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Final report of the land-based testing of the Hyde-Guardian™ -System, for Type Approval according to the Regulation D-2 and the relevant IMO Guideline

(April – July 2008)

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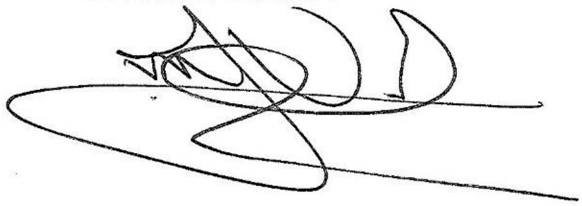

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1 Executive summary

The Hyde-Guardian™-system, a ballast water management system was tested according to the standard as set out in regulation D-2 of the “International Convention for the Control and Management of Ships’ Ballast Water and Sediments,” hereafter referred to as the “D2-Standard” and consistent with the IMO Guidelines for Type Approval testing (G8). The tests were conducted in the spring and early summer of 2008 in the harbour of the Netherlands Institute for Sea Research.

In general most of the requirements for testing which concern water characteristics and abundance of organisms were met and in most test runs environmental conditions were far more challenging than strictly needed. The Hyde-Guardian™-system is designed as a UV-light disinfection system (UV-reactor) with a mechanical filter as a pre-treatment step. The water is treated at intake and at discharge, i.e a two-fold disinfection of the ballast water. In total 10 test runs in a row were successful for all relevant parameters and the BWT system performed on average much better than stated in Regulation-D2 by achieving values for organisms well below the requirements of the D2 Standard. For many size classes of organisms the residual number of organisms was often one order of magnitude lower than the number in D2-Standard. This system should therefore be regarded as an effective way of treating ballast water in ships, thereby minimizing the risk of new invasions originating from the ships ballast water.

The sediment load was reduced in a pre-treatment step (self-cleaning disk filters) and the organisms were deactivated by ultraviolet light disinfection.

Although numbers of phytoplankton and bacteria were also largely reduced by the ultraviolet light disinfection step not all cells were fully disintegrated. Complementary tests indicated that the remaining organisms possessed a reduced viability and were (partly) deactivated.

Treated water, which was transferred to favourable conditions, showed no regrowth within a period of 10 days. Environmental acceptability tests however showed that the growth of organisms, mainly plankton, was not limited by the discharge water indicating that the discharged water was still vital.

2 Zusammenfassung

Das Hyde-Guardian™-system zur Behandlung von Ballastwasser wurde im Frühjahr und Sommer 2008 gemäß der Regularien-D2 (D2-Standard), sowie der IMO Richtlinien über Tests für eine Typzulassung (G8) im Hafen des Königlich Niederländischen Meeresforschungsinstitut (NIOZ) getestet. Generell wurden die allermeisten Anforderungen bezüglich der abiotischen Parameter des Testwassers und zur Organismendichte erfüllt. In den meisten Tests waren die Bedingungen sogar schwieriger, als in den Richtlinien verlangt. Das Hyde-Guardian™-System arbeitet mit UV-Licht als Desinfektionseinheit (UV-Reaktor). Zusätzlich verfügt es über einen mechanischen Filter als Vorabreinigungsstufe. Das Waser wird bei Aufnahme und Abgabe behandelt, d.h. es findet eine Doppelbehandlung des Ballastwassers statt. Es wurden 10 voneinander unabhängige Tests nacheinander, erfolgreich durchgeführt. Das System erfüllte alle Anforderungen der Regularien-D2 und der Richtlinie G8. Die Organismenanzahlen nach der Behandlung lagen deutlich unter den Anforderungen des D2-Standards, für einige Gruppen im Durchschnitt sogar um eine Größenordnung. Das System sollte demzufolge als effektive und sichere Möglichkeit zur Behandlung von Ballastwasser betrachtet werden, die dazu beitragen kann, weitere biologische Invasionen zu verhindern.
Die Sedimentfracht wurde in einem Vorbehandlungsschritt reduziert (selbstreinigende Scheibenfilter) und die Organismen durch UV-Bestrahlung inaktiviert.
Obwohl die Anzahl von Phytoplanktern und Bakterien durch die UV-Behandlung stark reduziert wurden, wurden nicht alle Zellen vollständig zerstört. Ergänzende Versuche ergaben, dass ein Teil dieser Zellen noch eine eingeschränkte Vitalität zeigte und nur teilweise inaktiviert war. Dennoch wurde in Inkubationsexperimenten über Zeiträume von mehr als 10 Tagen unter vorteilhaften Umweltbedingungen kein Planktonwachstum im behandelten Wasser festgestellt. Gleichzeitig durchgeführte Umweltverträglichkeitstests (Zugabe von Zeigeorganismen und Planktonkulturen, Verdünnungsreihen) mit dem behandelten Wasser ergaben jedoch keine negativen Auswirkungen auf das Planktonwachstum und zeigten somit, dass das behandelte Wasser selbst das Wachstum gesunder Organismen nicht beeinflusst.

3 Summary table with results for the Type Approval Certificate of the Hyde-Guardian™-BWT-System

Land-based tests NIOZ	**Reference & Treated**			**Reference**			**Treated**			
salinity 22.1 PSU	**Intake**			**Discharge**			**Discharge**			**Discharge***
natural plankton	Average	min.	max.	Average	min.	max.	Average	min.	max.	Average
total bacteria [counts/mL]	3.3+E6	1.3+E6	6.6+E6	2.1+E6	0.73+E6	3.6+E6	2.4+E6	0.38+E6	5.2+E6	0.31+E6
E. coli [cfu/mL]	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Enterococci [cfu/mL]	<1	< 1	<1	< 1	< 0.1	1	< 0.1	< 0.1	< 0.1	< 1
plankton <10 µm [counts/mL]	5446	235	13646	589	62	1939	<10	<10	<10	12
plankton 10-50 µm [counts/mL]	1028	304	1784	147	140	156	<10	<10	<10	2.3
plankton >50 µm [counts/m^3]	25.5+E5	10.1+E5	48.3+E5	3.95+E3	617	10.8+E3	2.9	n.d	7.3	-

Land-based tests NIOZ	**Reference & Treated**			**Reference**			**Treated**			
salinity 31.9 PSU	**Intake**			**Discharge**			**Discharge**			**Discharge***
natural plankton	Average	min.	max.	Average	min.	max.	Average	min.	max.	Average
total bacteria [counts/mL]	4.2+E6	0.72+E6	6.3+E6	2.1+E6	0.33+E6	5.4+E6	0.88+E6	0.24+E6	1.2+E6	0.64+E6
E. coli [cfu/mL]	<0.1	< 0.1	<0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Enterococci [cfu/mL]	8.3	< 1	17	< 0.1	< 0.1	1	< 1	< 1	< 1	< 1
plankton <10 µm [counts/mL]	2366	1194	3754	290	173	632	<10	<10	<10	1.9
plankton 10-50 µm [counts/mL]	1378	514	2090	140	121	151	<10	<10	<10	1.0
plankton >50 µm [counts/m^3]	1.58+E5	0.52+E5	4.11+E5	1.54+E4	0.25+E4	0.84+E4	n.d.	n.d.	n.d.	-

Summary table (average and minimum and maximum) of collected data covering the major groups of organisms at two series of 5 test-runs at a low and high salinity range, respectively. n.d.: not detectable in sample; -: no data; * numbers as determined in bottle incubations of discharge water.

4 Acknowledgements

The authors thank the technical staff of the NIOZ and in particular Anna Noordeloos, Eveline Garritsen, Swier Oosterhuis, Siem Groot, Robert Lakeman, Santiago Gonzalez and Pieter van Kralingen. Their cooperation was essential for the successful completion of this certification project.
Special thanks also to Yolanda van Iperen for providing the species list of plankton in the Wadden Sea.
We also thank Mr. Rolf von Ostrowski, Mrs. Karin Sigler and Dr. Kai Trümpler of the Bundesambt für Seeshifffahrt und Hydrographie, acting on behalf of the German Administration, for their excellent collaboration during this whole certification process.

5 Introduction

Ships transport 5-10 billion tons of ballast water annually all over the globe (Endresen et al. 2004). The ballast water is loaded with particulate sediment and an enormous variety of (living) organisms, which ranges from juvenile stages, larvae and eggs of fish and larger zooplankton (Williams et al. 1988; Carlton & Geller 1993) to macroalgae, phytoplankton (Hamer et al. 2000; Hallegraeff et al. 1997), bacteria and viruses (Gollash et al. 1998). In general these organisms belong to the natural ecosystem in and around the port of origin but they might not be occurring naturally in the coastal waters and port of destination at the end of a ship's voyage. In hundreds of cases around the world, this has resulted in severe damage to the receiving ecosystem and to human health, because these non-native organisms developed into a plague. This often has a high impact on the ecosystem and can cause economical damage (Hoagland et al. 2002), as it results in a decrease of stocks of commercially valuable fish and shellfish species and occasionally outbreaks of diseases such as cholera (Ruiz et al. 2000; Drake et al. 2001). If action is not taken, the problem of invasive species will increase in an exponential manner for several reasons. Ships are getting larger, faster and the amount of traffic across the oceans is expected to increase rapidly during the coming decades, and therefore also the chance of non-indigenous organisms to have large enough numbers for settling and expanding. The problem of invasive species is considered as one of the 4 major threats of the world's oceans next to land-based marine pollution, overexploitation of living marine resources, and physical alteration/destruction of habitats

To minimize these risks for the future, the International Maritime Organization (IMO) of the United Nations has adopted the Ballast Water Convention in 2004 (Anonymous 2005). The Convention states that finally ALL ships (>50,000 in number) should install proper ballast water treatment (BWT) equipment on board between 2009 and 2016. As a temporary and intermediate solution for the time being ship may reduce the risk of invasive species by performing ballast water exchange during their voyage when passing deep water (>200 m depth and 200 NM from the coast. Ballast water exchange faces many problems as to feasibility, safety and efficacy For a large part of ships' voyages the required depth and/or distance to shore requirements are never met; BW exchange can affect the ships construction stability and in rough seas exchange is not possible because of the risk to ship and crew. Treatment of ballast water is therefore considered to be the best solution of reducing the risk of invasive species.

During the recent years numerous solutions for treatment of ballast water have been mentioned and tested with the ultimate goal to reduce the amount of organisms in ballast water (Rigby & Taylor 2001). However, next to a high efficacy there is more needed for a BWT system to be a good system. Next to biologically effective the system should be practicable, environmentally acceptable and also cost effective.

Despite the fact that the treatment technology for drinking-, waste- and process water is well-developed none of these techniques is directly applicable to ballast water (Rigby & Taylor 2001; MEPC 49/2/13, 2003). Besides reducing the load of organisms the sediment load should be reduced as well. There are also considerable differences in ships operation, types of ships, the amount of space available for a ballast water treatment system on board and the way ships are operated. Ballast water treatment will develop into a new field of technology of its own with a commercial market estimated for the next 10 years in the order of 8 billion Euro (Haskoning 2001).

As a primarily scientific research institute NIOZ is defining its role in the certification process as to study

1) the **numerical abundance** and **biodiversity** of organisms prior, during and after a treatment with the Hyde-Guardian™- Ballast Water Treatment system (efficacy of the BWT system),
2) to determine the **viability** status of the remaining organisms during discharge,
3) to assess the **environmental acceptability** of discharging ultraviolet light disinfected ballast water by measuring residual effluent toxicity in order to determine latent effects, since ballast water treated with this specific UV-system does not need a complete G9 procedure (environmental impact).

This research strategy allows for more in depth testing, while it includes ALL organisms and not only the size classes as specified in the Convention D2-standard.

6 Description of the treatment facility

6.1 NIOZ Royal Netherlands Institute for Sea Research

NIOZ Royal Netherlands Institute for Sea Research is the National Oceanographic Institute of the Netherlands. NIOZ is part of the Netherlands Organization for Scientific Research (NWO). The institute employs around 200 people and the annual budget is approximately €20 million.
The mission of NIOZ is to gain and communicate scientific knowledge on seas and oceans for the understanding and sustainability of our planet. The institute also facilitates and supports marine research and education in the Netherlands and in Europe.

In order to fulfil its mission, the institute performs tasks in three specific fields.
Research: The emphasis is on innovative and independent fundamental research in continental seas and open oceans. The institute also carries out research based on societal questions when this merges well with its fundamental work. The senior scientists at NIOZ all participate in international research projects.
Education: The institute educates PhD and other students of universities and schools for professional education. Together with universities NIOZ also organises courses for PhD students and master students in the marine sciences. A number of our senior scientists of NIOZ is also appointed as professor at Dutch and foreign universities.
Facilitary services: NIOZ invites marine scientists from Dutch and foreign institutes and universities to write scientific proposals involving the institute's research vessels, laboratories, and the large research equipment, which is often designed and built by the institute's own technical department.
The basic oceanographic **disciplines** studied at NIOZ are physics, chemistry, biology and geology. Multidisciplinary research is regarded as one of the main strengths of NIOZ.

More information on www.nioz.nl

Figure 1: aerial view of the NIOZ harbour (lower right), NIOZ laboratories (upper left) and TESO ferry (top).

6.2 Portrait of HYDE-MARINE/LAMOR (producer of the HYDE-GUARDIAN™-ballast water management system)

More information on: www.hydemarine.com

Ballast water treatment - The Hyde-Guardian™-system
Hyde Marine, Inc. traces its origins back to the Hyde Windlass Company, established in Bath, Maine in 1865. Hyde Windlass was the largest supplier of anchor windlasses, winches, capstans, steering gear and specialty deck machinery in the United States before, during and after the First and Second World Wars.

Hyde Marine has been involved in ballast water treatment (BWT) since 1996, when it teamed with the Univ. of Michigan to study potential technologies for BWT, particularly for ships operating in the Great Lakes St. Lawrence Seaway System. This led to Hyde's participation, beginning in 1997, as the engineering contractor for the Great Lakes Ballast Technology Demonstration Project (GLBTDP). The GLBTDP was one of the first BWT research programs in North America and conducted tests of filtration technology for BWT aboard a Canadian Great Lakes bulk carrier, the *Algonorth*. After a season aboard the *Algonorth*, the test equipment was transferred to a barge in Duluth Superior Harbor (Lake Michigan), where additional testing of the filtration system and other BWT technologies was conducted, also under the GLBTDP.

Hyde first became involved in its own BWT equipment in 2000, when it installed its initial full scale first generation system aboard the US based cruise ship *Regal Princess*. In 2001 Hyde installed four additional systems, two on cruise ships and one each on a container ship and chemical tanker. In 2003, after the requirements were better defined, Hyde installed a state-of-the-art filtration and UV disinfection system aboard the *Coral Princess*. This system, named the Hyde-Guardian™-system, was tested extensively on land based installations and on board the *Coral Princess* in the fall of 2004. The on-board tests demonstrated the Hyde Guardian's capability to meet the IMO BWT Convention requirements. This system is now first in line for acceptance into the U.S. Coast Guard STEP program. In the fall of 2006 an essentially identical system was installed aboard RCCL's Celebrity *Mercury*. It was commissioned early in 2007. The Hyde-Guardian™-systems aboard the *Coral Princess* and *Mercury* were granted interim approval for use in Washington State waters by the State of Washington in 2004 and 2007 respectively. All seven systems were commercial transactions and the newest version, the Hyde-Guardian™-system, has been fully commercially available since early 2003.

6.3 The test facility

The land-based tests were carried out at the Royal Netherlands Institute for Sea Research (NIOZ), Landsdiep 4, 1797 SZ 't Horntje, Texel, the Netherlands, from March till July 2007 (www.nioz.nl).

The NIOZ test site is equipped with 3 coated concrete tanks of 300 m³ volume each to simulate the ballast water tanks of the ship (Figure 2). The tanks were (steam) cleaned after each run. Water samples can be taken from bypasses of the standard piping used to fill and to empty the tanks or directly from the tank at outflow at ca. 1 m from the bottom.

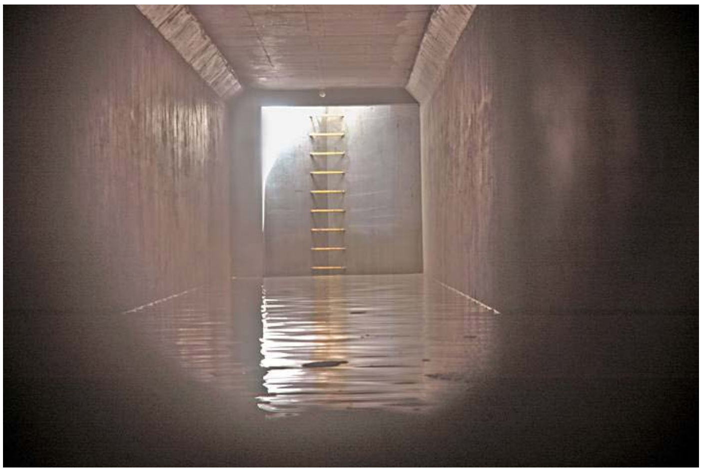


Figure 2:
Inside view of one of three subterranean water tanks

According to the requirements of the Guidelines G8, sampling points are fitted before the treatment system and directly after the system. Samples varying in volume from 500 mL up to 1 m^3 were taken using clean sampling containers. Sampling containers and all further handling of the samples were separated in a control and a treated set to avoid cross contamination by the active substance. The basic handling, such as concentrating, filtration and chemical analysis was done at the test site. Different samples (1 to 10 L) were transported to the institute's laboratories for further special analysis. For re-growth experiments 10 L of sample was transported (Nalgene bottle) to a climate room for incubation experiments (ca. 12 – 15 ºC; a light;dark regime of 16:8 h and 100 μmol quanta. $m^{-2}.s^{-1}$)
The Hyde-Guardian™-system is connected to a typical ballast water pump (capacity of 250 m^3/h) which was located in the NIOZ harbour. This is a pristine harbour with a direct access to the Wadden Sea and the origin of the test water changes with the tide. Furthermore, provision were made to allow the addition of salt water and / or freshwater in order to adjust the salinity of the natural water of the NIOZ harbour to the required test conditions of brackish water and marine water with a minimum of 10 PSU difference. A detailed description of the test installation is presented in figure 3.

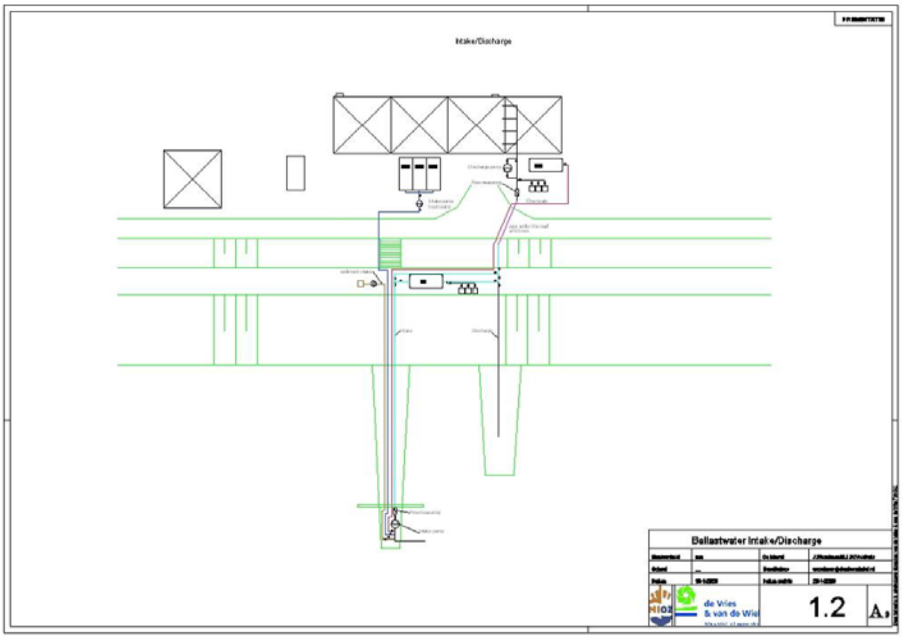


Figure 3: P&I diagram of the Ecochlor and Hyde-Guardian™-system installation at NIOZ with points of sampling

6.4 Technical description of the Hyde-Guardian™-System

The Hyde-Guardian™-system operates in-line during the uptake of the ballast water. It is based on a modular concept which includes a physical separation as well as disinfection with a UV-light source resulting in permanent deactivation of organisms.

The Hyde-Guardian™-system used during the tests at NIOZ has been build to the same standards as the double 250 m³/h containerised Hyde-Guardian™-system that is installed on the test ship (fig. 4).

Figure 4: The Hyde-Guardian™ skid mounted unit

The system consists of a UV-reactor (BersonInLine), a medium-pressure ultraviolet light disinfection system with a typical output of UV-C (Fig 5; 200 – 300 nm wavelength) combined with a pre-filter. The advantage of a pre-filter (Spin Klin® discs) is to reduce the number of larger organisms of which is known that UV is not a very effective way of deactivation (Raikow et al. 2007). In addition filtration reduces the load of (larger) particles and increases therefore the transparency of the water, hence the effectiveness of the UV irradiation.

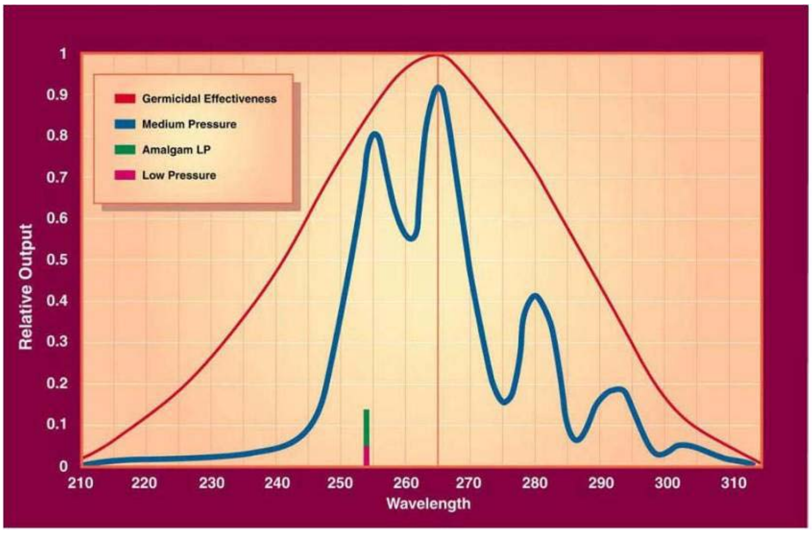


Figure 5: spectral composition of the medium pressure (MP) lamp of the BersonInLine UV-system used in the Hyde-Guardian™-system.

Unlike active substances the working of ultraviolet radiation (UV) is based on transferring electromagnetic energy to molecular components of organisms, resulting e.g. in DNA damage. This will prevent cell replication (growth and reproduction) but also causes (immediate) disintegration of cells, morphological changes, discoloration, cellular membrane leakage and cell damage.

To disinfect municipal water UV is commonly used because in general it does not significantly contribute to Disinfection By-Products (DBP; (Malley et al. 1995; Chin & Bérubé 2005).

The UV lamps in the Hyde-Guardian™-system are of the medium pressure type and run at a nominal of 100 W per centimeter in length. This produces an average intensity of 140 mW/cm^2. The end of lamp life is not when a lamp fails to light but rather when its output has decreased by 30%. The physics inside a UV lamp does not change over time, i.e. at the heart of the lamp the produced UV remains constant. As the amount of UV that is emitted decreases it is necessary to increase the power applied to the lamp to keep the UV intensity above the minimum levels. The Hyde-Guardian™-system uses a UV sensor to continuously monitor the output of the lamps. Besides monitoring the output of UV-C (200 – 280 nm) the UV sensor can also monitor the cleanliness of the sleeves and indirectly the water quality.

Because the power delivered to the UV lamp is not infinitely adjustable the majority of the time the system will be overdosing the UV. This is probably the only disinfection technology that can safely be overdosed. The control system will keep the amount of energy used to a minimum but given the discrete steps available for power control a new lamp will be running about 130% of the required UV.

The Hyde-Guardian™-system differs from other BWT-systems as the system is not only treating during intake (disc filter and UV-reactor) but also at discharge (only UV-reactor). Assessment of the efficacy of the BWT system was therefore immediately at intake (T0), at discharge after a holding period of 5 days (T5) and 5 days after discharge (T10)

In addition to sampling the large holding basins for a period of 5 days, subsamples (with a volume of 10 L) of the reference and treated tank were taken at intake and discharge and incubated in a climate room under optimal growth conditions for the plankton community present in the test water. In case the treatment is insufficient and residual viable organisms remain present, or there is germination of resting stages or cysts, plankton growth will be stimulated under these favourable conditions. This outgrow experiment also allows studies on the effect of the treatment over a period longer than 5 days (up to 20 days). A sufficiently high level of nutrients will be maintained, favouring phytoplankton growth and possible cyst germination throughout the incubation experiments.

The applied test protocols were communicated with the Maritime Coastguard Agency (MCA) as well as with the German Administration (Federal Maritime and Hydrographic Agency of Germany; BSH) and a brief description of the various methods is included in the next section. During the certification process the whole practical procedure of intake and discharge has been witnessed on 4 occasions (intake and discharge) by a classification society (Lloyds Register, London and Rotterdam) as well as national and international agencies.

NIOZ is currently acting as an official certification organization for land-based tests for the German and the UK administration according to approved protocols and final reports will be made public at the moment of awarding the certificate.

7 Requirements to meet the D2-Standard

According to the D2-Standard of the IMO/MEPC Convention of 2004 ((Anonymous 2005)) ships that meet the requirements of the Convention by meeting the ballast water performance standard must **discharge**:

1) Less than 10 viable organisms per cubic metre greater than or equal to 50 micrometers in minimum dimension;
2) Less than 10 viable organisms less than 50 micrometers in minimum dimension and greater than or equal to 10 micrometers in minimum dimension and
3) Less than the following concentrations of indicator microbes, as a human health standard:
    1. Toxicogenic *Vibrio chlorerae* (serotypes O1 and O139) with less than 1 colony forming unit (cfu) per 100 milliliters or less than 1 cfu per 1 gramme (wet weight) of zooplankton samples:
    2. *Escherichia coli* less than 250 cfu 100 milliliters;
    3. intestinal Enterococci less than 100 cfu per 100 milliliters.

The present D2-Standard is defined as a standard for the water characteristics at **discharge**. Furthermore, with exception of some indicator microbes (point 3) organisms < 10 µm are completely excluded. This is certainly an omission since this size class in particular includes numerous phytoplankton species characterized as Harmful Algal Blooms (HABs).
Nevertheless, the standard is clear with respect to the maximum number of organisms remaining present. On the other hand a proper definition of the dimensions of organisms is still subject of (academic) discussion. Moreover, as an operational definition for viable organisms the IMO is using: *"organisms and any life stages thereof that are living"*, but a more adequate (scientific) definition is: an organism that is able to complete it's life-cycle, including reproduction (DNA replication). In the case of UV it is known that for certain organisms cell may be (genetically) damaged but remain active for a considerable period. The organisms however, lack the ability to reproduce.

In addition to the (basic) requirements for the D2-Standard we have adopted a variety of methods and techniques to determine abundance, sizes and the viability status of different types of organisms. This includes also plankton < 10 µm other than bacteria (i.e. phytoplankton and viruses). Moreover, we extended our research effort to examine not only the fate of the organisms in the large-scale holding tanks but also took subsamples for incubation under optimal growth conditions in our to study the growth potential of remaining (viable) organisms and/or survival stages such as eggs, cysts or dormant cells over a longer period than the recommended 5 days. These later experiments allowed us also to study potential latent toxicity effects (environmental acceptability).

7.1 Requirements to meet: guideline G8

Next to the D2-Standard two guidelines were developed by the IMO/as a framework for approval of ballast water treatment systems (G8) and approval of the use of active substances in ballast water treatment systems (G9). For land-based testing MEPC 53/Annex 3 Anonymous 2005; and the revision of MEPC 58) was compiled of which the most relevant parts will be presented below. These guidelines were generically designed to meet the conditions of a broad range of potentially effective treatment techniques to be tested in typical port and environmental conditions found across the globe. Most test protocols therefore require extensions of the test design to cover the specific aspects of the treatment. The land-based testing serves to determine the biological efficacy of the BWT systems under consideration for Type Approval under more or less controlled and replicable conditions. This is intended to ensure that the efficacy of the equipment is consistent and can be shown repeatedly. The test set-up should therefore be representative of the characteristics of the arrangements used and the type of environment the BWT system was designed for.

One of the main criteria in the G8 test requirements is the salinity range and related to this the differences in Total Suspended Solids (TSS), Particulate Organic Carbon (POC) and Dissolved Organic Carbon (DOC). This resulted in three main categories of test conditions (Table 1).

Table 1: Three different salinity ranges and minimum concentrations of TSS, POC and DOC in the water.

	Salinity			
Parameter	> 32 PSU	3 – 32 PSU	< 3 PSU	unit
Total Suspended Solids	> 1	> 50	> 50	mg/L
Particulate Organic Carbon	> 1	> 5	> 5	mg/L
Dissolved Organic Carbon	> 1	> 5	> 5	mg/L

In general UV-reactors are not sensitive to changes in the salinity of the water except that water with a high alkalinity, iron and/or manganese will increase fouling of the UV-light source (Gundry 2007). Historically UV-reactors are widely used in treating (fresh) drinking water and sewage water and its use as a tool for disinfection is well studied. It was for this reason that the Type Approval tests were conducted at the intermediate (3 – 32 PSU) and high salinity (>32 PSU) regions. Moreover, the only difference in composition of the test water between the freshwater and intermediate salinity water is the presence or absence of (sea)salt. All other minimum requirements for TSS, POC and DOC for these two water types were identical (Table 1).
A further requirement is that the difference between the two salinity regimes should be at least 10 PSU. The test water, originating from the Wadden Sea, and the actual sampling did vary with the tide and as a result salinity was subject to variations. To assure the 10 PSU salinity difference it was decided to have the possibility of adding fresh water and upgrade coastal water of the North Sea water by enhancing the salinity (brine solution of commercially available sea salt; ca. 18%). As target number the freshwater addition was adjusted to a salinity of ca. 23 PSU for the low salinity regime and ca. 33 for the high salinity regime. In practice ca. 15 % (v/v) of freshwater was added during the low salinity tests and about 4% of brine solution (Instant Ocean®), during the high salinity test runs. In order to compensate the dilution of the TSS by the freshwater some extra sediment (taking from a nearby mudflat) was added as well. These additions were made close at the pump site, to ensure proper mixing, with a constant flow rate and done during filling of the control and the treated ballast tank.

Biology

The guideline G8 also defines criteria for the number and diversity of the organisms to be met during Type Approval testing (Table 2). These criteria should be met for all three salinity regions.

Table 2: Minimal numbers and species diversity required at intake for different size classes and groups of organisms.

Influent test water		
Parameter	**unit**	**Remarks**
organisms ≥ 50 micron	> 10^5/ m^3	at least 5 species from at least 3 different phyla/divisions
10 ≤ organism size ≤ 50 micron	> 10^3/ mL	at least 5 species from at least 3 different phyla/divisions
heterotrophic bacteria	> 10^4/ mL	not further defined

The test water should contain minimum densities of plankton which are typical densities encountered in the Wadden Sea during the annual spring bloom in April/May. With respect to the species diversity, the Wadden Sea is known for its natural richness in organisms and during the test period (April – July) indeed a large diversity in organisms, adults, juveniles, eggs, etc. was encountered.
An important aspect, so far not recognized in the guidelines (G8), when dealing only with natural populations of organisms in the influent of the test water is the natural seasonality of species and blooms. The actual onset of the spring bloom is characterized by a dominance in phytoplankton, but usually lacks high zooplankton abundance. Only at a later stage zooplankton starts to increase in abundance, subsequently due to predation it will diminish the numerical abundance of (smaller size) phytoplankton component.
Furthermore, for the high salinity range, the composition of the organisms in the water resembles that of a typical oceanic environment. This implies an increase of smaller sized cells, down to the micrometer scale, and also a dramatic decline in the number of larger (>10 µm) organisms. So far this shift in community structure has not been accounted for when using natural plankton for testing.

Human pathogens

Table 3: Maximum allowed numbers of 3 groups of indicator microbes in the effluent test water on discharge. cfu: colony forming units

Effluent test water		
Parameter	**unit**	**Remarks**
Toxicogenic *Vibrio chlorerae*	< 1 cfu/100 mL or < 1 cfu/ g wet weight of zooplankton	serotypes O1 and O139
Escherichia coli	<250 cfu/ 100 mL	
intestinal Enterococci	<100 cfu/ 100 mL	

Within the group of prokaryotic microbes only bacteria and more specifically the heterotrophic group (Table 2) has been defined by the standard but for completeness this should include all bacteria and presently also Archeae. While these microbes are part of the natural community in the aquatic environment the indicator microbes (Table 3), i.e. the human pathogens, are introduced as part of human activity and often associated with

sewage discharge. In the present research all microbes have been included as a bulk parameter, the number of heterotrophs as a viable component as well as the viability of the whole microbial community has been determined.
Within the whole microbial community the number of heterotrophic bacteria was determined as well as *E. coli* and total enterococci. The test area of the institute is part of a tidal estuary of the Wadden Sea, which is essentially a pristine environment. Moreover, waste water treatment is highly developed in the Netherlands. Therefore, numbers of these human pathogens during the tests were to be expected to be low for most of the sampling period. On the other hand during the different treatment steps a significant amount of particulate organic material is transferred into dissolved organic carbon (DOC) which acts as an excellent substrate stimulating growth of (heterotrophic) bacteria.

7.2 Experimental design

A variety of methods was applied to examine the biological efficacy of the Hyde-Guardian™-system for the different categories of organisms during the two test series. For detailed description we refer to read the outline of the official test protocols for the Hyde-Guardian™-system (Anonymous 2008). Sample handling and volumes were according to the description of the guideline for BWT testing (G8) or described in detail when these guidelines were insufficient or other considerations were taken into account, e.g. in the case of sampling and incubation of samples at discharge. Subsamples were taken at randomly or throughout the whole filling procedure of the tanks. As indicated previously there was great emphasis on analysing of the freshly taken samples and having multiple methods to examine numerical abundance and viability. Besides various biological samples there was also a basic set of physical and chemical parameters which were monitored prior, during and after discharge. A short description of each parameter and how it has been analysed is given below.

Physical and chemical properties of test water

Temperature

The water temperature was measured using a calibrated thermometer.

pH

The pH-level is measured using a calibrated pH-meter.

Salinity

For salinity ca. 250 - 500 mL water is sampled and stored at room temperature (glass bottles) until analysis by direct measurement in the laboratory at NIOZ. Salinity of the water was measured after each test cycle using a refractometer (Atago) calibrated against 0 and 33 PSU standard (sea)water. The accuracy of the salinity measurement is 0.5 PSU.

Dissolved Oxygen

The spectrophotometric method of the Winkler method (Winkler, 1888; Pai et al. 1993; Reinthaler 2006) was used to determine the oxygen concentration in the water. Samples were taken using gastight tubing which was specially fitted to the sampling tubing that was used to sample the ballast simulating tanks. The coded glass bottles are flushed at least three times their volume (ca. 120 mL) with water.
The sample bottles were stored in a dark container filled with water of the same temperature as the samples until further analysis at the laboratory. In the laboratory 1 mL H_2SO_4 is added prior to measuring the OD at 456 nm with a Hitachi U-3010

Spectrophotometer. The oxygen concentration was calculated using standards and expressed as µM O_2/L (or mg O_2/L; = µM O_2 * 0.032)

DOC

The concentration of dissolved organic carbon (DOC) was measured according to Reintaler & Herndl (Reinthaler & Herndl 2005). Samples for DOC (15 mL) were filtered through GF/C filters and sealed in pre-combusted glass ampoules after adding 50 µl of phosphoric acid (H_3PO_4). Sealed ampoules are stored at 4 °C. The DOC concentration was determined in the laboratory by the high temperature combustion method using a Shimadzu TOC-5000 analyzer. Standards were prepared with potassium hydrogen phthalate (Nacalao Tesque, Inc, Kioto, Japan). The mean concentration of triplicate injections of each sample (three in total) is calculated. The average analytical precision of the instrument is < 3 %.

TSS / POC (total suspended solids and particulate organic Carbon)

For TSS/POC pre-weighted glass fibre filters (GF/C) are used. Each filter was coded and stored in a clean Petri dish. The filtered volume was dependent on the particle load and concentration and type of organisms present in the water. The higher the total particle load in the sample, the smaller was the volume that could be filtered before the filter clogs. Practical volumes were between 100 and 1000 mL per sample.
After filtration the filter was rinsed with fresh water (MiliQ) to remove sea salt. Filters were dried overnight at 60 °C and allowed to cool in a vacuum exicator before weighing. The total amount of suspended solids was calculated from the weight increase of the filter and averaged for the three replicates (mg/L).
Next, the filter is combusted at 500°C (overnight) and allowed to cool in a vacuum exicator and weighted again. The POC was calculated from the weight decrease between this measurement and the TSS weight.

Figure 6:
Sampling point at tank 3

Biology

The majority of the large size fraction (>50 µm) consists of zooplankton, while the majority of the small size fraction (10-50 µm) consists of phytoplankton. Organisms > 50 µm are retained as recommended in MEPC 54/Inf.3 (using a Hydrobios net).

Samples for the 10 - 50 µm fraction were collected as whole undisturbed samples. These samples were then filtered over a 50 and a 10 µm sieve and fixated.
A second set of samples for this size class was taken and not separated from the organisms < 10 µm in order to include the fate of the smaller sized (phyto)plankton community as well and to avoid further damage of the plankton. The results of these samples were compared

to the ones from the double filtered samples to evaluate the loss of organisms caused by processing the samples.

Sample sizes

During the land-based tests containers from 1 to 1000 L were used for sampling and/or storage. Samples were taken continuously and evenly during the whole process of filling or emptying the ballast water tanks. These containers were thoroughly rinsed or heat-treated prior to use. Samples for the human pathogens were taken in sterile (bar-coded) bottles provided by the bacterial test laboratory.

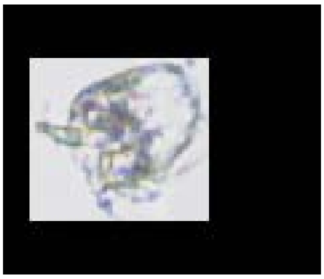
Figure 7:
1000 L container with a 50 µm Hydrobios sampling net

Organisms > 50 µm

The samples are pre-concentrated over a Hydrobios 50 µm net resulting in an end volume of approximately 100 to 200 mL. The samples were transferred to the lab directly after sampling and Neutral Red was added in a ratio that yields an end concentration of 1:50,000. Staining time is 2+ hours. Neutral Red stains living organisms (Crippen 1974; Fleming & Coughlan 1978) distinctively and quite rapidly (less than one hour, figure 6). Therefore the viability assessment remains unaffected by the possible death of organisms during the staining or during sample analysis.
It is assumed that dead but physically intact organisms will also be found. Consequently a detailed inspection of each intact individual is needed to assess viability. This includes the staining as well as the detection of internal (heart, gills) movement. Organisms which were not intact are assumed to be dead.

Neutral Red is a reliable staining method for all major groups of organisms but inconsistent staining was found for bivalves. For this latter group movement (including internal such as heart and gills for juvenile mussels) has to be used obligatorily to determine viability. This is dependent on the expertise of the person analyzing the samples. Therefore the same person analyzed all samples.


Figure 8: (top) live copepod (left) and nauplia (right) from a Neutral Red stained sample. (bottom) dead copepod (left) and nauplia (right) from a NR stained sample. Pictures made with the FlowCam.

The samples are analyzed manually using a binocular with a 20x magnification for counting and up to 50x for species identification and measurements when necessary. For inter comparison a subset of samples was also analyzed using a semi-automated tool (FlowCam, Fluid Imaging Technologies; (Anonymous 2001)). Organisms need to be counted according to their size. Here organisms of 50 µm in minimum dimension are relevant. Several tests have shown that a single size bar is not efficient as viable organisms move in the counting chamber. Better results are achieved when the entire field of view is equipped with a size grid. The minimum dimension to measure will be adjusted to the specific organism groups.

Organisms < 50 µm

Samples for visual inspection of species number and diversity were pre-concentrated using a sieve made of a Hydrobios 10 µm mesh net using the 50 µm prefiltered sample (effective size range is 10 – 50 µm). The retained organisms were flushed into 50 mL Greiner tubes using filtered seawater and fixed with Lugols solution. Sample analysis was conducted by microscopic count with an inverted microscope at 200x magnification (method by Utermöhl). Since the Utermöhl is not suitable to asses viability counting was restricted to the structural integrity of organisms and therefore the presence of intact cells (Paerl 1978). This method works for both zoo- and phytoplankton. This size fraction was also covered by flow cytometry on basis of a single cell measurement (Veldhuis & Kraay 2000) and PAM fluorometry, as a bulk parameter (Schreiber et al. 1993), using the intact and undisturbed samples. Besides numbers and sizes these two methods can be used to assess the cell viability (Veldhuis et al. 2001; Veldhuis & Brussaard 2006) or in case of the PAM fluorometry also the photosynthetic efficiency of the phytoplankton.

Flow cytometry: For total organisms counts 3 mL of unfiltered sample water (reference and treated, each in triplicate) were analyzed using a calibrated flow cytometer. This yields the total number of particles (dead and live organisms as well as detritus) as well as their size range and the presence or absence of chlorophyll. For the counts exactly 1 mL was analyzed.
The size of the plankton was determined by comparison to standardized beads (10 and 50 µm). These beads were also used as standards to calibrate the performance of the flow cytometer.
For organism viability testing, on the level of the individual cell, SYTOX Green was added to 1 mL of sample water (control and treated, each in triplicate). After 15 minutes samples were analyzed using the flow cytometer for the presence of dead and/or life organisms (cf. Veldhuis et al, 2001).

Figure: 9(A) Epifluorescence microscopic picture of a life phytoplankton cell. The red signal is due to the presence of chlorophyll and (B) a dead phytoplankton cell with a yellow/green fluorescence of the nucleus after staining with SYTOX Green.

PAM fluorometry: The photochemical efficiency of photosystem II of phytoplankton (providing an estimate of the general health of the algae) can be addressed using Pulse-Amplitude Modulated fluorometer (PAM-fluorometry) WALTZ- water PAM (Schreiber et al. 1993). For this 3 mL of unfiltered sample water (control and treated, each in triplicate) are filled into a glass cuvette and analysed using the Pulse-Amplitude Modulated fluorometer. The instrument was calibrated against filtered seawater and a healthy fast-growing population of phytoplankton.

Next to cell specific analysis Plant-pigments and chlorophyll *a* were determined to assess the fate of the whole phytoplankton community (Jeffrey et al. 1997; Kraay et al. 1992). For this purpose water samples of 0.2 to 1.5 L (GF/C filters) were taken. The samples were frozen until further analysis.

The system used is a Dionex HPLC system equipped with a C18 separation column. The different algal pigments can be separated according to their polarity. The following solvents are used as elutes in the HPLC gradient: A 0.5M ammoniumacetate in methanol and water (85:15), B acetonitril and water (90:10) and C ethylacetate 100%.

Bacteria

The classical method for counting bacteria in many applications is based on plating on selective media. Unfortunately, for studies in the aquatic environment this approach is by far insufficient for various reasons (Gasol & Giorgio 2000). As a result total bacteria were now determined by flow cytometry, using DNA-specific stains to get a more accurate bacteria

number. In addition samples were taken at discharge for specific human pathogens and heterotrophic bacteria using a plate method.
A 1.5 mL water sample was taken and pipetted in a Cryovial (in triplicate) and formaldehyde was added as a preservative. Samples were frozen and stored at -50 ºC until further analysis.
Upon analysis the sample is allowed to thaw completely. A subsample of 100 μl is taken, diluted with a TE-buffer, and the nucleic acid dye PicoGreen (MP) was added. Within 5 to 15 minutes after the addition of the stain the sample is analyzed using a flow cytometer (cf. Gasol & Del Giorgio 2000; Veldhuis et al. 1997). A known bacterial standard is used for calibration and counting.
The number of total heterotrophic bacteria was determined using a plate method as the number of colony forming units (cfu's) after incubation of the water at intake and discharge according to an international standard (NEN-EN-ISO 6222:1999).

Human pathogens

The samples for microbiological analysis are taken in special bottles of 600 mL and send to a special laboratory for further analysis. This laboratory was "Vitens laboratory bv" at Leeuwarden (accreditation certificate: NEN/ISO/IEC 17025; lab. no. L043). All analysis' are carried out according to NEN-EN-ISO standards.
These samples are sent to the laboratory immediately after sampling using a cooled transport container (4 °C). The analysis is carried out according NEN-EN-ISO 7899-2 for intestinal enterococci and NEN-EN-ISO 9308-1 for *E. coli* and related bacteria of the coli group as adopted for surface and waste water analysis in the Netherlands.

8 Results

The present section is a compilation of all relevant information needed for Type Approval Certification tests according to the Guidelines (G8), but also includes some relevant results of experiments conducted to assess the environmental acceptability of the applied UV-reactor and/or its potential by-products in the environment upon discharge. Data are presented as averages or ranges separated for the two salinity regimes tested. In Annex 1 a detailed species list of observed organisms is presented.

Figure 10: overview of the Hyde-Guardian™- Ballast Water Treatment System in the NIOZ harbour. In front of the container are the three IBCs with the 50 µm inline sampling nets.

The tests were carried out at two different salinity regimes (Tables 4 and 5) hereafter referred to as low and high salinity test series.

8.1 Physical and chemical parameters

Table 4: Average salinity and temperature of water at intake during the first low salinity tests of control and treated tanks for test runs 1 – 5.

test run	salinity [PSU]	s.d.	temperature [°C]
1 - 5	22.1	0.92	8.5 - 10.4

To the low salinity test cycles brackish water from the NIOZ harbour was collected during low tide and fresh water was added to a maximum of 15% (v/v).

Table 5: Average salinity and temperature of water at intake during the high salinity tests of control and treated tanks for test runs 6 – 10.

test run	salinity [PSU]	s.d.	temperature [°C]
6 - 10	31.9	1.3	14.3 - 17.5

To the high salinity test cycles coastal water from the North Sea was collected during high tide and a brine solution made from natural sea salt was added to a maximum of 4 % (v/v).

In terms of difference in salinity the variation between both salinity regimes is slightly less than 10 PSU. However, given the standard variation and the fact that tidal effects and prevailing winds showed occasionally disturbed the ideal situation the minor deviation from the ideal situation is considered to be of minor importance. Moreover, the present configuration of the Hyde-Guardian-BWT-system is not affected by changes in the salinity.

Low salinity

A summary of the results of the basic parameters (oxygen concentration, pH, TSS, POC and DOC) is presented in table 6 for the reference and treated water sample at intake and discharge.

Table 6: average or range of oxygen, pH, inorganic nutrients, TSS, POC and DOC concentration of 5 test series run at low salinity at intake and discharge of the reference and treated tank; [1]: range of values

Parameter	Intake	s.d.	Discharge (day 5)	s.d.	unit
O_2 reference[1]	9.3-12.9		7.9-10.7		mg/L
O_2 treated[1]	9.4-13.0		8.1-11.5		mg/L
pH reference	8.17	0.10	8.19	0.12	µM
pH-treated	8.12	0.12	8.09	0.08	µM
NO_3 reference	42.5	10.3	41.5	11.1	µM
NO_3 treated	41.5	8.00	36.8	10.7	µM
NO_2 reference	0.48	0.07	0.51	0.07	µM
NO_2 treated	2.99	1.13	4.62	0.52	µM
NH_4 reference	2.8	1.03	7.63	1.15	µM
NH_4 treated	2.9	1.24	5.38	1.63	µM
PO_4 reference	0.16	0.03	0.22	0.07	µM
PO_4 treated	0.24	0.03	0.17	0.05	µM
TSS-reference	42.5	21.90	9.60	2.30	mg/L
TSS-treated	29.9	11.80	11.10	5.70	mg/L
POC -reference	16.1	6.80	5.40	1.30	mg/L
POC-treated	10.5	3.21	5.80	2.63	mg/L
DOC -reference	5.82	3.67	3.03	0.27	mg-C/L
DOC-treated	4.87	2.97	3.36	0.37	mg-C/L

Table 6 clearly demonstrates that as far as the basic parameters are concerned the values were in accordance with the criteria as indicated in the guidelines (G8, Table 1). As far as the oxygen values of the reference tanks are concerned values at intake were closely corresponding to a saturation values (100%) for the given salinity and temperature. Since the water temperature increased steadily during the test series values are presented as a range and not averaged. At discharge, on day 5, the oxygen concentration in the reference tank declined by as much as 20% compared to intake values. In contrast oxygen concentration in the treated tank was less affected, due to reduced bacterial activity, resulting in higher values (14% decline relative to intake) as compared to the reference tank.

The total suspended solids (TSS) and particulate organic carbon (POC) concentrations were sufficiently high at intake but these values varied considerably among the different test runs. At discharge sediment concentration declined largely, mainly because of sedimentation in the storage tank during the holding period of 5 days. The applied filtration step effectively reduced the amount of TSS at intake and partly also the POC, on average by 30 and 35%, respectively. The remaining suspended material remained in suspension at discharge and half of its weight consisted of organic material.

In contrast to the particulate fraction the amount of dissolved organic carbon (DOC) was hardly affected by the ballast water treatment system. Mainly due to microbial mineralisation of this water for 5 days resulted in a reduction of the DOC concentration in the reference and treated tank by as much as 48% and 40%, respectively.

With respect to the concentration of inorganic nutrients (NO_3, NO_2, NH_4 and PO_4) there were with a few exceptions, only minor differences between the reference and treated ballast water tank. At the low salinity test series only the nitrite (NO_2) concentration increased by a factor 6.2 after the UV-reactor. It is known that in presence of nitrate UV-photolysis produces nitrite (Buchanan et al. 2006). The observed elevated concentration was nevertheless within the range typical for the challenge water used and therefore not excessively high. For comparison the observed concentrations of nitrite were lower than the US (EPA) and EU maximum allowed concentration for drinking water of 71.4 and 7.9 µM (1000 µg-N/L and 110 µg-N/L), respectively (Sharpless et al. 2003).

In this respect it should be noted that the prolonged dark incubation also increased the nitrite as well as the ammonia concentration, in both the reference and treated tank.

High salinity

Table 7: average or range of oxygen, pH, inorganic nutrients, TSS, POC and DOC concentrations of 5 test series run at high salinity at intake and discharge; [1]: range of values

Parameter	Intake	s.d.	Discharge (day 5)	s.d.	unit
O_2 reference[1]	4.7-9.2		2.6-6.1		mg/L
O_2 treated[1]	4.2-8.8		2.9-6.7		mg/L
pH reference	8.07	0.05	8.12	0.09	µM
pH-treated	8.05	0.09	8.10	0.12	µM
NO_3 reference	0.46	0.12	0.45	0.12	µM
NO3 treated	0.84	0.40	1.33	0.63	µM
NO_2 reference	6.87	4.55	6.28	4.63	µM
NO_2 treated	6.85	4.09	6.00	3.96	µM
NH_4 reference	7.33	4.67	6.74	4.75	µM
NH_4 treated	7.69	4.48	7.32	4.59	µM
PO_4 reference	0.41	0.10	0.56	0.29	µM
PO_4 treated	0.48	0.18	0.82	0.25	µM
TSS-reference	33.9	37.10	9.70	3.10	mg/L
TSS-treated	14	3.60	10.00	5.10	mg/L
POC -reference	10.2	5.30	4.80	1.30	mg/L
POC-treated	7.2	1.58	4.50	1.29	mg/L
DOC -reference	4.0	1.02	3.3	0.51	mg-C/L
DOC-treated	4.3	0.83	4.1	0.93	mg-C/L

The results of the basic parameters at the high salinity range were, with a few exceptions, on the whole the same as for the low salinity range (Table 7). At intake the oxygen concentrations were lower than observed for the low salinity test runs. This was mainly due to the temperature increase. Nevertheless, the ambient water was nearly saturated with oxygen and at discharge oxygen concentrations declined but they were still far from depleted. Partly because of the increase in seawater from the open North Sea sediment, POC and DOC values were lower as compared to the low salinity test series but still considerably higher than the minimum requirements according to the G8 guidelines. Again the filtration step turned out to be an effective manner of reducing the particle load. Both the TSS content and the POC content declined by a 59% and 30%, respectively.
Other differences were in the concentrations of the inorganic nutrients and in particular those containing nitrogen. While the nitrate concentration was very low, the level of nitrite and ammonia were elevated already in the water at intake. In fact these natural concentrations were much higher than the enhanced nitrite concentration caused by the UV-reactor. In contrast to the observations at the low salinity test runs the ammonia and nitrite concentration remained virtually constant during the 5 days of dark incubation.

8.2 Biology

Organisms > 50 µm

For the land based tests natural plankton was used and the required diversity of organisms (5 species of at least 3 different phyla) was easily fulfilled for all tests (Table 8). On average at least 10 different species of 4 to 5 different phyla were present in each sample (full details on species present see Annex 1).
Regarding the required minimal numbers of organisms per volume (Table 2), the values for the low salinity series (test run 1 -5) were all well above the minimum requirements. On average numbers in the >50 µm size fraction were 25 times higher than the minimum required number of 100,000 m^{-3}. At the high salinity range the water at intake was on average for the 5 test runs above the minimum numbers of organism but contained in two cases not sufficient numbers (test run 9 and 10). Test run 8 was slightly less than the required minimum of 100,000 organisms per m^{-3}. As explained earlier this is mainly due to the fact that natural seawater was used without any addition of (surrogate) organisms and that on average numbers of larger sized organisms are much lower in high saline water than brackish water.
On discharge the number of organisms had declined dramatically in the treated tank, to an average value for the low and high salinity test series of 2.9 and 2.4 organisms m^{-3}, respectively. It should however, be noted that the observed organisms, although still seemingly alive did receive prior to analysis a second UV-treatment. Since the applied BWT technology is known to have a delayed effect the organisms may be dying in the following day(s). Due to the very low concentration of organisms this could not be tested with sufficient statistical accuracy.
In fact during most test series at direct inspection immediately after intake already virtually no living organisms were observed. On average numbers in the treated tank were on discharge at day 5 well below the D2-standard of 10 organisms m^{-3}. In contrast numbers of organisms in the reference tank were still significantly above the G8 requirement and visual inspection and viability measurement indicated that these organisms were both intact and viable.

Table 8: Number of organisms (> 50 µm) of individual test runs and averages for the salinity range at intake (T0) and on discharge at day 5 (T5) of the reference (C) and treated (HG) tank. For test run 4 the same control was used, since this was a combined test run. All numbers are presented per m^3.

total plankton > 50 micron			numbers/m^3
low salinity	intake	reference	treated
	C-T0	C-T5	HG-T5
I	1.24E+06	1.08E+04	3.0
II	4.83E+06	6.17E+02	1.7
III	1.01E+06	2.18E+03	0.0
IV	3.12E+06	2.18E+03	2.7
V			7.3
average	2.55E+06	3.95E+03	2.9
s.d.	1.79E+06	4.65E+03	2.7

Table 8: continued

total plankton > 50 micron			
high salinity	intake	reference	treated
	C-T0	C-T5	HG-T5
VI	4.11E+05	1.31E+04	2.7
VII	2.75E+05	1.67E+04	0.3
VIII	8.57E+04	2.45E+03	1.7
IX	5.15E+04	1.73E+04	1.7
X	6.85E+04	2.76E+04	5.7
average	1.78E+05	1.54E+04	2.4
s.d.	1.58E+05	9.05E+03	2.0

Organisms 10 – 50 µm

This size class was dominated by phytoplankton, although heterotrophic organisms like ciliates and flagellates were occasionally present. For that reason the flow cytometric analysis included chlorophyll *a* fluorescence as an extra selection parameter. Moreover, the photosynthetic efficiency of the phytoplankton community was measured, as a tool to determine the efficacy of the treatment more specifically, on this group of organisms. Finally, a 10 L subsample of both the reference and treated water was collected at intake and for the treated water also after the second UV-irradiance disinfection at discharge and incubated under optimal growth conditions. Results of plankton numbers in the 10 – 50 µm of both reference and treated incubations are included in the table for comparison with the actual measurement of the tanks (Table 9).

Table 9 shows that regarding the number of organisms at intake for both salinity regimes the test water contained sufficient plankton numbers, on average 50% higher numbers than required according to the Guidelines G8. Prolonged incubation of the plankton community in the dark (5 days) resulted in a considerable reduction in the number of plankton in the 10 – 50 µm size range, on average by a factor 10. Nevertheless, the plankton community of the reference tanks still contained sufficient numbers of viable organisms to meet the G8 requirements for discharge of the reference tank. Moreover, the bottle incubations, which were ran parallel to the tank incubations, showed, with two exceptions (series 3 and 4) a considerable increase in numerical abundance of the plankton within 5 days, i.e. clearly indicative of the presence of viable population of mainly phytoplankton.

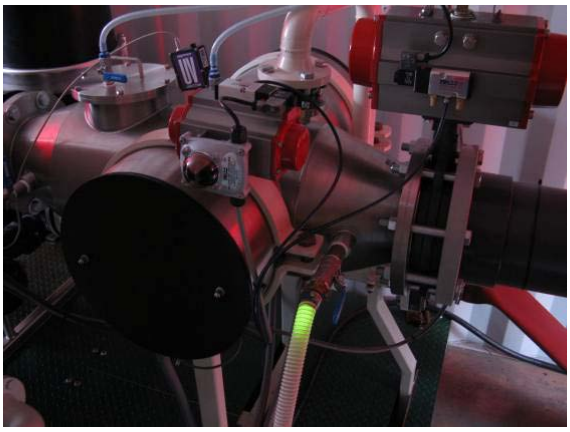


Figure 11: internal view of Hyde-Guardian™-system showing the UV-reactor. The UV-light is clearly visible in the open sampling line (yellow/greenish).

In contrast, treatment resulted in an efficient removal of plankton although in terms of numerical abundance numbers remained fairly high, certainly in the test series at the high salinity range, after the first treatment. After 5 days in the tanks residual plankton cells were still detected flow cytometrically. However, analysis of the viability using SYTOX Green and the photosynthetic efficiency measurements clearly demonstrated that these cells, although intact, were no longer viable. The incubated experiments confirmed the low abundance of intact cells and on average the number of viable cells after the first treatment step was < 1 cell/mL. Because the Hyde-Guardian™-system also included a second UV-irradiance treatment at discharge this water was incubated for another period of 5 to 7 days to determine the efficacy of the second passing of the UV-reactor over a longer period. Measurement showed that there was indeed a delayed effect. The original numbers in the tank at discharge (HG-T5) were further reduced to average values of 2.3 and 1.0 (HG-Dis-Tx) for the low and high salinity ranges, respectively. Also compared to the bottle incubations of the intake samples the second UV-irradiance disinfection resulted in a further decline in numbers of organisms.

Table 9: number of total plankton (10 – 50 µm size range) of each test run and average for the salinity range at intake (T0), reference (C) and treated tank (HG), reference and treated incubated samples of the intake (Inc.) at day 5 (T5) during discharge and of the incubated discharge samples (Dis-Tx). Test run 4 and 5 share the same reference since this was a combined test run. All numbers are presented per mL as total counts or of viable cells. Tx= varying sampling day (5 to 7 days).

10- 50 µm cell numbers		Reference			Treated				cells/mL
low salinity					total	viable	total	viable	x=5-7 days
test run	C-T0	C-T5	C-inc-T5	HG-T0	HG-T5	HG-T5	HG-inc-T5	HG-inc-T5	HG-Dis-Tx
I	1103	140	11184	677	242	<10	31.8	<1	0.0
II	1719	156	18706	836	21.5	<10	1.5	<1	10.9
III	1530	144	1149	483	396	<10	28.9	<1	0.0
IV	1784	147	765	304	103	<10	4.4	<1	0.0
V				309	169	<10	6.7	<1	0.7
average	1534	147	7951	522	186	<10	15	<1	2.3
high salinity									
VI	1657	121	9391	1091	53.3	<10	11.1	<1	0.0
VII	1657	149	6007	1453	68.8	<10	3.7	<1	2.2
VIII	2090	149	7774	2111	106	<10	6.7	<1	2.2
IX	1008	130	5754	514	7.4	<10	5.2	<1	0.7
X	1148	151	5941	1056	44.4	<10	3.7	<1	0.7
average	1512	140	6973	1245	56	<10	6.1	<1	1.0

In order to have a more complete insight of the fate of all organisms, i.e. also the planktonic fraction < 10 µm in size diameter, a single species of phytoplankton in the size range of ca. 6 µm (*Phaeocystis globosa)* was also monitored in the control and treated water (Table 10). Although no clear criteria are, yet, defined for this size class it was clear that the Hyde-Guardian™-system was also effective in reducing organisms in this size range. In general the response of *Phaeocystis* in terms of numbers and viable cells showed great similarity with the plankton in the 10 to 50 µm size class. Flow cytometry showed the presence of intact plankton in relatively high numbers in the treated tanks even after 5 days. This was confirmed by classical analysis of the chlorophyll and associated pigment content (data not shown). Viability tests indicated that the remaining cells were non-viable. This was also demonstrated in the incubation experiments. These bottle experiments showed that with exception of the first test run (204 cells/mL) numbers were already very low after the first treatment series. As with the 10 to 50 µm size range (Table 9), numbers of *Phaeocystis* cells also declined further after applying a second UV-irradiance disinfection step.

Table 10: number of a dominant phytoplankton species present (*Phaeocystis globosa*; ca. 6 µm in size) at each test run and average for the salinity range at intake (T0), reference (C) and treated tank (HG), reference and treated incubated samples of the intake (Inc.) at day 5 (T5) during discharge and of the incubated discharge samples (Dis-Tx). Test run 4 and 5 share the same reference since this was a combined test run. All numbers are presented per mL as total counts or of viable cells. Tx= varying sampling day (5 to 7 days).

***Phaeocystis* ~ 6 µm**		**Reference**			**Treated**				**cells/mL**
low salinity					**total**	**viable**	**total**	**viable**	**x=5-7 days**
test run	**C-T0**	**C-T5**	**C-inc-T5**	**HG-T0**	**HG-T5**	**HG-T5**	**HG-inc-T5**	**HG-inc-T5**	**HG-Dis-Tx**
I	**7807**	**179**	**10170**	**13646**	**5865**	**<10**	**204**	**<1**	**0**
II	**2661**	**62**	**17175**	**235**	**88**	**<10**	**0.7**	**<1**	**57.7**
III	**5460**	**178**	**3314**	**6850**	**1559**	**<10**	**0**	**<1**	**0**
IV	**7738**	**1939**	**4491**	**3832**	**332**	**<10**	**0**	**<1**	**0**
V				**322**	**425**	**<10**	**9.6**	**<1**	**2.2**
average	**5916**	**589**	**8788**	**4977**	**1654**	**<10**	**43**	**<1**	**12**
high salinity									
VI	**3754**	**632**	**9458**	**3475**	**493**	**<10**	**8.1**	**<1**	**0.7**
VII	**2227**	**204**	**4364**	**2611**	**463**	**<10**	**6.7**	**<1**	**3.7**
VIII	**2038**	**204**	**6650**	**3015**	**957**	**<10**	**3.0**	**<1**	**3.7**
IX	**1252**	**236**	**21473**	**1194**	**158**	**<10**	**1.5**	**<1**	**1.5**
X	**1395**	**173**	**14440**	**2705**	**665**	**<10**	**4.4**	**<1**	**0.1**
average	**2133**	**290**	**11277**	**2600**	**547**	**<10**	**4.7**	**<1**	**1.9**

Photosynthetic efficiency

Another, fast and reliable approach to gain insight in the physiology and growth response of the **whole** phytoplankton community (2 to 50 µm in cells size) is by measuring the photosynthetic efficiency (Fv/Fm) of the phytoplankton cells. This can be done for the whole community or after filtration for different size classes when needed. For a clear interpretation it must be noted that values of Fv/Fm > 0.4 are indicative of a healthy phytoplankton population; a Fv/Fm < 0.4 indicates that the phytoplankton community is experiencing severe stress and a value < 0.1 is typically observed in decaying phytoplankton populations.
Table 11 shows that during intake the whole phytoplankton community of the Wadden Sea (low salinity range) and coastal waters of the North Sea (high salinity range) was physiologically in a healthy condition, i.e. containing mostly photosynthetic active and therefore viable cells. Only at test run 7 the phytoplankton population showed some indication of stress. Prolonged incubation in the dark (5 days of dark stress) of phytoplankton in the reference tanks resulted in a severe reduction in the photosynthetic efficiency. Although the phytoplankton community did not enter the decay phase the majority of cells were far from healthy. In contrast the photosynthetic efficiency of the phytoplankton from the same water mass but incubated under optimal growth conditions showed that the cells

possessed a healthy status (C-inc-T5). This is not surprising since phytoplankton also increased in numerical abundance (Tables 9 and 10).
After the first treatment, at intake, most of the phytoplankton community was immediately dead resulting in Fv/Fm values of 0 (HG-T). A holding time of 5 days in the tank did not change this pattern. Also in the incubated sample no recovery of the phytoplankton population was observed. This results did not change after the second UV-irradiance disinfection step at discharge and a second incubation period of 5 to 7 days (HG-Dis-Tx).

Table 11: Photosynthetic efficiency (F_v/F_m) of the whole phytoplankton community (2 - > 50 µm size range) at each test run and average values for the salinity range at intake (T0), reference (C) and treated tank (HG), reference and treated incubated samples of the intake (Inc.) at day 5 (T5) during discharge and of the incubated discharge samples (Dis-Tx). Test run 4 and 5 share the same reference since this was a combined test run. All numbers are presented per mL as total counts or of viable cells. Tx= varying sampling day (5 to 7 days). nd: not determined

low salinity	Reference			Treated			
F_v/F_m	C-T0	C-T5	C-inc-T5	HG-T0	HG-T5	HG-inc-T5	HG-Dis-Tx
I	0.65	0.16	0.69	0.00	0.01	0.00	nd
II	0.51	0.19	0.62	0.00	0.00	0.00	0.00
III	0.58	0.10	0.70	0.00	0.00	0.16	0.00
IV	0.51	0.12	0.81	0.00	0.00	0.00	0.00
V				0.01	0.01	0.00	0.00
average	0.56	0.14	0.71	0.00	0.00	0.03	0.00
s.d.	0.07	0.04	0.08	0.00	0.00	0.07	0.00
high salinity							
VI	0.59	0.13	0.63	0.00	0.02	0.00	0.00
VII	0.44	0.11	0.65	0.00	0.04	0.00	0.00
VIII	0.67	0.16	0.60	0.00	0.00	0.01	0.05
IX	0.62	0.28	0.72	0.00	0.00	0.00	0.00
X	0.66	0.13	0.64	0.03	0.01	0.00	0.00
average	0.60	0.16	0.65	0.01	0.01	0.00	0.01
s.d.	0.09	0.07	0.04	0.01	0.01	0.01	0.02

Bacteria

For the microbial community the presence/absence of two types of human pathogens was monitored prior to and after treatment, while the response of the whole microbial community was also assessed.
Table 12 shows that even during intake the number of the target microorganisms was well below the standard as indicated in the D2-Standard. Only in three cases the numbers of human pathogens were above the detection limit (test runs 7, 8 and 9). The reason for this is that the NIOZ harbour is located in a pristine environment with little or no urban activity. Subsequently, also the number of human pathogens in the reference and treated tanks were below detection limit during discharge.

Table 12: plate counts of the human pathogens *E. coli* and total enterococci of each test run and averages for the salinity range at intake (T0), reference (C) and treated tank (HG), reference and treated incubated samples of the intake (Inc.) at day 5 (T5) during discharge and of the incubated discharge samples (Dis-Tx). Test run 4 and 5 share the same reference since this was a combined test run. All numbers are presented per mL as total counts or of viable cells. Tx= varying sampling day (5 to 7 days).

human pathogens	Reference				Treated			counts/mL
	C-T0	C-T0	C-T5	C-T5	HG-T5	HG-T5	HG-Dis-Tx	HG-Dis-Tx
low salinity	*E.coli*	Enterococci	*E.coli*	Enterococci	*E.coli*	Enterococci	*E.coli*	Enterococci
I	<0.1	<1	<0.1	<1	<0.1	<1	<0.1	<1
II	<0.1	<1	<0.1	<1	<0.1	<1	<0.1	<1
III	<0.1	<1	<0.1	<1	<0.1	<1	<0.1	<1
IV	<0.1	<1	<0.1	<1	<0.1	<1	<0.1	<1
V	<0.1	<1	<0.1	<1	<0.1	<1	<0.1	<1
average	<0.1	<1	<0.1	<1	<0.1	<1	<0.1	<1
high salinity								
VI	<0.1	<1	<0.1	<1	<0.1	<1		<1
VII		1		<1		<1	<0.1	<1
VIII	<0.1	17	<0.1	<1	<0.1	<1	<0.1	<1
IX	<0.1	7	<0.1	<1	<0.1	<1	<0.1	<1
X	<0.1	<1	<0.1	1	<0.1	<1	<0.1	<1
average	<0.1	8.3	<0.1	<1	<0.1	<1	<0.1	<1

In contrast to the almost complete absence of human pathogens, the typical marine microbial community was abundantly present. The total bacteria community is well studied in the Wadden Sea, using a method based on staining the nucleic acid of the cells, for many years. The currently observed numbers ranging from 0.72 to 6.6 10^6 per mL (variation of factor 9.1) are typically observed in spring and early summer (Table 13). Of this total bacterial population only a very small fraction could be identified as heterotrophic using the plate assay method (<10 to 60 per mL). Therefore, if only plating would be used as criteria for heterotrophic bacteria numbers this would result in a severe underestimation of the actual bacteria numbers. After 5 days of incubation total bacteria numbers on average declined in the control tank but there was considerable variation between the different test runs (C-T5).

As opposed to the tank total bacterial numbers increased, with exception of test run 2, in the incubated water of the reference tank (C-inc-T5) for both salinity regimes. This indicates that a significant fraction of the bacteria was viable.

In the treated tanks and in the incubation bottles of the same water the total bacteria numbers at discharge varied from the original numbers during intake in an inconsistent manner. In nearly half of the test runs there was an actual increase whereas in the others there was a decrease from the numbers at intake. These variations were nevertheless moderate and never exceeded the original values by more than a factor of 1.9. In a similar manner the numbers of total bacteria of the incubated sample were comparable with those of the holding tank samples. A clear reduction in bacteria number was observed after the second UV-disinfection step during discharge (HG-Dis-Tx) resulting in a decline in number by one order of magnitude relative to the number measured in the tank at day 5.

In terms of cultivable heterotrophic bacteria the treated water showed some remarkable differences with the water at intake and the reference. During the low salinity test runs the numbers of colony-forming heterotrophs increased on average by a factor 5 after 5 days of incubation but the second UV-irradiance disinfection step reduced the number of cfu's to below the detection limit.
At all test runs at the high salinity range there the number of colony forming units was roughly identical to the water at intake. In contrast to the low salinity test runs the number of cfu's increased in 3 out of 5 test runs at the high salinity tests after the second UV-irradiance disinfection step.

Table 13: Total bacteria number (numbers per mL) and colony forming heterotrophs (cfu/mL) of each test run and average for the salinity range at intake (T0), reference (C) and treated tank (HG), reference and treated incubated samples of the intake (Inc.) at day 5 (T5) during discharge and of the incubated discharge samples (Dis-Tx). Test run 4 and 5 share the same reference since this was a combined test run. All numbers are presented per mL as total counts or of viable cells. Tx= varying sampling day (5 to 7 days). nd: not determined

total bacteria											numbers/mL
heterotrophic bacteria				Reference			Treated				cfu/mL
low salinity	C-T0	C-T0	C-T5	C-inc-5	C-T5	HG-T0	HG-T5	HG-inc-T5	HG-T5	HG-Dis-Tx	HG-Dis-Tx
test run	total	colony	total	total	colony	total	total	total	colony	total	colony
I	4.5E+06	20	2.3E+06	8.3E+06	80	4.1E+06	4.8E+06	7.4E+05	250	nd	10
II	6.6E+06	60	3.6E+06	2.4E+06	20	3.4E+06	5.2E+06	3.9E+06	20	2.1E+05	<10
III	3.0E+06	30	1.8E+06	4.8E+06	60	3.8E+06	9.6E+05	2.1E+06	300	1.3E+05	<10
IV	1.3E+06	30	0.7E+06	4.9E+06	<10	1.4E+06	3.8E+05	8.8E+05	375	9.4E+04	<10
V		20				1.0E+06	7.4E+05	4.0E+05	225	7.5E+05	<10
average	3.9E+06	32	2.1E+06	5.1E+06	43.0	2.8E+06	2.4E+06	1.6E+06	234	3.0E+05	<10
s.d.	2.3E+06	16.4	1.2E+06	2.4E+06	30.6	1.4E+06	2.4E+06	1.5E+06	132.6	3.1E+05	
high salinity											
VI	5.6E+06	20	0.9E+06	6.1E+06	10	6.3E+06	1.0E+06	3.6E+05	10	4.3E+05	<10
VII	0.7E+06	50	5.4E+06	8.2E+06	20	5.2E+06	7.1E+05	3.8E+05	70	3.1E+05	20
VIII	4.9E+06	20	1.7E+06	5.1E+06	30	4.3E+06	1.2E+06	2.5E+06	50	1.2E+05	120
IX	2.4E+06	30	0.3E+06	3.0E+06	<10	3.4E+06	2.4E+05	3.8E+06	10	1.2E+06	170
X	5.3E+06	10	2.4E+06	6.3E+06	60	4.2E+06	1.3E+06	3.3E+06	<10	1.2E+06	>1000
average	3.8E+06	26	2.1E+06	5.7E+06	30	4.7E+06	8.8E+05	2.1E+06	35	6.4E+05	<10->1000
s.d.	2.1E+06	15.2	2.0E+06	1.9E+06	21.6	1.1E+06	4.2E+05	1.6E+06	30.0	5.0E+05	

9 Environmental acceptability

Ballast water treatment systems applying active substances, but also systems that do not use active substances should demonstrate according to the revised guidelines G8 (MEPC58, October 10th) that the treated water upon discharge is not harmful to the environment and organisms.
Although officially not obligatory for the Hyde-Guardian™-system a series of studies were conducted to examine long-term (20 days) regrowth and vitality. In total 2 long-term incubation experiments, one at each salinity regime, were conducted with treated water which was collected in a clean container (10 L) and incubated under optimal growth conditions in a climate room. The experiments were done for treated water collected at intake (pre-filtration and UV-reactor) and at discharge (second UV-irradiance disinfection step). The second series of (discharge) samples were stored in the dark tank for 5 days prior to the incubation.
To stimulate regrowth of planktonic organism extra nutrients were added (nitrate; 30 µM, phosphate; 2 µM, silicate; 20 µM). In particular at the peak of the spring bloom and thereafter the ambient nutrient concentration (mainly silicate and phosphate) can be extremely low, which may prevent growth of phytoplankton and also bacteria.

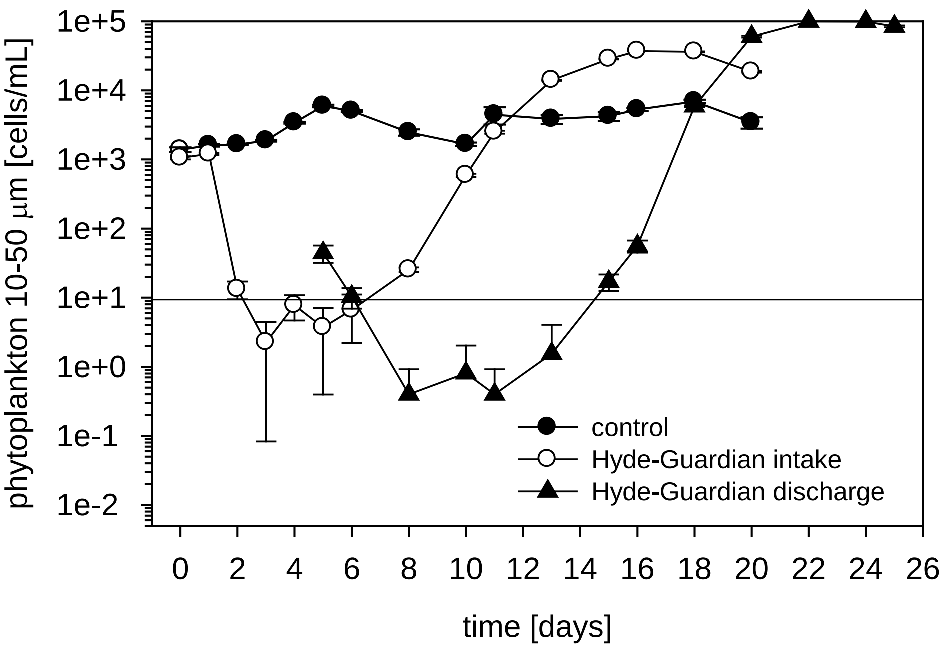


Figure 12: time course of phytoplankton numbers (10 – 50 µm size range) during a 25 day incubation period, control and treated water incubated at intake and treated water also incubated at discharge. Test run X; values are mean of three replicates. Line marks the D2-Standard value of 10 cells/mL.

Figure 12 shows that when incubated under optimal growth conditions the total cell numbers of phytoplankton in the 10-50 µm declined in the first three days. The same was observed for the smaller phytoplankton (data not shown). Regrowth of phytoplankton only occurred after 7 days of incubation. This observation indicates that the treatment did not deactivate the entire plankton community. Some cells survived as resting stages, cysts or did recover from the UV-irradiance disinfection step. Although figure 12 only shows the results for the larger fraction a similar trend was observed for the smaller phytoplankton as well (data not shown). Nevertheless, even under favourable conditions recovery, resulting in cell number exceeding the minimum number of 10/mL, was observed after 7 days.
For this reason a second UV-irradiance disinfection step was applied at discharge. This resulted in a further reduction of the total number of particles by an order of magnitude.

Similar to the first UV- disinfection treatment there was a delayed effect of ca. 3 days (figure 12). After this period the plankton community stabilized and regrowth started 14 days after the initial intake of the water. The second UV- irradiance disinfection step was certainly effective in this respect but the this water was still not entirely free of living plankton.
In terms of vitality it should be noted that regrowth of phytoplankton was observed and the final numbers achieved in these experiments, exceeded even the numbers of the control bottles (Figure 9). Therefore, potential by- or end-products generated by the UV- reactor did not affect the water quality in such a manner that growth of phytoplankton in the receiving environment would be inhibited.

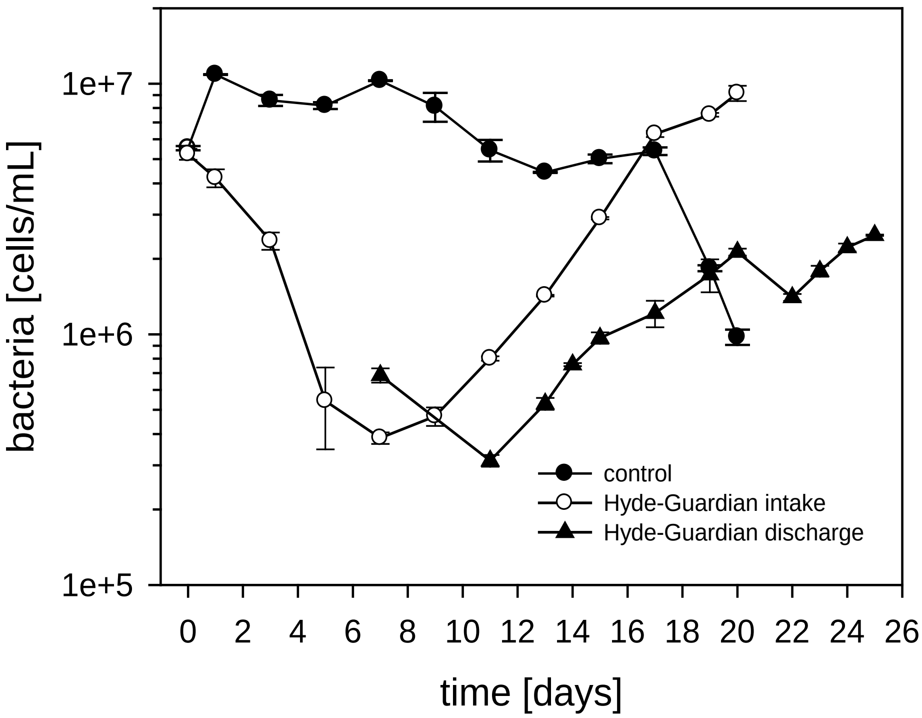


Figure 13: time course of bacteria numbers during a 25 day incubation period, control and treated water incubated at intake and treated water also incubated at discharge. Test run X; values are mean of three replicates.

With respect to total bacteria numbers the control and the two treated water samples showed a trend over time which was basically similar to that of development of the phytoplankton community over time (fig. 13). In the control bottle bacteria increased on the first day, remaining at a constant level for 8 days and declining gradually till the last day of sampling (day 20). The final numbers at the end were one order of magnitude lower than the original values at intake.
In contrast bacteria numbers in the treated water at intake declined rapidly by a factor 10 in the first 5 days of incubation. From day 7 onwards there was rapid growth and at the day 20 numbers had increased to a factor 2 higher than the initial numbers.
Similar to the phytoplankton (figure 9) the second treatment with the UV-reactor at discharge resulted in a further reduction of the total amount of bacteria present. A recovery, in terms of bacterial growth was delayed until day 11. The bacterial growth rate was reduced, compared to that of the bacteria at intake, and final numbers after 25 days of incubation were only 50% of the original bacteria numbers at intake.

10 Discussion and evaluation of results

The presented data show that the experimental design, type of test protocols used and additional experiments provide a solid data base of information on the performance of the Hyde-Guardian™-system under semi-*in situ* conditions. The initial idea of a treatment systems based on the (permanent) deactivation of organisms through ultraviolet light disinfection, both at intake and discharge turned out to be effective even in surface water with an extremely high density of mucilage containing organisms and a high turbidity. As a first step to reduce the particle load the coarse filter effectively removed the larger fraction including organisms.

With only some minor deviations the present 10 test runs were conducted according to the guidelines G8 of the IMO. The basic chemistry and physical properties of the water were not altered by the treatment and no factors could be detected effecting the vitality of the treated water upon discharge.

The treated water remained stable for most of the parameters over the whole holding period of 5 days. Concentrations of dissolved oxygen (DO) and dissolved organic carbon (DOC) hardly varied. Only the concentration of total suspended solids and the particulate organic carbon declined slightly mainly as a result of sedimentation. In this respect it should be noted that the application of the self-cleaning filter effectively removed larger particles and the load of sediment in the treated tanks was only minimal compared to that in the control tank.

With respect to the water chemistry a slight increase in nitrite concentration was observed during the low salinity test runs in the treated water (cf. Sharpless & Linden 2001). This apparent increase was still 50% of the natural nitrite concentrations observed during the second test series at high salinity and should therefore be considered as non hazardous. Moreover, the values were still lower than the maximum allowed concentration for drinking water (Sharpless et al. 2003).

The stable DO and DOC over time was indicative of a reduced biological activity during the holding period of 5 days. Numbers of organisms were in compliance with the D2-Standard at discharge but the second UV-disinfection effectively reduced the number of viable organism further. The treated water did not contain toxic or growth inhibiting substances. Neither were chemicals produced as by-products. On the contrary this water was rich in growth stimulating nutrients (nitrogen, phosphorus and silicate) for phytoplankton and bacteria.

With apparently some living cells present and ample growth stimulating resources present, phytoplankton and bacteria could start blooming in the presence of light. Still even under favourable conditions growth was delayed by a much as 10 days.

During the testing period we encountered some issues which need attention for future testing and legislation.

1. The number of organisms in the > 50 μm fraction, and to some extend also in the 10 – 50 μm size range, cannot (easily) be met in the salinity range > 32 PSU. The addition of cultured organisms is very difficult and poses a variety of problems. With increasing salinity the seawater will have the characteristics of typical open ocean water. This implies low to extremely low numbers of the organisms in the above indicated size ranges. For that reason we have extended our focus to the organisms < 10 μm since there is no biologically relevant reasoning why tests should exclude this size fraction.
2. The long-term incubations (ca. 20 days) and the environmental acceptability studies provide good insight in the response of the whole community to the growth potential/limitation of the treated water when released into environment. These experiments should be conducted next to the standard set of toxicology and residual chemistry tests of the treated ballast water.
3. Testing for the presence of human pathogens strongly depends on the natural abundance of these microbes in the natural environment. Since these pathogens cannot be supplemented for safety reasons accurate testing is therefore not possible.

Moreover, viability tests of the total bacteria community showed that not all bacteria were effectively deactivated. Therefore, at least in theory the human pathogens would remain a potential risk. This is a factor of concern for the land-based tests as well as for the ship-board trials when the ship remains in fairly clean ports for intake and discharge. Although UV systems, including the one applied in the present BWT-system, in general have been proven to effective in the disinfection of (drinking) water no data test are available.

4. The Guidelines G8 and the update version as adopted at MEPC 58 (10 October 2008) are incomplete with respect to treatment systems including a second treatment at discharge. In line of the tank and sample volume for treatment at intake a similar protocol should be developed for treatment at discharge. However, considering the expected number of organisms at discharge and statistical errors associated with the actual counts a second sample tank to collect and store the discharge would be required with a volume two orders of magnitude larger than the present tank. i.e. 20,000 m^3. As an alternative for these large volume incubations, but restricted to the smaller sized plankton, the bottle (10 L) incubations either in the dark or in a more favourable condition could be applied.

In conclusion, the present configuration of the Hyde-Guardian™-system offers a reliable and environmentally safe cleaning of the ballast water resulting in organism numbers well below the Standard of the IMO Regulation-D2.

11 References

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Appendix 1

Phytoplankton Marsdiep April-July 2008 ***data by Jolanda van Iperen***	***+: present but rare, ++: present; +++: dominant;*** ***++++: very abundant; +++++: massively present***	
group	***species name***	***relative dominance***
autotrophic flagellate	*Phaeocystis globosa* colony cell	+++++
	Phaeocystis globosa colonies < 50 µm	++++
	Phaeocystis globosa colonies > 50 µm	+++++
autotrophic flagellate	*Phaeocystis globosa flagellate cell*	+++++
heterotrophic flagellate	*parasite cyst of Ochromonas* group	++++
diatom pennate	*Pseudonitzschia delicatissima* group	++++
heterotrophic flagellate	*heterotrophic flagellate* indet. <10 µm	++++
autotrophic flagellate	*Prymnesiales* indet. <10 µm	++++
diatom centricate	*Thalassiosira* spp. 10 µm	++++
diatom centricate	*Chaetoceros socialis*	++++
autotrophic flagellate	*Hemiselmis* group	+++
diatom centricate	*Skeletonema "costatum"* group	+++
autotrophic flagellate	*Plagioselmis* group	+++
heterotrophic flagellate	*Paulinella* spp.	+++
heterotrophic flagellate	*heterotrophic flagellate* indet. 10-30 µm	++
autotrophic flagellate	*Pyramimonas* spp. <10 µm	++
heterotrophic flagellate	*Choanoflagellatea* indet.	++
hetero/auto flagellate	*Cryptophyceae* "light" group	++
autotrophic flagellate	*Teleaulax acuta* group	++
heterotrophic dinoflagellate	*Oxyrrhis marina*	++
diatom centricate	*Leptocylindrus minimus*	++
diatom centricate	*Minutocellus* group	++
diatom centricate	*Thalassiosira* spp. 10-30 µm	++
heterotrophic dinoflagellate	*Katodinium glaucum*	++
diatom centricate	*Guinardia delicatula*	++
freshwater green alga	*Pediastrum* spp.	++
heterotrophic flagellate	*Ciliophrys* group	++
autotrophic flagellate	*Prasinophyceae* indet. <10 µm	++
hetero/auto dinoflagellate	*Gymnodiniaceae* indet. 10-30 µm	++
diatom centricate	Various species	++
autotrophic flagellate	*autotrophic flagellate* indet. <10 µm	++
diatom centricate	*Chaetoceros* spp. <10 µm solitairy cells	+
autotrophic flagellate	*Chlorophyceae, Telonema* spp.	+
heterotrophic flagellate	*Chrysophyceae* indet.	+
freshwater green alga	*Crucigenia* spp., *Chlorophyta*	+
diatom centricate	*Skeletonema "costatum"* lenses	+
freshwater green alga	*Oocystis* spp.	+
diatom pennate	*Asterionellopsis glacialis*	+
autotrophic flagellate	*Rhodomonas* group	+
heterotrophic flagellate	*Bodo* group	+

Species list zooplankton and some larger (atypical) plankton

Appendix 1 continued

Phylum	Class	Subclass, Order, etc.	species no.	Identified genera	most likely present
Sarcomastigophora		Dinoflagellida	*2*	*Noctiluca, Protoperidinium*	
Bacillariophyceae			*3+*	*Bidulphia, Coscinodiscus*	
Cnidaria	Hydrozoa		*2+*	*Obelia*	
	Scyphozoa		*2*	*Aurelia, Cyanea*	
Ctenophora			*2+*		*Pleurobrachia, Beroe, Mnemiopsis*
Nemathelminthes	Rotatoria		*1+*		*Asplanchna*
	Nematoda		*1+*		
Annelida	Polychaeta		*2+*		
Arthropoda	Crustacea	Order Calanoida	*4+*	*Temora, Acartia, Centropages, Calanus and/or Pseudocalanus*	*Oithona*
		Order Harpacticoida	*2+*		*Tigriopus*
		Subclass Cirripedia	*1+*		*Semibalanus*
		Suborder Cladocera	*2*	*Podon, Evadne*	
		Subclass Malacostraca	*2+*	*Carcinus (zoea larvae)*	
Mollusca	Gastropoda		*1+*		*Littorina*
	Lamellibranchia		*2+*	*Cerastoderma*	*Mya*
Echinodermata	Ophiuroidea and/or		*2+*		*Ophiothrix, Echinocardium*
	Echinoidea				
Urochordata	Larvacea		*1*	*Oikopleura*	
Minimum number of species encountered (10 phyla):			*32*		