Environmental Science
Processes & Impacts

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

rsc.li/process-impacts

The formation of various kinds of DBPs in marine aquaria raised serious concerns on their health threats to aquarium animals.

Environmental impact: Recirculating mariculture system (RMS) has been widely employed in marine aquaria around the world, but hazardous disinfection byproducts (DBPs) formed during its seawater treatment have long been neglected. 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 were proposed accordingly. This study provides useful and timely information to mariculture industry regarding the occurrence and control of DBPs.

Environmental Science
Processes & Impacts

Cite this: DOI:

www.rsc.org/jem

PAPER

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

Zhimin Qiang [*a], Haiting Zhang [a], Huiyu Dong [a], Craig Adams [b], Gang Luan [c], and Lei Wang [c]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI:

Disinfection is commonly employed in recirculating mariculture systems (RMS) to control animal diseases and improve seawater quality, but little is known about the occurrence of disinfection byproducts (DBPs) formed therein. 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, but emerging 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 with 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 greatly exceeded the MCL regulated by the Sea Water Quality Standard of China for mariculture industry (Class II) by about 1100 times. Undoubtedly, the presence of various DBPs posed serious health threats to aquarium animals. To solve these problems, potential control measures for DBPs were proposed accordingly.

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 system (RMS) has 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) technologies 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 provide 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 needed to maintain continuous disinfection ability. Meanwhile, O_3 can readily oxidize chloride (Cl^-) and bromide (Br^-) ions in seawater to free chlorine ($HOCl/OCl^-$) and free bromine ($HOBr/OBr^-$), thus not only causing respiratory and osmoregulatory dysfunctions in fish,[8] 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^- with concentrations of up to 100 μg L^{-1}.[10,11] The natural or artificial seawater in marine aquaria has a high salinity (ca. 3.5%) and contains approximately 19 g L^{-1} Cl^- and 67 mg L^{-1} Br^-,[12] which favors the formation and accumulation of harmful DPBs inside.

ClO_2 disinfection will produce 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

[a] *Key Laboratory of Drinking Water Science and Technology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China. E-mail: qiangz@rcees.ac.cn; Fax: +86 10 628 496 59; Tel:+86 10 628 496 32*
[b] *Department of Civil and Environmental Engineering, Utah State University, Logan, UT 84322, US.*
[c] *Water Quality Analysis and Control Center, Department of Life Support Equipments for Aquatic Animals, Beijing Aquarium, Beijing, 100081, China.*

effective against a wide range of pathogens and capable of providing necessary residual protection in water distribution systems. However, Cl_2 reacting with DOM will produce harmful THMs and HAAs. For example, it was found that uneaten food, and animal feces and urine all 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 effective inactivation of pathogens, little formation of DBPs, small space occupancy, and easy operation.

It is also noted that NO_3^- is often generated in RMS at levels far greater than that normally found in natural seawater due to microbial nitrification, chemical oxidation, and addition of microalgae as a food source for filter-feeding organisms.[14] For example, the concentration of NO_3^- was measured to be 9700 μM (i.e., 601 mg L^{-1}) in the Main Tank of The Living Seas artificial aquarium in Florida[15] and 9500 μM (i.e., 589 mg L^{-1}) in the Ocean Tank of New Jersey State Aquarium[16]. However, Bower and Turner[17] suggested an acceptable level of 88.6 mg L^{-1} NO_3^- for mariculture.

The formation of DBPs has been extensively studied in drinking water; however, to date little is known about their occurrence in marine aquaria. Therefore, this study aimed to determine the concentration levels of DBPs formed in various animal tanks of Beijing Aquarium, and then 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

The chemicals 2,6-dimethylphenol (2,6-DMP), 4-chloro-2,6-dimethylphenol, 4-bromo-2,6-dimethylphenol, and 2,4,6-trichlorophenol (2,4,6-TCP) were obtained from Alfa Aesar (Haverhill, MA, US). The standards of THMs and HAAs were purchased from Accustandard (New Haven, CT, US). Sodium hypochlorite, sodium chlorite, sodium chlorate, potassium bromide, and potassium bromate were products of Beijing Chemical Reagents Company (Beijing, China). All these 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 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

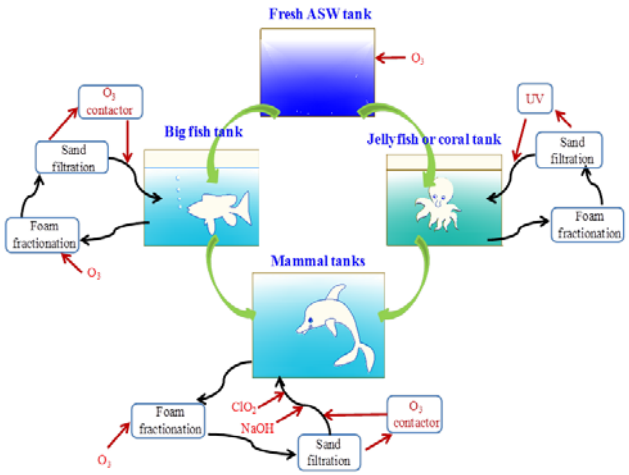

Fig. 1 Schematic diagram of seawater treatment processes for the recirculating mariculture system of Beijing Aquarium.

to ozone treatment before being supplied to non-mammal (e.g., big fish, jellyfish, coral) and mammal (e.g., dolphin, sea lion, white whale) tanks in sequence, to make up for the volume loss during treatment and recycling (Fig. 1). Every month, about 20–25% of seawater in the animal tanks is replaced by the fresh ASW. All animal tanks in Beijing Aquarium are indoor with seawater temperature controlled 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. Specifically, O_3 is first injected online into the foam fractionation tank, where suspended solids are removed by air floatation and a certain portion of DOM is decomposed by O_3 oxidation. The penetrating solid particles are further removed by sand filtration. Afterwards, a side stream of the sand-filtered seawater is contacted with O_3 for about 5 min (i.e., bypass treatment) and 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 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 approximately ranges from 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 Beijing Aquarium from June 2012 to March 2013. Specifically, on a sampling day, two samples were collected from each tank in the morning and afternoon separately. 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-μm 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 then the specific UV absorbance (SUVA) value was 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-μm 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 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 filtered through an OnGuard II Ag cartridge and an OnGuard II H cartridge in sequence, 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 reach a final concentration of 20 mg L^{-1} (as Cl_2). The sample was placed in the dark for 72 h at a controlled temperature (21 ± 2 °C), quenched with $Na_2S_2O_3$ solution, and analyzed for the concentrations of THM4 and HAA5 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 Beijing Aquarium are listed in Table 1. The results show that the pH of different tanks varied only in a narrow range of 7.86–8.12, similar to that of natural seawater (i.e., pH 7.8–8.2). The fresh ASW tank had a low DOC concentration of 1.3 mg L^{-1}, which mainly came from the tap water used for 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., 8.0–9.7 mg L^{-1}) than other tanks because of two facts: (1) these tanks receive the used seawater from the non-mammal tanks (Fig. 1); and (2) mammals have more intake of food 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 (0.012–0.022 cm^{-1}) and mammal tanks (0.084–0.089 cm^{-1}). The SUVA of the target tanks ranged from 0.71 to 1.11 L m^{-1} mg^{-1}, implying that the samples mainly contained hydrophilic DOM with a low aromaticity.[19] In addition, all tanks had low turbidities (i.e., 0.10–0.44 NTU), indicating the high efficiency of the foam fractionation and sand filtration processes on the removal of suspended particles. The clarity of the seawater 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 in four typical animal tanks were monitored once per week throughout 2012 and 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 as 4860, 3480, 3000, and 900 colony forming units per 100 mL of sample [CFU (100 mL)$^{-1}$] in summer, and 3340, 2520, 2490, and 210 CFU (100 mL)$^{-1}$ in winter, respectively. Spring and summer are breeding seasons for most kinds of fish, and the presence of larvae provides a favorable condition for bacteria 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, the concentration of MHB 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 manifested 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

Table 1 Major characteristics of seawater in seven representative tanks of Beijing Aquarium (mean ± standard deviation, $n = 5$ [a]).

Sampling tanks	pH	DOC (mg L^{-1})	UV_{254} (cm^{-1})	SUVA (L m^{-1} 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).

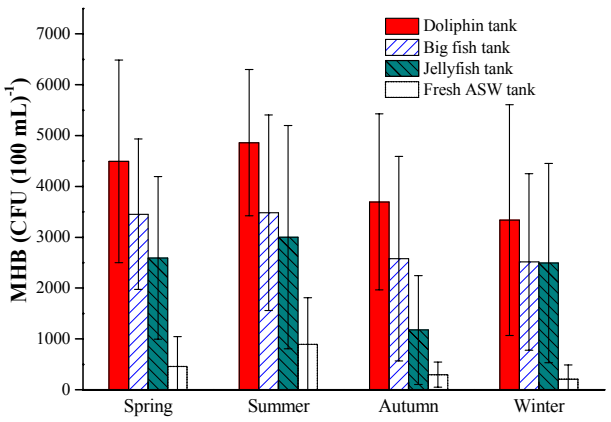

Fig. 2 Inactivation of marine heterotrophic bacteria in four typical animal tanks in different seasons (n = 13).

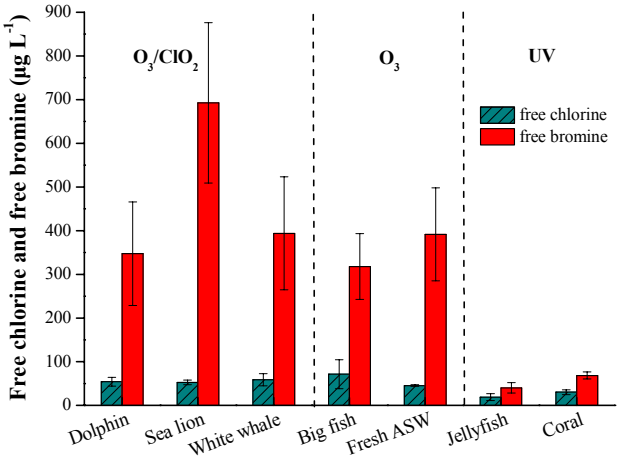

Fig. 3 Free chlorine and free bromine concentrations in various animal tanks (n = 5).

that the fresh ASW tank had the highest turbidity among the four tanks, which arose mostly from the undissolved salt particles. After these particles were effectively removed by the foam fractionation and sand filtration processes, the turbidity of seawater dropped obviously 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., 1×10^4–5×10^6 cells mL^{-1}),[21] demonstrating that the disinfection processes adopted in Beijing Aquarium (i.e., O_3, UV, and O_3/ClO_2,) are highly effective in controlling bacteria 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. 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 μg L^{-1}, respectively. Despite of a much 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 O_3 (i.e., k_{O_3,Cl^-} = 0.003 M^{-1} s^{-1}) is by far smaller than that between Br^- and O_3 (i.e., k_{O_3,Br^-} = 160 M^{-1} s^{-1}).[22,23] Moreover, free chlorine can also react fast with Br^- (k_{HOCl,Br^-} = 6.77 × 10^3 M^{-1} 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 O_3/ClO_2 and O_3 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 O_3 dose (ca. 0.5 mg O_3 per mg DOC) was initially applied to improve both physicochemical and microbial aspects of the sweater 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] by 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 those 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 BrO_3^-, ClO_3^-, ClO_2^-, and NO_3^-, in various animal tanks are 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 μg 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). Since 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. In the meantime, 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 O_3 was continuously applied therein. Note that the BrO_3^- concentration in each tank was all above the maximum contaminant level (MCL) regulated by USEPA for drinking water (10 μg L^{-1}), particularly those in the mammal tanks exceeded by 19–25 times. A former study on eastern oysters reported significant reduction in embryo development at a BrO_3^- concentration of 50 μg 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 μg 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 will be 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

Table 2 The concentrations of DBPs in seven representative tanks of Beijing Aquarium (mean ± standard deviation, $n = 5$ [a]).

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

[a] As same as in Table 1; [b] ND: not detected.

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]

The 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 then 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 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 of 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 Beijing Aquarium to resolve. By 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 studied animal tanks, respectively. Their 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 will in turn promote the formation of THMs and HAAs.[11,18] Our monitoring results show that the concentrations of THM4 and HAA5 were both below the USEPA-regulated MCLs for drinking water, i.e., 80 and 60 μg L^{-1}, respectively (Table 2). It is not surprising because THM4 and HAA5 are primarily generated during Cl_2 disinfection. In addition, the low SUVA values (< 1.2) in all studied animal tanks implied that hydrophilic DOM predominated in the seawater (Table 1), which was unfavorable for the formation of THM4 and HAA5. Hua and Reckhow[31] found that hydrophobic DOM is the major precursor of THMs and HAAs in natural waters.

The highest concentrations of THM4 (22.3 μg L^{-1}) and HAA5 (17.2 μg L^{-1}) were detected in the fresh ASW tank. These chlorinated DBPs could be carried over from 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, since they contained much 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 by the secondary oxidants (i.e., free chlorine and free bromine), thus leading to an obvious decrease in their concentrations. The mammal tanks also showed low concentrations of THM4 (2.0–3.1 μg L^{-1}) and HAA5 (5.8–9.1 μg L^{-1}), 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.

3.6 Potential control measures for DBPs

As described above, the serious problems that Beijing Aquarium is facing with include the formation of secondary oxidants at health-relevant levels, especially free bromine (40.2–692.5 μg 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 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 seems indispensible due to 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] and some shellfish as well (informed by the animal life support professionals in Beijing Aquarium). In fact, some US marine aquaria adopting the RMS have long been operated under low Br^- concentrations (e.g., 4.3 mg L^{-1}),[11] and no discernable negative effects to aquarium animals have been reported. According to our monitoring results, the management of 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 drop from 66 to 3 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 another more suitable

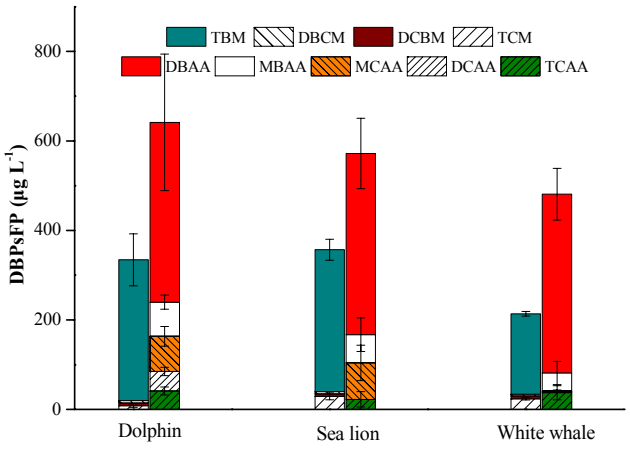

Fig. 4 The formation potential of chlorinated DBPs (THM4 and HAA5) in three mammal tanks ($n = 3$).

disinfectant. Cl_2 tends to be an alternative since it has long been employed for 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 μg L^{-1} for the dolphin tank, 356 and 571 μg L^{-1} for the sea lion tank, and 213 and 481 μg 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 (O_3-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 about DOC removal by O_3-BAC is currently carried out in Beijing Aquarium.

To control the NO_3^- concentrations in animal tanks, heterotrophic denitrification process has been evaluated at pilot-scale in Beijing Aquarium with externally supplied methanol as carbon source. The long-term continuous operation of two pressurized heterotrophic denitrification reactors has shown about 70% removal of NO_3^-. Alternatively, autotrophic denitrification process, which uses elemental sulfur as electron donor and NO_3^- as electron acceptor, seems to be a better choice for NO_3^- removal in the RMS.[37] On the one hand, autotrophic denitrification process does not require an external supply of carbon source (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 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 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 while) of Beijing Aquarium. Based on the monitoring results, the following conclusions can be drawn:

- The seawater treatment processes currently adopted by 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 the seawater quality.
- The secondary oxidants, free chlorine and free bromine, were generated from ozonation with concentrations of 19.0–71.5 and 40.2–692.5 μg L^{-1}, respectively. Their occurrence 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 animal tanks ranged broadly from 30.1 to 262.0 μg 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). The mammal tanks had 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 had extremely high concentrations of NO_3^- (1448–1572 mg L^{-1}), which greatly exceeded the MCL regulated by the Sea Water Quality Standard of China for 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 μg L^{-1} THM4 and 1.6–9.7 μg L^{-1} HAA5), because Cl_2 was not used as disinfectant in Beijing Aquarium.
- To solve the serious problems caused by DBPs of high concern, potential control measures were proposed accordingly, 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 heterotrophic or autotrophic denitrification process. Our future study will investigate the operational feasibility of these control measures, expecting to establish a clean, safe, and sustainable living environment for aquarium animals eventually.

Acknowledgements

This work was financially supported by the People Programme (Marie Curie Actions) of the European Union's Seventh Programme FP7/2007-2013 under a REA grant (Agreement No. 318926) and the National Natural Science Foundation of China (51221892).

References

1 M. J. Sharrer, Y. Tal, D. Ferrier, J. A. Hankins and S. T. Summerfelt, *Aquacult. Eng.*, 2007, **36**, 159–176.

2 L. R. Pomeroy and W. J. Wiebe, *Aquat. Microb. Ecol.*, 2001, **23**, 187–204.

3 N. Leonard, J. P. Guiraud, E. Gasset, J. P. Cailleres and J. P. Blancheton, *Aquacult, Eng.*, 2002, **26**, 111–127.

4 J. J. Wang, A. T. Chow, J. M. Sweeney and J. A. K. Mazet, *Water Res.*, 2014, **59**, 219–228.

5 A. A. Gonçalves and G. A. Gagnon, *Ozone-Sci. Eng.*, 2011, **33**, 345–367.

6 S. T. Summerfelt, *Aquacult. Eng.*, 2003, **28**, 21–36.

7 M. S. Tango and G. A. Gagnon, *Aquacult. Eng.*, 2003, **29**, 125–137.

8 D. P. Middaugh, L. E. Burnett and J. A. Couch, *Estuaries*, 1980, **3**, 132–141.

9 U. von Gunten, *Water Res.*, 2003, **37**, 1469–1487.

10 E. Agus, N. Voutchkov and D. L. Sedlak, *Desalination*, 2009, **237**, 214–237.

11 H. L. Shi, Z. M. Qiang and C. Adams, *Chemosphere*, 2013, **90**, 2485–2492.

12 D. R. Kester, I. W. Duedall, D. N. Connors and R. M. Pytkowic, *Limnol. Oceanogr.*, 1967, **12**, 176–183.

13 D. Couri, M. S. Abdel-Rahman and R. J. Bull, *Environ. Health Persp.*, 1982, **46**, 13–17.

14 P. R. Muir, D. C. Sutton and L. Owens, *Mar. Biol.*, 1991, **108**, 67–71.

15 G. Grguric and C. J. Coston, *Water Res.*, 1998, **32**, 1759–1768.

16 G. Grguric, C. J. Sondey and B. M. DuVall, *Sci. Total Environ.*, 2000, **247**, 57–69.

17 C. E. Bower and D. T. Turner, *Aquaculture*, 1983, **34**, 85–92.

18 Z. M. Qiang, Y. J. Jiang, W. W. Ben, C. Adams and H. Y. Dong, *Anal. Methods*, 2012, **4**, 3646–3652.

19 L. Liang and P. C. Singer, *Environ. Sci. Technol.*, 2003, **37**, 2920–2928.

20 Y. X. Wu, T. Clevenger and B. Deng, *Appl. Environ. Microb.*, 2005, **71**, 4140–4143.

21 F. Azam, T. Fenchel, J. G. Field, J. S. Gray, L. A. Meyer-Reil and F. Thingstad, *Mar. Eco-Prog. Ser.*, 1983, **10**, 257–263.

22 W. R. Haag and J. Hoigne, *Environ. Sci. Technol.*, 1983, **17**, 261–267.

23 W. R. Haag and J. Hoigne, *Water Res.*, 1983, **17**, 1397–1402.

24 A. Bousher, P. Brimblecombe and D. Midgley, *Water Res.*, **1986**, 20, 865–870.

25 V. Uyak and I. Toroz, *J. Hazard. Mater.*, 2007, **149**, 445–451.

26 D. J. Fisher, D. T. Burton, L. T. Yonkos, S. D. Turley and G. P. Ziegler, *Water Res.*, 1999, **33**, 760–768.

27 M. E. Stewart, W. J. Blogoslawski, R. Y. Hsu and G. R. Heiz, *Mar. Pollut. Bull.*, 1979, **10**, 166–169.

28 C. Collivignarelli and S. Sorlini, *J. Water Supply Res. Technol.*, 2004, **53**, 159–168.

39 C. S. Furman and D. W. Margerum, *Inorg. Chem.*, 1998, **37**, 4321–4327.

30 I. N. Odeh, J. S. Nicoson, K. E. H. Hartz and D. W. Margerum, *Inorg. Chem.*, 2004, **43**, 7412–7420.

31 G. H. Hua and D. A. Reckhow, *Environ. Sci. Technol.*, 2007, **41**, 3309–3315.

32 K. M. S. Hansen, R. Zortea, A. Piketty, S. R. Vega and H. R. Andersen, *Sci. Total. Environ.*, 2013, **443**, 850–856.

33 W. Fenical, *J. Phycol.*, 1975, **1**, 45–259.

34 S. Andersson, L. Kautsky and N. Kautsky, *Mar. Biol.*, 1992, **114**, 661–665.

35 J. S. Yang, D. X. Yuan and T. P. Wen, *Desalination*, 2010, **263**, 271–278.

36 W. H. Chu, N. Y. Gao, D. Q. Yin, Y. Deng and M. R. Templeton, *Chemosphere*, 2012, **86**, 1087–1091.

37 A. Koenig and L. H. Liu, *Water Environ. Res.*, 2004, **76**, 37–46.

38 D. J. Wan, H. J. Liu, J. H. Qu, P. J. Lei, S. H. Xiao and Y. N. Hou, *Bioresource Technol.*, 2008, **100**, 142–148.