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Formation of disinfection byproducts in a recirculating mariculture system: emerging concerns

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Disinfection is commonly employed in recirculating mariculture systems (RMS) to control animal diseases and improve seawater quality; however, little is known about the occurrence of disinfection byproducts (DBPs) formed in such RMS. Beijing Aquarium is a typical RMS with artificially prepared seawater and mainly adopts a decentralized treatment strategy for different animal tanks, including sand filtration, foam fractionation, and disinfection (O_3 , UV, and O_3/ClO_2). This study reveals that the adopted disinfection processes were highly effective in controlling marine heterotrophic bacteria; however, some concerns were raised on the formation of various kinds of DBPs, including secondary oxidants, inorganic oxyanions, and hazardous organic species. Free chlorine and free bromine were generated from ozonation at health-relevant concentrations. High concentrations of BrO_3^- and ClO_3^- were formed in mammal tanks, which exceeded the USEPA-regulated maximum contaminant level (MCL) for drinking water by 19–25 and 52–54 times, respectively. Extremely high concentrations of NO_3^- were detected in mammal tanks, which considerably exceeded the MCL regulated by the Sea Water Quality Standard of China for the mariculture industry (Class II) by about 1100 times. Undoubtedly, the presence of various DBPs poses serious health threats to aquarium animals. To solve these problems, potential control measures for DBPs are proposed.

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Recirculating mariculture systems (RMS) have been widely employed in marine aquaria around the world. Hazardous disinfection byproducts (DBPs) formed during the seawater treatment of RMS has been neglected for a long time. This study investigated the formation of various kinds of DBPs (*i.e.*, secondary oxidants, inorganic oxyanions, and hazardous organic species) in animal tanks of a typical RMS, which raised serious concerns on the health threats of the detected DBPs to aquarium animals. To solve these problems, potential control measures for DBPs are proposed. This study provides useful and timely information to the mariculture industry regarding the occurrence and control of DBPs.

1. Introduction

A recirculating aquaculture system treats polluted water within a closed loop, offers improved environmental control, and allows aquatic animal production in water-deficient areas.¹ As a result, recirculating mariculture systems (RMS) have been widely employed in marine aquaria around the world. Heterotrophic bacteria are ubiquitously present in marine ecosystems,² but their metabolic byproducts and the substances released from cellular lysis may cause animal diseases in the

RMS.³ Hence, appropriate disinfection is of essential importance to inactivate pathogens and protect the health of marine animals.⁴ However, disinfection byproducts (DBPs) formed in different disinfection processes have long been neglected in marine aquaria, which can also pose serious threats to animal health.

Currently, ozone (O_3), chlorine dioxide (ClO_2), chlorine (Cl_2), and ultraviolet (UV) have been employed commonly to disinfect natural or artificial seawater (ASW) in the RMS. O_3 has multiple functions such as decomposing dissolved organic matter (DOM) and controlling algae, color, odor and taste,⁵ and thus is mainly used to improve the physicochemical aspects of water quality rather than disinfection.^{6,7} Because O_3 cannot provide a persistent residue in water distribution systems, a secondary disinfectant (*e.g.*, Cl_2 , NH_2Cl , or ClO_2) is required to maintain continuous disinfection ability. Moreover, O_3 can readily oxidize chloride (Cl^-) and bromide (Br^-) ions in seawater to free

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chlorine (HOCl/OCl^-) and free bromine (HOBr/OBr^-), respectively, thus not only causing respiratory and osmoregulatory dysfunctions in fish⁸ but also reacting with other inorganic and organic materials to produce harmful DBPs (e.g., bromate ion (BrO_3^-), trihalomethanes (THMs), and haloacetic acids (HAAs)).⁹ It was reported that ozonation of salt waters could produce BrO_3^- at concentrations of up to $100 \mu\text{g L}^{-1}$.^{10,11} The natural or artificial seawater in marine aquaria has a high salinity (ca. 3.5%) and contains approximately $19 \text{ g L}^{-1} \text{ Cl}^-$ and $67 \text{ mg L}^{-1} \text{ Br}^-$,¹² which favors the formation and accumulation of harmful DBPs.

ClO_2 disinfection produces harmful chlorite (ClO_2^-) and chlorate (ClO_3^-) ions,¹³ which can accumulate in the RMS because they are very difficult to remove once formed. As the most commonly used chemical disinfectant, Cl_2 is highly effective against a wide range of pathogens and is capable of providing necessary residual protection in water distribution systems. However, reaction of Cl_2 with DOM produces harmful THMs and HAAs. For example, it was found that uneaten food, and animal feces and urine contributed to the formation of THMs at The Marine Mammal Center (Sausalito, California, US) whose seawater was primarily disinfected by O_3 with supplemental Cl_2 and Br_2 .⁴ UV is another disinfection method employed in the RMS because of its distinct merits such as the effective inactivation of pathogens, low formation of DBPs, small space occupancy, and easy operation.

It should also be noted that NO_3^- is often generated in the RMS at levels far greater than that normally found in natural seawater because of microbial nitrification, chemical oxidation, and the addition of microalgae as a food source for filter-feeding organisms.¹⁴ For example, NO_3^- concentration was measured to be $9700 \mu\text{M}$ (i.e., 601 mg L^{-1}) in the Main Tank of The Living Seas artificial aquarium in Florida¹⁵ and $9500 \mu\text{M}$ (i.e., 589 mg L^{-1}) in the Ocean Tank of the New Jersey State Aquarium.¹⁶ However, Bower and Turner¹⁷ suggested an acceptable level of $88.6 \text{ mg L}^{-1} \text{ NO}_3^-$ for mariculture.

The formation of DBPs has been extensively studied in drinking water; however, to date, little is known regarding their occurrence in marine aquaria. Therefore, this study aims to determine the levels of DBPs formed in various animal tanks of the Beijing Aquarium, and propose potential control measures for the DBPs of high concern. The studied DBPs consisted of secondary oxidants (free chlorine and free bromine), inorganic oxyanions (BrO_3^- , ClO_2^- , ClO_3^- , and NO_3^-), and hazardous organic species (THMs and HAAs).

2. Experimental

2.1 Chemicals and standards

2,6-Dimethylphenol, 4-chloro-2,6-dimethylphenol, 4-bromo-2,6-dimethylphenol, and 2,4,6-trichlorophenol were obtained from Alfa Aesar (Haverhill, MA, US). THMs and HAAs standards were purchased from Accustandard (New Haven, CT, US). Sodium hypochlorite, sodium chlorite, sodium chlorate, potassium bromide, and potassium bromate were from Beijing Chemical Reagents Company (Beijing, China). All the chemicals were of at least analytical grade and the highest purity available.

All aqueous solutions were prepared with ultrapure water produced by a Milli-Q system (Advantage A10, Millipore, Billerica, MA, US). High performance liquid chromatography grade hexane and methyl-*tert*-butyl ether were purchased from Fisher Scientific (Houston, TX, US), and diethyl-*p*-phenylenediamine test kits were supplied by Hach Company (Loveland, CO, US).

2.2 Aquarium seawater treatment processes

Beijing Aquarium is a typical RMS with a total seawater volume of $18\,000 \text{ m}^3$. Fresh ASW is prepared in a separate tank and all major ions are externally added to simulate the ion ratios of natural seawater with a total salinity of 3.0–3.5%. The concentrations of chloride and bromide are about 19.4 g L^{-1} and 66 mg L^{-1} , respectively. The freshly prepared ASW is subjected to ozone treatment before being sequentially supplied to non-mammal (e.g., big fish, jellyfish, coral) and mammal (e.g., dolphin, sea lion, white whale) tanks to make up for the volume loss during treatment and recycling (Fig. 1). Every month, about 20–25% of the seawater in the animal tanks is replaced by the fresh ASW. All animal tanks in Beijing Aquarium are indoor with seawater temperature maintained in the range of 20–25 °C. The dolphin, sea lion, and white whale tanks house 4, 4, and 2 animals, respectively.

Beijing Aquarium mainly adopts a decentralized treatment strategy for different animal tanks, which generally includes sand filtration, foam fractionation (with O_3 addition), and disinfection (O_3 , UV, and O_3/ClO_2). The big fish tank only uses O_3 for disinfection. Typically, O_3 is first injected online into the foam fractionation tank, where suspended solids are removed by air floatation and a certain portion of the DOM is decomposed by O_3 oxidation. The penetrating solid particles are further removed by sand filtration. Afterward, a side stream of the sand-filtered seawater is contacted with O_3 for about 5 min (i.e., bypass treatment), which then merges with the main stream to return to the big fish tank. Because jellyfish and coral cannot tolerate any residual chemical oxidant, their tanks are disinfected by UV (254 nm) through bypass treatment. The

Fig. 1 Schematic diagram of seawater treatment processes for the recirculating mariculture system of Beijing Aquarium.

seawater from the big fish, jellyfish, and coral tanks further flows into the mammal tanks, where O_3 is used as the primary disinfectant followed by ClO_2 as the secondary disinfectant to provide a persistent residue (*ca.* 0.04 mg L^{-1}). The hydraulic retention time (HRT) of each tank ranges from approximately 0.5 to 3 h (Table 1).

2.3 Analytical methods

Seawater samples were collected five times from each of the seven representative tanks (*i.e.*, fresh ASW, big fish, jellyfish, coral, dolphin, sea lion, and white whale) of the Beijing Aquarium from June 2012 to March 2013. Typically, on a sampling day, two samples were separately collected from each tank in the morning and afternoon. For each sample, seawater from the surface, middle, and bottom layers of a target tank was collected and mixed together. The concentration of dissolved organic carbon (DOC), after filtering the sample through $0.45 \mu\text{m}$ glass fiber filters, was determined with a total organic carbon analyzer (TOC-Vcph, Shimadzu, Japan). The UV absorbance at 254 nm (UV_{254}) was measured using a Hach DR5000 UV-Vis spectrophotometer (Loveland, CO, US), and the specific UV absorbance (SUVA) was then calculated from the UV_{254} and DOC concentration.

To analyze marine heterotrophic bacteria (MHB), seawater samples (100 mL each) were collected at about 20 cm below the surface of target animal tanks once per week using aseptic sampling bags (Whirl-Pak, Fort Atkinson, WI, US). Thereafter, 15 mL of each sample was withdrawn and filtered through a $0.45 \mu\text{m}$ membrane installed in a Millipore Microfil Funnel (Millipore, Billerica, MA, US). The membrane was cultured at 25°C in Marine Broth 2216 (BD Difco, Erembodegem, Belgium) for 48 h , and then the number of MHB was counted.

Free chlorine and free bromine were derivatized (*i.e.*, fixed) on site immediately after sampling, and then the samples were transferred to the laboratory and analyzed following our earlier developed method.¹⁸ THM4 and HAA5 were determined according to the United States Environmental Protection Agency (USEPA) methods 551 and 552.2, respectively. The inorganic oxyanions including BrO_3^- , ClO_2^- , ClO_3^- , and NO_3^- were analyzed by an ICS-2000 ion chromatograph (Dionex, Sunnyvale, CA, US). Prior to analysis, each sample was sequentially filtered through an OnGuard II Ag cartridge and an OnGuard II H cartridge to remove the predominant Cl^- ions

and the released Ag^+ ions, respectively. Ammonia and nitrite were determined by Hach methods 8155 and 8507 (Hach Company, Loveland, CO, US), respectively.

To test the disinfection byproducts formation potential (DBPsFP), a desired amount of NaOCl was added into a seawater sample (200 mL) to achieve a final concentration of 20 mg L^{-1} (as Cl_2). The sample was placed in dark for 72 h at a controlled temperature ($21 \pm 2^\circ\text{C}$), quenched with $Na_2S_2O_3$ solution, and analyzed for THM4 and HAA5 concentrations with the methods described above.

3. Results and discussion

3.1 Major characteristics of seawater in representative tanks

The major characteristics of seawater samples collected from seven representative tanks of the Beijing Aquarium are listed in Table 1. The results show that the pH of different tanks varied in a narrow range of $7.86\text{--}8.12$, similar to that of natural seawater (*i.e.*, pH $7.8\text{--}8.2$). The fresh ASW tank had a low DOC concentration of 1.3 mg L^{-1} , which mainly arose from the tap water used for the ASW preparation. The coral, jellyfish, and big fish tanks also exhibited low DOC concentrations ranging from 1.3 to 2.7 mg L^{-1} . The mammal tanks had obviously higher DOC concentrations (*i.e.*, $8.0\text{--}9.7 \text{ mg L}^{-1}$) than other tanks because of two facts: (1) these tanks receive used seawater from the non-mammal tanks (Fig. 1); and (2) mammals have larger food intake and thus more excretion of urine and feces than non-mammals. The UV_{254} exhibited a similar ascending trend from the fresh ASW tank (0.010 cm^{-1}), to non-mammal ($0.012\text{--}0.022 \text{ cm}^{-1}$) and mammal tanks ($0.084\text{--}0.089 \text{ cm}^{-1}$). The SUVA of the target tanks ranged from 0.71 to $1.11 \text{ L m}^{-1} \text{ mg}^{-1}$, implying that the samples mainly contained hydrophilic DOM with a low aromaticity.¹⁹ In addition, all tanks had low turbidities (*i.e.*, $0.10\text{--}0.44 \text{ NTU}$), indicating the high efficiency of the foam fractionation and sand filtration processes for the removal of suspended particles. Seawater clarity is necessary for visitors to enjoy the sight of ornamental animals in marine aquaria.

3.2 Inactivation of marine heterotrophic bacteria

The concentration of MHB reflects the microbial aspect of the seawater quality in the RMS, which influences the disinfectant dose to ensure a suitable environment for marine animals. The MHB concentrations of four typical animal tanks were

Table 1 Major characteristics of seawater in seven representative tanks of Beijing Aquarium (mean $\pm$ standard deviation, $n = 5$)^a

Sampling tanks	pH	DOC (mg L^{-1})	UV_{254} (cm^{-1})	SUVA ($\text{L m}^{-1} \text{ mg}^{-1}$)	Turbidity (NTU)	HRT (h)	Disinfection process
Fresh ASW tank	8.01 ± 0.03	1.3 ± 0.2	0.010 ± 0.002	0.77	0.38 ± 0.07	3.0	O_3
Big fish tank	7.86 ± 0.05	2.7 ± 0.5	0.022 ± 0.005	0.81	0.07 ± 0.01	1.0	O_3
Jellyfish tank	8.10 ± 0.04	1.7 ± 0.3	0.012 ± 0.003	0.71	0.12 ± 0.01	0.5	UV
Coral tank	8.12 ± 0.10	1.3 ± 0.1	0.012 ± 0.005	0.92	0.10 ± 0.01	0.5	UV
Dolphin tank	8.02 ± 0.05	9.7 ± 0.3	0.084 ± 0.002	0.86	0.17 ± 0.06	2.0	O_3/ClO_2
Sea lion tank	7.98 ± 0.03	9.4 ± 0.2	0.085 ± 0.003	0.90	0.44 ± 0.11	2.0	O_3/ClO_2
White whale tank	7.99 ± 0.04	8.0 ± 1.0	0.089 ± 0.001	1.11	0.21 ± 0.06	2.0	O_3/ClO_2

^a Seawater samples were collected in 2012 (Jun. 1, Oct. 18 and Dec. 19) and 2013 (Jan. 14 and Mar. 19).

monitored once per week throughout 2012 and are presented in Fig. 2. UNIANOVA of SPSS (Version 18.0) was employed to analyze the difference in MHB concentration among different seasons. The results show that the MHB concentrations in spring and summer were considerably higher than those in autumn and winter for all the four tanks ($p < 0.05$). For example, the mean concentrations of MHB in the dolphin, big fish, jellyfish, and fresh ASW tanks were detected to be 4860, 3480, 3000, and 900 colony forming units per 100 mL of sample [CFU (100 mL)⁻¹] in the summer, and 3340, 2520, 2490, and 210 CFU (100 mL)⁻¹ in the winter, respectively. Spring and summer are breeding seasons for most fishes, so the presence of larvae provides a favorable condition for bacterial growth.

The lowest and highest mean concentrations of MHB were detected in the fresh ASW and dolphin tanks, respectively. This is not surprising because mammals excrete more bacteria in urine and feces than non-mammals. Moreover, MHB concentration is partially related to the turbidity of seawater because they can hide in or adhere to the surface of suspended particles, and thus become more resistant to disinfectants than free-living bacteria.²⁰ As presented in Table 1, the dolphin tank had a relatively higher turbidity (0.17 NTU) than the big fish and jellyfish tanks (0.07 and 0.12 NTU, respectively). It is also noted that the fresh ASW tank had the highest turbidity among the four tanks, which was mostly because of undissolved salt particles. After these particles were effectively removed by the foam fractionation and sand filtration processes, the turbidity of seawater obviously decreased in the non-mammal tanks (Table 1).

The MHB concentrations in various animal tanks are significantly lower than those commonly found in natural marine environments (*i.e.*, 1×10^4 to 5×10^6 cells per mL),²¹ demonstrating that the disinfection processes adopted in the Beijing Aquarium (*i.e.*, O₃, UV, and O₃/ClO₂) are highly effective in controlling bacterial growth.

3.3 Formation of secondary oxidants

The mean concentrations of the secondary oxidants (free chlorine and free bromine) in different tanks are shown in Fig. 3.

Fig. 2 Inactivation of marine heterotrophic bacteria in four typical animal tanks in different seasons ($n = 13$).

The results indicate that free chlorine and free bromine were present in the concentration ranges of 19.0–71.5 and 40.2–692.5 $\mu\text{g L}^{-1}$, respectively. Despite a considerably higher concentration of Cl⁻ than Br⁻ in seawater, free chlorine exhibited obviously lower concentrations than free bromine because the reaction rate constant between Cl⁻ and O₃ (*i.e.*, $k_{\text{O}_3, \text{Cl}^-} = 0.003 \text{ M}^{-1} \text{ s}^{-1}$) is significantly smaller than that between Br⁻ and O₃ (*i.e.*, $k_{\text{O}_3, \text{Br}^-} = 160 \text{ M}^{-1} \text{ s}^{-1}$).^{22,23} Moreover, free chlorine can also rapidly react with Br⁻ ($k_{\text{HOCl}, \text{Br}^-} = 6.77 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$) to produce additional free bromine.²⁴ Except the obviously higher free bromine concentration detected in the sea lion tank, there was no significant difference in the concentrations of free chlorine or free bromine between the O₃/ClO₂ and O₃ treatment processes ($p > 0.05$), implying that the two secondary oxidants were primarily formed during ozonation. In particular, their concentrations detected in the fresh ASW tank were similar to those in the dolphin and white whale tanks because a relatively high O₃ dose (*ca.* 0.5 mg O₃ per mg DOC) was initially applied to improve both physicochemical and microbial aspects of seawater quality. It is also noted that the jellyfish and coral tanks, although disinfected by UV, still contained a low level of free chlorine and free bromine, which could primarily come from the supplementary fresh ASW.

Previous studies have shown that HOBr is about 20 times more reactive than HOCl with respect to the formation of THMs and HAAs.²⁵ The acute toxicity of chlorine-produced oxidants is less than 1–46 μM , measured as 48 h LC50 for certain fishes;¹³ in contrast, bromine-produced oxidants are two to five times more toxic than their chlorine analogues.²⁶ Our monitoring data clearly reveal that the aquarium animals (particularly oxidant-sensitive species such as big fish, jellyfish, and coral) are under a severe health threat. Hence, the formation of free chlorine and free bromine is one of the serious problems for marine aquaria that adopt ozone treatment.

3.4 Formation of inorganic oxyanions

The mean concentrations of inorganic oxyanions, including BrO₃⁻, ClO₃⁻, ClO₂⁻, and NO₃⁻, in various animal tanks are

Fig. 3 Free chlorine and free bromine concentrations in various animal tanks ($n = 5$).

listed in Table 2. Although to date, DBPs are only regulated for drinking water, more attention should be given to marine aquaria where aquatic animals live all the time. The results indicate that the BrO_3^- concentrations ranged from 30.1 to 262.0 $\mu\text{g L}^{-1}$, with a descending order of O_3/ClO_2 (dolphin, sea lion, and white whale tanks) > O_3 (big fish tank) > UV (jellyfish and coral tanks). Because UV does not produce BrO_3^- from Br^- , the jellyfish and coral tanks showed similar BrO_3^- concentrations to that in the fresh ASW tank. Moreover, the BrO_3^- concentration kept increasing along the seawater flow path (*i.e.*, from the fresh ASW tank to the big fish tank, and then to the mammal tanks) because of continuous application of O_3 . Note that the BrO_3^- concentration in each tank was above the maximum contaminant level (MCL) regulated by USEPA for drinking water (10 $\mu\text{g L}^{-1}$), particularly, those in the mammal tanks exceeded by 19–25 times. A previous study on eastern oysters reported a significant reduction in embryo development at a BrO_3^- concentration of 50 $\mu\text{g L}^{-1}$.²⁷

As expected, ClO_2^- and ClO_3^- were only detected in the mammal tanks where ClO_2 was applied in addition to O_3 for disinfection. The ClO_2^- concentrations were in a low range of 22.1–26.5 $\mu\text{g L}^{-1}$, while the ClO_3^- concentrations reached as high as 53.2–55.1 mg L^{-1} . Typically, for drinking water disinfection, approximately 60% of the applied ClO_2 is converted to ClO_2^- and only 10% to ClO_3^- .²⁸ However, under a constant oxidative stress in the mammal tanks disinfected by O_3/ClO_2 , the initially generated ClO_2^- could be further oxidized to ClO_3^- by both O_3 and free bromine,^{29,30} thus accumulating to a high level. The ClO_3^- concentrations in the mammal tanks exceeded the MCL (1.0 mg L^{-1}) regulated by USEPA for drinking water by 52–54 times. It was reported that ClO_2^- and ClO_3^- could induce erythrocyte morphology alteration and osmotic fragility, and at higher dosages mild hemolytic anemia would occur.⁸

NO_3^- concentration increased rapidly along the seawater flow path, specifically, from 32–67 mg L^{-1} in the fresh ASW, jellyfish and coral tanks to 257 mg L^{-1} in the big fish tank, and to 1448–1572 mg L^{-1} in the mammal tanks. According to the Sea Water Quality Standard of China (GB 3097–1997), NO_3^- is regulated with an MCL of 0.3 mg N L^{-1} for the mariculture industry (Class II). Static bioassays showed that significant mortality of larval *Penaeus monodon* (Fabricius) occurred within 40 h at a NO_3^- concentration as low as 1 mg L^{-1} , and sublethal effects at this concentration resulted in changes to ganglionic neuropiles and muscles; at higher NO_3^- concentrations (10 and

100 mg L^{-1}), additional tissues were affected, including hypodermis, midgut, and proventriculus.¹⁴ Apparently, the extremely high concentrations of NO_3^- in the mammal tanks present an urgent problem for the Beijing Aquarium that needs to be resolved. In contrast, the concentrations of NH_3 and NO_2^- were measured to be only 0.01–0.06 and 0.03–0.26 mg L^{-1} in all the studied animal tanks, respectively. These negligible concentrations could arise from a constant oxidative stress induced by disinfectants in the RMS.

3.5 Formation of organic DBPs

It is known that O_3 , UV, and ClO_2 produce less harmful DBPs than Cl_2 . However, ozonation leads to the formation of free chlorine and free bromine in seawater, which in turn promotes the formation of THMs and HAAs.^{11,18} Our monitoring results show that the concentrations of THM4 and HAA5 were below the USEPA-regulated MCLs for drinking water, *i.e.*, 80 and 60 $\mu\text{g L}^{-1}$, respectively (Table 2). This is not surprising because THM4 and HAA5 are primarily generated during Cl_2 disinfection. In addition, the low SUVA values (<1.2) in all the studied animal tanks implied that hydrophilic DOM predominated in the seawater (Table 1), which is unfavorable for the formation of THM4 and HAA5. Hua and Reckhow³¹ reported that hydrophobic DOM is the major precursor of THMs and HAAs in natural waters.

The highest concentrations of THM4 (22.3 $\mu\text{g L}^{-1}$) and HAA5 (17.2 $\mu\text{g L}^{-1}$) were detected in the fresh ASW tank. These chlorinated DBPs could be carried over from the municipal tap water, which was disinfected by Cl_2 and used to prepare the fresh ASW. The jellyfish and coral tanks showed the lowest concentrations of THM4 and HAA5 because they contained considerably less free chlorine and free bromine than other animal tanks (Fig. 3). Furthermore, UV irradiation may also decompose THM4 and HAA5.³² In the big fish tank, the transformation and/or volatilization of THM4 and HAA5 during ozone treatment appeared to overwhelm their formation *via* secondary oxidants (*i.e.*, free chlorine and free bromine), thus leading to an obvious reduction in their concentrations. The mammal tanks also showed low concentrations of THM4 (2.0–3.1 $\mu\text{g L}^{-1}$) and HAA5 (5.8–9.1 $\mu\text{g L}^{-1}$), which are comparable to those in the big fish tank. In brief, the chlorinated DBPs are not a problem in the RMS adopting O_3 , ClO_2 , and UV for disinfection.

Table 2 Concentrations of DBPs in seven representative tanks of Beijing Aquarium (mean $\pm$ standard deviation, $n = 5$)^a

Sampling tanks	BrO_3^- ($\mu\text{g L}^{-1}$)	ClO_3^- (mg L^{-1})	ClO_2^- ($\mu\text{g L}^{-1}$)	NO_3^- (mg L^{-1})	THM4 ($\mu\text{g L}^{-1}$)	HAA5 ($\mu\text{g L}^{-1}$)
Fresh ASW tank	42.2 $\pm$ 11.8	ND ^b	ND	40 $\pm$ 10	22.3 $\pm$ 9.6	17.2 $\pm$ 6.4
Big fish tank	162.3 $\pm$ 9.2	ND	ND	257 $\pm$ 36	2.0 $\pm$ 0.6	9.7 $\pm$ 2.2
Jellyfish tank	47.8 $\pm$ 8.2	ND	ND	67 $\pm$ 33	1.5 $\pm$ 0.6	1.6 $\pm$ 1.4
Coral tank	30.1 $\pm$ 11.4	ND	ND	32 $\pm$ 8	1.3 $\pm$ 0.5	2.1 $\pm$ 1.6
Dolphin tank	208.0 $\pm$ 14.6	53.2 $\pm$ 7.6	26.5 $\pm$ 7.5	1448 $\pm$ 33	2.0 $\pm$ 0.7	8.7 $\pm$ 2.1
Sea lion tank	203.8 $\pm$ 7.9	54.5 $\pm$ 6.3	25.5 $\pm$ 8.2	1518 $\pm$ 91	2.1 $\pm$ 0.4	5.8 $\pm$ 2.5
White whale tank	262.0 $\pm$ 22.6	55.1 $\pm$ 6.3	22.1 $\pm$ 8.1	1572 $\pm$ 58	3.1 $\pm$ 1.0	9.1 $\pm$ 2.4

^a Same as in Table 1. ^b ND: not detected.

3.6 Potential control measures for DBPs

As described above, the serious problems that the Beijing Aquarium is facing include the formation of secondary oxidants at health-relevant levels, especially free bromine ($40.2\text{--}692.5\ \mu\text{g L}^{-1}$); high concentrations of BrO_3^- and ClO_3^- (with their concentrations in the mammal tanks exceeding the USEPA-regulated MCLs by 19–25 and 52–54 times, respectively); and extremely high concentrations of NO_3^- (with its concentrations in the mammal tanks exceeding the MCL regulated by the Sea Water Quality Standard of China for the mariculture industry (Class II) by about 1100 times).

Free bromine and BrO_3^- are primarily generated from the reactions between O_3 and Br^- . For the RMS seawater treatment, O_3 appears to be indispensable because of its multiple functions in decomposing DOM, improving water clarity, and controlling odor and taste. Hence, to reduce the concentrations of free bromine and BrO_3^- in the RMS, the only possible way is to reduce the concentration of Br^- in the fresh ASW. To the best of our knowledge, Br^- only affects the growth of marine brown and red algae,^{33,34} as well as some shellfish (informed by the animal life support professionals in the Beijing Aquarium). In fact, some US marine aquaria adopting the RMS have long been operated under low Br^- concentrations (e.g., $4.3\ \text{mg L}^{-1}$),¹¹ and no discernable negative effects to aquarium animals have been reported. According to our monitoring results, the management of the Beijing Aquarium has decided to stop the external addition of Br^- ions into the fresh ASW after careful consideration. Expectedly, the Br^- concentration in animal tanks will gradually reduce from 66 to $3\ \text{mg L}^{-1}$ (background concentration from sea salts) in the future. The effects of reducing Br^- concentration on the formation of free bromine and BrO_3^- in animal tanks and on the health of aquarium animals are being closely monitored in our ongoing study.

Because ClO_3^- is unceasingly accumulating in the mammal tanks, ClO_2 must be replaced by more suitable disinfectants. Cl_2 tends to be an alternative because it has long been employed for the disinfection of RMS; however, the formation of chlorinated DBPs should be evaluated first. Our DBPsFP test results show that the mean concentrations of THM4 and HAA5 were 334 and $641\ \mu\text{g L}^{-1}$ for the dolphin tank, 356 and $571\ \mu\text{g L}^{-1}$ for the sea lion tank, and 213 and $481\ \mu\text{g L}^{-1}$ for the white whale tank, respectively (Fig. 4). In each mammal tank, TBM and DBAA were the predominant species for THM4 and HAA5, respectively. It is seen that if ClO_2 is replaced by Cl_2 , the formation of chlorinated DBPs will cause a new problem. To make Cl_2 disinfection applicable, the precursors of chlorinated DBPs (*i.e.*, DOM) in the mammal tanks should be significantly reduced prior to the addition of Cl_2 . Ozone-biological activated carbon ($\text{O}_3\text{-BAC}$) appears to be a feasible choice because this combined treatment process can effectively remove DOC, turbidity, and UV_{254} .^{35,36} A pilot-scale study regarding DOC removal by $\text{O}_3\text{-BAC}$ is currently being carried out in the Beijing Aquarium.

To control NO_3^- concentrations in animal tanks, a heterotrophic denitrification process has been evaluated at pilot-scale in the Beijing Aquarium with externally supplied methanol as the carbon source. The long-term continuous operation of two

Fig. 4 Formation potential of chlorinated DBPs (THM4 and HAA5) in three mammal tanks ($n = 3$).

pressurized heterotrophic denitrification reactors has shown approximately 70% removal of NO_3^- . Alternatively, an autotrophic denitrification process, which uses elemental sulfur as an electron donor and NO_3^- as an electron acceptor, seems to be a better choice for NO_3^- removal in the RMS.³⁷ On the one hand, the autotrophic denitrification process does not require an external carbon source supply (e.g., methanol), thus reducing the treatment cost and enhancing the operation safety (*i.e.*, avoiding the potential leak of harmful methanol to animal tanks). On the other hand, the production and subsequent release of SO_4^{2-} into the treated seawater, which seriously limits the application of this process to groundwater denitrification,³⁸ is not a problem for the RMS because the SO_4^{2-} concentration can be easily adjusted when preparing the fresh ASW with sea salts. Our future study will investigate the autotrophic denitrification process for the removal of NO_3^- in the Beijing Aquarium.

4. Conclusions

This study investigated the occurrence of various kinds of DBPs in seven representative tanks (*i.e.*, fresh ASW, big fish, jellyfish, coral, dolphin, sea lion, and white whale) of the Beijing Aquarium. Based on the monitoring results, the following conclusions can be drawn:

- The seawater treatment processes currently adopted by the Beijing Aquarium, including sand filtration, foam fractionation and disinfection (O_3 , UV, and O_3/ClO_2), could fairly well control both physicochemical (*i.e.*, pH, DOC, UV_{254} , SUVA, and turbidity) and microbial (*i.e.*, MHB) aspects of seawater quality.
- The secondary oxidants, free chlorine and free bromine, were generated from ozonation with concentrations of $19.0\text{--}71.5$ and $40.2\text{--}692.5\ \mu\text{g L}^{-1}$, respectively. Their presence not only posed a serious threat to the health of aquarium animals, but also enhanced the formation of other DBPs (e.g., BrO_3^- , THMs, and HAAs).
- The BrO_3^- concentration in the animal tanks ranged broadly from 30.1 to $262.0\ \mu\text{g L}^{-1}$, with a descending order of

O_3/ClO_2 (dolphin, sea lion, and white whale tanks) $> \text{O}_3$ (big fish tank) $> \text{UV}$ (jellyfish and coral tanks). The mammal tanks exhibited the highest BrO_3^- concentrations, which exceeded the USEPA-regulated MCL for drinking water by 19–25 times.

- High concentrations of ClO_3^- (53.2–55.1 mg L^{-1}) were only detected in the mammal tanks where ClO_2 was applied, which exceeded the USEPA-regulated MCL for drinking water by 52–54 times.

- The mammal tanks exhibited extremely high concentrations of NO_3^- (1448–1572 mg L^{-1}), which considerably exceeded the MCL regulated by the Sea Water Quality Standard of China for the mariculture industry (Class II) by about 1100 times.

- The chlorinated DBPs were formed in animal tanks at low concentrations (*i.e.*, 1.3–3.1 $\mu\text{g L}^{-1}$ THM4 and 1.6–9.7 $\mu\text{g L}^{-1}$ HAA5) because Cl_2 was not used as a disinfectant in the Beijing Aquarium.

- To solve the serious problems caused by DBPs of high concern, potential control measures are proposed such as stopping the external addition of Br^- ions into the fresh ASW, replacing ClO_2 with Cl_2 , decomposing the major precursor of THMs and HAAs by O_3 -BAC, and removing NO_3^- by a heterotrophic or autotrophic denitrification process. In the future, we will investigate the operational feasibility of these control measures to establish a clean, safe, and sustainable living environment for aquarium animals.

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