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Ozone Application in Recirculating Aquaculture System: An Overview

Alex Augusto Gonçalves,[1] and Graham A. Gagnon[2]

[1]Federal University of Semi Arid, Animal Science Department, Mossoró, RN, Brazil
[2]Center of Water Resources Studies, Dalhousie University, Halifax, NS, Canada B3J 1Z1

In recirculating aquaculture systems (RAS), particulates (including feces, uneaten feed, bacteria, and algae) can cause several problems, in that they may harbor pathogens, can physically irritate the fish, and upon decomposition, release ammonia and consume oxygen. Mechanical filters, foam fractionators, and other engineered devices are used to remove particles quickly from aquaculture systems, in order to improve fish health and decrease the load on biofilters and oxygenators. Ozone is used in RAS as a disinfectant, to remove organic carbon, and also to remove turbidity, algae, color, odor and taste. Ozone can effectively inactivate a range of bacterial, viral, fungal and protozoan fish pathogens. But the effectiveness of ozone treatment depends on ozone concentration, length of ozone exposure (contact time), pathogen loads and levels of organic matter. In spite of ozone is a very effective oxidizing agent, higher ozone concentrations are a risk to cultured fish stocks causing gross tissue damage and stock mortalities, and also are a risk to bacterial films on the biofilter.

Keywords Ozone, Aquaculture, Re-circulating systems, Disinfection, Water quality, Toxicity

INTRODUCTION

Aquaculture will be a critical component of future seafood production to supply the ever-expanding human population and is continuing to expand worldwide, but such growth is dependent on the availability of high-quality water. The use of recirculating culture systems is one means of using available water more efficiently (Bullock et al., 1997; Kim, 2000; Bai, 2007), and provides potential advantages over pond or cage-based forms of aquaculture. These include flexibility in site selection, reduced water usage, lower effluent volumes, better environmental control, and higher intensity of production. However, as stock densities and levels of water re-use increase, wastes accumulate rapidly and environmental control becomes more difficult. Sophisticated systems capable of removing both particulate and dissolved organic wastes become necessary (Read, 2008; Pfeiffer et al., 2008).

Despite the advantages, recirculating aquaculture systems (RAS) pose a latent disease and public health risk. Part of the biological filtration necessary for removal of harmful toxins involves the biofilm that forms on all components of a recirculating system (King, 2001). Because the water is reused, pathogens introduced into the system could remain through incorporation into the biofilm, leading to recurring exposure of fish to pathogens and the presence of asymptomatic carriers.

Maintaining healthy fish in a recirculating system involves establishing adequate dissolved oxygen levels, removal of solid wastes, sufficient ammonia nitrification (King, 2001; Ebeling and Timmons, 2009) and also for fish sensorial quality, i.e., removal off-flavors from MIB and geosmin from water (Westerhoff et al., 2006). Increasing the daily water exchange rate in an RAS will remove accumulated colloidal solids, refractory organics and nitrite, to the detriment of water budgets and the cost of heating or cooling the system. The alternative method of removal is to break down these organic wastes using an oxidizing agent, such as ozone (Tango and Gagnon, 2003; Sharrer and Summerfelt, 2007; Read, 2008).

Ozone has been shown to be efficient in eliminating most pathogens affecting seafood in freshwater and seawater aquaculture systems (salmon, halibut, tilapia, shrimp, etc.). Moreover, ozone can improve water quality by reducing biochemical oxygen demand (BOD), ammonia and nitrite (Hunter, 2000; Meunpol et al., 2003; Summerfelt, 2003; Tango and Gagnon, 2003; Buchan et al., 2005; Coman et al., 2005; Ritar et al., 2006; Sharrer and Summerfelt, 2007), to disinfect incoming hatchery water (Tipping, 1987; Crisp and Bland, 1990), to disinfect hatchery wastewater (Majumdar and Sproul, 1974; Conrad et al., 1975) and to disinfect fish

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Address correspondence to Dr. Alex Augusto Gonçalves, Post doctoral fellow at Dalhousie University, (Present address) Federal University of Semi Arid (UFERSA), Animal Science Department, Av. Francisco Mota, 572, Costa e Silva 59625-900, Mossoró, RN, Brazil. E-mail: alaugo@gmail.com

eggs (Mimura et al., 1999; Grotmol and Totland, 2000; Ben-Atia et al., 2001; Su et al., 2001; Grotmol et al., 2003). Although direct exposure of aquatic organisms to ozone and the oxidants formed in ozonated seawater can be lethal (Wedemeyer et al., 1979a, 1979b; Coler and Asbury, 1980; Fisher et al., 1999); but fertilized fish eggs can tolerate varying levels of dissolved ozone. Specific exposure levels need to be determined for each species (Grotmol et al., 2003), and reliable methods to measure ozone in sea water are therefore needed to ensure that lethal limits are not exceeded. According to this information, this review intends to provide information of ozone use in aquaculture systems and also its safety to cultured organisms.

OZONE

Chemistry of Ozone

Ozone is a very unstable molecule and, after injection into raw water, it decomposes very rapidly. The pathway of the ozone reaction depends on the properties and concentrations of the other compounds, as well as the quality of the water, including pH, bicarbonate level, the level of total organic carbon and temperature. These factors affect the decomposition of ozone and therefore the effective oxidation power and oxidation rate of ozone (Lawson, 1995; Summerfelt and Hochheimer, 1997; Rakness, 2005).

In the presence of organic impurities, the half-life of ozone is reduced to minutes (Duvivier et al., 1996). The rate of decomposition also increases with increasing pH; and at low pH (<7), molecular ozone (O_3) is the dominant species; as pH increases, O_3 turns into the very short-lived (microseconds) hydroxyl radicals (OH•) (Rice and Wilkes, 1992; Rakness, 2005).

The chemistry of ozone in seawater is considerably different than that in brackish and freshwater. This difference in chemistry has a profound effect on disinfection. The most important difference with ozone chemistry in seawater compared to freshwater is due to the presence of bromide ion (Br^-) in seawater (Oemcke and van Leeuwen, 1998). Bromide ion catalytically decomposes ozone (Westerhoff et al., 1998; von Gunten 2003a, 2003b). In seawater, the primary brominated compounds formed by ozone are hypobromous acid (HOBr), which is in equilibrium with hypobromite (OBr^-). These compounds have disinfection properties (Herwig et al., 2006; Perrins et al., 2006). Bromine has disinfectant properties and is quantified as total residual oxidant (TRO), usually in units of mg Br_2 L^{-1} or mg Cl_2 L^{-1} (White, 1999).

Typical bromide ion concentrations of 60–70 mg L^{-1} in seawater give a high formation potential of bromo-oxides, even at a common oxidation ratio of 10%. Correspondingly, chloro-oxy anions may be formed, but these are limited by a higher oxidation-reduction potential barrier (Grguric et al., 1994). The following reactions of ozone with bromide ion (Br^-) and hypobromite ion (OBr^-) have been proposed (Grguric et al., 1994; Bonacquisti, 2006):

$$O_3 + Br^- \rightarrow O_2 + OBr^-$$

$$O_3 + OBr^- \rightarrow 2O_2 + Br^-$$

$$2O_3 + OBr^- \rightarrow 2O_2 + BrO_3^-$$

The first reaction shows that ozone will oxidize the bromide ion in seawater to hypobromite ion. The hypobromite ion will hydrolyze into hypobromous acid, which is a weak acid with a pK_a of 8.8 at 20 °C ($HOBr \leftrightarrows OBr^- + H^+$). The sum of HOBr and OBr^- is the biocidal bromine. In seawater with a typical pH of 8, hypobromous acid will predominate and be the most important disinfectant with a half-life of hours to days dependent on light conditions and water quality characteristics (Westerhoff et al., 1998; Legube et al., 2004; Liltved et al., 2006).

In freshwater, ozone decomposes rapidly to oxygen after application. By introducing ozone in aquacultural seawater systems, a series of redox-reactions take place and several reactive intermediates are formed (Liltved et al., 2006). The halogen ions in seawater are oxidized by ozone to halo-oxy anions. The specific formation potential is highest for iodine and bromine and somewhat lower for chlorine species. Although the iodide ion may be completely oxidized, the low iodide concentration in seawater (<1 mg L^{-1}) makes the succeeding oxidation products less important.

System and Application

The design of the ozone reactor or contact vessel is very important for safe, successful ozonation. There are a range of reactors available using various designs to transfer ozone to the water. Designs include fine bubble diffusers, turbine contactors, injectors, deep u-tube reactors, packed columns, static mixers and spray contact chambers. Some designs are also used for oxygen transfer or aeration. Each design has advantages and disadvantages not discussed here, but some important considerations when choosing a reactor include: i) ozone transfer efficiency; ii) leak-free design and construction; and, iii) construction with ozone resistant materials (Hunter, 2000; Rakness, 2005; Read, 2008).

Ozone can be applied continuously, as a series of treatments per day or as a single batch treatment per day (Hunter, 2000; Rakness, 2005). Application in most situations can be linked to the feeding strategy employed in the culture system. Three to 4 h after feeding fish, the concentrations of ammonia, dissolved organics and other wastes products reach a maximum. If fish are fed several times during the day, a series of ozone treatments can be introduced after each feed to target the associated rise in waste levels. If feed is introduced 24 h per day, water quality degrades continuously and so ozone application should be continuous. A single-batch ozone treatment can be used to target rises in waste levels in the system associated with a moderate feed event or to treat batches of exchange or inlet water from the supply source. Continuous

ozonation is beneficial when compared to batch and serial treatments because water quality remains relatively stable. However, the lower costs of serial and batch ozonation make these treatments regimes viable management options (Hunter, 2000; Read, 2008).

The required amount of ozone for treatment in a RAS is usually calculated according to the daily feed rate. Rates of 10–15 g ozone per kg feed are generally recommended to reduce accumulated organics. Any background organic loadings of the source water used for the RAS should also be taken into account (King, 2001).

Ozone Residual (in-Water) Measurement

Accurate measurements of ozone residual are required to properly determine disinfection credit. The accuracy of the ozone residual measurement is affected by the sampling system layout for both grab sample tests and continuous readings from on-line meters. Successful ozone residual measurement is enhanced by sampling systems that: i) minimize detention time in the sample line; ii) allow for easy collection of grab samples that can be taken without affecting the on-line instrument measurement; iii) are properly configured such that consistent and sufficient flow is available to an on-line instrument; and, iv) provide flexibility for measuring ozone residual at a variety of sampling locations within the ozone contactor (Rakness and Hunter, 2000; Rakness, 2005; Lee et al., 2008).

The successful use of ozone in the aquaculture industry also requires a reliable, simple and fast method for its measurement that can be conducted on-site at a hatchery, under non-laboratory conditions. The test must allow multiple readings during the course of a treatment to ensure that constant concentrations are maintained to prevent an unnecessary overexposure and high mortalities (Buchan et al., 2005). Although direct exposure of fish and other organisms to ozone and the oxidants formed in ozonated sea water can be lethal (Wedemeyer et al., 1979a, 1979b; Coler and Asbury, 1980; Fisher et al., 1999), most of them can tolerate varying levels of dissolved ozone. Specific exposure levels need to be determined for each species (Grotmol et al., 2003), and reliable methods to measure ozone in sea water are therefore needed to ensure that lethal limits are not exceeded. However, no single method is consistently used by the scientific community or industry, making comparisons and standardization of exposures difficult (Buchan et al., 2005).

The most important reaction is the oxidation of bromide ions (Br^-), forming hypobromite ions (OBr^-), which can then either be reduced back to Br^- or further oxidized to form bromate ions (BrO_3^-) (Grguric et al., 1994; Buchan et al., 2005; Liltved et al., 2006). The residual oxidant (OBr^-) interferes with reagents used to measure ozone as it reacts with the reagents as if it were ozone. Because of this, it is important to be careful of the units used to report dissolved ozone concentrations in seawater. The values obtained by measuring "ozone" will not only include any ozone present, but also any other oxidants in the sample. However, dissolved ozone concentrations in seawater can be expressed in mg L^{-1} of total residual oxidants (TRO), or ozone produced oxidants (OPO), or residual ozone concentration (ROC) and ozone (Buchan et al., 2005).

Comparative ozone residual (in-water) measurement methods. Five methods for measuring ozone are cited regularly in the literature, often using different measurement units and with little to no explanation as to why a particular method was chosen. These methods (summarized in Buchan et al., 2005) are: (1) Neutral buffered iodometric method (Shechter, 1973) where ozone concentrations is reported in mg L^{-1} of TRO; (2) Modified neutral buffered iodometric method (Sugita et al., 1992; 1996), reported in mg L^{-1} of TRO; (3) Iodometric titration method (Franson, 1989), usually used to measure chlorine levels, and ozone concentrations using this method are reported in mg L^{-1} of TRO or OPO; (4) *DPD* (N,N-diethyl-p-phenylenediamine) *colorimetric method* (Franson, 1976), ozone concentrations using this method are reported in mg L^{-1} of ROC or TRO; (5) Oxidation/reduction potential (ORP), an ORP probe attached to a meter is placed in the water sample, giving the ORP in mV. This technique was used by Tango and Gagnon (2003).

Also, ozone residual can be measured by gravimetric method (Yates and Stenstrom, 2000), i.e., determining volume based on weight. This procedure is easy to implement in the field and is highly accurate when properly performed. Buchan et al. (2005) compared commonly cited methods of ozone measurement for their ability to measure dissolved ozone and the ease with which they can be applied on-site in an aquaculture facility. Among the different units for expressing dissolved ozone concentrations examined, it is recommended that under non-laboratory (i.e., hatchery) conditions, a DPD "total chlorine test" be used to measure dissolved ozone levels, and the results be reported in TRO (mg L^{-1} as Cl_2). The TRO concentrations (mg L^{-1}) were also calculated and expressed as equivalent concentrations of bromine (Br_2; 1 mol $Cl_2 = 0.44$ mol Br_2) (Jones et al., 2006; Perrins et al., 2006; Lee et al., 2008).

Similar to the TRO measurement, AccuVac® Ampoules were used with freshly collected samples and analyzed using a water quality laboratory spectrometer. This is a commercially available modification of the indigo colorimetric method produced by Hach Chemical Company, Loveland, Colorado, USA (Hunter and Rakness, 2002). Powdered indigo reagent is enclosed in a small glass under vacuum. When the tip of the ampoule is broken under the water surface, a specific volume of the sample water is drawn into the ampoule and mixed with a specific amount of indigo powder (i.e., low-, medium-, and high-range measurement). Low-range ampoules are used when the ozone residual is less than 0.25 mg L^{-1}; medium-range ampoules are used to measure ozone residual between zero and 0.75 mg L^{-1}; and high-range ampoules are used to measure ozone residual between zero and 1.5 mg L^{-1}, with

a sensitivity of 0.1 mg L^{-1} (Rakness, 2005; Herwig et al., 2006). This method is more simple and easy to use.

To compare the official Indigo Colorimetric Method for ozone residue determination in water to Accuvac® test kit, an experiment was performed using Milli-Q® water (Gradient System A10, Millipore™, Billerica, MA, USA) as a control (Salinity 0‰), brackish water (Salinity 5‰) and seawater (Salinity 25‰). A laboratory semi-bath apparatus method was used for this study was carried out at the Center of Water Resources Studies Laboratory, Dalhousie University (Halifax, NS, Canada). Compressed air is passed into the ozone generator (VMUS-4, AZCO Industries Ltd., Langley, B.C. Canada) where high voltage corona discharge causes the breakdown of oxygen molecules into oxygen radicals which combine with the oxygen molecules and form ozone. The produced ozone is passed into the 10 L Glass through the tubing (rate of 0.3434 $mgO_3\ L^{-1}\ min^{-1}$) for 60 minutes and turned off. For the collection of residual ozone in the off gas system, a 2% potassium iodide solution was used in a flask as shown in Figure 1.

The temperature and pH during each experiment were maintained constant and the average was for temperature 25.05±0.28 (Salinity 0‰ - control), 25.18±0.12 (Salinity 5‰) and 25.10±0.21 (Salinity 25‰); and for pH 5.48±0.79 (Salinity 0‰ - control), 7.41±0.49 (Salinity 5‰) and 8.05±0.56 (Salinity 25‰). Ozone concentrations, total residual oxidants (TRO), bromate and bromoform were monitored each 5 min during 60 min of ozonation and 60 min after stopped ozonation, and were performed in duplicate using standard methods (APHA, 1995) as follows:

i. *Residual aqueous ozone concentration* ($mgO_3\ L^{-1}$): was measured using the official Indigo Colorimetric Method for ozone determination in water (4500-O_3: Ozone residual) (APHA, 1995) and Accuvac® test kit (Hach Co., Loveland, CO, USA). Freshly collected samples were analyzed by HACH DR/4000U Spectrophotometer (Method 8311). The ampoules had a range of 0–1.5 mg L^{-1}, with a sensitivity of 0.1 mg L^{-1} ozone;
ii. *Total residual oxidant* (TRO): ozone quickly reacts with bromide ion in seawater, forming hypobromous acid that is in equilibrium with hypobromite. Together, these compounds are referred to as bromines and they constitute TRO measure in ozonation seawater (Herwig et al, 2006). TRO was determined using a standard DPD colorimetric analysis for bromine (measured as $mgBr_2\ L^{-1}$), analyzed by HACH DR/4000U Spectrophotometer (Method 8016);
iii. *Oxidation/reduction potential* (ORP): was measured in mV using a Fisher Scientific Accumet Excell XL60 Meter;
iv. *Bromate* ($mgBrO_3^-\ L^{-1}$): was measured using a 761 Compact Ion Chromatography and 788 IC Filtration Sample Processor (Metrohm AG, Herisau, Switzerland). Calibration verification standard was performed ($R^2 = 0.9996$) for all analyses in accordance with their stringent QA/QC (Quality Assurance and Quality Control) program. The percent recovery of each compound was calculated and recorded on the quality control chart. The

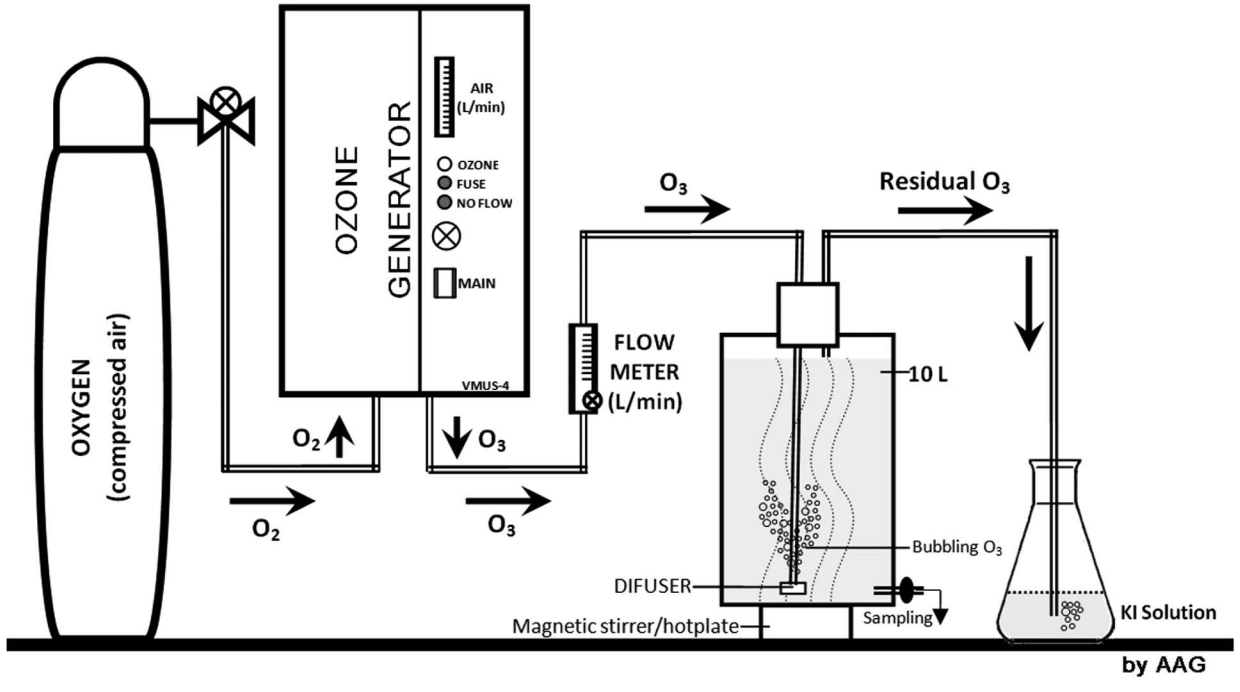

FIGURE 1. Schematic of the laboratory scale semi-batch apparatus during ozone treatment.

calibration curve was: y = 3.7225x + 0.2499 (R^2 = 0.9996);

v. *Bromoform* ($\mu gCHBr_3\ L^{-1}$): was measured using a CP3800 VARIAN Gas Chromatograph and CP8400 Auto Sampler System (VARIAN Inc., Palo Alto, CA, USA). The calibrations were done (R2 = 0.9923) for all analyses in accordance with their stringent QA/QC (Quality Assurance and Quality Control) program. The percent recovery of each compound was calculated and recorded on the quality control chart. The calibration curve was: y = 0.5358x + 0.6198 (R^2 = 0.9923).

The results were presented in Figure 2 and showed similar results using both methods suggesting that we can use each one to check ozone concentration in water. This experiment only reinforces the need to be cautious when comparing different ozone concentrations made using different measuring techniques. The Indigo colorimetric method must to be done carefully because you need to prepare indigo stock solutions, standardize indigo reagents, and when necessary, diluted samples, which increase the variability in the final results.

Interestingly, the residual ozone concentration was lower in water with 25‰ of salinity when compared to 5‰ and Milli-Q water, which has lower concentration of organic matter and could react with ozone. But the main concern was the residual ozone concentration after 60 minutes of the ozonation was stopped. Some authors (Summerfelt, 2003; Summerfelt et al., 2004; Liltved et al., 2006) comment the importance of the presence of a treatment unit for removal residual ozone and others oxidants, which could be toxic for aquatic organisms. This issue is discussed later.

A major encumbrance in the use of ozonation of seawater is that it is difficult to measure accurately the formation of ozonation by-products (OBP), particularly inorganic OBP such as bromate and other brominated compounds (Tango and Gagnon, 2003). The measurement of OBP is further complicated because the chemistry of seawater is variable especially inshore and in estuaries, often subject to fluctuations in salinity and turbidity due to wave action and terrestrial run-off. The content of OBP may be estimated indirectly via oxidation–reduction potential (ORP, or redox potential), which measures the potential of the seawater to oxidize or reduce, and is thus an indication of its ability to disinfect against microorganisms or kill aquaculture animals (Tango and Gagnon, 2003).

ORP may be used to control ozone addition to seawater but is not necessarily equivalent to the ability of the treated water to disinfect (Tango and Gagnon, 2003). Rather than measure ozone directly, an ORP probe measures the total capacity, in millivolts (mV), or various oxidants in a solution to oxidize an electrode. By keeping ORP measurements within a certain range, the levels of total oxidants can be controlled, which gives indirect control over ozone. A safe ORP level of freshwater fish culture is generally considered to be 300 mV (Read, 2008).

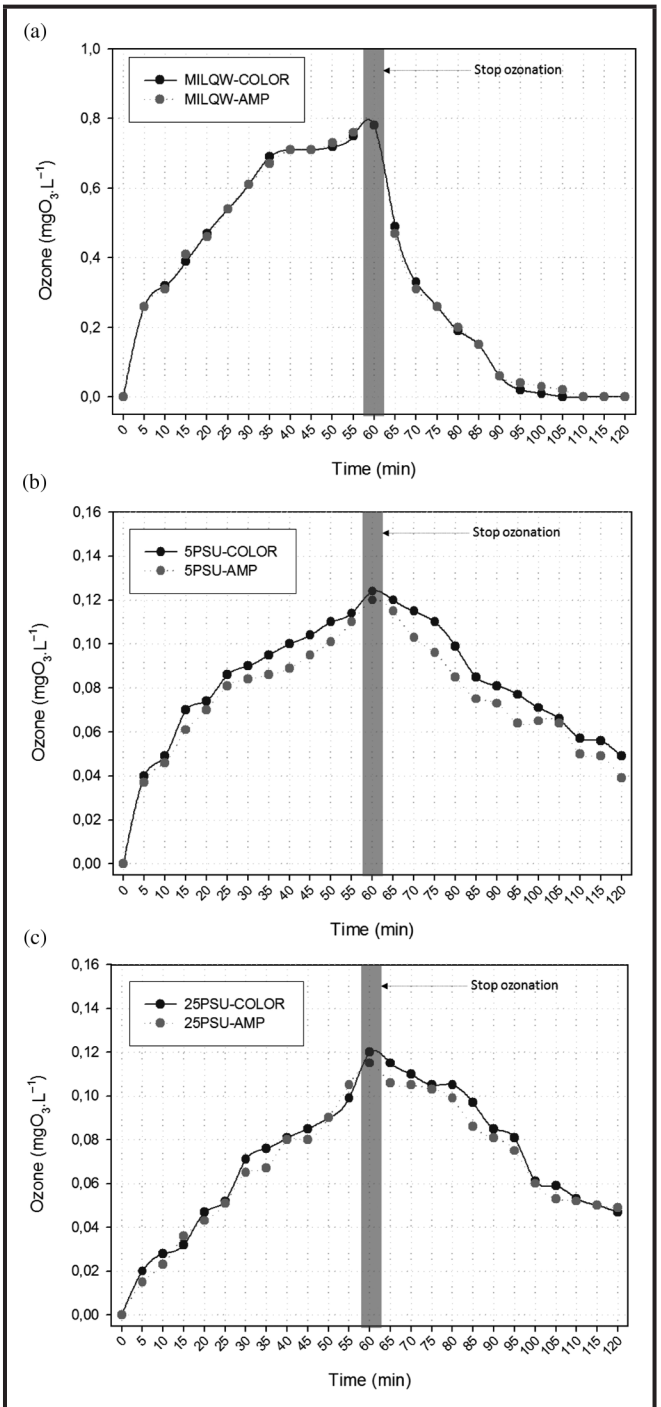

FIGURE 2. Ozone measurements during and after ozonation using the official indigo method (COLOR) and Accuvac® test kit (AMP); MILQW (Milli-Q water), 5 PSU (5 practical salinity units or 5‰), 25 PSU (25 practical salinity units or 25‰).

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Results presented in Figure 3 showed final ORP values below to 250 mV and 150 mV in brackish water (salinity of 5‰) and Milli-Q water (0‰), respectively. However, in sea water (25‰) the ORP values have remained above 500 mV, which could be dangerous to aquatic organisms. According to Tango and Gagnon (2003), this indirect measure of residual ozone (ORP) could be an indication of process water's potential to disinfect or to kill fish, and moreover, can be used to control ozone addition and thus ensure the desired treatment objective.

OZONE APPLICATION IN RECIRCULATING AQUACULTURE SYSTEMS

Ozone application within aquaculture systems requires ozone generation, ozone transfer into solution, contact time for ozone to react, and possibly ozone destruction to ensure that no ozone residual makes it into the culture tanks (Summerfelt and Hochheimer, 1997; Summerfelt et al., 2001; Summerfelt, 2003; Summerfelt et al., 2004; Liltved et al., 2006; Sharrer and Summerfelt, 2007). Ozone off-gas needs to be destroyed too.

Effective transfer of ozone into water is important because the cost of producing ozone is not insignificant, especially if the ozone is carried within purified oxygen feed gas that is either purchased or produced on site. The rate of ozone transfer and the subsequent rate of ozone decomposition depend upon the contact system efficiency and the reaction rates of ozone with constituents in the water. The ozone reaction rate depends on the water temperature and on the concentration and type of constituents contained in the water (Summerfelt, 2003; Summerfelt et al., 2004).

Ozone is used in aquaculture systems to improve water quality and overall system performance (King, 2001). Ozonation has proven useful in aquaculture systems in promoting the removal of solid matter (Rueter and Johnson, 1995; Tango and Gagnon, 2003), stabilization of water quality in recirculating systems (Reid and Arnold, 1994; Summerfelt et al., 1997), water clarification and dissolving nonbiodegradable organic material (Paller and Lewis, 1988; Summerfelt and Hochheimer, 1997; Summerfelt et al., 2004), is advantageous in disease control (Liltved and Landfald, 1995; Liltved

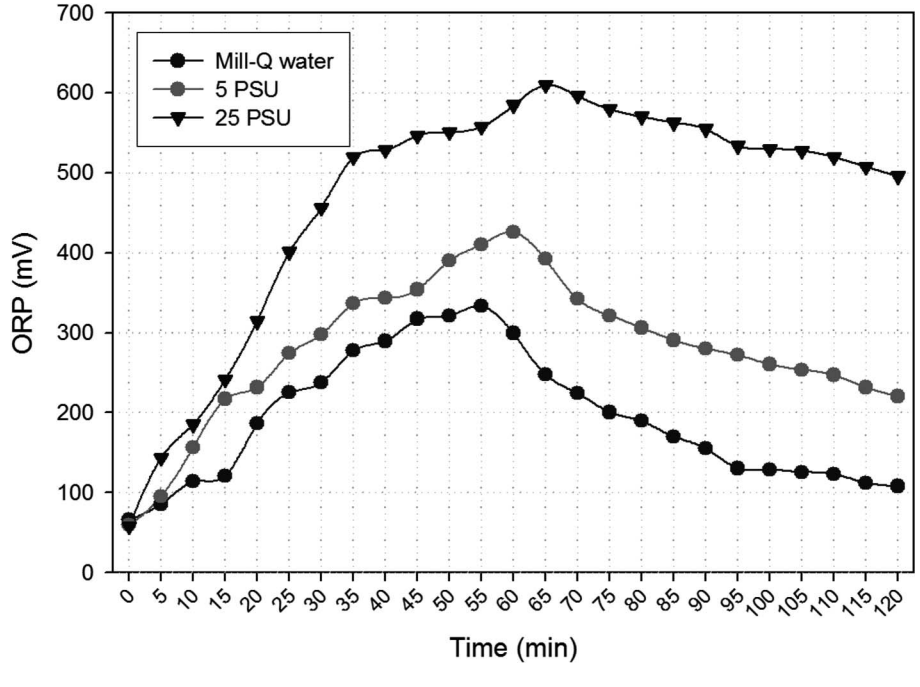

FIGURE 3. ORP measurements during ozonation (60 min) and after stopping ozonation.

et al., 1995), and ozonation of the water appeared to remediate the fish health decline (Good et al., 2009). This indicates that ozonation could find a place in the intensive culture, but also a potential candidate for commercial aquaculture (Jobling et al., 1993; Ritola et al., 2000).

In general, ozone is an effective bactericide, parasiticide, and virucide (Lohr and Gratzek, 1984; Colberg and Lingg, 1978; Liltved et al., 1995; Bullock et al., 1997; Liltved, 2002) by disrupting cell membrane function, entering the cell and destroying the nuclear chemistry of the cell (Lawson, 1995; Sharrer and Summerfelt, 2007); however, some viruses have shown high resistance to ozonated seawater (Liltved et al., 2006). This effectiveness is a function of dosage and contact time (Lawson, 1995). The target organism and water quality determine the required concentration of ozone and the necessary contact time (Lawson, 1995; Summerfelt and Hochheimer, 1997). Microbial reductions are limited by the ability to maintain a specific ozone concentration for the time needed (Summerfelt and Hochheimer, 1997).

In spite of the apparent advantages of ozone in aquaculture, its physiological effects on the fish are still largely unknown since unexpected deaths have occurred even at low O_3 concentrations (Bullock et al., 1997). This issue will be discussed later in this paper.

Reduction of Organic Matter

Besides its primary use in disinfection, the ozonation process has been observed to be associated with flocculation in waters containing particulate matter. Moreover, an essential ingredient for the success of any hatchery is a suitable water supply having both sufficient flow rate and appropriate quality. The presence of fine particulate matter can cause problems, i.e., shielding of pathogens, a decreased oxygen transfer rate to eggs and fry, and the deposition of sediment in rearing zones. Rueter and Johnson (1995) found in their study that the use of ozone prior to sedimentation or filtration improved the removal of suspended solids and concluded that ozone's multiple uses for disinfection, water aeration, removal of metabolic by-products, along with the improvements in suspended solids removal shown in this and other research, make it an especially appropriate treatment process for hatcheries.

Summerfelt et al. (1997) applied ozone to water in a recirculating rainbow trout (*Oncorhynchus mykiss*) culture system just prior to the culture tanks in order to oxidize nitrite and organic material, improve overall water quality, and assist removal of solids across the microscreen filter. Adding ozone (25–39 g ozone/kg feed fed) reduced the mean concentration of Total Suspended Solids (TSS) by 35%, Chemical Oxygen Demand (COD) by 36%, Dissolved Organic Carbon (DOC) by 17%, and color by 82% within the water entering the culture tanks. Additionally, ozone reduced the mean nitrite concentration by 82% within the culture tanks; and, reduced bacterial gill disease associated mortalities and chemical treatments required to control bacterial gill disease (BGD) epizootics.

Ozonation leads to coagulation of smaller particles into larger ones, and the large particles are removed from the system by the screen filter, the question remains as to the effect of ozone on the particle size distribution of the remaining particles. With continual production and continuous breakdown, aggregation, and removal of particles, it is difficult to predict the net effect of ozone on the particle distribution in a recirculating system. The study conducted by Krumins et al. (2001b) addresses this question by quantifying the effect of ozonation on the particle size distribution in RAS. Surprisingly, regardless of ozone dose, there was no significant difference in the slope of the power law fit for the particle size distribution. In over one-half of the experimental trials when no ozone was added, the particle size distributions were distinctly bimodal.

Edwards et al. (1993) note that ozone can act to remove carbon (Total Organic Carbon – TOC) in two ways, by oxidizing dissolved organic matter or by improving coagulation of organic-containing particulates. They found that ozonation improved particle removal for doses of less than about 0.7 mg ozone per mg TOC. Rosenthal (1981) reported that ozone doses of approximately 7–10 mg L^{-1} (approximately 0.1–0.2 mg ozone per mg TOC, this author's calculation) in a RAS increased the biological oxygen demand (BOD_5) of the water.

According to Krumins et al. (2001a), recent work on ozone application in RAS gives guidelines as to the daily ozone dose as a function of feed rates, but does not indicate whether the ozone should be added continuously throughout the day or in shorter, more intense doses. Their study examined the effect of adding the same total amount of ozone (15 g ozone/kg feed) 24, 12, and 6 h per day, compared with a control (without ozone). The ozone treatments significantly ($p<0.05$) reduced TOC, turbidity, and total ammonia nitrogen (TAN) compared with the control, but surprisingly did not significantly reduce average nitrite concentrations. They concluded that cycling ozone *on* and *off* throughout the day can help to maintain a stable population of nitrifying bacteria.

Water Disinfection

Recirculation of water is practiced when the water supply is limited or when energy saving is demanded. Disinfection seems to be required at high degrees of recirculation in order to suppress the general microflora, and to prevent spreading of pathogens with the effluent from special aquacultural activities, like as scientific laboratories working with infected fish and fish pathogens, and processing industry for farmed fish (Liltved et al., 1995; Bullock et al., 1997; Buchan et al., 2005).

Recirculating systems for salmonids can require exceptional water quality and tight biosecurity to reduce the likelihood of restricted fish growth and increased mortality (Noble and Summerfelt, 1996). To optimize water quality, recirculating systems will use water treatment processes that effectively

and rapidly remove fecal matter and waste feed, because rapid removal of organic matter can minimize the amount of fine particulates, soluble organic compounds, and ammonia that they would release if given the opportunity to degrade within the recirculating system (Summerfelt et al., 2004).

Bacterial reduction and viral inactivation may be desirable within recirculating systems. However, to disinfect recirculating systems water with ozone could be very expensive due to: (1) the much higher ozone loading required to overcome the organic demand and to sustain a residual that would be sufficient to achieve significant bacterial and viral reductions; and (2) the need to strip any remaining residual ozone from the water before it is returned to the culture tank (Bullock et al., 1997).

According to Bullock et al. (1997) adding ozone at a lower rate (25 g ozone/kg feed) to water in a recirculating rainbow trout (*Oncorhynchus mykiss*) culture system just before it entered the culture tanks could provide about the same benefits as a higher dosing rate (36–39 g ozone/kg feed fed) e.g., reduced bacterial gill disease (BGD) associated mortalities and no required use of non-approved chemical treatments to control BGD epizootics. In the sequence of this study, Summerfelt et al. (1997) showed that ozone can improve overall water quality at the same ozone rate. 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.

Greater reductions in bacteria within the recirculating system, with its high oxidation demand, would have required ozone loading rates greater than those used by Bullock et al. (1997) (i.e., >39 g ozone/kg feed), which would be difficult to achieve without: (1) wasting excess oxygen to carry more ozone to the low-head oxygenators (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–l0% 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.

Ozone has a unique characteristic that distinguishes it from other disinfectants: it is highly volatile and disappears shortly after contact with organic material suspended in water. Unlike other disinfectants, ozone can be continuously applied to a water stream within a culture system if there is sufficient residence time for its dispersion between the treatment location and the pond intake (USEPA, 1999; Schuur, 2003; Newman, 2006; Lee et al., 2008).

Studies from Meunpol et al. (2003) showed that degree of bacterial inhibition also depend on the concentration of ROC at first contact. Longer ozonation resulted only in longer suppression period but not greater bacterial reduction.

The total dose of ozone is commonly expressed as a CT value, which is the product of the ozone concentration (C) and exposure time (T). Several studies have found that the toxicity of ozone generally increases with ozone dose (CT value) (Davis and Arnold, 1997; Theisen et al., 1998; Su et al., 2001). However, the individual contributions of C and T to the toxicity of the ozone dose can only be assessed in studies involving factorial combinations of C and T (Rakness, 2005).

Ozone oxidation can kill microorganisms, but disinfecting the water requires maintaining a certain dissolved ozone concentration for a given contact time. Thus, disinfecting efficiency depends on the product of the ozone residual concentration multiplied by its contact time. An ozone contact vessel should provide the time necessary for the ozone residual to react with and inactivate the target microorganism(s). Disinfecting water can require maintaining a residual ozone concentration of 0.1–2.0 mg L^{-1} in a plug-flow type contact vessel for periods of 1–30 min, depending upon the target microorganism (Summerfelt, 2003). Ozone at a concentration not exceeding 0.5 mg L^{-1} (to minimize bromate production) can be used to treat seawater in batches for periods up to 10 minutes (Lee et al., 2008).

If disinfection is the primary goal of ozonation, the amount of ozone necessary is largely dependent on the background organic loading of the water to be treated (Summerfelt, 2003). In pure water, residual concentrations of 0.01–0.1 mg L^{-1} ozone for periods as short as 15 sec can be effective in reducing bacterial loads. However, in water with organic loadings the residual ozone concentration and/or contact time of ozone must be increased to produce significant disinfection. Natural waters (seawater, brackish and freshwaters) generally require residual concentrations of between 0.1–0.2 mg L^{-1} ozone and contact times of 1–5 min for disinfection. Aquaculture effluent generally requires between 0.2–0.4 mg L^{-1} residual ozone for 1–5 min for significant disinfection to occur after oxidation of organics (Read, 2008).

There is growing awareness of the need to disinfect water entering and leaving aquaculture systems. Improvement of the microbial standard of inlet water and stringent microbiological restrictions on effluent water are in many cases needed to control diseases in the fish farming industry. Different types and volumes of water to be disinfected and different target pathogens may influence the choice of method, dose requirements and other design criteria for disinfection units (Liltved et al., 1995; Bullock et al., 1997).

In freshwater hatcheries, the necessity of new methods is mainly for fungi pathologies control. Ozone (O_3) is a very unstable allotrope state of oxygen producing O native characterized by high bactericide activity (Forneris et al., 2003). In some cases, almost the complete elimination of pathogenic bacteria such as *Aeromonas salmonicida*, *Vibrio anguillarum*, *Vibrio salmonicida* and *Yersinia ruckeri* has been obtained (99.9%). The disappearance of viral diseases such as infectious pancreatic necrosis virus (IPNV) in both fresh and marine water fish (Colberg and Lingg, 1978; Liltved et al., 1995) was also observed.

Nodavirus is the causative agent of viral nervous necrosis (VNN), also known as viral encephalopathy and retinopathy (VER), or fish encephalopathy. Nodavirus infections are a worldwide problem affecting over 30 species of marine finfish including Atlantic halibut (*Hippoglossus hippoglossus*),

sevenband grouper (*Epinephelus septemfasciatus*), Japanese flounder (*Paralichthys olivaceus*), red drum (*Sciaenops ocellatus*), winter flounder (*Pseudopleuronectes americanus*), Atlantic cod (*Gadus morhua*) and haddock (Buchan et al., 2006). Arimoto et al. (1996) assessed the effectiveness of ozone (as a total residual oxidant) to inactivate striped jack nervous necrosis virus (SJNNV) and 0.1 $\mu g\ L^{-1}$ was required to inactivate SJNNV for 2.5 minutes. Also, washing fertilized eggs and the treatment of sea water with ozone decrease the rate of occurrence of viral nervous necrosis (VNN).

Due to few published data on virus inactivation in seawater, Liltved et al. (2006) initiated a study to supply fish farmers, consultants and manufacturers with more precise dose requirements of ozone and UV irradiation to accomplish inactivation of viruses important in aquacultural systems. Such data are crucial to establishing a firm basis for the design of disinfection systems and for better operation and control of existing installations. The viruses studied were IPNV, Atlantic halibut nodavirus (AHNV) and infectious salmon anaemia virus (ISAV) and the results demonstrated a wide span in UV and TRO resistance among them. ISAV was sensitive to both methods, while high resistance in AHNV and IPNV were experienced. The TRO resistance in IPNV and AHNV contradict earlier published results and suggests reconsideration of existing ozonation practice to inactivate these viruses in seawater (Liltved et al., 2006).

Among the numerous viruses of penaeid crustacea, several, notably Taura Syndrome Virus (TSV), White-spot Syndrome Virus (WSSV), and Yellow-head Virus (YHV), are responsible for epizootic incidents in commercial shrimp ponds that have resulted in high mortality and economic losses. WSS was first reported in 1992 in *Penaeus japonicus* cultured in northeastern Taiwan and continues to cause substantial losses in the aquaculture industry in many countries; any practical means to eradicate or to inactivate the virus in the culture environment would be of enormous practical benefit (Chang et al., 1998; Schuur, 2003).

Chang et al. (1998) investigated the efficacy of some commonly used chemical and physical disinfectant methods for inactivation of white spot syndrome bacilovirus (WSBV) in juvenile black tiger prawn (*Penaeus monodon*) and concluded that the effective concentration for ozone to reduce WSBVs infectivity to zero was 0.5 mg L^{-1} as a total residual oxidant for 10 min at 25 °C.

In controlled bioassay conditions a concentration-time (CT) product sufficient to deactivate most viruses including WSSV is about 5.0 (e.g., 1.0 mg L^{-1} for 5 min or 0.5 mg L^{-1} for 10 min). A nominal design value for ozone disinfection is a CT product of 1.5–2.0 times the bioassay value. A CT product of 7.5–10 is therefore suggested as an estimate for crustacean viruses (Schuur, 2003).

The causative virons have been detected in eggs, larvae and broodstock of striped jack (*Pseudocaranx dentex*), indicating that spawners can be a source of infection (Arimoto et al., 1992). Nodavirus is durable and tolerant to various environmental conditions (Frerichs et al., 2000). However, ozone has been shown to inactivate nodavirus, although specific VNN titers were not reported (Arimoto et al., 1996; Grotmol and Totland, 2000). Fertilized eggs from different species of fish can tolerate varying levels of dissolved ozone, so specific exposure levels need to be determined for each species (Grotmol et al., 2003).

Considering these concerns, Buchan et al. (2006) investigated the tolerance of newly fertilized haddock eggs to dissolved ozone and to determine if this exposure is sufficient to disinfect against piscine nodavirus. Ozone can successfully disinfect fertilized haddock eggs against nodavirus. It is recommended that fertilized haddock eggs be disinfected with an ozone dose of 3.0 mg L^{-1} TRO for 3.3–6.7 min (10–20 CT units).

Treatment with ozone has also shown a positive effect against infection in abalone (Dixon et al., 1991) and in crustaceans, against viral pathologies (Chang et al., 1998) or against viral pathologies of the pancreas in Atlantic salmon (McLoughlin et al., 1996). The effect of ozone has also resulted positive in the treatment of ceratomyxosis in rainbow trout (Tipping, 1988). In adult fish, the use of ozone requires particular care as it is the cause of death, even at low concentrations, following the alteration of the branchial epithelium and the outermost layers of the epidermis (Wedemeyer et al., 1979b; Paller and Heidinger, 1980; Richardson et al., 1983). The effectiveness of ozone has instead proved to be limited in particular farming conditions, for example, when there is a high amount of suspended organic substances and even sometimes in recycling systems (Bullock et al., 1997; Summerfelt et al., 2004).

Saprolegniasis is a widespread mycotic infection in freshwater aquaculture and represents a serious problem affecting egg production in trout hatcheries (Forneris et al., 2003). The damage results in an average annual lack of production of about 20%, with peaks higher than 40%. Saprolegniasis is a secondary manifestation of a pathology suffered by developing embryos. In order to reduce the presence of water moulds, Forneris et al. (2003) studied the effectiveness of ozone as a fungicide to control the incidence of saprolegniasis in trout eggs incubation. From the results, it has emerged that the treatment with ozone is effective and the hatching eggs range from 42.6% to 49.1% dose of ozone from 0.01 to 0.2 mg L^{-1}.

Ozone is also a likely candidate for disease prevention and water quality management in shrimp culture (Rosenthal, 1980; Menasveta, 1980; Matsumura et al., 1998; Sellars et al., 2005). Despite ozone's potential benefits, few shrimp culturists embrace its use (Matsumura et al., 1998), perhaps because practical details are often lacking, and because of inconsistent results or the limited documentation on ozone's effectiveness to inactivate shrimp viruses (Sellars et al., 2005).

Meunpol et al. (2003) evaluated the effects of ozone on bacteria and black tiger shrimp postlarvae (*Penaeus monodon*), which included using different ozone exposures on shrimp as well as on harmful bacteria (*Vibrio harveyi* D331), and beneficial probiotic bacteria (*Bacillus* S11). They concluded that shrimp postlarvae exposed to 0.34–0.50 mg O_3

L^{-1} (8-h ozonation) caused loss of balance, immobility and destruction of gill lamellar epithelium, but damage of organisms by ozone is not only related to ozone concentration but exposure time.

Although ozonation has been proven non-toxic to post-larval shrimp at concentrations capable of eliminating viruses present in the culture systems (Blogoslawski et al., 1977; Jiang et al., 2001; Meunpol et al., 2003), protocols are yet to be established for using ozone as a virucidal treatment for shrimp embryos. An assessment of embryo tolerance to ozone is essential before investigations into ozone's capacity as a virucidal embryo treatment. Such studies highlight the requirement for comprehensive toxicity trials to be performed for each cultured species before ozone can be applied to improve water quality or deactivate pathogens in a culture system.

Sellars et al. (2005) investigated the tolerance of *Penaeus* (M*arsupenaeus*) *japonicus* embryos to ozone disinfection. The toxicity of ozone applied at different concentrations and periods of time was assessed at three developmental stages (post-spawning treatment times) for three separate families. This study shows considerable potential for using ozone as a virucidal agent for *P. japonicus* embryos as they can tolerate exposure to ozone at concentrations well above those reported to inactivate viral and bacterial pathogens of other marine species (CT 2 mg L^{-1}). Future studies investigating the ability of ozone to inactivate viral pathogens of *P. japonicus* embryos should use 2 mg L^{-1} ozone for 1 min applied at either 120 or 480 min post-spawning detection.

Following from previous work examining the tolerance of *P. japonicus* embryos to ozone disinfection (Sellars et al., 2005), Coman et al. (2005) assessed the hatch rates of *P. japonicus* embryos ozonated at several CT values, generated from different combinations of C and T, to determine the relative effect that C and T has on ozone toxicity, and suggested that the toxicity of ozone is more dependent on C than T. This suggests that CT value is a redundant measure of ozone toxicity. Protocols for ozonation in aquaculture should quote the individual values of C and T that provide effective disinfection for the cultured organism.

Ritar et al. (2006) examined different levels of ozonation of culture water, as determined by ORP levels, on the survival, growth and bacteriology of phyllosoma larvae of the southern rock lobster (*Jasus edwardsii*). The culture of phyllosoma to Stage IX using ozonated seawater was effective in controlling pathogenic bacteria to improve larval survival. However, excessive ozonation caused deformities and eventual death. Tolerance to ozonation declined at later stages of larval development. It is clear that ozonation needs to be monitored and controlled precisely to deliver an effective disinfection for the benefit of larval health, while avoiding the detrimental toxins causing deformities and death. Thus, a better understanding is needed of the changes in water chemistry during ozonation and improved technologies are required to accurately measure the levels of OBP in ozonated seawater.

Improvement of Taste and Odor

The occurrence of off-flavors in all types of aquaculture products is costly and continues to be a detriment to the growth of the aquaculture industry. Off-flavors also contribute to losses to the industry due to consumer dissatisfaction with the cultured product, which can result in the decreased likelihood of future purchases and inhibit expansion into new markets. Producers using recirculation or partial recirculation systems, in which off-flavored fish are present, have instituted depuration practices where by fish are held in clean water until any off-flavor disappears from the product (Tucker et al., 2000; Schrader et al., 2005).

Taste and odor of water is a common source of customer complaints to water utilities (Suffet et al., 1995). The odorants 2-methylisoborneol (MIB) and *trans*-1,10-dimethyl-trans-9-decalol (geosmin) often cause earthy/musty odors. The production of fish in recirculating aquaculture systems (RAS) continues to be hampered by problems with environmentally derived "off-flavor" that is best described as an "earthy" and/or "musty" taste of the fillet. Recent studies have determined that the presence of the odorous compounds geosmin and MIB in the flesh of RAS-cultured fish is responsible for these off-flavors (Schrader et al., 2005; Guttman and van Rijn, 2008; Schrader and Summerfelt, 2010).

Blue-green algae and actinomycetes present in surface waters produce MIB and geosmin, resulting in part-per-trillion odorant concentrations in water supplies (Schrader et al., 2005; Westerhoff et al., 2005). This evidence of MIB and geosmin accumulating is also common in recirculating systems (Masser et al., 1999; Schrader et al., 2005). So far, few studies have addressed the possible causes and prevention of off-flavor compounds' accumulation in these latter systems (Schrader et al., 2005; Guttman and van Rijn, 2009). The biofilters used in the water recirculating systems for culturing the white sturgeon may be a source of geosmin-producing actinomycetes. Research is ongoing to help determine the sources of geosmin and MIB in these recirculating-water systems (Schrader et al., 2005).

Ozone is widely used to achieve multiple water quality benefits (e.g., disinfection, trace organic removal, natural organic matter removal), also effectively oxidizes odorants such as MIB and geosmin (Westerhoff et al., 2006; Guttman and van Rijn, 2008). Over the past several decades, various municipal drinking water facilities in the United States have used ozonation to remove geosmin and MIB from the water via oxidation (Schrader et al., 2010).

Much of the information on MIB and geosmin oxidation by ozone is dose-response relationships, which are inherently difficult to extrapolate from one pilot study or water to other locations. A number of factors can impact the efficiency of ozone addition in the removal of geosmin and MIB including dosage (Koch et al., 1992; Westerhoff et al., 2006), water temperature (Westerhoff et al., 2006), organic matter content of the water (Bruce et al., 2002; Ho et al., 2004), pH (Westerhoff et al., 2006), and alkalinity (Ho et al., 2004).

Variable removal (35% to 95%) of both MIB and geosmin was observed during ozonation (1.5 mg L^{-1}) of surface waters spiked with 50 ng.L^{-1} of MIB and geosmin; a higher ozone dose (7 mg L^{-1}) removed >95% of the odorants (Lundgren et al., 1988). Ozonation of Colorado River water (4 mgO_3 L^{-1}) removed 78% of the MIB and 89%of the geosmin (Glaze et al., 1990).

High odorant removal during advanced oxidation processes suggests that hydroxyl radicals (HO•) play an important role in MIB and geosmin oxidation. Based on advanced oxidation process (AOP) studies, second-order rate constants have been estimated between HO• and MIB or Geosmin, but rate constants are not available for direct reactions of the odorants with ozone (O_3). Thus, Westerhoff et al. (2006) quantified the relative importance of O_3 and HO• oxidation pathways and rate constants for MIB and geosmin. Kinetics was evaluated for MIB and geosmin oxidation by ozone in surface waters with a range of water qualities. In addition, geosmin oxidizes faster than MIB (Westerhoff et al., 2006). The resistance of MIB to oxidation by O_3 may be due to greater steric hindrance based upon its chemical structure compared to geosmin (Ho et al., 2004). MIB and geosmin oxidation increases with greater ozone dose, higher pH, higher temperature or addition of H_2O_2.

The use of ozonation in RAS has recently been studied to determine the requirements to achieve adequate disinfection of recirculating water (Summerfelt et al., 2009). In addition, previous studies have determined that ozone addition in RAS will improve water quality by inducing microflocculation of fine particles [i.e., improving total suspended solids (TSS) capture and reducing TSS concentration] and oxidizing nitrite (i.e., reducing nitrite concentration) and undesirable organic molecules (e.g., reducing non-biodegradable and refractory compounds that stain the water) (Chen et al., 1994; Summerfelt et al., 1997, 2009; Krumins et al., 2001a, 2001b; Summerfelt, 2003).

Ozonation has been demonstrated to be effective in reducing geosmin and MIB concentrations in water. For example, ozone dosages of 1, 2, and 4 mg/L at a contact time of 12 min reduced an initial MIB concentration of 100 ng/L, in river water, by 58%, 65%, and 75%, respectively (Koch et al., 1992). In another study, Glaze et al. (1990) determined that 0.1 mg/L of ozone with a contact time of 20 min reduced initial geosmin and MIB levels of 100 ng/L in aqueduct water by 35% and 40%, respectively, and 0.2 mg/L of ozone (20 min contact) reduced 100 ng/L of geosmin and MIB by 86–92% and 73–83%, respectively (Schrader et al., 2010).

In the current study (Schrader et al., 2010), ozone addition was at significantly lower levels than those used in the above mentioned studies. The concentration and flow of ozone in the feed gas supplied to one of the three RAS receiving ozonation were measured and used to quantify that approximately 20–25 g of ozone were added to the recirculating flow for every 1 kg of feed fed daily, which was equivalent to an ozone dose of approximately 0.25–0.28 mg/L. This ozone dose was used to maintain an ORP of 248 mV, which extrapolates to less than 1μg/L of dissolved ozone residual according to data published by Summerfelt et al. (2009); in fact, an ozone residual concentration of 1μg/L is not expected until ORP reaches approximately 350 mV.

A dissolved ozone concentration below 1μg/L is safe for rainbow trout in freshwater (Bullock et al., 1997). Even though the recirculated water was subjected to the continuous addition of ozone, the ozone addition had no significant effect ($p > 0.05$) in reducing levels of geosmin and MIB in the water or trout fillets compared to RAS with no ozone addition. These results indicate that "low-dose" ozone addition with the intended goal of improving certain water quality parameters (e.g., TSS, color, etc.) will not provide benefits in the management of off-flavor problems related to geosmin and MIB.

Ozone in Shellfish Depuration

Filter-feeding molluscan shellfish accumulate microorganisms, such as bacteria and human viruses, when grown in sewage-polluted waters and can present a significant health risk when consumed raw or lightly cooked (Sobsey and Jaykus, 1991). Current regulations of shellfish and their growing waters are based on bacterial standards (fecal coliforms and *Escherichia coli*) and have prevented bacterial gastrointestinal infections. However, they are believed to have limited predictive value for viral pathogens such as enteroviruses (Jofre, 1992), Norwalk-like virus (NLV), hepatitis A virus (HAV) and hepatitis E virus (HEV) (Wanke and Guerrant, 1990; Desenclos et al., 1991; Holliman, 2005a, 2005b).

Ozone is very effective at inactivating both bacteria and viruses, and at a concentration not exceeding 0.5 mg L^{-1} (to minimize bromate production) can be used to treat seawater in batches for periods up to 10 min. This is undertaken in a separate tank to that used for depuration and then the residual ozone has to be discharged from the seawater before use so that it does not adversely affect the animals – this is achieved by aeration. There are two additional concerns with the use of ozone – the first is that bromates are formed when ozone is in contact with seawater and these are regarded as potential cancer forming compounds. The second is that residual levels of ozone may cause the shellfish to reduce or stop activity, thus reducing the effectiveness of the depuration process (Lee et al., 2008).

Several studies have revealed the differential rates of reduction of bacteria and viruses in depurating shellfish (Power and Collins, 1989; De Mesquita et al., 1991; Dore and Lees, 1995) and there is an urgent need for indicators of human-specific viral fecal pollution to improve the biological safety of shellfish. Then, to evaluate the period of depuration that ensures the microbiological safety of shellfish, Muniain-Mujika et al. (2002) analyzed the depuration rates of various parameters in naturally polluted shellfish mussel (*Mytilus galloprovincialis*) using highly efficient depuration equipment. Seawater of the depuration tank was disinfected by UV irradiation, ozone and passed through a skimmer and a biological filter; and in

this specific depuration system, 5 days may be necessary to assess the sanitary quality of shellfish.

Certain microalgae are also relatively insensitive to residual oxidants. The growth of the microalgae *Tetraselmis chuii* was found to be unaffected at levels up to 0.7 mg L^{-1}; at 1 mg L^{-1}, growth was impacted negatively (Zheng et al., 2002). The use of ozone to depurate shellfish was used in Europe as early as 1929 (Violle, 1929) and has been shown to reduce toxicity of *K. brevis* toxins (Blogoslawski et al., 1973, 1975). The use of ozone to detoxify shellfish or minimally, its utilization to treat incoming seawater to a depuration or wet storage facility could reduce the risk of cross contamination in areas where a bloom might have occurred (Schneider et al., 2003).

As for other organisms, the damage to the American oyster (*Crassostrea virginica*) by residual oxidants varied with their age. Even for adults, fecal matter accumulation was reduced at TRO levels as low as 0.05 mg L^{-1} (Richardson et al., 1982).

Removal of Algae and its Toxins

There have been several reports regarding the potential of use of ozone to kill algal cells (Sengco, 2009). Deeds et al. (2004) found that 0.4 mg L^{-1} led to lysis of *Karlodinium micrum* (>80% removal) and a reduction in hemolytic activity in fish farm operation in the Chesapeake Bay (Maryland, USA). Similarly, cultures of *Prorocentrium triestinum*, *Scrippsiella trochoidea*, and *Karenia diginata* were killed within 15 min after exposure to 1 g O_3 m^{-3} (Ho and Wong, 2004). The treatment also reduced concentrations of ammonium and total inorganic nitrogen while dissolved oxygen levels remained within acceptable levels.

Ozone was also tested against *Karenia brevis* and its toxins (Scheider et al., 2003). Direct treatment of *K. brevis* culture with 25 mg of ozone resulted in an 80% loss of cells within 10 s. All of the cells destroyed after 60 s. Similarly, free brevetoxins introduced into seawater were significantly reduced after a 10-min treatment. However, 135 mg of ozone was needed. The survival of *Cyprinodon variegates* in fish bioassays was inversely related to the time after ozone treatment, indicating a reduction in toxicity over time.

Other important issue that might be considered is the occurrence of red tide blooms which interrupts the culture, production, harvesting and subsequently the marketing of seafood products (Schneider et al., 2003). Shellfish toxicity has also been reported during the occurrence of Florida red tides and brevetoxins have been cited as the cause of sub-lethal human intoxications known as neurotoxic shellfish poisoning (NSP). Toxin levels normally found in shellfish during *Karenia brevis* blooms can be fatal to humans, although no deaths have been attributed to the consumption of affected shellfish (Baden et al., 1984). Ozonated seawater has been shown to be effective in inactivating crude toxins associated with dinoflagellate blooms, as well as in reducing the levels accumulated in shellfish (Thurberg, 1975; Blogoslawski et al., 1979).

Schneider et al. (2003) examined the effectiveness of ozone to reduce the numbers of Florida red tide organism (*Karenia brevis* Davis) and its associated toxins in an artificial seawater environment. The reduction in toxin concentration, as measured by high performance liquid chromatography (HPLC) analysis, displayed a positive correlation with the reduction of toxicity as determined by a fish (*Cyprinodon variegatus*) bioassay. Despite large total doses of ozone applied, as compared to levels that might be found at a commercial ozonation facility, some toxins were still recoverable by HPLC after ozone treatment.

Improvement of Quality of Live Food

The economic profitability of larval rearing in marine finfish hatcheries depends to a large extent on the continuous availability of high quality live food. In this respect, the demand for rotifers has gradually increased over the last years which can be explained by the relatively stagnating Artemia supply for an increased aquaculture production (Suantika et al., 2003).

Artemia, *Brachionus plicatilis*, is an excellent first food for fish and crustacean larvae, but there are still some problems related to its culture and use. The most stringent problems for automation of the production cycle reside in the unpredictability of the mass production and the variability in the quality of the product (Walz et al., 1997). The unstable production and low quality of rotifers produced in commercial hatcheries can mainly be explained by the static culture a procedure (batch cultures) in which water quality is degrading rapidly (Suantika et al., 2001, 2003).

Moreover, water quality was a first prerequisite for healthy rotifer cultures but no attention was made to the hygienic quality of rotifers (Yoshimura et al., 1994). However, it is assumed that rotifers, the first food administered to fish larvae, are the major carriers of bacteria causing poor survival and growth of fish larvae (Munro et al., 1994). For this reason, the reduction of bacteria and/or a method of controlling bacterial populations in rotifer cultures need to be considered in rotifer production units.

Suantika et al. (2001) evaluated the use of ozone as a disinfectant in a recirculation rotifer culture system as a tool to improve water quality and reduce the bloom of opportunistic bacteria. In general terms, it can be stated that supplementation of ozone in a closed recirculation system for rotifers considerably improves water quality (the ammonium levels were reduced by 67%, nitrite levels by 85% and nitrate levels by 67%), ensures stable and longer rotifer culture periods and controls bacterial proliferation.

Preliminary work (Tolomei, unpublished data) using ozone as a disinfectant has shown similar positive results, i.e., Artemia appear resilient to ozone, and bacterial levels in Artemia culture water were reduced by 99.9% within minutes of exposure to 4 mg L^{-1} ozone. To confirm these results, Tolomei et al. (2004) assessed the efficacy of various commercial and algal enrichment diets and ozone treatments to

reduce bacterial load in the intestine and on external surfaces of on-grown Artemia. Direct exposure to ozone at 4 mg L^{-1} for 5 min provided further bacterial reduction, resulting in a combined bacterial load reduction of 99.5% without compromising Artemia viability.

Risks

There are two additional concerns with the use of ozone – the first is that bromate and bromoform form when ozone is in contact with seawater and these are regarded as potential cancer forming compounds (Lee et al., 2008). Disinfection by-product (DBP) formation is associated with all disinfectants and oxidants; however, the major DBP of concern when using ozone is bromate (BrO_3^-), a DBP that forms from naturally occurring bromide (Br^-) in raw water (Legube et al., 2004). When sufficient ozone has been transferred to create disinfecting ozone residual concentration at the end of the contact chamber, then that residual will need to be removed before the water reaches aquatic organisms in the culture tanks. Residual ozone can be lethal to fish at concentrations as low as 0.01 mg L^{-1}, but the actual concentration depends upon species and life stage (Summerfelt, 2003). The fact that ozone reacts with bromide (Br^-) and chloride (Cl^-) ions to form oxidants in seawater strongly suggests that direct extrapolation of the results obtained in freshwater to applications in seawater is dangerous (Sugita at al., 1996).

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 (Bullock et al., 1997). Ozone is reported to be toxic to a wide range of fresh and salt-water organisms at residual concentrations between 0.01 mg L^{-1} and 0.1 mg L^{-1}. When deciding where to introduce ozone the effect of residual concentrations from the reactor on either the biofilter or fish stocks should be carefully considered. Direct treatment of the culture tank is not recommended. This method carries a high risk of exposing fish stocks to residual ozone concentrations (Read, 2008).

Residual oxidants vs. RAS health. Ozonation of estuarine (brackish) or marine waters can produce different by-products oxidants and significant amount of TRO which is highly toxic for aquatic organisms, i.e., bromate, bromoform, etc. (Holmes-Farley, 2006). Ozone reacts with bromide and chloride ions in saltwater to produce relatively stable oxidants that are toxic to aquatic organisms. Use of ozone in saltwater systems is usually restricted to batch treatment of water separate to the main recirculating flow.

The maximum safe level of chronic ozone exposure for salmonids is 0.002 mg L^{-1} (Wedemeyer et al., 1979a, 1979b). A compilation of results from several studies indicates that most fish exposed to ozone concentrations greater than 0.008–0.06 mg L^{-1} will develop severe gill damage that can result in serum osmolality imbalances and can kill fish immediately or leave them more susceptible to microbial infections (Bullock et al., 1997). Wedemeyer et al. (1979b) reported gill epithelial damage and death of rainbow trout (*Oncorhynchus mykiss*) exposed to 0.0093 mg O_3 L^{-1}.

Study conducted by Bullock et al. (1997), ozone was added to water in a recirculating rainbow trout (*Oncorhynchus mykiss*) culture system just before it entered the culture tanks 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. Rationale for ozone's success at preventing BGD mortalities were not fully understood but may in part be due to improved water quality. Use of the lower ozone dosing rate (25 kg ozone/kg feed) appeared to provide the same benefits as the higher dosing rate (36–39 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.

Recent study of histological evaluations in rainbow trout (*Oncorhynchus mykiss*) conducted by Good et al. (2009) revealed that fish in ozonated systems (RAS received water ozonation to an ORP of 250 mV) were significantly more likely to exhibit subclinical gill pathology (epithelial hyperplasia and hypertrophy) as well as hepatic lipidosis. The findings of this study indicate that, despite an increase in specific subclinical pathologies, water ozonation in low-exchange RAS can improve water quality and overall rainbow trout performance as they are reared to market size.

As discussed previously (ORP results, Figure 3) oxidation-reduction reactions occur during the disinfection process (after ozonation), and the residual oxidants remained after ozonation should be considered for aquatic organisms in RAS. According to Cooper et al. (2002) ozone toxicity tests have been conducted for several marine taxa, including microalgae, invertebrates and vertebrates; and analytical measurements taken in most tests were not specific to ozone, but rather are expressed as TRO, or "ozone-produced oxidants". Ozone toxicity is thus most correctly expressed as a function of TRO, rather than O_3 *per se*.

Figure 4 showed final TRO values below to 2 mg L^{-1} in sea water (salinity of 5‰) and zero for Milli-Q water (salinity of 0‰). However, in sea water (salinity of 25‰) the TRO values have remained between 8 and 10 mg.L^{-1}, which could be dangerous to aquatic organisms.

Substantial mortality (i.e., 50–100% mortality) was observed for microalgae, crabs and lobster at concentration from 0.14–1.0 mg L^{-1} of TRO. Ozone toxicity tests with striped bass and white perch were conducted using flow-through test systems to deliver more reliable and consistent ozone exposures. For striped bass, LC50s (i.e., concentration that kills 50 % of the organisms) ranged from 0.06–0.2 mg L^{-1}, depending on the life stage tested and length of exposure. Eggs were the most sensitive life stage when reared in freshwater (LC_{50} = 0.06 mg L^{-1}), but fingerlings were most sensitive in seawater if the test was run for 96 h (LC_{50}

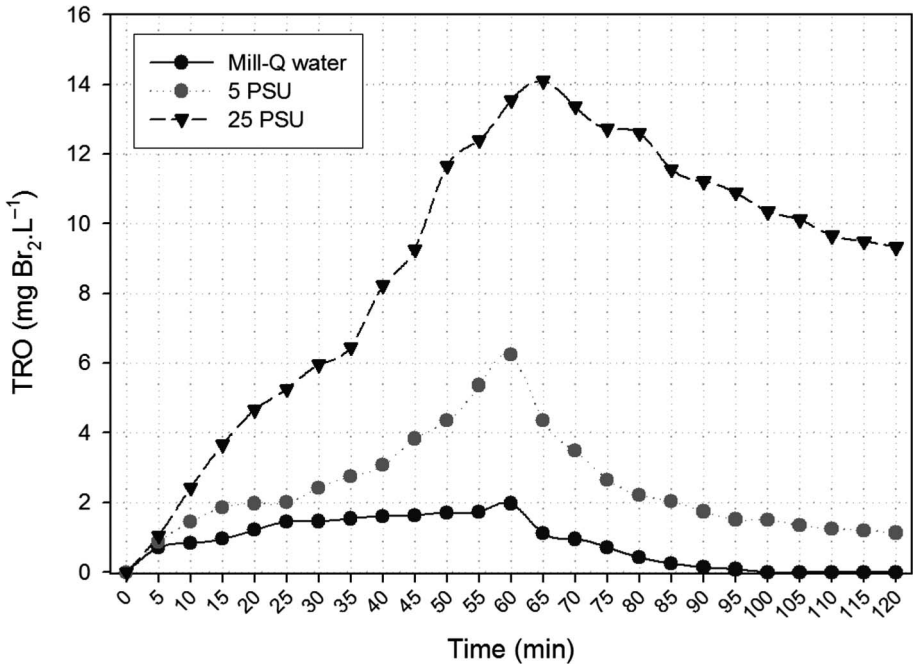

FIGURE 4. TRO measurements during ozonation (60 min) and after stop ozonation: 5 PSU (5 practical salinity units or 5‰), 25 PSU (25 practical salinity units or 25‰).

= 0.08 mg L^{-1}). Slightly higher concentrations (0.15–0.4 mg L^{-1}) induced 100% mortality (i.e., LC_{100}) to striped bass fingerlings. In contrast to striped bass, TRO was slightly less toxic to white perch with LC_{50} values ranging from 0.2–0.38 mg L^{-1}, and an LC_{100} of 0.8 mg L^{-1} after a 6-h exposure (Cooper et al., 2002).

In aquaculture the risk of transmission of fish pathogens via eggs is reduced by disinfection in ozonated seawater, but this treatment may delay or reduce hatching. Disinfection of the egg surface may reduce the negative effects of the microbial load and offer a barrier to the transfer of pathogens both between broodstock and their offspring and between geographical regions. Egg disinfection may thus contribute to a more stable production (Grotmol et al., 2003).

Larvae are, in general, more sensitive to TRO than are eggs (Asbury and Coler, 1980) adults or juveniles (Ozawa et al., 1991). Japanese flounder eggs were found to be impacted by residual oxidants to the extent that 50% did not hatch after 1 min of exposure to 2.2 mg L^{-1} TRO. Larvae aged 3–15 days were killed to the extent of 50% in 24 h at 0.02–0.05 mg L^{-1} TRO. Larvae aged 44 days were killed to the extent of 50% in 24 h at 0.15 mg L^{-1} TRO. In this case, the larvae were shown to have damage to their branchial tissues (Mimura et al., 1998).

The eggs and larvae of Japanese whiting (*Sillago japonica*) also have been tested for toxicity by residual oxidants. In this case, half of the eggs and larvae died in about 24 h when exposed to 0.18 and 0.23 mg L^{-1} TRO, respectively (Isono et al., 1993). The oxidants formed during sea water ozonation may also react with compounds in the eggshell (chorion), altering its functional properties and thus possibly influencing hatchability. Then, Grotmol et al. (2003) investigated the effects of disinfection with ozonated seawater on the hatchability of eggs of Atlantic cod, turbot and Atlantic halibut. Two milligrams O_3 per liter for 2 min and lower exposures ought to be sufficient to ensure an excess of oxidants for efficient inactivation of fish pathogens while avoiding negative effects on the hatchability of halibut, cod and turbot eggs.

Toxicity tests of residual oxidants on shrimp show them to be less sensitive than fish. *Penaeus chinensis* and *Paralichthys olivaceus* were found to live up to 48 h at TRO concentrations of more than 1 mg L^{-1}, while *Bastard halibut* (fish) in the same study lived only 3 h at 1 mg L^{-1} and 48 hours at 0.13 mg L^{-1} (Jiang et al., 2001).

The effect of residual oxidants on rotifers (*Brachionus plicatilis*) has also been determined (Davis and Arnold, 1997). No effect on survival was seen at less than 0.22 mg L^{-1}, but effects became significant above that level. The authors point out those bacteria and other pathogens can be killed at that level, so rotifer cultures can be used with that amount of continuous ozone to reduce bacterial contamination.

Leynen et al. (1998) investigate the possible adverse impact of discharged dissolved ozone in terms the acute toxicity to fish larvae (*Cyprinus carpio*, *Leuciscus idus* and *Clarias gariepinus*) and *Daphnia magna*. Results indicate that ozone is very toxic to aquatic organisms. Neonates of *D. magna* are

more susceptible to ozone than are fish larvae. No major difference in toxicity of ozone for daphnids was observed at the different test temperatures, and all three species of fish larvae are similarly sensitive to ozone. The 48-h LC_{50} for fish larvae *C. carpio* (at 27 °C), *L. idus* (at 27 °C), and *C. gariepinus* (at 32 °C) ranges between 30 and 45 $\mu g\ L^{-1}$, and the 48-h No Observed Effect Concentration (NOEC) for *D. magna* is 11 $\mu g\ L^{-1}$ at 21 °C and 16 $\mu g\ L^{-1}$ at a test temperature of 27 °C.

Bromate and bromoform formation (BrO_3 vs. ozonation time). In recent years, bromate has become known as a contaminant of potable water supplies and in aquariums due to its formation from naturally occurring bromide during ozonation. Evidence supports the view that bromate is a possible human carcinogen and is therefore strictly controlled in drinking water (Krasner et al., 1993b; Weinberg et al., 1993; Bull and Cotruvo, 2006). Ozonation has become increasingly important in water treatment across the world as an oxidizing agent and disinfectant due to its strong oxidation potential (von Gunten, 2003a, 2003b; Jarvis et al., 2007).

Disinfection by-product (DBP) formation is associated with all disinfectants and oxidants (Sohn et al., 2004; Jarvis et al., 2007); however, the major DBP of concern when using ozone is bromate (BrO_3^-), a DBP that forms from naturally occurring bromide (Br^-) in raw water (Legube et al., 2004). Toxicity testing on experimental animals has consistently shown bromate to induce cancer in rats, mice and hamsters through damage to genetic material (Chipman et al., 1998; Bull and Cotruvo, 2006).

The bromide ion (Br^-) is defined as an inorganic ion found in surface water and ground water and caused by (i) sea intrusion, (ii) the impact of connate, or (iii) industrial and oil-field brine discharge (Symons, 1999). When oxidized by chlorine (Cl_2 or HOCl) or ozone (O_3), it can result in the formation of organic and inorganic bromine-substituted disinfection by-products.

The bromate ion (BrO_3^-) is the highest oxidation state of the bromide ion. The bromate ion can be formed during the ozonation of bromide-containing waters (Symons, 1999). Bromate is formed through a complex web of pathways with several bromine-containing intermediates that undergo reactions with both molecular ozone and hydroxyl radicals (Glaze and Weinberg, 1999). Ozone oxidizes bromide to form hypobromite ion (OBr^-). Hypobromite continues to be oxidized to form bromate or to form an unidentified species, possibly BrO_2^- that regenerates bromide ion (Glaze and Weinberg, 1999).

Brominated by-product formation in ozonated waters is influenced by bromide ion concentration, the source and concentration of natural organic matter (NOM), pH, ozone dose, and reaction time. It is important to note that ozonation under higher pH conditions produces higher bromate concentrations, such that with sufficient bromide and ozone applied to meet an ozone residual for disinfection; tens of micrograms per liter of bromate can be formed (Faust and Aly, 1998).

Ozone reacts with bromide ions in brackish and seawater systems to form the oxidants hypobromous acid (HOBr) and hypobromite ion (OBr^-), which is relatively stable and toxic to fish and shellfish (Blogoslawski and Perez, 1992; Keaffaber et al., 1992). Prolonged ozonation can further oxidize hypobromite ion to bromate (BrO_3^-), which is another persistent and toxic compound (Marhaba and Bengraïne, 2003; Jarvis et al., 2007).

The bromate ion cannot be further oxidized and will be the final product of the oxidation of bromide ion in seawater. Bromate ion is a stable compound and not acutely toxic to aquatic animals (Marhaba and Bengraïne, 2003; Liltved et al., 2006; Jarvis et al., 2007). Unfortunately, the production conditions and toxicity towards aquatic animals of these ozonation by-products are not well understood (Summerfelt, 2003).

Bromate formation has been the major barrier in the use of ozone for water treatment where the source water contains bromide, particularly given the challenging targets set for the maximum allowable bromate concentration (Magazinovic et al., 2004). The formation of bromate during ozonation is strongly dependent on the characteristics of the water to be treated and the amount of ozone contacting the water. The following are important variables for bromate formation: bromide concentration, pH, applied ozone concentration and contact time, DOC concentration, alkalinity, ammonia concentration, and temperature (Marhaba and Bengraïne, 2003; Sohn et al., 2004; Jarvis et al., 2007).

The most stable by-products of seawater ozonation typically are bromate ion and bromoform and both may persist long after ozone treatment is terminated (Cooper et al., 2002). However, the limited available toxicity data set suggests that these compounds are not acutely toxic with LC_{50} values 1–2 orders of magnitude higher than either TRO or bromine.

The data for the bromate and bromoform in the three experiments are presented in Figure 5. For sea water (25‰) the concentration of bromate and bromoform increased with the time of ozonation and remained at higher levels. For lower water salinity (5‰) the bromate formation was lower and bromoform formation increase slowly during ozonation time and remained constant to the end of the experiment (lower than the salinity of 25‰). These results showed the importance of the control of by-product formation during the ozonation for aquatic organisms, and these results should be considered for others studies and/or applications in RAS.

The most sensitive species to bromate ion is the mysid shrimp *Neomysis awatschensis* with an acute LC_{50} of 176 mg bromate ion L^{-1}, and the most sensitive species to bromoform is the sheepshead minnow with 96-h LC_{50} values ranging from 7.1–18 mg bromoform L^{-1}. Therefore, even if bromate ion and/or bromoform are produced as by-products of seawater ozonation, they are not likely to be of toxicological concern (Cooper et al., 2002).

Bromate formation and control has been the focus of intensive research efforts since the early 1990s when bromate

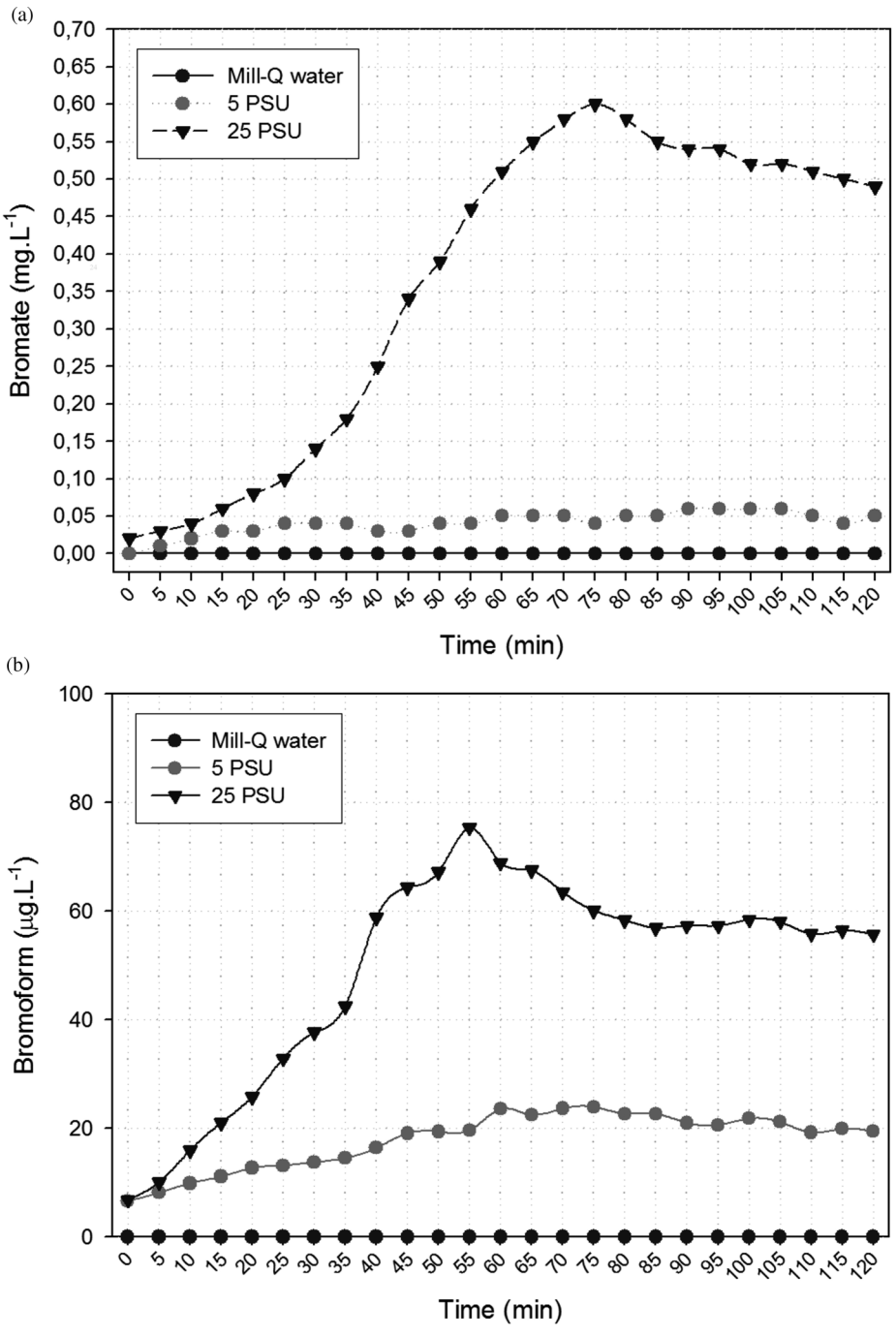

FIGURE 5. Bromate (BrO_3^-) and Bromoform ($CHBr_3$) measurements during ozonation (60 min) and after stop ozonation: 5 PSU (5 practical salinity units or 5‰), 25 PSU (25 practical salinity units or 25‰).

(BrO_3^-) was implicated as a potential carcinogen (Buffle et al., 2004). Whilst there is no data demonstrating that bromate is carcinogenic to humans, it is plausible to assume that the mechanisms resulting in tumor formation in laboratory animals could occur in humans. For this reason the World Health Organization (WHO) has set a provisional guideline concentration of 10 $\mu g\ L^{-1}$ (0.01 $mg\ L^{-1}$) bromate in drinking water (WHO, 2004). European Union law specifies that all member states must enforce a maximum bromate concentration of 10 $\mu g\ L^{-1}$ by 2008 (European Drinking Water Directive, 1998). In the United Kingdom, the legislation enforcing this standard came into effect in 2003. In the United

States (United States Environmental Protection Agency), regulations also specify a maximum value of 10 μg L^{-1} (USEPA, 1998).

Few studies have examined the toxicity of excess bromate itself to marine organisms (Hutchinson and van Wijk, 1998). Bromate is classified as carcinogenic to human health by the IARC (International Agency for the Research on Cancer) and the USEPA (United States Environmental Protection Agency) and is a known toxin to fish and other aquatic life, causing respiratory and osmoregulatory dysfunction (Grguric et al., 1994). It is also probably toxic to crustaceans such as phyllosoma larvae (Ritar et al., 2006).

Bromate toxicity tests on marine animals indicate the levels of bromate produced by chlorination or ozonation of power plant cooling waters are not acutely toxic. The LC_{50} ranged from 30 mg L^{-1} bromate for Pacific oyster (*Crassostrea gigas*) larva to several hundred mg L^{-1} for fish, shrimp and clams (Lugo-Fernandez and Roscigno, 1999). Toxicity studies carried by Burton and Richardson (1981) showed that the concentrations of bromate which theoretically could be formed in an ozonated discharge were not toxic to the early life stages of striped bass (*Morone saxatilis*) and juvenile spot (*Leiostomus xanthurus*).

An individual study showed that Pacific oysters (*Crassostrea gigas*) had abnormal larval development at bromate levels of 30–300 mg L^{-1} (Hirtle and Mann, 1978). Fertilized eggs of the oyster *Crassostrea virginica* were killed at 1 mg L^{-1} (Stewart et al., 1979). The clams *Protothaca staminea* (littleneck) and *Macoma inquinata* (bent-nosed) were killed by 880 mg L^{-1} (Hirtle and Mann, 1978).

The marine dinoflagellate *Glenodinium halli* showed changes in population growth at 16 mg L^{-1}; the marine microalgae *Isochrysis galbana* showed changes in population growth at 8 mg L^{-1}; the marine diatom (*Skeletonema costatum*) showed changes in population growth at 0.125 to 16 mg L^{-1}; and the marine diatom *Thalassiosira pseudonana* showed changes in population growth at 16 mg L^{-1} (Erickson and Freeman 1978). The salmon *Oncorhynchus keta* was killed at 500 mg L^{-1} and the perch *Cymatogaster aggregata* at 880 mg L^{-1} and shrimps (*Pandalus danae* and *Neomysis awatschensis*) were killed at 880 and 176 mg L^{-1}, respectively (Hirtle and Mann, 1978).

Although there are limited data for several important taxonomic groups, the majority of available data suggest that bromate is non-toxic to many aquatic organisms with $E(L)C_{50}$ values being generally greater than 100 mg BrO_3^- L^{-1}. Although the toxicity of bromate has been addressed using a relatively wide range of organisms (4 algae, 8 invertebrates, and 4 fish species), the most sensitive organisms appear to be marine fish larvae, with a 96-h LC_{50} of 31 mg BrO_3^- L^{-1} (Richardson et al., 1981b). For toxicity data from this range of aquatic species, it is customary to apply a factor of 10 to extrapolate from an acute to a safe chronic level of a given substance. On this basis, the most conservative data available (marine fish larvae) suggest that to protect aquatic organisms from long-term adverse effects, surface water concentrations should not exceed approximately 3.0 mg BrO_3^- L^{-1} (Hutchinson et al., 1997).

Removal of disinfection by-products (DBP) residuals. The minimization of DBPs can be approached differently. First, it would be logical to remove the precursors and second, to control the BrO_3^- formation (Grecelius, 1977; Richardson et al., 1981a; Wajon and Morris, 1982; Haag and Hoigné, 1983, 1984; Krasner et al., 1991; Siddiqui and Amy, 1993; von Gunten and Hoigné, 1992, 1994; von Gunten et al., 1993). This can be done by pH depression, ammonia addition, hydrogen peroxide addition, or modifications to ozone contactor design and operation. However, these strategies are not effective especially in the presence of natural organic matter (NOM). The alternative would be to eliminate BrO_3^- once formed (Marhaba and Bengraïne, 2003).

Legube et al. (1995) showed that pH, Br^- concentration, and O_3 dosage control the formation of BrO_3^-. Higher pH and temperature favor the formation of BrO_3^- due to the decrease in the pKa of HOBr and OBr^-. Therefore, O_3 concentration should be kept low and contact time extended (Marhaba and Medlar, 1993). Krasner et al. (1993b) concluded that the appropriate staging of O_3 through two or three chambers has the potential to minimize O_3 residual and BrO_3^- formation while still meeting the CT criterion. Further, as the pH of ozonation was lowered, the O_3 dosage necessary to meet the CT criterion dropped and less BrO_3^- was produced (Krasner et al., 1993a).

Due to the acute toxicity of residual ozone and others oxidants (i.e., bromine) to aquatic organisms, a treatment unit for removal of residual bromine has to be included when seawater is ozonated in aquacultural systems. Activated carbon filtration, addition of a reducing agent, UV radiation, or passing through a sand filter or biofilter or by air stripping prior to fish cultural systems or discharge to surface waters will reduce or eliminate these residues (Liltved et al., 1995; Summerfelt, 2003; Summerfelt et al., 2004; Liltved et al., 2006; Read, 2008).

Summerfelt et al. (2004) determined the ultraviolet (UV) irradiation dosages required o destroy dissolved ozone in a commercial-scale recirculating salmonid culture system operated at a constant 13–15 °C. The results showed that dissolved O_3 removal across the UV irradiation unit could be modeled using first-order kinetics and was dependent upon the inlet O_3 concentration and the retention time within the irradiation chamber. At a temperature of 13–15 °C, UV irradiation doses of 80.4±2.6 mW s cm^{-2} and 153.3±2.1 mW s cm^{-2} consistently removed 100% of the dissolved O_3 when the inlet O_3 concentration was ≤0.30 mg L^{-1}.

Sharrer and Summerfelt (2007) assessed the degree of total heterotrophic and total coliform bacteria inactivation using ozone alone (at several ozone dosages) and to determine if a synergistic effect is seen in the disinfection of microorganisms from process water in a fully recirculating fish culture system when UV irradiation is applied directly after ozonation. Combining ozone dosages of only 0.1–0.2 min mg L^{-1}

with a UV irradiation dosage of ≥50 mJ cm^{-2} provides an advanced oxidation process that could consistently produce post-treatment water nearly free from total coliform and total heterotrophic bacteria colony forming units.

CONCLUSION

Ozone is a powerful oxidizing agent that has seen wide use in aquaculture applications for achieving both disinfection and water quality improvements. Ozone is added to aquaculture system waters to inactivate fish pathogens, oxidize organic wastes (including color) and nitrite, or supplement the effectiveness of other water treatment units like UV light, filters and biofilters.

Care must be used when determining the effective ozone dose that must be supplied to achieve disinfection. Certain pathogens may require much higher ozone *c* x *t* values in order to achieve inactivation. Applying ozone to disinfect aquaculture system influents or effluents can be quite complex and costly, yet disinfection is still necessary in many situations to control pathogen introduction.

For maximum benefit from ozonation, the user have to first know the purpose of applying ozone so as to determine the correct techniques and ozone dosages. It is critical that all ozone disinfection systems are pilot tested and calibrated prior to installation to ensure the true output and concentration.

Further researches are required into the potential disinfectant-by-product risks associated with ozone and their impacts on aquatic health.

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LIST OF ABBREVIATIONS

AHNV	Atlantic Halibut Nodavirus
AOP	Advanced Oxidation Process
BGD	Bacterial Gill Disease
BOD_5	Biological Oxygen Demand
Br^-	Bromide ion
BrO_3^-	Bromate ions
COD	Chemical Oxygen Demand
CT	value ozone concentration (C), exposure time (T)
DBP	Disinfection By-Product
DOC	Dissolved Organic Carbon
DPD	N,N-diethyl-p-phenylenediamine
FE	Fish Encephalopathy
GEOSMIN	Trans-1,10-dimethyl-trans-9-decalol
HAV	Hepatitis A Virus
HEV	Hepatitis E Virus
HOBr	Hypobromous acid
HPLC	High Performance Liquid Chromatography
IPNV	Infectious Pancreatic Necrosis Virus
ISAV	Infectious Salmon Anaemia Virus
LC_{100}	100% lethal concentration
LC_{50}	50% lethal concentration
MIB	2-methylisoborneol
NLV	Norwalk Like Virus
NOM	Natural Organic Matter
NSP	Neurotoxic Shellfish Poisoning
OBP	Ozonation By-Products
OBr^-	Hypobromite ion
OPO	Ozone Produced Oxidants
ORP	Oxidation/Reduction Potential
RAS	Recirculating Aquaculture System
ROC	Residual Ozone Concentration
SJNNV	Striped Jack Nervous Necrosis Virus
TAN	Total Ammonia Nitrogen
TOC	Total Organic Carbon
TRO	Total Residual Oxidants
TSS	Total Suspended Solids
TSV	Taura Syndrome Virus
USEPA	U.S. Environmental Protection Agency
VER	Viral Encephalopathy and Retinopathy
VNN	Viral Nervous Necrosis
WHO	World Health Organization
WSBV	White Spot Syndrome Bacilovirus
WSSV	White Spot Syndrome Virus
YHV	Yellow Head Virus

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