Evaluating the biological efficacy of a ballast water management system using filtration and electro-catalysis with an accurate definition of holding time

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ABSTRACT

We employed a mesoscale experimental system and enriched natural plankton communities to investigate the efficacy of a type of ballast-water management system (BWMS) that uses a combination of filtration and electro-catalysis as the treatment technology. The water samples were collected immediately after treatment and at discharge to measure the biological efficiency of this BWMS. The main hydrochemical parameters, the TRO concentration and the plankton species composition before and after the ballast treatment process, were measured. After treatment, the concentration of TRO remained at a high level of 1.690 ± 0.573 (SD) mg/L. The biological efficacy of ballast water after treatment at holding times of 10 min, 20 min, 30 min, 40 min, and 50 min were measured. Holding time significantly impacted the biological efficacy. The discharged, treated water satisfied the D-2 standard of the International Maritime Organization (IMO) after 50 minutes of holding time.

Key words: ballast-water management system, electro-catalysis treatment, holding time, total residual oxidant

HIGHLIGHTS

• A mesoscale experimental system and cultured nature plankton communities were employed to investigate the efficacy of a ballast water management system (BWMS).
• The BWMS used a combination of filtration and electro-catalysis that proved to be effective.

1. INTRODUCTION

Ships that carry little cargo exhibit instability and vulnerability under conditions of strong winds and high waves. For the past century, the solution to this problem has involved taking onboard large quantities of ballast water in watertight compartments in order to lower the ships’ waterlines. Ships often take on ballast water at the beginning of the voyage, and they discharge it at the end of the voyage to load more cargo. Ship load and unload ballast water for various other reasons, such as adjusting the ship’s trim and increasing propulsion efficiency (Yonsel et al. 2014).

Ballast-waters have potentially hazardous consequences at the destination ports. A variety of aquatic organisms can be spread through the discharge of untreated ballast water, including bacteria and viruses; microalgae; protists; eggs, larvae, and small invertebrates; and the spores, seeds, and cysts of plants (Yonsel et al. 2014).

To date, more than 100 million tons of sediments and 10 billion tons of ballast water have transferred between ports over the world (Tamelander et al. 2010). The International Maritime Organization (IMO) initially recommended that vessels release the ballast they take onboard in coastal waters and replace it with oceanic water during the course of their voyages. In 2014, the IMO adopted the Ballast Water Management (BWM) Convention, which requires ships to conduct ballast-water exchanges with a volumetric efficiency of at least 95% (Molina & Drake 2016). However, open-ocean exchange proved to be limited in that it could not prevent the invasion of imported pathogens and exotic species (Molina & Drake 2016). In response, the Ballast Water Treatment (BWT) Convention was enacted by the IMO in 2017 (Molina & Drake 2016). This requires that existing ships with renewal surveys after 8 September 2019 must meet the D-2 standard by the time of their surveys.

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BWT can largely eliminate the spread of harmful aquatic organisms carried in ballast water, but the shipping industry will face huge economic and regulatory challenges. The total cost of retrofitting and operation for existing ships has been estimated as high as 74 billion USD (Aravossis & Pavlopoulou 2013). It is imperative that the onboard systems be affordable, and easy to use and install.

According to the requirements, ballast water treatment facilities on land must also meet the IMO’s G8 guidelines. IMO MEPC has adopted the D-2 discharge standard (Table 1) as uniform specifications for testing and performance of ballast water management systems (BWMS). This ensures the desired treatment effect before being discharged into the aquatic environment. Wherever ballast water is treated by ballast water reception facilities, it should at least meet the ballast-water performance standard specified in standard D-2 of the Convention before being discharged into the aquatic environment. At present, electro-chlorination is one of a number of BWMS technologies approved by the IMO and the United States Coast Guard (Goncalves & Gagnon 2012; Guney & Yonsel 2013). As of July 10th, 2019, there were 81 types of BWMS products approved by the IMO. Twenty-four of these products used this technique (G8 MEPC174 (58) Class NK EQD 18 March 2021). The technology has also been studied for several decades and found to be effective in municipal and industrial wastewater treatment (Yavuz et al. 2010). It is reliable, with low energy consumption in saline water (Li et al. 2002). There were 81 types of BWMS products approved by IMO till July 10th, 2019. Twenty-four of these products used the technique, according to ClassNK EQD as of 18 March 2021, Latest Information of Approval of Ballast Water Management System (G8 MEPC174 (58)).

Both direct and indirect oxidation processes can destroy the cells of organisms in ballast water (Guney & Yonsel 2013). In electro-chlorination, chlorine, sodium hydroxide and hydroxyl ions are simultaneously generated as active substances of the disinfection process, together referred to as ‘total residual chlorine’ (TRC) (Guney & Yonsel 2013). In the process, some other oxidants are produced that are also capable of inactivating organisms, such as hydroxyl radicals (OH), hydrogen peroxide (H$_2$O$_2$), and ozone (O$_3$). All the halogen elements in the +1 oxidation state and the total ozone are referred to as ‘total residual oxidant’ (TRO) (Jung et al. 2014). They decay with time, and the decay process is influenced by factors that include light, salinity, TRO concentration, and source-water chemical characteristics (Jung et al. 2014).

Previous studies have suggested the initial TRO concentration is primarily affected by conductivity and electrolysis time, and that its decay time is affected by temperature, organic compounds, inorganic compounds, and other factors (Hwang et al. 2010; Yavuz et al. 2010; Kim et al. 2012). The essential TRO dosage concentration depends on the pretreatment apparatus, properties of the species in the ballast water, and water conditions such as temperature and turbidity (Kim et al. 2012). The maximum allowable dosage of TRO is critical to the efficacy of BWMS utilizing electrolysis approaches (Kim et al. 2016). When TRO decays and the concentration TRO falls below 0.5–1 mg/L, the bacterial population begins to multiply (Perrins et al. 2006).

As the initial dosage of TRO and holding time are the two most important factors determining the efficacy of disinfection of ballast water (Perrins et al. 2006), optimizing the combination of these two factors remains the primary challenge in the design and testing of BWMS. The initial dosage of TRO had been discussed for many years. One report proposed that the concentration should be above 1.85 mg/L to ensure the disinfection effect (Perrins et al. 2006). Nielsen (2006) argued that the concentration of TRO should be higher than 3.0 mg/L for disinfection. Another report concluded that the TRO dosage of approved treatment systems ranged extensively from 1 to 15 ppm (Kim et al. 2012). However, the allowable dosage of TRO must be restricted in order to reduce the corrosion of marine carbon-steel (Song et al. 2009; Wang et al. 2013) and the required amount of neutralizer added prior to discharge. Prolonging the holding time will potentially increase a ship’s berthing time, whereas enhancing the initial dosage of TRO will increase energy consumption (Zhang et al. 2018). Therefore, shortening the holding time and reducing the initial dosage of TRO simultaneously should be a goal of BWMS design.

Previous studies have often utilized several indicator species to evaluate the efficiency of the treatment system (Hwang et al. 2010; Tao et al. 2017). However, the IMO requires evaluating impacts on the natural plankton community for approval of ballast water management systems (BWMS CODE) (IMO 2018). Evaluating the natural plankton communities is more useful and practical for developing treatment techniques and improving the design of treatment machines. Moreover, although sufficient holding time is essential to the efficacy of UV treatment systems, studies of the BWMS relationship between removal efficiency, initial TRO concentration and holding time of BWMS using electro-catalysis are scarce. Thus, we employed a mesoscale experimental system and cultured natural plankton communities to test the biological efficacy of a treatment system using filtration and electrolytic chlorination. The main operating conditions of the system, the treatment efficiency of plankton at different reaction times, and the species composition of plankton in the outlet-water were investigated to examine the relationship between holding time and efficiency of the treatment system.
2. METHODS

2.1. Ballast-water management systems and definition of T0

A BWMS was installed in the test facility at the Ballast Water Detecting Lab of Shanghai Ocean University (SHOU-BWDL) (Figure 1). This BWMS uses a fully automatic back flushing sand filter to remove most of the biological and non-living particles. Following the filter there is an electro catalysis ultra-treatment (EUT) unit. This unit uses high-performance semi-conductive catalysis materials to produce large numbers of active substances such as hydroxyl radicals. The current and voltage from the rectifier during the treating process remained at 346 A, 8.4–8.5 V. In all salinities, this was expected to give the target TRO of 2.0 mg/L. The BWMS uses a neutralization unit to neutralize the discharge water.

The definition of zero holding time, T0: In order to control holding time accurately neutralization was performed manually during sampling. The neutralizing agent was Na₂S₂O₅ and the primary reaction was:

\[ 5H_2O + Na_2S_2O_3 + 4Cl_2 \rightarrow Na_2SO_4 + H_2SO_4 + 8HCl \]

We utilized a neutralizing agent to control holding time, exploring the minimum holding time based on our definition of T0, which corresponded to the different designs of BWMS.

We define the start of holding time, T0, as the time the water is discharged from the electrolysis unit. For a BWMS with a system design limitation (SDL) of holding time, the neutralizing agent needs to be used at the end of the holding time and before discharging the ballast water to the sea. For a BWMS that can instantly discharge ballast water, the holding time is zero, and the neutralizing agent should be added at time T0. Based on the two situations, we designed the treatment track and the control track to explore the biological efficacy of the BWMS.

2.2. Experimental procedure

Experiments were conducted in August–September of 2019, and performed at a flow rate of 260 m³/h. Sea water was collected from the sea area near Yangshan Port. Brine was added to the natural water in the feed tank (Figure 2) to increase the salinity to 29 PSU. This and other chemical parameters were monitored and adjusted until all the parameters met the IMO and USCG standards. The densities of organisms in the inlet waters were monitored continuously until they were also adequate (IMO 2016). These parameters were monitored in the Inlet sample (Figure 2) taken before the filter and

Figure 1 | The mesoscale experimental system used in the experiment, which is located at the Ballast Water Detecting Lab of Shanghai Ocean University (SHOU-BWDL).
2.2.1. The treatment track
To explore the effect of the BWMS, we designed a treatment track and a control track. In the pipeline of the treatment track (Figure 2), 100-L samples of the treated water were collected continuously from a sampling valve (IMO 2016). Na₂S₂O₃ was added to the sampling buckets in advance. In this and all the following samples, neutralization was ensured by adding a 1.8-times higher dosage of Na₂S₂O₃ than needed to neutralize a TRO of 2.5 mg/L. This sample, T₀-AN (after neutralization) was the immediate discharge from the electrolysis unit. It represents discharged water after treatment by the BWMS but with no holding time. The purpose of this experiment was to simulate the model in which the treated water was discharged immediately. Neutralization was ensured by adding a 1.8-times higher dosage of neutralizer than needed to neutralize a TRO of 2.5 mg/L.

According to the requirements of the IMO type approval test of BWMSs, discharge of treated ballast water may first start after 120 minutes of holding time in the tank. Therefore, another time-integrated 100 L sample, T₁₂₀-AN, was collected after 120 minutes. Na₂S₂O₃ had been added by the BWMS’ neutralizing unit prior to sampling. Therefore, another time-integrated 100 L sample was collected after 120 minutes (shown in Figure 2 as T₁₂₀-AN, after neutralization). The T₁₂₀-AN sample represents the situation in which TRO is neutralized after the required holding time and the treated water is then discharged. From both T₀-AN and T₁₂₀-AN, 3/L subsamples were collected at early, middle, and late stages of the discharge process for determination of chemical parameters. Another 1 L subsample was taken from each 100-L water sample. This subsample was analyzed for living organisms. The T₁₂₀-AN sample represents the situation in which TRO is neutralized after ballast treatment and before being discharged.

2.2.2. Measuring the effect of holding time
The treatment track was used to determine the minimum holding time necessary to achieve the IMO limits. A 50-L water sample was collected after the electrolysis unit but without Na₂S₂O₃ in the sampling bucket. Then 1-L sub-samples were taken from this 50-L water sample at 0 min (T₀-BN, before neutralization), 10 min (T₁₀-AN), 20 min (T₂₀-AN), 30 min (T₃₀-AN), 40 min (T₄₀-AN), and 50 min (T₅₀-AN). Na₂S₂O₃ had been added to the AN sub-sampling bottles in advance. 1 L samples were also taken from each of the 5 subsamples. These were analyzed for living organisms (>50 μm). The neutralizing agent (Na₂S₂O₃) had been added to the sub-sampling bottles in advance for neutralizing TRO immediately. As above, the neutralization was ensured by adding a 1.8-times higher dosage of neutralizer than that needed to neutralize a TRO of 2.5 mg/L, representing the situation in which TRO was neutralized after ballast treatment and before being discharged.
2.2.3. Control track
Simultaneously, inlet water was pumped into a control track and was held for 2 hours without any BWMS treatment (Figure 2). Before and after the holding time of 2 hours, 2 x 100-L samples (Inlet-C and Discharge-C) were collected. From each 100-L sample, 3 x 1-L subsamples were collected at early, middle, and late stages of the discharge process. These subsamples were analyzed for the chemical parameters. 2 x 1-L subsamples were also collected from Inlet-C and Discharge-C. These subsamples were analyzed for living organisms.

2.3. Survey of living organisms
All the 1-L living organism subsamples were inverted several times to mix them. Then, 3 x 1 mL water from the 1-L samples collected from the Inlet and inlet-C samples and 6 x 1 mL water from the 1-L samples collected from the discharge samples (all the T samples plus Discharge-C) were extracted and placed in 2-mL centrifuge tubes (IMO 2016). The 1-mL samples were mixed with 5-chloromethylfluorescein diacetate (CMFDA; 2.5 μM final concentration) and fluorescein diacetate (FDA; 5 μM final concentration) (Blatchley et al. 2018) and incubated in the dark for 10 minutes. Each 1-mL, dyed water sample was transferred onto a 1–4 mL gridded Sedgewick Rafter chamber, and the chamber was placed under the epifluorescence microscope with a mesh microscale to examine and enumerate the organisms. The diameters of organisms counted were 10–50 μm. For the inlet samples, at least 100 cells were counted.

After counting had been done under fluorescence 3 times, the microscope was turned to the ordinary light mode (halogen lamp) and the samples were counted under this light 3 times. If the appearance of an organism was vague under fluorescence, the taxon could be determined by switching to ordinary light. For microbial samples, E. coli, Enterococci and Vibrio Cholerae samples were tested. For E. coli samples, 10 ml of sample liquid and 90 ml of sterilized water were taken into a 100 ml sterilized glass bottle then added a Colilert-18 kit (IDEXX laboratories Inc., Westbrook, ME, USA). For Enterococci samples, 10 ml of sample liquid and 90 ml of sterilized water were taken into a 100 ml sterilized glass bottle then added an Enterolert kit (IDEXX laboratories Inc., Westbrook, ME, USA). The IDEXX Colilert kit and the IDEXX Enterolert kit were used according to the manufacturer’s protocol. For Vibrio Cholerae samples, they were analyzed using the method in ETV. All samples were taken back to the laboratory on the day of sampling. Analysis of organisms ≥50 μm and 10–50 μm were finished within 6 hours after sampling, microbials were transformed in 2–6 °C and incubation was started in 6 hours after sampling, and each microbial sample was analyzed in triplicate. The concentrations of the organisms are listed in Table 2.

2.4. Chemical parameters
All of the 1-L chemical parameter samples were analyzed as follows. The TRO concentration was determined using a colorimetric DPD method following US EPA method 330.5. Other chemical parameters were also determined (Table 3). The measurements of TSS, POC, DOC, and UV-T were carried out. Temperature (T), salinity (SAL), turbidity and dissolved oxygen (DO) were measured when the water was sampled from the valve. Measurements were taken three times with a WTW Multi3630 (Germany) with a galvanic dissolved oxygen sensor. All sensors were calibrated beforehand.

2.5. Analytical procedures
The average density of living organisms in a water sample was calculated using the function:

\[ CSO_{\text{avg}} = \frac{\sum_{i=1}^{n} (CSO_i + CHO_i)}{n} \]

where \( CSO_{\text{avg}} \) was the average abundance of the living organisms in the sample (cell/mL), \( CSO_i \) was the abundance of the dyed organisms in the subsample, and \( CHO_i \) was the abundance of heterotrophic organisms in the subsamples. The differences between the concentrations of living organisms is shown in Figures 3–5. The graphs were created using GraphPad Prism 8 and MS Excel.

3. RESULTS
3.1. TRO and other chemical parameters
All the chemical parameters measured showed little change after both the treatment and neutralization reactions (Table 3). Chemical parameters of the inlet and discharge waters conformed to IMO regulations.
In the experiment exploring necessary holding time, neutralizer was added to the sampling container after disinfection. TRO concentration was determined as shown in Figure 3. The TRO concentration of inlet water was as low as ~0.01 mg/L. In the experiment evaluating the effectiveness of ballast-water treatment, the concentration of TRO decreased from 1.690 ± 0.573 SD (T0-AN) to 0.437 ± 0.005 (T120-AN) (Figure 3).

Figure 3 | Changes in TRO concentration in the experiments.

Figure 4 | Changes in cell concentrations of living organisms in the experiments.

Figure 5 | Cell concentrations of organisms after TRO treatment. Dashed red line shows D-2 standard approved by the IMO. The full colour version of this figure is available in the online version of this paper, at http://dx.doi.org/10.2166/wst.2021.410.

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3.2. Removal of living organisms
In the control track, the inlet water contained high concentrations of living organisms (1,499 ± 14.7 SD cell/mL). After 2 hours of holding time without any treatment, the concentration of living organisms in water samples of Inlet-C and Discharge-C, without any treatment, remained at a high level of 1,082.7 ± 45.1 cell/mL (Figure 4).

In the treatment track, the inlet water was filtered by the BWMS (Figure 2). After filtration, the cell-abundance decreased acutely. The cell concentration of T0-water decreased sharply but remained as high as 45.7 ± 4.19 cell/mL, not satisfying the D-2 standard. After 2 hours of holding time, the cell concentration of T120-AN decreased to 3.0 ± 1.2 cell/mL (Figure 2), which met the D-2 standard. All the concentrations of organisms we measured are shown (Table 2).

3.3. The removal of living organisms at different holding times
In the samples T0-BN to T50-AN, the concentration of living organisms decreased with time (Figure 4). The concentration met the requirement of IMO after 50 min. The results implied that the death rate and removal efficiency of living organisms increased with holding time.

3.4. Species composition in the inlet water
In all the treatment track samples, the species compositions of waters with the same salinity were similar. The most abundant plankton species in the enriched seawater were *Oscillatoria* sp. (Cyanophyta), *Pleurosigma* sp. (Chlorophyta), and *Gymnodinium* sp. (Pyrrophyta) (Tables 4 and 5). On the other hand, microalgae heterotrophs were scarce. The main species composition before and after treatment were also similar (Tables 4 and 5).

4. DISCUSSION
We employed filtration before electrolytic treatment to reduce the organism concentration in the inlet water because the efficiency of treatment is affected by the abundance of plankton (Hwang et al. 2010). Filtration reduced most plankton substantially, according to our results. However, pre-filtration cannot remove all ballast-water organisms due to intrinsic limitations (Gregg et al. 2009). It can, however, improve the removal efficiency of the electro-catalysis approach, reducing the minimum dosage of TRO (Kim et al. 2012). As a consequence, the necessary holding time and the required initial concentration of TRO may be reduced.

Disinfection was ensured when the filtration and electrolytic units were integrated, even when the dosage of TRO was less than 1.5 ppm (Matousek et al. 2006). Perrins et al. (2006) conducted a series of mesoscale experiments evaluating the biological efficacy of ozone treatment for ballast water. They used unfiltered water in the experiments, and suggested initial dosage of

### Table 1 | The IMO D-2 standard for ballast-water discharge

| Microorganism category                  | IMO standard                      |
|-----------------------------------------|-----------------------------------|
| >50 μm zooplankton                      | <10 viable cells /m³               |
| 10-50 μm phytoplankton                  | <10 viable cells /mL               |
| *Vibrio cholerae* bacterium             | <10 cfu/100 mL or <1 cfu/1 gram (wet weight) zooplankton samples |
| *E. coli* bacterium                     | <250 cfu/100 mL                    |
| Intestinal enterococci bacterium        | <100 cfu/100 mL                    |

### Table 2 | Concentrations of the living organisms

|               | Inlet | Inlet-C | Discharge | Discharge-C |
|---------------|-------|---------|-----------|-------------|
| ≥50 μm        | 125,000 | 108,815 | 0.33      | 89,333      |
| *E. coli*     | 442    | 414     | 10        | 439         |
| Enterococci   | 125    | 103     | <10       | 111         |
| *Vibrio cholerae* | Absent | Absent | Absent | Absent      |
TRO fluctuated among 2 mg/L to about 4.5 mg/L. Correspondingly, the holding time of ballast water was as long as tens of hours under certain dosages in laboratory studies examining the decay rate of TRO (Perrins et al. 2006). In our mesoscale experiments, the TRO concentration at T0 was $1.690 \pm 0.573$ SD mg/L (T0-AN, Figure 3). The TRO concentration decreased during 2 hours of holding time (Figure 4). The fact that the results met the D-2 standard (Figures 4 and 5) suggest that the

| Category | Inlet water | T0-AN | T120-AN |
|----------|------------|-------|---------|
| Pyrrophyta | Goniasulax polyedra | $88.00 \pm 6.48$ | $2.33 \pm 1.37$ | $0.33 \pm 0.47$ |
| Bacillariophyta | Chaetoceros sp. | $10.00 \pm 1.63$ | $5.67 \pm 1.89$ | |
| Cyanophyta | Chroococcus sp. | $358.67 \pm 28.29$ | $8.33 \pm 1.49$ | $1.17 \pm 0.69$ |
| Chlorophyta | Cosmarium sp. | $6.67 \pm 0.47$ | |
| Xanthophyta | Tribonema sp. | |
| Cryptophyta | Chroomonas acuta | |
| Euglenophyta | Euglena caudata | |
| Chrysophyta | Ochromonas sp. | |

Table 3 | Chemical parameters of inlet water and discharge water

| Parameter | Inlet water | Treated, T0-AN | Inlet-C water | Treated, T120-AN | Discharge-C |
|-----------|-------------|----------------|---------------|-----------------|------------|
| Temperature (°C) | 27.267 ± 0.047 | 27.267 ± 0.047 | 27.200 ± 0.047 | 27.433 ± 0.047 | 27.333 ± 0.047 |
| Salinity (PSU) | 29.300 ± 0.000 | 29.300 ± 0.000 | 29.333 ± 0.047 | 29.067 ± 0.014 | 29.300 ± 0.000 |
| pH | 8.470 ± 0.029 | 8.347 ± 0.012 | 8.449 ± 0.011 | 8.333 ± 0.002 | 8.439 ± 0.002 |
| DO (mg/L) | 8.000 ± 0.037 | 8.137 ± 0.048 | 8.000 ± 0.102 | 8.020 ± 0.014 | 7.977 ± 0.068 |
| Turbidity (NTU) | 18.710 ± 0.566 | 20.323 ± 1.297 | 15.377 ± 0.760 | 12.977 ± 0.984 | 14.320 ± 0.123 |
| TSS (mg/L) | 65.133 ± 1.569 | 65.367 ± 1.558 | 65.867 ± 1.342 | 66.100 ± 0.698 | 61.133 ± 1.320 |
| POC (mg/L) | 6.945 ± 0.380 | 6.975 ± 0.123 | 6.232 ± 0.170 | 6.740 ± 0.153 | 3.463 ± 0.201 |
| DOC (mg/L) | 13.063 ± 0.229 | 11.590 ± 0.360 | 13.783 ± 0.336 | 11.963 ± 0.276 | 12.783 ± 0.389 |
| MM (Mineral matter) (mg/L) | 58.188 ± 1.682 | 58.392 ± 1.487 | 59.635 ± 1.512 | 59.360 ± 0.686 | 57.670 ± 1.373 |
| UV-T (%) | 63.790 ± 0.615 | 68.477 ± 1.429 | 67.520 ± 0.984 | 71.543 ± 0.264 | 68.417 ± 0.111 |
| UV-T (%) (filtered) | 74.260 ± 0.147 | 77.073 ± 0.300 | 73.563 ± 0.483 | 75.543 ± 0.387 | 73.397 ± 0.246 |

Table 4 | Plankton concentrations of inlet water and samples collected at T0 (T0-AN) and T120 (T120-AN)
Table 5 | Plankton concentrations in samples collected at different times

|                | T0-BN |       |       |       |       |       |       |       |
|----------------|-------|-------|-------|-------|-------|-------|-------|-------|
|                | Test  | 1     | 2     | 3     | Test  | 1     | 2     | 3     |
|                |       | Mean ± |       |       |       |       |       |       |
|                |       | SD    |       |       |       |       |       |       |
| Pyrrophyta     |       |       |       |       |       |       |       |       |
| Gymnodinium    | 1     | 0     | 0     | 0.33 ±| 0.47  | 0     | 1     | 0     | 0.33 ±| 0.47  | 1     | 1     | 0     | 0.67 ±| 0.47  |
| aeruginosum    |       |       |       |       |       |       |       |       |
| Gonadulax      |       |       |       |       |       |       |       |       |
| polyedra       |       |       |       |       |       |       |       |       |
| Bacillariophyta| 5     | 7     | 12    | 8.00 ±| 2.94  | 6     | 10    | 4     | 6.67 ±| 2.49  | 8     | 2     | 9     | 6.33 ±| 5.09  |
| Pleonosigma sp.|       |       |       |       |       |       |       |       |
| Chlorophyta    |       |       |       |       |       |       |       |       |
| Platymonas sp. | 1     | 0     | 1     | 0.67 ±| 0.47  | 0     | 0     | 1     | 0.33 ±| 0.47  |       |       |       |       |
| Cyanophyta     | 9     | 2     | 13    | 8.00 ±| 4.55  | 6     | 8     | 8     | 7.33 ±| 0.94  | 11    | 4     | 10    | 8.33 ±| 5.09  |
| Oscillatoria sp.|       |       |       |       |       |       |       |       |
| Heterotrophs   | 1     | 0     | 0     | 0.33 ±| 0.47  |       |       |       |       |       |       |       |       |       |
| Total (cells/mL)| 16    | 9     | 25    |       |       | 13    | 18    | 13    |       |       | 19    | 7     | 19    |       |       |
| Average        |       |       |       |       |       |       |       |       |
| concentration  |       |       |       |       |       |       |       |       |
| (cells/mL)     | 16.67 | ±6.55 |       |       |       | 14.67 | ±2.36 |       |       |       | 15.00 | ±5.66 |       |       |       |
|                |       |       |       |       |       |       | 13.67 | ±1.70 |       |       | 10.67 | ±1.70 |       |       |       |
|                |       |       |       |       |       |       |       |       | 7.00 ±| 1.41  |       |       |       |       |       |
initial dosage of TRO was effective for disinfection of ballast water in our experiments. This experiment also showed that the removal efficiency of living organisms changed with holding time. The minimum disinfection time of 50 min is shorter than the IMO minimum holding time of 2 hours, implying that the IMO time might be shortened further.

After being generated, TRO disintegrates over short time intervals (Hwang et al. 2010). As the maximum allowable discharge of TRO must be controlled, the discharged water needs to be neutralized (Culin & Mustac 2015). A BWMS using active substances can neutralize the treated water either before or after a holding time. In this experiment’s treatment track, the TRO in the T0 sample was neutralized immediately after the electrolysis unit. Therefore, T0 refers to zero holding time. When water samples were collected at T0 as T0-AN sample, the TRO-disinfection process was stopped immediately and the aquatic organisms were sampled and assessed immediately. Our results indicated that the T0 samples were not in compliance with the D-2 standard. In our experiment aimed at exploring the minimum holding time with neutralization after the ballast treatment, T0 was referred to be the start time of holding time (Figure 2). Our results indicated the T0 samples likewise did not satisfy the D-2 standard. Subsequently, the concentration of living organisms in the water samples decreased gradually (Figure 4). By a minimum holding time of 50 minutes, the concentration of organisms satisfied the D-2 standard. The reason might be that the organisms treated by the TRO were immediately inactivated and incapable of reproducing, but their esterase activity should have continued for hours or even days due to continuing cell apoptosis. Previous reports have suggested that cumulative mortality caused by the persistence of TRO after treatment and during holding is essential for biological efficacy of BWMS (Perrins et al. 2006).

In recent years, reducing holding time has become critical in the design of BWMS; however, minimal or no holding time raises questions of sufficient disinfection and operational compliance. The revised G8 guidelines introduced the concept of System Design Limitations (SDL): documenting critical parameters to describe the limitations of design. Manufacturers should identify the SDL of a given BWMS according to its design, test procedure and type approval (TA). Our results imply that BWMS employing electro-catalysis as the treatment technology will require different procedures depending on whether neutralization occurs before or after the ballast treatment, including differences in initial TRO concentration, holding time, and verification sampling, consequently producing different SDLs.

Some previous studies took several or tens of days to evaluate the efficiency of the treatment for assuring that pathogens and/or plankton did not regrow (Casas-Monroy et al. 2016). Other studies aimed at testing and evaluating various treatments after several hours (Tao et al. 2017). Ballast-water treatments need to be implemented in different ways: before or after debal- lasting, before or after the ballasting procedure, and so on (Goncalves & Gagnon 2012). In consequence, a variety of treatment approaches with different holding times for different purposes need to be developed.

In the control track, without any treatment, the concentration of living organisms in water from Discharge-C without treatment retained concentrations >1,000 cells/mL. The cell concentrations before and after holding were not significantly different (Table 5). The samples before electro-catalysis treatment (T0-AN) and after treatment (T120-AN) were significantly different, indicating that treatment was effective. In the experiments, the plankton cells of the samples (<10 μm) were enumerated using fluorescence microscopy for distinguishing the dead cells. Although epifluorescence microscopy is time-consuming, it remains the most useful and the only reliable approach widely accepted by researchers for compliance testing (Casas-Monroy et al. 2016).

Discrepancies in the efficiencies measured in these studies might be due to taxon-specific differences in resistance to different treatment approaches (Kim et al. 2012). More species and natural aquatic communities should be employed for exploring the efficacies of different treatment techniques and for assessing the efficiency of BWMS.

### CONCLUSION

Since the dosage of TRO and the holding time are crucial for the effectiveness of BWMS using filtration and electro-catalysis techniques, the definition of zero holding time, T0, was fundamental for evaluation of biological effectiveness. We have evaluated the biological effectiveness of a BWMS based an accurate definition of T0. Holding time significantly impacted the biological efficacy, and was essential for the effectiveness of the system. Combining the filtration and electrolysis process (the peak value of TRO was 2.0 mg/L), the treated water could reach the discharge standard.

In this investigation combining filtration and electrolysis units (the peak value of TRO was 2.0 mg/L), the treated water reached the IMO discharge standard after a holding time of 50 min. However, due to taxon-specific differences in resistance...
to different treatments, this BWMS must be tested with other species and natural aquatic communities before a final assessment of its efficacy.

DATA AVAILABILITY STATEMENT
All relevant data are included in the paper or its Supplementary Information.

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