Non-native aquatic species dispersed via ships’ ballast water create a threat for local marine ecosystems throughout the world. Increasing maritime traffic and faster ships have increased the risk for species introductions during the last decades and effort has been made to develop reliable methods for ballast water management. While various technologies such as ultraviolet irradiation and ultrasonication have shown promising results, the efficacy of any treatment depends on various chemical, physical and biological properties of water such as turbidity, salinity and the size and type of the organisms. Although many of the major harbours are located in estuaries with a wide range of salinity, technologies developed so far have not been tested in brackish environments. In this study, we assessed the efficacy of four potential ballast water treatment technologies – ozonation, ultraviolet irradiation (UV), ultrasonication (US) and hydrogen peroxide ($\text{H}_2\text{O}_2$) – on indigenous brackish water zooplankton (copepods, cladocerans and rotifers as the main groups). All technologies showed promising results and high kill percents (>99 %) were achieved in these low-saline conditions. Different taxa responded differently to the various technologies but basically the most effective treatments were the combinations of US+UV and UV+ $\text{H}_2\text{O}_2$. Many issues, however, need to be addressed in order to define the feasibility of the technologies in full-scale applications.

Keywords: ballast water treatment, ultraviolet, ultrasound, hydrogen peroxide, ozone, mesozooplankton, brackish water
INTRODUCTION

Invasions of nonindigenous species transported via ships’ ballast water have been recognized throughout the world as a considerable threat for native aquatic flora and fauna. Uncontrolled dispersal of non-native species is leading to a worldwide homogenization of aquatic ecosystems and to an increasing loss of biogeographical integrity (Lovel, 1997; Leppäkoski & Olenin, 2001). Today more ships from more regions are carrying larger volumes of ballast water in less time than ever before (Ruiz et al., 1997). Aquatic organisms can survive for a considerable time in ships’ ballast tanks and some are even able to reproduce during the journey (Gollasch et al., 2000). Shipping has become the most important vector for the introduction of aquatic organisms; at any time several thousand species have been estimated to be hitchhiking in ballast tanks to new habitats (Carlton & Geller, 1993; Gollasch, 1996).

The Baltic Sea, the world’s largest brackish water region, forms a unique but challenging aquatic environment for its inhabitants: vertical and horizontal gradients in hydrological and biological factors such as temperature, salinity and primary production vary spatially and temporally. Severe environmental conditions combined with a short evolutionary history (the recent stage has lasted only ca 7500 years) result in a relatively low number of species, many of which live at the limits of their distribution. On the other hand, due to a rapid eutrophication during the last decades together with a lesser degree of competition, the abundance of the surviving taxa can reach extreme numbers (e.g. the invasion history of Marenzelleria cf. viridi; Stigzelius et al., 1997; Zettler 1997; Daynus et al., 1999). These characteristics make the Baltic Sea ecosystem extremely vulnerable for large-scale biological changes caused by non-native species. Recent invasions include the spionid polychaete M. cf. viridi and the predatory cladoceran Cercopagis pengoi, both transported via ships and potentially having a wide impact on the benthic and pelagic food webs (Leppäkoski & Olenin, 2000 and 2001). So far, species extinctions caused by invaders have not been demonstrated, but this possibility cannot be precluded in the future. Particularly, in the Gulf of Finland the maritime traffic has been predicted to increase two-fold by 2010–2015, and the total oil transportation will be three times higher in 2010. These numbers are largely due to the rehabilitation projects of old ports together with construction of new harbours in Russia and the Baltic States (Rytkönen et al., 2002).

As an attempt to control new species introductions via ballast water discharges, the International Maritime Organization (IMO) recently adopted International Convention for the Control and Management of Ship’s Ballast Water and Sediments (IMO, 2004), outlining general standards for ballast water management. Mid-ocean exchange of ballast water, currently the only generally applied method, is claimed unreliable and also involves a safety risk for ships (Locke et al., 1993; Rigby & Hallegraeff, 1994; Zhang & Dickman, 1999; Oemcke & van Leeuwen, 2003a). A number of alternative technologies have been tested in order to develop a cost effective, safe and environmentally friendly ballast water treatment strategy. Exposure to ozone (Davis & Arnold, 1997; Oemcke & van Leeuwen, 2004), ultraviolet light (Sutherland et al., 2001; Waite et al., 2003) or hydrogen peroxide (Bolch & Hallegraeff, 1993; Ichikawa et al., 1993; Kuzirian et al., 2001), among others, have
been reported to have lethal effects on various planktonic taxa such as bacteria, algal cells or cysts, and zooplankton. In addition, ultrasonic technology has shown promising results (e.g. Buchholz et al., 1998). The efficiency of different technologies vary, however, depending on the species and taxonomic levels and moreover, on the physical and chemical characteristics of water (Oemcke & van Leeuwen, 2003b). Although many if not most of the major harbours of the world are located at river mouths with wide salinity ranges, there are, to our knowledge, no studies concerning ballast water treatments on natural plankton communities in brackish-water conditions.

The aim of the present study was to determine and compare the efficacy of four potential future technologies – ozonation, ultraviolet irradiation, ultrasonication and hydrogen peroxide treatment – applied to natural zooplankton assemblage in low-saline coastal surface water (5-6 psu) having the potential to be loaded as ballast water. This paper is a contribution to the project “On Board Treatment of Ballast Water and Application of Low-sulphur Marine Fuel (MARTOB)”, funded by European Commission (2001-2004) under the Competitive and Sustainable Growth (GROWTH) Programme. Our work was focused on three objectives defined for the MARTOB project: (1) investigation of methodologies for preventing the introduction of nonindigenous species through ships’ ballast water, (2) development of treatment equipment to be used in the further development of ballast water treatment techniques, and (3) assessment of the effectiveness of current and newly developed methods.

**MATERIAL AND METHODS**

Technical descriptions and operational parameters

**Ultraviolet light**

Ultraviolet light (UV) treatment is a validated technological option for the disinfection of water. Irradiation induces photochemical changes of biological components in microorganisms or more specifically, degradation of chemical bonds in DNA and RNA molecules and cell proteins. The UV device used in the onshore test trials was provided and manufactured by Berson Milieutechniek BV, Netherlands. The Berson InLine 5 UV disinfection unit has one 316 l stainless steel irradiation chamber (length 460 mm, diameter 56 mm). Inside the chamber, one medium pressure mercury gas discharge lamp (B410 Berson MultiWave 350W) is mounted perpendicular to the flow and enclosed by a quartz sleeve. The UV output is 200-400 nm or germicidal UV output is 210-320 nm. UV output power is 58 W and operation gas pressure 2-3 bar.

Both the UV and ultrasound (US) technologies consisted of a flow-through system in which unfiltered seawater was conducted through the devices. Water samples were taken near the inlet (pre-treatment sample) and near the outlet (post-treatment sample). Due to an elaborate piping arrangement, some plankton seemed to remain stuck into the system. To minimize this source of technical error, the device was flushed with seawater for approximately 1 h between each replicate before sampling. UV treatments were run at flow rates of 200 to 800 l h⁻¹, corresponding to 562–141 mJ cm⁻³. Trials at 800 l h⁻¹ were run as a part of the hurdle technology combining UV with H₂O₂.
Ultrasound

Ultrasound treatment is a relatively new technology in ballast water management. Two types of US exist, low intensity, which is not used for disinfection, and power US. Ultrasound is generated by a transducer, which converts the mechanical or electrical energy into high frequency vibration, causing rapid formation and collapse of microscopic gas bubbles in the exposed liquid, and leading to rupture of cell membranes and collisions with other organisms. The intensity of US is influenced by frequency, power density and exposure time. Ultrasonic technology has been utilised in various applications in the water treatment and food industries in order to control microorganisms.

The US device used in the onshore test trials was designed and constructed by Acomarin Engineering Ltd, Finland. The device (effective output power 2000 W) is equipped with the dr. Hielscher UIP 2000 Ultrasonic Processor, including a generator, a transducer and a sonotrode. The amplitude is adjustable and equipped with an automatic frequency scanning system. As in UV treatments, the device was flushed for 1 h between the replicates. US treatments were run at flow rates of 200 to 800 l h⁻¹ and at four amplitudes (25 to 100 % of maximum oscillation).

FIGURE 1
A schematic layout of the test arrangements with the combination of hydrogen peroxide and ultraviolet light.
Ultrasound with ultraviolet

In addition to testing UV and US as single technologies, the effect of the combination of US and UV (US+UV) was investigated. In order to enable flexible test arrangements, the UV and US devices were built in the same test rig. Untreated seawater was conducted first through the US and then through the UV system. Trials were run at the flow rate of 520 l h⁻¹, at US amplitudes of 50 and 100 %.

Hydrogen peroxide with ultraviolet light

Hydrogen peroxide (H₂O₂) has both strong reductive and oxidizing properties. Direct reactions or (spontaneous) dissociations in various products (radicals) result in a number of molecular additions and substitutions or oxidations and reductions with a variety of organic and inorganic compounds. In solution, H₂O₂ will slowly decay into H₂O + O₂ which may be accelerated by the presence of various multi-valent metal cations or the catalase enzyme in solution, or the contact of the solution with metallic surfaces.

In this study, the combination of UV and H₂O₂ (UV+ H₂O₂) was tested. These hurdle treatments were conducted with two options, A and B, combining UV treatment (at a flow rate of 800 l h⁻¹) as described above with 48 h incubation with H₂O₂. Figure 1 shows the test design specifying the samples (S1 to S10) taken at different phases. In option A, the pre-UV sample (S1) was taken near the inlet and the post-UV sample (S2) near the outlet of the UV device. S2 was then poured in a 30 l container and incubated with H₂O₂ (S3: 15 mg l⁻¹; S4: 30 mg l⁻¹ and R1: 0 mg l⁻¹) for 48 h. In option B, untreated water was conducted in a 360 l tank from which the pre-treatment sample (S5 and S8) was taken. H₂O₂ (15 or 30 mg l⁻¹) was then added and the water was run through the UV device. The post-UV samples were taken immediately after UV (S6 and S9) or after 48 h incubation (S7 and S10).

Ozone

Ozone (O₃) has been used so far mainly in onshore applications for water treatment. In industrial applications, O₃ is not used to eliminate microbial populations, but rather to limit the population growth. O₃ is unstable at atmospheric pressure and thus it must be generated onsite. It is also a greenhouse gas and is toxic in high concentrations. When O₃ is introduced into water, three main processes affect the release of ozone: reaction with water impurities, decomposition, and stripping to the atmosphere.

Instead of a flow-through arrangement, the O₃ treatments were carried out in 60 and 360 l contact tanks. The O₃ device (Ozonfilt® OXVa Type 1) was designed and manufactured by ProMinent Dosierotechnik GmbH, Germany. Ozone, produced from ambient air by a generator, was fed to the bottom of the contact tank through a plastic pipe. The tank was equipped with a mixer (speed ca. 200 and 60 rpm in the 60 and 360 l tanks, respectively), to keep the water column homogenized.

Before introducing O₃, seawater was fed into the tank and a pre-treatment sample taken from this untreated water. Water was then incubated at 17 mg O₃ l⁻¹ (60 l tank) for 1 to 6 h (post-treatment samples taken at 1 h intervals) or at 7 mg O₃ l⁻¹ (360 l tank) for 1 to 24 h (post-treatment samples taken after 1, 2, 3, 5, 8 and 24 h).

Methods

Field experiments were carried out on the coast of the Hanko Peninsula, SW Finland, during September-October 2002 and August-
TABLE 1
Zooplankton densities in the pre-treatment samples in all replicate trials or incubations in UV, US, US+UV and H2O2 treatments. In H2O2, the sample codes S1 (pre-UV) and R2 (pre-H2O2) in option A and R5 and R8 (pre-H2O2) in option B refer to the pre-treatment samples specified in Figure 1. Cop: copepod adults and copepodites, Cn: copepod nauplii, Clad: cladocerans, Rot: rotifers, Cir: barnacle nauplii. (*) denotes densities <1 ind. l-1, i.e. densities below which the species group in question has been precluded from the analysis. In bold: the highest density of the treatment.

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September 2003. All experiments were conducted with unfiltered inshore seawater (average salinity 5 to 6 psu), either run through the devices or incubated in contact tanks. Samples were taken before and after treatment, i.e. a pre-treatment sample (10 to 15 l) and a post-treatment sample (15 to 60 l). The sample volume was determined on the basis of the ambient zooplankton density.

Sample water was poured into a 100 µm sieve, washed into 0.9 l plastic containers with filtered seawater (<10 µm) and transferred immediately to ca. +13°C for 2 to 5 h, to determine whether the paralysed organisms would revive rapidly, or conversely, whether the individuals survived would hereafter die. After the recovery time, the unpreserved samples were studied under stereomicroscope, allowing actively swimming and damaged individuals to be counted separately. The discrimination between active and damaged individuals was made visually, on the basis of swimming behaviour and morphological characters; i.e. organisms with normal swimming and escape responses were classified as “active” while those with clear physical injuries or evident changes in locomotion were judged “damaged”. Species were classified into four groups: adult copepods and copepodites (Copepoda), copepod nauplii (C. nauplii), cladocerans (Cladocera) and rotifers (Rotifera). In addition, planktonic larvae of barnacles (Cirripedia) and bivalves (Bivalvia) were included in the results when abundant (initial density >1 ind. l⁻¹, see below).

Three replicate trials (two replicates with UV+ H₂O₂) were conducted for each treatment combination, one replicate in 2002 and two replicates in 2003. Since the experimental water was directly taken from the sea, some species groups were occasionally rare. To eliminate stochastic effects of rare species in the flow-through test designs (i.e. treatments utilising UV and US devices), only groups with a minimum density of 1 ind. l⁻¹ in the pre-treatment sample of a given replicate trial were included in the analysis. Therefore, in some treatments with UV and US for some species groups the final number of replicates is less than three (Table 1).

To determine the mortality or loss of individuals caused by the devices themselves, without treatment doses, three replicate control trials (control incubations in O₃ treatments) for all technologies (in UV and US treatments separately at each flow rate) following the same procedure as with treatment trials (zero doses) were carried out. The fraction of organisms killed in a
treatment was determined by comparing the ratios of live planktonic organisms before and after treatment and before and after control:

\[
\text{Kill rate} = 1 - \frac{N_1}{N_0} / \frac{C_1}{C_0} \quad \text{(Eq. 1)}
\]

where

- \(N_0\) = density of live individuals before treatment
- \(N_1\) = density of live individuals after treatment
- \(C_0\) = density of live individuals before control
- \(C_1\) = density of live individuals after treatment

In addition, the percent of damaged and active individuals after treatment was determined as the ratio of damaged and active individuals in the post-treatment sample to all live individuals in the pre-treatment sample. The sum of the proportions of damaged and active organisms forms the total of live organisms after treatment (as a percentage of live organisms before treatment).

As the composition of zooplankton communities fluctuates between the seasons (Viitasalo et al., 1995), an additional water sample of 11 to 21 l was taken at the test area weekly to determine the ambient zooplankton density and species composition. The samples were preserved in Lugol's solution and counted under a binocular microscope.

Statistics

Data on kill percents were analyzed using the statistical package SPSS 11.0 for Windows. The difference in kill rates between the species groups, various treatment parameters (flow

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**FIGURE 2**
The composition of mesozooplankton community at the study site in September to October 2002 (left) and August to September 2003 (right). Others include e.g. larvae of bivalves, gastropods and polychaetes. Note the different scales on the y-axis.
rates, amplitudes etc.) and different treatments were tested with Mann-Whitney U-test or Kruskal-Wallis non-parametric ANOVA, followed by Tukey’s HSD.

RESULTS

Mesozooplankton community and water properties

Total mesozooplankton abundance in the preserved zooplankton samples varied largely, from less than 8 ind. l⁻¹ in October 2002 to more than 170 ind. l⁻¹ in September 2003 (Figure 2). In 2002 the average mesozooplankton density remained at a moderately stable level (~10 ind. l⁻¹), except in the beginning of this period when there was a higher copepod density (adults, copepodites and nauplii). Overall, zooplankton abundances were higher in 2003 than in 2002, mainly resulting of very high rotifer densities (maximum density 156 ind. l⁻¹). Also copepod nauplii were more abundant in the latter year, while the abundances of the other groups were closer to those in 2002. Taxa observed include the copepods Acartia bifilosa, Eurytemora affinis, Temora longicornis, Cyclops spp., Harpacticoida sp.; the cladocerans Pleopsis polyphemoides, Bosmina l. maritima, Evadne nordmanni, Polyphemus pediculus, Cercopagis pengoi, Ceriodaphnia sp., Daphnia cf. cucullata, Chydroridae spp.; the rotifers Keratella cochlearis, Keratella quadrata, Keratella cruciformis var. eichwaldi, Synchaeta monopus, Synchaeta baltica, Euchlanis sp.; the barnacle Balanus improvisus (nauplii and cypris larvae); and others (larvae of bivalves, gastropods and polychaetes). During both periods, copepod nauplii and rotifers were the most abundant groups in the pre-treatment samples, and the only groups represented in all treatments (Tables 1 and 2). In these groups the main species were the copepods E. affinis and A. bifilosa and the rotifers Synchaeta spp. and Keratella spp.

Temperature ranged widely, in 2002 from 6.6 to 11.1°C and in 2003 from 8.6 to 22.1°C, reflecting the prevailing weather conditions. Total organic carbon (TOC) was measured in treatments with ozone (3.0–3.3 mg l⁻¹) and total suspended solids (TSS) in treatments with UV (3.9–4.7 mg l⁻¹).

Treatment effects

Ultraviolet light

Experiments with UV light consisted of four flow rates (200, 400, 520 and 800 l h⁻¹). The average kill rates ranged between 83 and 100 %, 84 and 100 %, 76 and 77 %, 98 and 100 % and 66 and 94 % for copepod adults.
and copepodites, copepod nauplii, cladocerans, rotifers and barnacle nauplii, respectively (Figure 3A). The treatment effects varied depending on the species group (Kruskal-Wallis $H = 11.493$, df = 3, $p = 0.009$) and the flow rate (Kruskal-Wallis $H = 16.372$, df = 3, $p = 0.001$). Rotifers were more severely affected than copepod adults and copepodites and barnacle nauplii (Tukey’s HSD $p = 0.000–0.028$) while barnacle nauplii were the least affected (Tukey’s HSD $p = 0.000–0.022$). Kill rates were higher at 200 l h$^{-1}$ than 520 l h$^{-1}$ (Tukey’s HSD $p = 0.026$) and 800 l h$^{-1}$ (Tukey’s HSD $p = 0.036$). Cladocerans appeared the most tolerant group, but the lack of replicates precludes them from analysis.

Figure 3B shows the average proportions of actively swimming and damaged organisms after treatment (in the post-treatment samples) as percentages of all live organisms before treatment (in the pre-treatment samples). The proportion of active individuals decreased with the flow rate; at the lower flow rates the majority of live individuals were damaged.

**Ultrasound**

Four flow rates (200, 400, 520 and 800 l h$^{-1}$) and four amplitudes (25, 50, 75 and 100 %) were tested in US experiments. The average kill rates ranged between 87 and 99 %, 82 and 99 %, 92 and 99 %, 58 and 85 % and 61 and 97 % for copepod adults and copepodites, copepod nauplii, cladocerans, rotifers and barnacle nauplii, respectively (Figure 4). Differences were found between the species groups (Kruskal-Wallis $H = 42.079$, df = 4, $p = 0.000$), rotifers being the most tolerant group (Tukey’s HSD $p = 0.000$ for rotifers vs. all other groups). Higher kill rates were observed at lower flow rates (Kruskal-Wallis $H = 11.486$, df = 3, $p =$
Although kill rates appeared higher at higher US amplitudes, no statistical differences were found (Kruskal-Wallis H = 3.087, df = 3, p = 0.378).

In US treatments, the proportions of damaged individuals were small in all treatments (Figure 5), i.e. the majority of the surviving organisms were active after treatment.

**Ultrasound with ultraviolet**

Trials with the combination of US and UV were run at 800 l h⁻¹, at US amplitudes of 50 and 100 %. Kill rates were high, ranging between 94 and 100 % (Figure 6). In addition to copepods, rotifers and barnacle nauplii, bivalve veligers were abundant in single trials at US amplitude of 50 % (initial density 1.5 ind. l⁻¹) and 100 % (1.4 ind. l⁻¹). US+UV turned out to be equally efficient against all groups (Kruskal-Wallis H = 4.316, df = 3, p = 0.229) and at both amplitudes (Kruskal-Wallis H = 0.002, df = 1, p = 0.964). The proportions of actively swimming as well as damaged individuals

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**FIGURE 4**

Kill rates in trials with ultrasound (US) at amplitudes of 25, 50, 75 and 100 % for (A) copepod adults and copepodites (n = 1–3), (B) copepod nauplii (n = 3), (C) cladocerans (n = 1–2), (D) rotifers (n = 2–3) and (E) barnacle nauplii (n = 1–2).
after treatment of all live individuals before treatment were very low, 0.4 % and 0.3 % (at 50 % amplitude) and 0.2 % and 0.5 %, respectively.

**Hydrogen peroxide with ultraviolet light**

In UV+H$_2$O$_2$, the kill rates are determined separately for UV (S2), UV followed by 48 h incubation with H$_2$O$_2$ at 0 mg l$^{-1}$ (R1), 15 mg l$^{-1}$ (S3) and 30 mg l$^{-1}$ (S4), incubation with H$_2$O$_2$ at 15 mg l$^{-1}$ (R3) and 30 mg l$^{-1}$ (R4), H$_2$O$_2$ at 15 and 30 mg l$^{-1}$ followed by UV without (S6 and S9) and with 48 h incubation (S7 and S10) (see Figure 1). In option A, when UV was followed by H$_2$O$_2$, the kill rates ranged between 90 and 100 %, 94 and 100 % and 98 and 100 % for copepod adults and copepodites, copepod nauplii and rotifers, respectively (Figure 7A). In incubations with H$_2$O$_2$ (no UV), kill rates were 100 for copepod adults and copepodites, copepod nauplii and rotifers with both doses (15 and 30 mg l$^{-1}$), but only 80 to 91 for cladocerans (Figure 7B). In option B, when H$_2$O$_2$ was followed by UV, the kill rates ranged between 87 and 100 %, 99 and 100 and 89 and 100 for copepod nauplii, rotifers and barnacle nauplii, respectively (Figure 7C). In option A, kill rates after UV (S2) were lower than after UV+H$_2$O$_2$ (S3, S4 and R1) (Kruskal-Wallis H = 19.272, df = 3, p = 0.000, Tukey’s HSD p = 0.000), i.e. the treatment effect was intensified by 48 h incubation, but independent of the H$_2$O$_2$ dose (Tukey’s HSD p = 0.650–1.000). All species groups were equally affected (Kruskal-Wallis H = 0.403, df = 2, p = 0.817). In incubations with H$_2$O$_2$ (no UV), cladocerans were less affected than other groups (Kruskal-Wallis H = 14.619, df = 3, p = 0.002, Tukey’s HSD p = 0.000). Also in this case, the effect of hydrogen
peroxide was independent of the dose (Mann-Whitney U = 30.0, p = 0.783). In option B, S9 (30 mg l\(^{-1}\) H\(_2\)O\(_2\) + UV, no incubation) was less efficient than treatments with incubation (Kruskal-Wallis H = 7.157, df = 2, p = 0.028).

After UV treatment (with or without 48 h incubation), a considerable proportion of live organisms was damaged (Figure 7D: S2, R1, S6, S9) whereas after incubation in H\(_2\)O\(_2\) practically all individuals were dead (Figure 7D: R3, R4, S7, S10).

**Ozone**

In O\(_3\) experiments, no mortality was observed during the control incubations (Figure 8A and B). The death rates observed in treatment incubations (\(1 - \frac{N_f}{N_0}\); cf. Eq. 1) are therefore directly presented as % kill caused by the treatment. Two experiments were carried out, a 6-h incubation at 17 mg O\(_3\) l\(^{-1}\) (O\(_3\)-6) and a 24-h incubation at 7 mg O\(_3\) l\(^{-1}\) (O\(_3\)-24) to determine the tolerance of different species groups to a high dose in a short-term versus a low dose in an extended exposure. In both experiments, kill rates increased with time and finally achieved 100 % in nearly all cases (Figure 8C and D). In O\(_3\)-6, >99 % kill was achieved in only 2 h (and in 1 h for rotifers) for all groups except cladocerans whereas in O\(_3\)-24, >99 % kill was
achieved in 5 h (rotifers), 8 h (copepod adults and copepodites, copepod nauplii and barnacle nauplii) and 24 h (cladocerans). Comparisons of kill rates between the two experiments after 1, 2, 3 and 5 h incubation revealed that the 6-h experiment was more lethal (Mann-Whitney $U = 19.0–61.5$, $p = 0.000–0.050$). In both experiments, the treatment effect differed between the species groups (Kruskal-Wallis $H = 34.498$, df = 4, $p = 0.000$ in the 6-h experiment and $H = 16.696$, df = 4, $p = 0.002$ in the 24-h experiment),
cladocerans being the most tolerant group in both cases (Tukey’s HSD $p = 0.000–0.019$).

In O$_3$-6, the proportions of both active and damaged individuals were low (<5 %) already after the first hour (Figure 9A). Instead, in the 24-h experiment, the proportion of actively swimming individuals dropped more slowly, but towards the end of the experiment the majority of live individuals was damaged (Figure 9B).

**DISCUSSION**

Variation between the replicates results mainly from the heterogeneous nature of the experimental material, i.e. the indigenous zooplankton assemblage in the sea. Despite the fluctuating zooplankton community and the temperature variations between the seasons, each technology yielded high kill rates (>99 %) at least for one species group and
each species group was efficiently eliminated at least by one technology. Evidently, the use of natural seawater is advantageous as the water characteristics (pH, salinity, suspended solids, rubbish etc.) affect the disinfection potential of any technology, and those of typical ballast water may differ largely from industrial process waters.

The benefits of hurdle technologies are obvious, as the combination of US and UV were more efficient than both treatments separately, and UV combined with H$_2$O$_2$ was more efficient than UV alone. Typically, kill rates increased in all treatments with increasing dosage (or amplitude) and exposure times (decreasing flow rates). Also the variability tended to decrease with increasing doses, indicating that the high elimination levels achieved did not depend on the initial zooplankton density. In conclusion, >99 % kill was achieved in UV treatments for copepod adults (200 to 520 l h$^{-1}$), copepodites, copepod nauplii and rotifers; in US treatments for copepod adults and copepodites (200 l h$^{-1}$, 50 and 100 % amplitudes); in US+UV for copepod nauplii, rotifers and bivalve veligers (50 and 100 % amplitudes); in UV+H$_2$O$_2$ for copepod adults, copepodites, copepod nauplii, rotifers and barnacle nauplii (S3, S4, R1, R3, R4, S7, S9 and S10); and in O$_3$ treatments for all species groups, rotifers being the first and cladocerans the last to be eliminated. In the current experiments, only mesozooplankton was included. However, in preliminary trials with the same devices, the technologies utilising O$_3$, UV and US proved lethal (up to 80 % kill) to the dinoflagellate *Alexandrium tamarense*. In addition, indications of reduced bacterial growth rates were found with UV (MARTOB, 2004).

Species-specific differences were found; throughout the tests, rotifers appeared to be the most susceptible group (>99 % kill in all
treatments but US) while cladocerans were the least affected (>99 % kill only in O₃ treatments after 24 h incubation, although low abundances precluded cladocerans from the calculations in many cases, involving a degree of uncertainty into these comparisons). Interspecific differences in tolerance to UV radiation have been observed in crustacean plankton (Leech & Williamson, 2000). Photoprotective compounds such as melanin, carotenoids and mycosporine-like amino acids are more abundant in some species than in others (Hebert & Emery 1990, Rhode et al. 2001, Goncalves et al. 2002) which might explain the differences observed in UV treatments. US appeared to eliminate cladocerans more efficiently (maximum 98.7 % kill) than the other technologies. This may be due to their considerably large body size (~400 to 1000 µm), making them liable to be hit by the ultrasonic “axe”. Rotifers, on the other hand, were obviously less tolerant to irradiation or to various chemical stresses, while small body size (~ 100 to 400 µm) could make them less susceptible to US.

Technologies utilising UV have been found efficient killing some diatom species at flow rates as high as 350 m³ h⁻¹ (Sutherland et al., 2001) and on bacteria at 5.7 m³ h⁻¹ (Waite et al., 2003). Waite et al. (2003) failed to find any effect of UV on copepods at 60 mJ cm⁻² whereas in this study, the effect on copepods was evident at flow rates of 800 to 200 l h⁻¹, corresponding to 141 to 562 mJ cm⁻². However, particularly for copepods (adults, copepodites and nauplii), the treatment effect (kill rates) was notably depressed as the flow rate was doubled. In a full-scale application with flow rates of up to 5000 m³ h⁻¹, a remarkably high UV output power would be required, increasing the capital costs. In addition, water turbidity and high concentrations of suspended particles decrease the effect of UV (in the current experiments, the measured TSS values of 3.9 to 4.7 mg l⁻¹ were within the range that is found to have no implications for the use of UV; Oemcke & van Leeuwen, 2003b). The shading effect of suspended particles is probably more pronounced on smaller organisms (e.g. bacteria). Instead of treating the water during ballasting or deballasting, circulation of water from ballast tank to treatment process and then back to the tank during the voyage could enable a more cost effective design.

Data on ultrasonic technology are scarce but Buchholz et al. (1998) reported some evidence on the effect of US on viruses, bacteria, nematodes and zebra mussel larvae (Dreissena polymorpha) at a flow rate much higher (2.2 m³ h⁻¹) than in this study (200 to 800 l h⁻¹). While copepods and cladocerans (>200 µm) were efficiently killed also at the highest flow rate, smaller organisms (in this study rotifers <200 µm were the smallest) may need additional treatment. In contrast to UV technology, suspended inorganic or organic matter in water increase the effect of US due to collision and friction between the particles and organisms. Thus, the less particulate matter there is in the water the lower the effect of US. The use of US at high flow rates in full-scale application involves the same energy requirements and cost aspects as the use of UV. A combination of US and UV would lower energy demands and improve the treatment efficiency.

Kuzirian et al. (2001) reported that hydrogen peroxide at concentrations as low as 1 mg l⁻¹ would kill zooplankton in only 30 min. A much higher concentration (100 mg l⁻¹) inhibited the germination of cysts of Polykrikos schwartzii, a red tide
Also Bolch and Hallegraeff (1993) found an effect of H₂O₂ on germination of dinoflagellate cysts by only at very high concentrations (5000 mg l⁻¹). Results from the current experiment show that H₂O₂ at 15 mg l⁻¹ was sufficient to kill all the tested mesozooplankton species in 48 h. High efficiency was also found in similar experiments with the copepod *Acartia tonsa* and larvae of the polychaete *Nereis virens* (93 and 100 % kill for *A. tonsa* and *N. virens*, respectively) (MARTOB, 2004), indicating that H₂O₂ is more efficient against zooplankton than against phytoplankton cysts. Energy requirements of technologies with hydrogen peroxide are minimal as compared with those with US or UV. As H₂O₂ degrades to oxygen and water, it leaves no harmful residues for the environment.

Certain aspects need to be addressed when assessing the feasibility of ozonation as ballast water treatment. Firstly, owing to the highly reactive nature of ozone gas, large loads of organic or inorganic matter decrease the efficiency of ozonation, increasing the energy requirements. The combination of high concentrations of iron and organic carbon creates a risk of poor disinfection efficacy (Oemcke & van Leeuwen, 2003b), and ozone induced corrosion of iron calls for a need for corrosion resistant materials in the ballast tanks. Further, ozonation is affected by pH – the effect of ozone is stronger in pH values near 7 than in values near 8 (Oemcke & Van Leeuwen, 2004). Finally, the discharge of residual oxidation products such as bromate may cause toxicity to marine organisms (Crecelius, 1979). The length of the time bromine residuals persist after ozonation thus needs to be determined.

The relatively long contact times needed may constrain the use of ozone in ballast water management systems (Hallegraeff, 1998; Oemcke & Leeuwen, 1998). Ozone dosages of 9 mg l⁻¹ (pH 7) to 14 mg l⁻¹ (pH 8.2) yielded lethal effects on *Bacillus subtilis* in 24 h (Oemcke & Leeuwen, 2004). Also in this study, up to 24 h were required to yield >99 % kill at a dosage of 7 mg l⁻¹ (pH values near 8 are typical of the study area; Niemi, 1975). However, O₃ at 17 mg l⁻¹ proved to almost totally eliminate rotifers, copepods and barnacle nauplii in 1 to 2 h. It must be noted that the zooplankton densities tended to increase during the control incubations, indicating that the homogenization was incomplete despite the mechanical mixing. This possibly patchy distribution of zooplankton may have resulted in an underestimation of the amount of live organisms and thus an overestimation of death rates during the early part of the experiments. Nevertheless, the effect of the lowered ozone dose on the exposure time required becomes evident when comparing the results of the shorter (17 mg l⁻¹) vs. the longer (7 mg l⁻¹) experiment. Ballast water treatment by ozonation during (de)ballasting may not be commercially viable. Instead, the optimum application might consist of one or several ballast tanks equipped with ozone injection equipment, to be used as contact tanks during the voyage.

In some treatments, although high kill rates were not achieved, a sublethal effect on zooplankton was evident. A noticeable proportion of surviving individuals was damaged after UV and O₃. Damaged individuals have weaker escape responses and will be more susceptible to predation. In addition to decreased survival, physical injuries may affect reproduction. Interestingly, while UV at 800 l h⁻¹ resulted in
only 84 to 99 % kill, depending on the species group, the same treatment combined with 48 h incubation (without H2O2) yielded 100 % elimination in all groups tested, indicating that individuals which apparently survived the irradiation were killed during the succeeding 48 h. Assessing the viability of damaged individuals (IMO, 2004) requires laborious and time-consuming post-treatment growth and reproduction experiments and would not be applicable to onboard testing procedures. Nevertheless, the capability of distinguishing between viable and non-viable individuals seems an important aspect of assessment in ballast water trials.

According to the Ballast Water Performance Standards (IMO, 2004), ships are allowed to discharge no more than 0.01 ind. l⁻¹ of viable organisms of size ≥ 50 μm. In the present study, organisms were >100 μm and the initial zooplankton densities were considerably high (total densities of up to 100 ind. l⁻¹ in pre-treatment samples, i.e., a kill % of >99.99 should be achieved to meet the standards). Since the maximum volume of the post-treatment samples was 60 l, already a single viable organism in a sample resulted in the final density of 0.017 ind l⁻¹. However, in treatments with O₃ (7 mg l⁻¹, 24 h incubation) and UV+H₂O₂ (15 and 30 mg l⁻¹, 48 h incubation), 100 % kill was achieved (the final density of live organisms 0 ind. l⁻¹) thus qualifying the IMO standards. In order to ensure the required disinfection level for all organism groups and keep the energy costs reasonable, it may be necessary to combine two or more of our technologies or use a pre-treatment such as filtration or cyclonic separation (particularly with UV and O₃), removing larger organisms and particulate matter from the water.

Invasion history of the Baltic Sea includes a number of examples of planktonic species – the predatory cladoceran *Cercopagis pengoi*, the copepod *Acartia tonsa*, the dinoflagellate *Alexandrium tamarense* and the diatom *Thalassiosira punctigera*, among others – that has been introduced by shipping (Baltic Sea Alien Species Database, www.ku.lt/nemo/alien_species_directory.html). In addition, the barnacle *Balanus improvisus*, the bivalve *Dreissena polymorpha* and the polychaete *Marenzelleria cf. viridis*, also introduced by shipping, all have planktonic larvae. The onshore trials with UV, US, H₂O₂ and O₃ treatment options demonstrated that all the technologies have potential for ballast water management, aiming to control new species introductions. Each technology proved its efficiency against the target organisms – natural mesozooplankton assemblage – in a natural low-saline environment of the brackish Baltic Sea. The real-life conditions onboard a ship set, of course, further requirements to the treatment systems. For instance, vibrations, ships’ motion, accelerations, salty water atmosphere, flow rates and pressure drops must be considered when designing devices robust enough for onboard installations. In addition, the duration of the voyage is an important parameter: the shorter the time for treatment, the higher the dose of disinfectant or energy will be required, and the higher the capital and operational costs. Considering all these aspects, the development of a cost effective and reliable full-scale operation system will be difficult without thorough studies on the disinfection potential of various technologies with various operational parameters on a variety of marine organisms in changing environmental conditions.
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