permit (EUP) from the EPA and appropriate state regulatory agencies to conduct experimental applications of CO2 or inorganic nitrogen compounds, singularly or in combination, to enhance winter kill conditions in natural rearing ponds to remove populations of unwanted fish.
8. Compile data into final study reports suitable for submission to the EPA to support potential approval of CO_2 or inorganic nitrogen compounds to enhance winter kill conditions.
9. Summarize results into appropriate extension materials for dissemination to NCR aquaculturists.

Project Summary

Fish culturists are often left with a pond containing a few undesirable fish at the end of a year. These undesirable fish can lead to decreased yields and profits during subsequent years. Current strategies to control these undesirable fish require culturists to use expensive chemicals and to properly remove carcasses. These studies determined thresholds of the combined effects of readily available CO_2/inorganic nitrogen compounds on survival of fishes to enhancing or manage winterkill conditions for fish culturists. It required 0.37 mg/L ammonium chloride when oxygen levels were very low to achieve total mortality in some fishes, or required 495 mg/L CO_2 to achieve total mortality. The use of diffused gases and/or inorganic nitrogen compounds appear to offer alternatives to commonly used toxicants for aquaculture operations where undesirable fish species need to be controlled. The use of CO_2 would cost approximately \$244 and ammonia chloride would cost \$264 to treat an acre foot of water, while rotenone would cost \$140 to treat that same volume. However, since fish exposed to rotenone are classified as hazardous, there is an additional cost of removal of the carcasses following a rotenone treatment. Using either inorganic nitrogen or CO_2 may be a viable alternative to the use of chemicals for fish control.
Currently, the registration of CO_2 as a fish control is underway.

Technical Summary and Analysis

Objective 1. — A manuscript is in the advance stage of development. The first draft was prepared as introduction section to the dissertation by T. Parker which was defended in August 2013. The manuscript focuses on toxicity of ammonia nitrogen compounds at different oxygen levels in common carp and discusses, among other aspects, the changes to the gill tissues associated with the ammonia/hypoxia exposure. Data obtained for common carp is being finalized into a separate manuscript prepared for submission to "Aquatic Toxicology". Toxicity of ammonia to carp, percids, salmonids, and catfish will be compiled and presented with a literature review to make a white paper to be reported to NCRAC.

The literature review for the use of CO_2 as a control agent for undesired fish species is currently under review by U.S. Geological Survey and U.S. Fish and Wildlife Service partners for submission for publication to Fisheries. This literature review incorporates findings from preliminary experimental work with multiple fish species and gives a comprehensive description of CO_2, its use and efficacy.

Objective 2. — The cost per acre foot for pond treatment was calculated the amount of CO_2 to produce mortality observed in pond trials (Objective 5). The cost of CO_2 gas was \$0.34/kg (\$0.16/lb) while liquid CO_2 was \$0.23/kg (\$0.11/lb). Therefore, the costs to treat an acre foot of pond with CO_2 gas was \$361.35 and \$244.16 if treated with liquid CO_2. The actual cost for fish producers will be dependent upon local distributors.

The cost per acre foot for pond treatment was calculated for the amount of ammonia chloride to produce mortality observed in pond trials (Objective 5). The cost of ammonia chloride was \$1.36/kg (\$0.62/lb). The cost to treat an acre foot of pond with this ammonia chloride was \$105.95; however, 100% mortality was not achieved. A 2.5 times of the dose used in our trials (i.e. adding 62.5 kg [28.41 lb] rather than 25 kg [11.37 lb]) would be expected to produce 100%

mortality. This would increase the costs to $264.88 for treating an acre foot of pond with ammonia chloride to produce 100% mortality. However, the actual cost will be dependent upon local distributors.

For comparison, the cost per acre foot for pond treatment was calculated for the amount of rotenone to produce 100% mortality. This piscicide would cost approximately $140.00 to treat an acre foot of pond. However, the cost of removal and disposal of rotenone-exposed fish are not included. These additional costs could substantially increase the total costs for use of this chemical and would be minimized in using CO_2 or ammonia chloride. Thus, both CO_2 and ammonia chloride may be viable alternatives to the use of rotenone for the removal of fish remaining in ponds during late winter.

Objective 3. — Completed.

Objective 4. —
CO2 - Studies have been completed. A significant interaction between treatment and species was observed. The interaction was a result of differences in mortality between species at specific treatment levels. In the common carp (CAP) and channel catfish (CCF) trials, mortality in both the 380 ppm CO2 and 495 ppm CO2treatment groups was significantly higher than in the control, 75, 150, 225, and 300 ppm CO2 groups, but mortality in the 495 ppm CO2treatment was higher than in the 380 ppm CO2 treatment. There was no difference in mortality between the 225 ppm CO2 and 300 ppm CO2 treatment groups in the CCF trials. There were no differences in mortality in any treatment groups between CAP and CCF. Inorganic

N - Studies have been completed. There was significant interaction between ammonia concentration and dissolved oxygen is significant in yellow perch which indicates that ammonia toxicity is increased by reduced oxygen content in the water for yellow perch.
Yellow perch mortality for fish exposed to 0.37 ± 0.01 ppm NH3-N was 6.25 ± 6.25%, 93.75 ± %, and 100.0 ± 0.0% in normoxia, moderate-hypoxia, and severe hypoxia, respectively. When exposed to 0.23 ± 0.03 ppm NH3-N mortality for the three oxygen treatments, in the same order, were 2.50 ± 2.50%, 22.5 ± 13.1%, and 72.5 ± 24.3%. All rainbow trout survived during exposure to 0.15 ± 0.02 ppm NH3-N. However, during exposure to 0.26 ± 0.02 ppm NH3-N, rainbow trout experience significantly higher mortality in severe hypoxia. Rainbow trout experienced 6.25 ± 8.84%, 7.81 ± 7.86%, and 48.4 ± 25.7% in normoxia, moderate- hypoxia, and severe hypoxia, respectively.

Objective 5. — Studies have been completed. A controlled outdoor pond study to assess the use of CO_2 and ammonia chloride for enhancement of winter kill conditions was performed at the
U.S. Geological Survey Upper Midwest Environmental Sciences in La Crosse, Wisconsin. In early October of 2012, eight fish-free 0.04-ha (0.1-acre) outdoor rectangular ponds were filled with well water to a mean depth (± SD) of 96 ± 4 cm (37.8 ± 1.57 in). On October 11, 2012, each pond was stocked with 100 common carp (5.0 - 7.5 cm [2.0 - 3.0 in]) and 100 channel catfish (7.5 - 20.3 cm [3.0 - 8.0 in]). Ponds were left to freeze under natural conditions.

On February 19, 2013, a 12.7 cm (5 in) diameter hole was drilled through the ice in the center of each pond. Temperature and dissolved oxygen were measured at the surface and bottom of each pond and pH was measured at the bottom of each pond. Carbon dioxide was measured with a commercially available titrant kit.

On March 7, 2013, one 12.7 cm (5 in) hole was drilled in the northeast corner and a similar size second hole in the northwest corner of each pond. One hole was used to pump water (approximately 0.264 L/min [30 gal/min]) from the bottom of the pond, treated with the chemical and then returned through the second hole just below the ice. Each of three randomly selected ponds was injected with ammonia chloride (Prince Agri Products, Inc., Quincy, IL). At the side of the pond, 55.12 lbs (25 kg) ammonia chloride was dissolved in approximately 208 L (55 gal) of pond water and injected into each of the at approximately 113 L/m flow (29.85 gal/min). This amount of ammonia chloride was used to achieve approximately 85 ppm ammonia, which is more than double the 31.7 ppm required to reach 0.85 ppm NH_3-N at a pH of approximately 9.0 at 4°C (39.2°F; Piper, 2010).

Between March 8 and March 10, 2013, three other randomly selected ponds were injected with 48.4 ± 2.9 kg (106.7 ± 6.39 lbs) of CO_2 delivered as a gas at a rate of 25 L (6.60 gal) at approximately 113 L/min flow (29.85 gal/min). This amount of amount of $CO_2$was used to achieve a nominal concentration of 150 ppm CO_2, which was expected to overcome alkalinity of the pond water and result in toxic levels of free CO_2, greater than 25 ppm.

On March 11, 2013, a 12.7 cm (0.12 ft) diameter holes were drilled through the ice in the center of the pond, one on the north end and one on the south end of each pond. Temperature and dissolved oxygen were measured at the surface and bottom of each pond and pH was measured for each pond. Dissolved CO_2 was measured with a commercially available titrant kit (CHEMetrics, Inc., Midland, VA). Ponds were then left to thaw under ambient conditions. On April 11, 2013, all ponds were free of ice and immediately drained. All live fish were harvested from each pond and counted.

All water quality parameters (i.e. pH, temperature, dissolved oxygen, ammonia and CO_2) and the number of surviving carp and catfish are reported as means with standard deviations. Water quality parameters were compared between treatments pre- and post-treatment using ANOVA. Initial, pre-treatment, water qualities were similar among ponds. Water temperatures were 3.11 ± 0.67°C (37.60 ± 1.38°F). All ponds were slightly basic, 9.14 ± 0.34. Ponds treated with CO_2 had the greatest pH, 9.57 ± 0.19 and differed from that in ponds treated with ammonia (8.83 ± 0.08) or left untreated (8.96 ± 0.08). Dissolved oxygen was found to be super saturated in all ponds,
219.5 ± 35.9%. No dissolved CO_2 or ammonia was detected in any of the ponds prior to treatment.

Differences were observed in water quality parameters post-treatment. Water temperatures were lower in all ponds when measured following treatments on March 11, 2013 then when measured during pre-treatment. Water temperatures were lowest in ponds treated with CO_2 (0.47 ± 0.12°C [32.85 ± 0.21°F]), but similar between control ponds and ponds treated with ammonia 1.58 ± 0.21°C (34.84 ± 0.38°F) and 1.43 ± 0.23°C (34.57 ± 0.42°F) respectively. pH did not change in either the control ponds or ponds treated with ammonia. The pH in ponds treated with CO_2 dropped from 9.57 ± 0.19 to 6.13 ± 0.11, thus the CO_2-treated ponds had lower pH than either the control ponds or ponds treated with ammonia. DO did not change in control ponds or ponds treated with CO_2, but increased to 32.94 ± 3.13 ppm in ponds treated with ammonia. No differences in DO were found between any of the treatments on March 11, 2013. Total ammonia was greatest in ponds treated with ammonia chloride, 12.60 ± 11.18 ppm, but was only a fraction of the 31.7 ppm needed to produce 100% mortality. Total ammonia levels in ponds treated with CO_2 or untreated had very low levels of total ammonia, 0.22 ± 0.17 ppm and 0.13 ± 0.12 ppm respectively. Dissolved CO_2 was detected in only those ponds treated with CO_2.

The number of fish surviving varied among treatments. No live common carp were found in one of the control ponds, while 90 channel catfish were alive in that same pond. Thirty-five common carp and 9 channel catfish were alive in the other control pond. Survival ranged from 0 to 35 for common carp and from 1 to 36 for channel catfish in ponds treated with ammonia. Only a single common carp and no channel catfish survived in all ponds treated with CO_2. Ponds treated with CO_2 did have greater total fish mortality than the control ponds. Ammonia chloride had no effect on mortality at levels used in our study.

Objective 6. — Completed - Long term ice cover was never present in central Ohio and therefore winterkill conditions were not observed.

Objective 7. — Completed - UMESC held an initial coordination meeting with EPA to discuss whether the use of CO_2 will require EUP to allow its use to enhance winterkill conditions.
Depending on the size and scope of the application, it is likely that EUP would be required prior to registration. As part of its efforts to assess the use of CO_2 in barriers to deny aquatic invasive species access to critical habitat, UMESC will be evaluating the required registration information required to register CO_2 for use to control aquatic nuisance species.

Objective 8. — Completed - UMESC is coordinating with EPA to determine the information required to register CO2 for uses to control aquatic nuisance species. UMESC and its collaborators on this project will compile data reports of project generated information to meet those registration requirements, where appropriate.

Objective 9. — Completed - Extension materials will be prepared pending coordination with EPA to determine registration requirements. Extension materials cannot be prepared and disseminated until a determination is made regarding the registration requirements of CO_2 or inorganic nitrogen compounds.

Principal Accomplishments
A simple method for delivering CO_2 or ammonia below ice was developed. The greatest return on investment for this project will be in the removal of undesirable fish from both public and private aquaculture ponds. Removal of remaining fish will enhance production in those ponds. Results of the experiments, where appropriate, will be presented at scientific meetings and extension workshops and may be published in scientific journals, extension bulletins, or NCRAC fact sheets and bulletins. Research results will also be disseminated

through the NCRAC Annual Progress Reports. These reports are available on the NCRAC Web site http://www.ncrac.org .

Impacts:
- The approval of CO_2 and/or ammonia for the removal of unwanted fish in natural rearing ponds will be a significant benefit to the public and private aquaculture producers. Removal of these fish will enhance production in these ponds.
- Approval of CO_2 and/or ammonia as a chemical for the control of unwanted fish species is contingent on providing evidence on the effectiveness of these chemicals to produce mortality in targeted fishes. Thus, an initial study required includes the establishment of efficacy of both CO_2 and ammonia. Trials have been completed.
- Both CO_2 and ammonia produced mortality in laboratory and field trials. Cost of both of these chemicals is greater than that required to treat ponds with the registered piscicide rotenone. However, the use of CO_2 or ammonia as a fish toxicant will not produce dead fish that will require special disposal like those killed with rotenone. The removal of these unwanted fish is critical to improvement of efficiency of public and private fish producers.
- CO_2 infusion under ice was effective in eliminating channel catfish and common carp during under ice exposures.

Recommended follow-up Activities: Future studies should focus on the development of an injection system for deliver CO_2 under ice. Studies should also look the relationship between dose time.

Publications, Manuscripts, Workshops, and Conferences
See the Appendix for a cumulative output for all NCRAC-Funded Other activities