Ozonation of a re-circulating rainbow trout culture system
I. Effects on bacterial gill disease and heterotrophic bacteria


Abstract:
Ozone was added to water in a recirculating rainbow trout (Oncorhynchus mykiss) culture system just before it entered the culture ranks in an attempt to reduce the numbers of heterotrophic bacteria in system water and on trout gills, and to prevent bacterial gill disease (BGD) in newly stocked fingerlings. During four 8-week trials, ozone was added to the system at a rate of 0.025 or 0.036-0.039 kg ozone/kg feed fed. In the control, where no ozone was added, and in previously published research, BGD outbreaks occurred within two weeks of stocking, and these outbreaks generally required three to four chemotherapeutant treatments to prevent high mortality. In three of four trials where ozone was added to the system, BGD outbreaks were prevented without chemical treatments, but the causative bacterium, Flavobacterium branchiophilum, still colonized gill tissue.

The one ozone test where BGD outbreaks required two chemical treatments coincided with a malfunction of the ozone generator. Although ozonation did reduce BGD mortality, it failed in all trials to produce more than a one log reduction in numbers of heterotrophic bacteria in the system water or on gill tissue. Failure of the ozone to lower numbers of heterotrophic bacteria or to prevent the causative BGD bacterium from occurring on gills was attributed to the short exposure time to ozone residual (35 sec contact chamber) and rapid loss of oxidation caused by levels of total suspended solids. Rationale for ozone's success at preventing BGD mortalities are not fully understood but may in part be due to improved water quality. Use of the lower ozone dosing rate (0.025 kg ozone / kg feed) appeared to provide the same benefits as the higher dosing rate (0.036-0.039 kg ozone / kg feed fed); however, the lower ozone dosing rate was less likely to produce a toxic ozone residual in the culture tank and would also reduce ozone equipment capital and operating costs.

Discussion:
Prior to ozonation, BGD was a constant problem among newly stocked fish. During an 11-month period previous to ozonation, five groups of rainbow trout were stocked, and up to 30% of each group died because of BGD or a secondary amoebic infection (Bullock et al., 1994) despite regular chemotherapeutic treatments. In the ozonation study, BGD associated mortalities also occurred on a regular basis when ozone was not added or insufficient ozone was added. Adding ozone appeared to lower total mortality and the number of clumps of BGD bacteria on gill tissue in tests one, three and four, compared to that in the control and test two, when the ozone generator failed.

A total of 14 treatments were required to reduce BGD mortality in the two tanks in the control and test two, while no treatments were needed in the other trials. After ozone
addition, only 1.7-4.1% of stocked fish died because of BGD, and chemical treatments were rarely required.

The benefits of adding ozone to our system were an overall improvement in water quality entering the culture tanks (Summerfelt et al., 1997) and, more importantly, a reduction of mortality due to BGD and a reduction in the need for chemotherapeutic treatments. The improvement in water quality from ozonation may, at least indirectly, affect mortality from BGD. MacPhee et al. (1995) found that feeding played an important role in BGD mortality; fish fed after being challenged with F. branchiophilum developed clinical signs of BGD and had high levels of mortality, while those that were not fed after the challenge developed only moderate clinical signs and were generally normal 72 h post challenge.

They proposed that feeding promotes active excretion of urea and ammonia which accumulates in the mucus and static water layer surrounding the gills, and this provides a nutrient-rich environment that allows colonization and growth of BGD bacteria on gill tissue. They also proposed that acidification of the mucous boundary layer of the gill, which can be produced from increased carbon dioxide excretion as a result of feeding, may play an important role in F. branchiophilum attachment and colonization of the gills. Because MacPhee et al. (1995) used a single-pass system, it is unlikely that deterioration of water quality or environmental stresses favored the development of BGD. Within our recirculating system, however, it is more likely that the nitrogenous and organic substrates in the water affected the growth of F. branchiophilum.

Better water quality (Summerfelt et al., 1997) and reduced BGD mortalities both appeared to result from system ozonation; but the connection between the two was not shown. Although limiting nutrients to F. branchiophilum may be a reason for reduced BGD mortality, other factors are probably involved.

Several factors contributed to the failure of ozone to eliminate F. branchiophilum and the general failure to reduce numbers of heterotrophic bacteria in our recirculating system by even one log10. Bacterial reduction can be predicted from the product of the dissolved oxidant concentration and the exposure times, as described by the Chick-Watson model (Watson, 1908). Within our system, ozone was co-transferred with oxygen in LHO™ units and short (35 s) contact times were provided for ozone reaction after transfer to the flow before entering the culture tank. Even the roughly 55 daily exposures of recirculated water to ozone within the LHO units did not offset the short contact time each pass.

The other factor that limited bacterial reductions was the low ozone residuals (means ranged from 0.02 to 0.180 mg/I) at the end of the ozone contact tank (Table 2). Within our recirculating system, ozone demand produced by suspended solids, nitrite, and color (dissolved organic molecules) reduced ozone's half-life to levels that were generally too short to measure. The longest half-lives measured were only 15 s. In contrast, the half-life of ozone in a solution of pure water is about 165 min at 20deg C (Rice at al., 1981). The ozone demand of the water in the recirculating system consumed the ozone's oxidative power and thus shielded the bacteria from direct oxidation. The shortened half-life reduced the effective concentration and the time of ozone contact within solution and
thus reduced the predictor of ozone disinfection power, the product of residual concentration and contact time.

The product of the contact time and range of ozone concentrations in these trials were less than those reported by others. In the studies by Owsley (1991), the water supply was treated with 0.2 mg/l ozone for 10 mm to kill infectious hematopoietic necrosis virus (IHN); after treatment, water was degassed in packed columns to reduce ozone to a safe level for the fish. Liltved et al. (1995) reported 99.99% inactivation (4 log reductions in viable count) of four bacteria (Aeromonas salmonicida subsp. salmonicida, Vibrio anguillarum, Vibrio salmonicida, and Yerkinia ruckeri) and the infectious pancreatic necrosis virus (IPNV) within 180 s at residual ozone concentrations of 0.15 to 0.20 mg/I within distilled water in bench-top studies. Tipping (1988) reported that a contact time and ozone concentration product of 1 mg/I * min was necessary to kill the protozoan Ceratomyxa shasta from the water entering a trout hatchery. And, Colberg and Lingg (1978) reported 99% kill of four bacterial fish pathogens (A. salmonicida subsp. Salmonicida, A. liquefaciens, Pseudomonas fluourecsens. and Y ruckeri) when exposed to 0.1 and 1.0 mg/I ozone for 60 s in simulated recirculating system water.

Greater reductions in bacteria within our recirculating system, with its high oxidation demand, would have required ozone loading rates greater than those used here (i.e., >0.039 kg ozone/kg feed), which would be difficult to achieve without: (1) wasting excess oxygen to carry more ozone to the LHO™ unit, and/or (2) replacing the ozone generator with a larger unit that could produce a higher ozone concentration in the oxygen feed gas (6-10% instead of 4-5%), and/or (3) installing an ozone removal unit (air stripper, UV light, or large hydraulic retention chamber) to prevent the increased ozone residual from reaching toxic levels in the culture tank.

One of the main reasons that ozone is not widely used in aquaculture is its toxicity and a manager's unwillingness to risk losing fish to an accidental overdose. Residual ozone is highly toxic to fish at low levels. Ozone destroys epithelium covering the gill lamella which results in a rapid drop in serum osmolality (Paller and Heidinger, 1979; Wedemeyer et al., 1979) and, if mortality does not occur immediately, can leave the fish highly susceptible to microbial infections (Paller and Heidinger, 1979). Wedemeyer et al. (1979) reported that an ozone residual of 0.002 mg/I would be a safe level or ozone when culturing rainbow trout. Based on the literature, the exact level of ozone that damages gills or kills rainbow trout is between 0.008-0.06 mg/I (Roselund, 1975; Wedemeyer et al., 1979). In our research, ozone concentration rose to lethal levels on five occasions when we attempted to maximize ozone dosages in trials three and four.

The high ozone concentrations were caused by variable ozone demand in the water and the short hydraulic retention time provided before each fish culture tank. Ozone levels as high as 0.08 mg/l were measured during fish mortalities; however, higher ozone levels probably occurred but were not measured because staff would first attempt to restore ozone-free water flow to protect the fish; measuring ozone residual was less important. Ozone mortalities were not observed in tests one and two, probably because the ozone dosing rate per unit feed fed was lower than those in tests three and four. Additionally,
we observed that when fish stopped feeding from the demand feeders after being stressed (for example, just after selective harvest of the fish greater than about 0.34 kg) ozone accumulated more readily within the region that was harvested. This indicated that the production of organic compounds during and after feeding affected the rate at which ozone reacted, which decreased ozone concentrations.

Occurrence of ozone produced mortalities illustrates a serious liability of ozone Technology - the lack of instrumentation to continually detect ozone at levels <0.1 mg/l and the lack of chemical tests to readily measure ozone in water grab samples at concentrations <0.01 mg/l. At present, there is no fail-safe system to directly measure and control ozone in solution. An indirect measure of residual ozone is the water's oxidation reduction potential (ORP), which is a measure of a water's potential to oxidize and is thus a measure of the water's potential to disinfect or to kill fish. ORP can be monitored and used to control ozone addition to ensure that the desired treatment objective has been achieved and to ensure that ozone residual is not in the fish culture tank. A safe ORP for freshwater appears to be between 300-350 mV, depending upon pH. Our attempts to indirectly measure ozone residuals by ORP control strategies were only partially successful. An ORP control system was identified that could prevent ozone residual from accumulating in the culture tank within the region of the ORP probe. However, because our recirculating system contained two culture tanks, each partitioned into two areas to isolate fingerlings from larger fish, a single ORP controller, no matter how accurate, could not prevent mortalities from occurring within a given region of a culture tank unless a probe was in that region. In a single completely mixed freshwater environment, a good automatic ORP controller could probably help to obtain maximum oxidative treatment with minimum toxicity to fish.

These results may indicate that adding ozone at a lower rate (0.025 kg ozone/kg feed) could provide about the same benefits as a higher dosing rate (0.036-0.039 kg ozone/kg feed fed): e.g., reduced BGD associated mortalities and no required use of non-approved chemical treatments to control BGD epizootics. Yet, the lower ozone dosage rate apparently did not kill fish from ozone toxicity because ozone had such a short half-life and its residual quickly reacted away. Accordingly, the lower ozone addition rate could allow use of a shorter ozone contact time before the completely mixed culture tanks and also avoid the use of ozone residual removal units and the dependence upon expensive and sometimes unreliable ORP control technologies. Hence, use of the lower dose could provide all of the benefits but also reduce capital and operating costs associated with the higher ozone dosing rate.