

Review

Can ozone be used to control the spread of freshwater Aquatic Invasive Species?

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Abstract

The introduction of aquatic invasive species to non-native habitats can cause negative ecological effects and also billions of dollars in economic damage to governments and private industries. Once aquatic invasive species are introduced, eradication may be difficult without adversely affecting native species and habitats, urging resource managers to find preventative methods to protect non-invaded areas. The use of ozone (O₃) as a non-physical barrier has shown promise as it is lethal to a wide range of aquatic taxa, requires a short contact time, and is relatively environmentally safe in aquatic systems when compared to other chemicals. However, before O₃ can be considered as an approach to prevent the spread of aquatic invasive species, its effects on non-target organisms and already established aquatic invasive species must be fully evaluated. A review of the current literature was conducted to summarize data regarding the effects of O₃ on aquatic taxa including fish, macroinvertebrates, zooplankton, phytoplankton, microbes, and pathogens. In addition, we assessed the practicality of ozone applications to control the movement of aquatic invasive species, and identified data gaps concerning the use of O₃ as a non-physical barrier in field applications.

Key words: dispersal, lethality, mortality, non-physical barriers

Introduction

Aquatic invasive species (AIS) are a major concern to resource managers and policy makers as they cause negative environmental and economic consequences to invaded habitats (Leung et al. 2002; Lodge et al. 2006; Bellard et al. 2016; Gallardo et al. 2015; Sala et al. 2000). AIS contribute to the estimated U.S. \$120 billion that invasive species cause annually in damages (Pimentel et al. 2005; Lodge et al. 2006), and can contribute to reductions in species abundances (e.g., Gallardo et al. 2015), biodiversity (e.g., Sala et al. 2000), and ecosystem services (e.g., Pejchar and Mooney 2009). Once established, AIS are difficult to eradicate because of their unique biological characteristics, which include high fecundity, a wide tolerance to environmental factors, and efficient resource use (Kolar and Lodge 2002; Ricciardi and MacIsaac 2011). In addition, aquatic habitats, specifically rivers and streams, are highly connected,

meaning that newly introduced species can broaden their range with little geographical resistance. These factors have motivated scientists to develop methods to prevent AIS dispersal within these systems (Leung et al. 2002; Lodge et al. 2006; Finnoff et al. 2007).

Reducing the connectivity between invaded and non-invaded water bodies by constructing permanent physical barriers is an obvious way to limit the distribution of AIS. However, it is often not feasible to construct permanent physical barriers due to the negative economic impacts it may have on commercial and recreational riverine transportation, as well as the disruption it may cause to the ecological functions of that area (Schwieterman 2010; Larinier 2001). With this in mind, the development of non-physical barriers are of great interest to resource managers to control the spread of AIS (Noatch and Suski 2012).

Non-physical strategies include piscicides, electricity, pulse pressure actuators, pheromones,

attractants, acoustic deterrents, strobe lights, dissolved gases, and bubble screens (Noatch and Suski 2012; Vetter et al. 2015; Romine et al. 2015; Zielinski et al. 2014). These methods largely control the movements of invasive fish and some have already been implemented in certain U.S. waterways. For instance, electric barriers are used in the Chicago Area Waterway System to prevent the transport of invasive species from the Mississippi River Basin to the Great Lakes region (Moy et al. 2011). Although many non-physical barrier methods have had limited success, most do little to prevent the spread of AIS taxa other than fish (Noatch and Suski 2012). This is a growing concern, as the U.S. Nonindigenous Aquatic Species (NAS) database has registered 1121 aquatic species as of January 2016 (Fuller 2016), including species of plants, amphibians, mollusks, crustaceans, and reptiles. There is an urgent need to develop novel ways to control all invasive species and taxa while still limiting the negative impacts for non-target species and habitats (Noatch and Suski 2012).

A recent literature review identified multiple chemicals as possible candidates for better AIS prevention (Hubert et al. 2016). Of these, dissolved ozone (O_3) has shown promise to be effective against a range of aquatic taxa. Ozone is highly lethal, requires a short contact time, has a short half-life within freshwater water, and has a minimal impact on the environment when compared to other chemicals (Hubert et al. 2016). In addition, the FDA classifies O_3 as GRAS (Generally Recognized As Safe) for drinking water applications and O_3 is used routinely in wastewater treatment and aquaculture facilities.

Ozone toxicity has been assessed in several studies (Gonçalves and Gagnon 2011; Summerfelt et al. 2008), but few synthesis papers look specifically at the effects of O_3 on freshwater biota or how practical its use would be in a large-scale application. The purpose of this synthesis, therefore, is to review the studies that have explored ozone's effect on aquatic biota, and to assess whether ozone may be used to limit the spread of AIS, either as a stand-alone approach or in tandem with other control and removal technologies. Using the database Web of Science™, a literature review was performed relating the search terms ozone, dissolved O_3 , and ozonation to a range of aquatic taxa including fish, invertebrates, zooplankton, phytoplankton, microbes, and fish pathogens. After the literature search was complete, titles and abstracts were screened for studies that described the direct effects of ozone on aquatic taxa in both freshwater and marine environments. This resulted in 45 papers that are referenced in this synthesis, the majority of which were produced from research in the aquaculture and shipping industries.

Ozone chemistry in freshwater

Ozone (O_3) is an inorganic, gaseous molecule, and a highly reactive oxidizer (Oakes et al. 1979). Dissolved O_3 neutralizes bacteria, oxidizes metals and organics, removes foul tastes and odors from water, and kills pathogens (Summerfelt and Hochheimer 1997). Ozone can be generated using several techniques, but most commercially available generators use high-voltage corona discharge that produces O_3 by creating an electric potential between two surfaces through which air passes. When air moves past this electric potential, O_2 molecules energize, resulting in the formation of O_3 . After its formation, O_3 can be efficiently dissolved into water either by a simple injection system (e.g., O_3 added through a ceramic diffuser), or by a more complex method (e.g., Venturi injector). Regardless of injection technique, the most efficient way to mass transfer O_3 into water is to create an O_3 -liquid mix with high surface area to volume ratio (i.e., many tiny bubbles). As is the case with other dissolved gases, the amount of O_3 that can be dissolved in a liquid follows Henry's law, and is proportional to the partial pressure of the gas. In addition, the saturation concentration is a function of temperature, pressure, pH, ionic strength of the liquid, and the amount of O_3 generated. Therefore, the maximum concentration of O_3 in liquid varies widely based on environmental conditions, as does the amount of O_3 needed to maintain concentrations. Ozone decomposition forms O_2 and hydroxyl radicals ($\cdot OH$), and the rate of O_3 decomposition is fastest at warm temperatures, in water with high pH (basic), at high carbonate and dissolved solid concentrations, and at high organic loads.

Ozone chemistry in Seawater

Although this review will focus on the use of ozone in freshwater, the degradation of ozone in seawater must also be introduced, as a sizeable portion of the literature describes the effect of ozone on marine species. This section will briefly describe the major chemical changes ozone undergoes in seawater. See Hoigné (1998) for an in-depth description of these processes.

When ozone is added into seawater, it will react rapidly with bromide to form hypobromous acid, hypobromite, bromate (a carcinogen), and other brominated compounds which may become sources of disinfectants (Perrins et al. 2006). Within this reaction, hypobromous acid and hypobromite are held in equilibrium at a pH of 8.8, but hypobromous acid will dominate should the pH fall lower than this. If this were to happen, hypobromous acid will become

the main disinfectant; able to persist within the water column for an extended period of time (Perrins et al. 2006; Gonçalves and Gagnon 2011). If nitrogenous compounds are also present, they will react with hypobromous acid and hypobromite to form monobromamine and dibromamine (Herwig et al. 2006). These chemicals can remain in the seawater or be broken back down to bromine by O₃. In all, the reaction rates and retention times of all the compounds listed will depend on water quality and the organic material present.

It should be stated that brominated compounds are the major toxic constituents to marine life when ozone is added into seawater. The majority of marine studies listed in this review have measured the amount of ozone added into seawater and not the amount of bromine compounds produced. Those that do identify the amount of bromine compounds produced, however, will have their concentrations listed as Total Residual Oxidants (TRO). Also, the delineation between studies using ozone in freshwater vs. seawater used in this review can be identified in Tables 1–7.

Biological effects of ozone

Research on the effects of O₃ to aquatic species can be grouped into three categories: 1) aquaculture sterilization (e.g., Gonçalves and Gagnon 2011); 2) ship ballast water disinfection (e.g., Herwig et al. 2006); and, 3) wastewater effluent treatment (e.g., Magdeburg et al. 2012). The following review focuses on literature describing ozone's use in aquaculture and ballast water treatments, and will show examples of ozone's effects on fish and fish life stages, zooplankton, phytoplankton, microbes, and fish pathogens (Tables 1–7). Wastewater effluent treatment will not be covered in this paper, unless otherwise indicated, as few studies address the direct toxicity of ozone to aquatic taxa.

1. Aquaculture

Beneficial effects

Ozone treatment has been used increase the health of economically important species (Summerfelt and Hochheimer 1997), such as fish, mussels, and lobsters. In various fish life stages, low doses of ozonation can reduce bacterial, viral, and fungal infections (Summerfelt and Hochheimer 1997; Tables 1 and 2). For instance, Powell et al. (2015) indicated an increased survival of turbot (*Psetta maxima* (Linnaeus,

1758)) exposed to low levels of O₃ (360 mV oxidation reduction potential (ORP)), when applied concurrently with antibiotic treatments to reduce bacterial loads. Li et al. (2015) demonstrated that the bacterium *Vibrio anguillarum* (Bergeman, 1909) could be reduced in the culture of European seabass (*Dicentrarchus labrax* (Linnaeus, 1758)) with exposures to O₃ (240–270 mV ORP), but also found that higher levels of O₃ (300–320 mV ORP) decreased both the feed intake and growth rate. The fungus saprolegniasis can be removed from brown trout eggs (*Salmo trutta* (Linnaeus, 1758)) treated with varying concentrations of O₃ (Forneris et al. 2003; Benoit and Matlin 1966). Buchan et al. (2006) reduced piscine nodavirus (not included in the International Committee on Taxonomy of Viruses (ICTV) Master Species List (2015)) infections in haddock (*Melanogrammus aeglefinus* (Linnaeus, 1758)) eggs treated with 3 mgL⁻¹ TRO for 3.3–6.7 min, and Ghomi et al. (2007) decreased fungal infections on sturgeon eggs (*Acipenser persicus* (Borodin, 1897)) with 0.15 mgL⁻¹ of O₃ while maintaining a 76.4% hatching success.

Low doses of ozonation are of value in culturing the European lobster (*Homarus gammarus* (Linnaeus, 1758)) (Table 3). Scolding et al. (2012) observed that O₃ significantly improved larval lobster survival, yet prolonged O₃ exposures resulted in a decline in larval weight and length. In addition, ozone is effective against fish-specific pathogens (Table 7). Wedemeyer and Nelson (1977) inactivated two bacterial pathogens (*Aeromonas salmonicida* (Lehmann and Neumann, 1896) and *Yersinia ruckeri* (Ewing et al., 1978)) with concentrations of 0.01 mgL⁻¹ O₃ for 10 min, and 0.01 mgL⁻¹ O₃ for 0.5 min, respectively. The Infectious Hematopoietic Necrosis Virus (IHNV) (ICTV Master Species List (2015): <https://talk.ictvonline.org/files/master-species-lists/m/msl>, Taxonomic History: http://ictvonline.org/taxonomyHistory.asp?taxnode_id=20151109) was inactivated with 0.01 mgL⁻¹ O₃ in 0.5 min and the Infectious Pancreatic Necrosis Virus (IPNV) (ICTV Master Species List (2015): <https://talk.ictvonline.org/files/master-species-lists/m/msl>, Taxonomic History: http://ictvonline.org/taxonomyHistory.asp?taxnode_id=20152049) could be inactivated with 0.01 mgL⁻¹ O₃ in 1 min (Wedemeyer et al. 1978). Other viruses destroyed by O₃ exposure include the Atlantic Halibut Nodavirus (AHNV) (not included in ICTV Master Species List (2015)), Infectious Salmon Anemia Virus (ISAV) (ICTV Master Species List (2015): <https://talk.ictvonline.org/files/master-species-lists/m/msl>, Taxonomic History: http://ictvonline.org/taxonomyHistory.asp?taxnode_id=20153233), and IPNV. Higher concentrations of O₃ were required for inactivation (Table 7; Liltved et al. 2006).

Table 1. Summary of selected studies on adult and larval fish exposed to ozone.

Study	Species	Endpoint	Concentration	Exposure Duration	Water Type
Coler and Asbury 1980	yellow perch larvae (<i>Perca flavescens</i> (Mitchill, 1814))	LC ₉₉	1.2 mgL ⁻¹ O ₃	0.5 min	Freshwater
	fathead minnow larvae (<i>Pimephales promelas</i> (Rafinesque, 1820))	LC ₅₀	< 0.1 mgL ⁻¹ O ₃	0.5 min	Freshwater
	bluegill larvae (<i>Lepomis macrochirus</i> (Rafinesque, 1819))	LC ₅₀	0.13–0.17 mgL ⁻¹ O ₃	0.25 min	Freshwater
da Costa et al. 2014	Zebrafish (<i>Danio rerio</i> (Hamilton, 1882))	80% mortality	1.44 mgL ⁻¹ O ₃	48 h	Freshwater
Fukunaga et al. 1992	Japanese charr (<i>Salvelinus leucomaenis</i> (Pallas, 1814))	100% mortality	0.7 mgL ⁻¹ O ₃	30 min	Freshwater
Hall et al. 1981	striped bass larvae (<i>Morone saxatilis</i> (Walbaum, 1792))	LC ₅₀	0.08 mgL ⁻¹ OPO	96 h	Seawater
Li et al. 2015	European seabass (<i>Dicentrarchus labrax</i> (Linnaeus, 1758))	18% mortality	300–320 mV ORP	7 days	Seawater
Jones et al. 2006	topsmelt larvae (<i>Atherinops affinis</i> (Ayres, 1860))	LC ₉₅	>0.9 mgL ⁻¹ TRO as Br ₂	0.5 h	Seawater
	sheepshead minnow juvenile (<i>Cyprinodon variegatus</i> (Lacepède, 1803))	LC ₉₅	> 0.42 mgL ⁻¹ TRO as Br ₂	0.5 h	Seawater
Leynen et al. 1998	ide (<i>Leuciscus idus</i> (Linnaeus, 1758))	LC ₅₀	0.036 mgL ⁻¹ O ₃	48 h	Freshwater
	common carp (<i>Cyprinus carpio</i> (Linnaeus, 1758))	LC ₅₀	0.031 mgL ⁻¹ O ₃	48 h	Freshwater
	African catfish (<i>Clarias gariepinus</i> (Burchell, 1822))	LC ₅₀	0.035 mgL ⁻¹ O ₃	48 h	Freshwater
Powell et al. 2015	turbot (<i>Psetta maxima</i> (Linnaeus, 1758))	~87% survivability	360 mV ORP	91 days	Seawater
Richardson and Burton 1981	Atlantic menhaden juvenile (<i>Brevoortia tyrannus</i> (Latrobe, 1802))	~25% mortality	0.3 mgL ⁻¹ OPO	4 days	Seawater
Richardson et al. 1983	white perch (<i>Morone americana</i> (Gmelin, 1789))	LC ₅₀	0.38 mgL ⁻¹ OPO	24 hour	Freshwater
Wedemeyer et al. 1979	rainbow trout (<i>Oncorhynchus mykiss</i> (Walbaum, 1792))	LC ₅₀	0.0093 mgL ⁻¹ O ₃	96 hour	Freshwater

Adverse effects

Ozone is injurious to the peripheral tissues in adult and larval fish, and can cause gill lamellar clubbing, hypertrophy, and necrosis (Jones et al. 2006; Leynen et al. 1998; Coler and Asbury 1980; Reiser et al. 2011; Paller and Heidinger 1980; Richardson et al. 1983). Japanese char (*Salvelinus leucomaenis* (Pallas, 1814)) succumbed to 0.7 mgL⁻¹ O₃ in 30 min due to gill lamella degeneration (Fukunaga et al. 1992). Similar results were seen in Pacific white shrimp (*Litopenaeus vannamei* (Boone, 1931)) exposed to 0.84 mgL⁻¹ OPO (Ozone-Produced Oxidants) in 24 hours (Schroeder et al. 2010). In addition to injury to peripheral tissues, exposure to high levels of O₃ (0.7 mgL⁻¹) impairs the oxygen binding capabilities of the red blood cells (RBC) (Wedemeyer et al. 1979), and may cause RBC lysis (Fukunaga et al. 1992).

Several reports on the adverse effects of ozone treatment to fish eggs were observed. Battaglione and Morehead (2006) found 95% mortality in striped trumpeter (*Latris lineata* (Forster in Bloch and

Schneider, 1801)) embryos exposed to 5 mgL⁻¹ of O₃ for 5 min (Table 2). Atlantic cod (*Gadus morhua* (Linnaeus, 1758)) and halibut eggs (*Hippoglossus hippoglossus* (Linnaeus, 1758)) exposed to 2.2 mgL⁻¹ O₃ for ≥3 min had only a 20% hatching success, but these same concentrations did not decrease turbot egg hatching success (Grotmol et al. 2003).

Dissolved ozone concentrations, ranging from 0.3–2 mgL⁻¹, are harmful to mussels, clams, lobsters, and shrimp (Table 3). Research on zebra mussel (*Dreissena polymorpha* (Pallas, 1771)) biofouling found that 100% mortality was achieved with 1.0 mgL⁻¹ O₃ after 5 h of continuous exposure (Van Benschoten et al. 1993). In the American oyster (*Crassostrea virginica* (Gmelin, 1791); straight-hinged larval life stage), 100% mortality was reached after 96 hour exposures to 0.3 mgL⁻¹ OPO (Richardson et al. 1982). These authors also revealed that O₃ reduced the shell growth in adult oysters, but caused minimal mortality to this life stage. Finally, exposure of European Lobster (*Homarus gammarus* (Linnaeus, 1758)) to 320 mV ORP of O₃ for 18 d resulted in a 15% survival after 31 d post treatment (Middlemiss et al. 2015).

Table 2. Summary of selected studies on fish eggs exposed to ozone.

Study	Species	Endpoint	Concentration	Exposure Duration	Water Type
Battaglione and Morhead 2006	striped trumpeter (<i>Latris lineata</i> (Forster in Bloch and Schneider, 1801))	~95% mortality after 5 days	5.0 mgL ⁻¹ O ₃	5 min	Seawater
Benoit and Matlin 1966	rainbow trout (<i>Oncorhynchus mykiss</i> (Walbaum, 1792))	84% of eggs survived to fry	26–65 ppm O ₃ ^a	17 days	Freshwater
Buchan et al. 2006	haddock eggs (<i>Melanogrammus aeglefinus</i> (Linnaeus, 1758))	20% mortality	3.0 mgL ⁻¹ TRO	10 min	Seawater
Cao et al. 2009	Japanese medaka (<i>Oryzias latipes</i> (Temminck and Schlegel, 1846))	52% mortality	4.0 mgL ⁻¹ O ₃	4 d	Freshwater
Coler and Asbury 1980	yellow perch (<i>Perca flavescens</i> (Mitchill, 1814))	LC ₉₉	7.5 mgL ⁻¹ O ₃	20 min	Freshwater
	white sucker (<i>Catostomus commersoni</i> (Lacepède, 1803))	LC ₉₉	11.8 mgL ⁻¹ O ₃	40 mins	Freshwater
	fat head minnow (<i>Pimephales promelas</i> (Rafinesque, 1820))	LC ₉₉	5.2 mgL ⁻¹ O ₃	40 mins	Freshwater
Ghomi et al. 2007	Iranian sturgeon (<i>Acipenser persicus</i> (Borodin, 1897))	76% hatching rate	0.15 mgL ⁻¹ O ₃	76 days	Freshwater
Grotmol et al. 2003	Atlantic cod (<i>Gadus morhua</i> (Linnaeus, 1758))	20% hatching rate	2.2 mgL ⁻¹ O ₃	3 min	Seawater
	Atlantic halibut (<i>Hippoglossus hippoglossus</i> (Linnaeus, 1758))	20% hatching rate	2.2 mgL ⁻¹ O ₃	3 mins	Seawater
Yan et al. 2014	Japanese medaka (<i>Oryzias latipes</i> (Temminck and Schlegel, 1846))	60% mortality from secondary effluents	0.26 mgL ⁻¹ O ₃	2–3 weeks	Freshwater

^a Indicates amount of O₃ placed into the test tank at beginning of study.

In summary, the potential negative effects from ozone exposure should illustrate that it is a chemical to be used with caution, regardless of the application. Although O₃ has been shown to cause mortality in a range of cultured species, lethal concentrations vary considerably from species to species (0.0093 mgL⁻¹–1.44 mgL⁻¹; Table 1). In general, for fish species, low levels of O₃ (< 300 mV ORP; Table 1) can have positive effects, whereas moderate to high levels of O₃ (~150 to >1000 mV ORP) cause severe physiological consequences and can cause mortality (Table 1).

2. Ballast water treatment

Ozone has also been used in the shipping industry as a decontamination/sterilization method for ballast water, mainly to protect against the transfer of invasive zooplankton, phytoplankton, and microbes (Tables 4–6). In zooplankton, a 96% reduction of two copepod species (*Pseudodiaptomus marinus* (Sato, 1913) and *Paracalanus* sp. (Boeck, 1865)) was achieved in 10 h with varying ozone concentrations (Herwig et al. 2006). Brine shrimp (*Artemia salina nauplii* (Linnaeus, 1758)) showed a 98.6% mortality in 3h when exposed to 10.9 mgL⁻¹ TRO (Juretić et al. 2011). Rotifer eggs

(*Brachionus plicatilis* (Mueller, 1786)) were inactivated after a 10 min of exposure to 1.63 mgL⁻¹ TRO (Davis and Arnold 1997).

Phytoplankton species also displayed adverse effects to ozone. In industrial biofouling treatments, *Mesocyclops* and *Schmackeria* copepods could be killed in drinking water filters with 5 mgL⁻¹ O₃, inactivating 95.2% of each species (Lin et al. 2012). Several species within the taxa of Dinophyceae, Raphidophyceae, and Euglenophyceae experienced a 0% growth rate when treated with 0.15 mgL⁻¹ of O₃ for 5 min (Honjo et al. 2001). Toxic cyanobacteria densities of *Anabaena* (St. Vincent, 1886), *Aphanizomenon* (Morren, 1888), *Microcystis* (Lemmermann, 1907), and *Pseudanabaena* (Lauterborn, 1915) were reduced 41–80% after exposure to O₃ (2–5 mgL⁻¹; Zamyadi et al. 2015). Perrins et al. (2006) found that a spectrum of phytoplankton could be removed from ballast water by O₃ concentrations of 2–5 mgL⁻¹ TRO as Br₂, and recommended persistent ozonation rather than a single treatment to control cyanobacteria.

Microbe populations in ballast water can be controlled with varying concentrations of ozone (Table 6). Wu et al. (2011) found that O₃ could inactivate 60% of *Amphidinium* sp. (Claparède and

Table 3. Summary of selected studies on invertebrates exposed to ozone.

Study	Species	Endpoint	Concentration	Exposure Duration	Water Type
Coman et al. 2005	penaeus embryos (<i>Marsupenaeus japonicus</i> (Bate, 1888))	0% hatch rate on eggs tested 16 mins after fertilization.	2 mgL ⁻¹ O ₃	2 min	Seawater
Harrington et al. 1997	zebra mussel (<i>Dreissena polymorpha</i> (Pallas, 1771))	95% mortality in water 32° C.	0.5 mgL ⁻¹ O ₃	5.78 h	Freshwater
Meunpol et al. 2003	black tiger shrimp (<i>Penaeus monodon</i> (Fabricius, 1798))	75% survival with probiotics	0.333–0.341 mgL ⁻¹ ROC (residual ozone concentration)	24 h	Seawater
Middlemiss et al. 2015	European lobster (<i>Homarus gammarus</i> (Linnaeus, 1758))	15% survival after 31 d	320 mV ORP	Test administered 18 d, mortality checked for 31 d	Seawater
Richardson and Burton 1981	blue crab juvenile (<i>Callinectes sapidus</i> (Rathbun, 1896))	100% mortality	0.6 mgL ⁻¹ OPO	6 d	Seawater
Richardson et al. 1982	American oyster (<i>Crassostrea virginica</i> (Gmelin, 1791))	100% mortality in straight hinged larvae stages	0.3 mgL ⁻¹ OPO	96 h	Seawater
	American oyster pediveliger larva (<i>Crassostrea virginica</i> (Gmelin, 1791))	30% mortality	0.3 mgL ⁻¹ OPO	96 h	Seawater
Ritar et al. 2006	southern rock lobster larva (<i>Jasus edwardsii</i> (Hutton, 1875))	100% mortality	600 mv ORP	18 d	Seawater
Schroeder et al. 2010	Pacific white shrimp (<i>Litopenaeus vannamei</i> (Boone, 1931))	LC ₅₀	0.84 mgL ⁻¹ OPO	24 h	Seawater
Van Benschoten et al. 1993	zebra mussel (<i>Dreissena polymorpha</i> (Pallas, 1771))	100% mortality	1 mgL ⁻¹ O ₃	5 h	Freshwater

Lachmann, 1859) in 5 min. 99.9% of all bacteria cultured from ballast water was inactivated 99.9% in a 10 h treatment (775–799 mV ORP; Herwig et al. 2006), and 92% of *Cryptosporidium* (Tyzzer, 1907) oocysts in wastewater can be inactivated following 10 min of exposure to 1.2 mgL⁻¹ of O₃ (Wohlsen et al. 2007). However, data suggests that O₃ treatments of ballast water should be carried out frequently, as bacterial counts can rebound to pre-treatment levels in as little as 3 d if ineffective concentrations are used (Hess-Erga et al. 2010).

Application of ozone as a non-physical barrier against AIS and Key knowledge gaps

Based on the toxic effects described in this review, O₃ treatments can be conducted at concentrations able to kill aquatic species of concern, such as zebra mussels (Van Benschoten et al. 1993, Harrington et al. 1997), algal (dinoflagellate) species that form red tide (Herwig et al. 2006), and species of cyanobacteria (Zamyadi et al. 2015). Ozone could be used to control many AIS, but few AIS species have been

specifically tested for mortality when exposed to O₃. Consequently, it is necessary to conduct research to determine how AIS of concern to freshwater riverine systems will respond to the deployment of an O₃ barrier. In addition, the behavioral and non-lethal physiological changes need to be further observed in aquatic species. In this review of literature, we found that ozone may be damaging to peripheral tissues and RBC in fish species, but there is a sparsity of literature describing any behavioral or other minute physiological effects induced by elevated ozone concentrations.

Because many non-physical barriers (e.g. water guns, bubble curtains, fish pheromones) are unlikely to block the advance of smaller, non-nektonic invasive species (e.g., spiny water flea (*Bythotrephes longimanus* (Leydig, 1860)), zebra mussel, Eurasian water milfoil (*Myriophyllum spicatum* L.)), ozone's powerful oxidizing properties may provide the additional protection needed against these kinds of AIS. Moreover, ozone's use in containable areas with minimal connections to the surrounding ecosystem, such as in lock systems or shipping canals, may reduce

Table 4. Summary of selected studies on zooplankton exposed to ozone.

Study	Species	Endpoint	Concentration	Exposure Duration	Water Type
da Costa et al. 2014	<i>Daphnia similis</i> (Claus, 1876)	60% mortality	1.44 mgL ⁻¹ O ₃	48 h	Freshwater
Davis and Arnold 1997	rotifer eggs (<i>Brachionus plicatilis</i> (Mueller, 1786))	100% mortality	1.63 mgL ⁻¹ TRO	10 min	Seawater
Herwig et al. 2006	<i>Pseudodiaptomus marinus</i> (Sato, 1913), <i>Paracalanus</i> sp. (Boeck, 1865)	> 96% mortality	5 mgL ⁻¹ TRO	10 h	Seawater
	Microflagellates	93-98% mortality	5 mgL ⁻¹ TRO	10 h	Seawater
Jones et al. 2006	mysid shrimp (<i>Americamysis bahia</i> (Molenock, 1969))	LC ₉₅	> 0.9 mgL ⁻¹ TRO as Br ₂	0.5 h	Seawater
	<i>Leptocheirus plumulosus</i> (Shoemaker, 1932)	LC ₉₅	> 0.65 mgL ⁻¹ TRO as Br ₂	0.5 h	Seawater
	<i>Rhepoxynius abronius</i> (J. L. Barnard, 1960)	LC ₉₅	> 0.48 mgL ⁻¹ TRO as Br ₂	0.5 h	Seawater
Juretić et al. 2011	<i>Artemia salina nauplii</i> (Linnaeus, 1758)	98.6% mortality	10.9 mgL ⁻¹ TRO	3 h	Seawater
Leynen et al. 1998	<i>Daphnia magna</i> (Straus, 1820)	100% mortality	0.14 mgL ⁻¹ O ₃	1 h	Freshwater
Perrins et al. 2006	Mesozooplankton	100% mortality	3.46 mgL ⁻¹ TRO as Br ₂	24 h	Seawater

Table 5. Summary of selected studies on phytoplankton exposed to ozone.

Study	Species studied	Endpoint	Concentration	Exposure Duration	Water Type
Herwig et al. 2006	dinoflagellates	82–100% mortality	5 mgL ⁻¹ TRO	10 h	Seawater
Honjo et al. 2001	<i>Cochlodinium polykrikoides</i> (Margalef, 1961)	100% mortality	0.15 mgL ⁻¹ O ₃	5 min	Seawater
	<i>Heterocapsa</i> sp. (Stein, 1883)				
	<i>Heterocapsa triquetra</i> (Stein, 1883)				
	<i>Prorocentrum minimum</i> (Schiller, 1933)				
Lin et al. 2012	copepod species	95% mortality	5 mgL ⁻¹ O ₃	20-25 mins	Freshwater
Sugita et al. 1992	<i>Pfiesteria piscicida</i> (Steidinger and Burkholder, 1996)	99% mortality	0.063 mgL ⁻¹ TRO	1 min	Seawater
Wu et al. 2011	<i>Amphidinium</i> sp. (Claparède and Lachmann, 1859)	60% mortality	0.48 mg/min O ₃	5 min	Seawater
Zamyadi et al. 2015	<i>Anabaena</i> sp. (St. Vincent, 1886)	41–80% mortality	2–5 mgL ⁻¹ O ₃	10 min	Freshwater
	<i>Aphanizomenon</i> sp. (Morren, 1888)				
	<i>Microcystis</i> sp. (Lemmermann, 1907)				
	<i>Pseudanabaena</i> sp. (Lauterborn, 1915)				

the risk of unwanted environmental effects. Ozone could also be considered for use in tandem with other barriers and control tactics as part of an integrated pest management plan. For example, if healthy, native fish populations are a requirement, but there is a need to control the spread of an invasive fish and zooplankton, barriers such as elevated CO₂ and electric barriers may be used to control fish movement, while low levels of O₃, which are not harmful to fish, could potentially be used to control the spread of zooplankton. Based on our review, an O₃ barrier would need to be constantly operated at moderate to high levels of O₃ if the goal of the barrier is to be effective against all aquatic taxa. If the barrier is used to protect against zooplankton,

plankton, microbes, or pathogens, lower levels of O₃ might be effective. Further toxicity testing with ozone will help to more clearly delineate this division.

Before O₃ is used to control movement or populations of AIS, several logistical, monetary, and structural considerations must be addressed. Foremost is the design of a gas diffuser system capable of injecting large volumes of O₃ into freshwater. To our knowledge, no studies have attempted to continuously treat large amounts of freshwater in a pulse discharge/renewal system with O₃, *in situ*. However, Summerfelt et al. (2008) treated large volumes (400–2,400 Lmin⁻¹) of surface water to be used as part of a fish culture treatment system to promote fish health. In their system, a residual concentration

Table 6. Summary of selected studies on microbes exposed to ozone.

Study	Species Studied	Endpoint	Concentration	Exposure Duration	Water Type
Austin 1983	<i>Flavobacterium</i> sp. (Bergey et al., 1923)	99% mortality	0.1 mgL ⁻¹ O ₃	4 min	Freshwater
Itoh et al. 1997	<i>Aeromonas hydrophila</i> (Chester, 1901)	99% mortality	0.1 mgL ⁻¹ TRO	0.5–2.0 min	Seawater
Sugita et al. 1992	<i>Enterococcus seriolicida</i> (Kusuda et al., 1991)	99% mortality	0.11 mgL ⁻¹ TRO	1 min	Seawater
	<i>Vibrio anguillarum</i> (Bergeman, 1909)	99% mortality	0.064 mgL ⁻¹ TRO	1 min	
Tripathi et al. 2011	Total coliform, fecal coliform, and <i>E. coli</i> (Migula, 1895)	98% mortality	10 mgL ⁻¹ O ₃	5 min	Freshwater
Wohlsen et al. 2007	<i>Cryptosporidium</i> oocysts (Tyzzer, 1907)	92% mortality	1.2 mgL ⁻¹ O ₃	10 min	Freshwater

Table 7. Summary of selected studies on fish pathogens exposed to ozone.

Study	Species studied	Endpoint	Concentration	Exposure Duration	Water Type
Arimoto et al. 1996	Striped Jack Nervous Necrosis Virus (SJNNV) (ICTV Master Species List (2015): https://talk.ictvonline.org/files/master-species-lists/m/msl , Taxonomic History; http://ictvonline.org/taxonomyHistory.asp?taxnode_id=20153205)	100% mortality	0.0001 mgL ⁻¹ TRO	2.5 min	Seawater
Chang et al. 1998	White Spot Syndrome Baculovirus (WSBV) (ICTV Master Species List (2015): https://talk.ictvonline.org/files/master-species-lists/m/msl , Taxonomic History; http://ictvonline.org/taxonomyHistory.asp?taxnode_id=20153193)	0% of species infected with WSBV when used.	0.0005 mgL ⁻¹ TRO	10 min	Seawater
Liltved et al. 1995	<i>Aeromonas salmonicida</i> (Lehmann and Neumann, 1896) <i>Vibrio anguillarum</i> (Bergeman, 1909) <i>Vibrio salmonicida</i> (Egidius et al., 1986) <i>Yersinia ruckeri</i> (Ewing et al., 1978)	99.99% mortality.	0.15–0.20 mgL ⁻¹ O ₃	3 min	Freshwater and Seawater
Liltved et al. 2006	Infectious Pancreatic Necrosis Virus (IPNV)	98.7% mortality	7.9 mgL ⁻¹ TRO	17 min	Seawater
	Atlantic Halibut Nodavirus (AHNV)	98% mortality	1.6 mgL ⁻¹ TRO	31.5 min	Seawater
	Infectious Salmon Anemia Virus (ISAV)	99% mortality	0.33 mgL ⁻¹ TRO	0.25 min	Seawater
Wedemeyer and Nelson 1977	<i>Aeromonas salmonicida</i> (Lehmann and Neumann, 1896)	100% mortality	0.01 mgL ⁻¹ O ₃	10 min	Freshwater
	Enteric Redmouth Bacterium (ERB) (<i>Yersinia ruckeri</i> (Ewing et al., 1978))	100% mortality	0.01 mgL ⁻¹ O ₃	0.5 min	Freshwater
Wedemeyer et al. 1978	Infectious Hematopoietic Necrosis Virus (IHNV)	100% mortality	0.01 mgL ⁻¹ O ₃	0.5 min	Freshwater
	Infectious Pancreatic Necrosis Virus (IPNV)	100% mortality	0.01 mgL ⁻¹ O ₃	1 min	

of 0.2 mgL⁻¹ O₃ was maintained in the presence of changing surface water quality and environmental conditions. Despite the success of this system to treat large volumes of surface water, the deployment of an O₃ barrier would be considerably larger in, for example, a navigational lock. Large volumes of water would be replaced several times a day and substantial amounts of O₃ would need to be generated and transferred to the water quickly. Furthermore, water quality would vary widely, with a range of water temperatures, high amounts of organic material, and potentially various amounts of carbonate. These factors would cause a rapid decline of dissolved ozone requiring frequent replenishment of the system. Other factors, such as the effects of bottom topography and hydrological characteristics, will impact if O₃ concentrations can be maintained (Noatch and Suski 2012). Currently, it is uncertain if

appropriate technology is available that will provide for sufficient generation of O₃ for deployment for the extended periods of time that will be needed to create an efficient non-physical barrier.

The costs associated with utilizing ozone will be a substantial factor when considering its use as a non-physical barrier. To our knowledge, there are no prior publications specifically addressing this, but Sassi et al. (2005) did review the cost of ozonating a ship capable of holding 45,000 cubic meters of ballast water. It was found that it cost 0.22 to 0.28 US dollars per cubic meter to treat the ballast with ozone. Sassi et al. (2005) did acknowledge, however, that this estimate was based on the cost of running a diesel generator as the ship's primary source of electricity.

The use of ozone in wastewater treatment facilities can also be a source of reference for the potential costs of an ozone barrier. Rosen (1973) stated that

for a facility capable of treating ten million gallons of wastewater per day with ozone, it costs 0.02 US dollars (USD) per cubic meter of wastewater. The costs associated with retrofitting facilities for ozone treatment has also been assessed. The Environmental Protection Agency (1999) stated that it costs 300,000 USD in capital, excluding contingencies, to construct an ozone system capable treating one million gallons of wastewater per day. Annually, the operation and maintenance costs associated with a facility this size is around 15,000 USD, excluding power consumption (Solomon et al. 1998).

Other externalities for the cost and practicality of using ozone additionally exist. It is important to consider that ozone is corrosive to certain metals (Wyllie and Duquette, 1998; Viera et al. 1999; Pehkonen 2001). Treatment areas will have to be constructed or restructured so that metals susceptible to degradation are not used, which may increase costs considerably. Also, ozone creates bromides when added to seawater, as previously described. Bromides can cause corrosion of metals over time, further complicating what materials can be associated with ozone treatment systems (Kutty et al. 1991). If ozone is used in any brackish or estuary area, even if leading to freshwater, the generation of bromides could be a problem. If it is not feasible to modify or change the materials associated with, or exposed to, an ozone treatment system, other non-physical barrier methods should be considered.

Finally, there are human health hazards associated with ozone treatment. Ozone is a respiratory irritant; a trait which has been well documented (Lippmann 1989, Devlin et al. 1991). Some O₃ will off-gas during its addition to water, creating the potential for human (occupational and incidental) exposure. The potential exposure levels from this application would need to be compared to the occupational exposure limits (e.g. 0.1 ppm O₃) to assess potential risk (OSHA 2016). The application of O₃ to achieve concentrations acutely lethal to aquatic organisms could result in off-gassing rates at levels above 0.1 ppm in the atmosphere above the application site. Personal protective equipment (i.e. respirators) and monitoring could manage the risk of O₃ exposure to workers at the site.

Identifying Key Knowledge Gaps:

Based on the above review of O₃ as a potential non-physical barrier to control the spread of AIS, the following is a list of key knowledge gaps suggested for future research:

1. Tolerances of O₃ on species that are currently of concern (e.g., bighead carp in the US Midwest).

There was a surprising lack of information on the biological responses of AIS to elevated levels of O₃. This information is needed to determine if O₃ is an appropriate non-physical barrier to control the spread of AIS. Researchers should be holistic in their approach to studying species tolerances and avoidance behaviour toward O₃ and consider a range of environmental conditions and life-stages.

2. Impacts of elevated O₃ to the multiple life stages of non-target species and the potential downstream effects on natural habitats should be considered prior to the approval of O₃ in a natural system.
3. Methods to combine O₃ with other non-physical barriers. Special attention should be given to enclosed areas that can be treated with high levels of O₃ for brief periods of time.
4. Methods for generating substantial amounts of O₃ in a field setting. Though systems have been developed for ballast water disinfection and wastewater treatment, this literature review suggests that there are clear limitations of using dissolved O₃ in natural systems. A feasible O₃ barrier will need to overcome a range of environmental conditions to be effective.
5. Effects ozone may have on the structural components of treatment systems, the costs associated with treating large amounts of water, and the human health hazards ozone may impose.

In conclusion, ozone's lethality to aquatic organisms make it a strong candidate as a potential non-physical barrier. There are, however, several key knowledge gaps that need further investigation before considering ozone as a tangible AIS solution, as stated here. If these knowledge gaps can be resolved, ozone could play an integral role in stopping the spread of AIS in the future.

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