Ministerie van Verkeer en Waterstaat

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Ballast water

An investigation into the presence of plankton organisms in the ballast water of ships arriving in Dutch ports, and the survival of these organisms in Dutch surface and port waters

On behalf of the North Sea Directorate, Rijswijk

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Preface

This study was carried out under the authority of the North Sea Directorate of the Ministry of Transport, Public Works and Water Management. From the North Sea Directorate, support was given by Mrs S. van Gool.

We acknowledge the many people who contributed in one or another way to this ballast water study:
-Ship owners and shipping agencies for permission to take ballast water samples on board their ships and for help in the extensive contacts with the ships before sampling;
-Officers and crews of the ships we boarded in order to obtain ballast water samples. They provided us with information about ship and ballast water and assistance during sampling;
-Mr E.A. van de Berg, port authority Zeeland Seaports, for allowing us to sample in the port of Vlissingen-Oost;
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The samples from the period November 1998 - January 2001 were analysed by AquaSense/Tripos (Amsterdam) and from the period April 2001 - November 2000 by Koeman & Bijkerk (Haren).

Samenvatting

Het wereldwijde transport van ballastwater blijkt een effectieve distributie vector te zijn van talrijke uitheemse organismen. Lozing van dit ballastwater kan leiden en heeft geleid tot de introductie van deze organismen in allerlei zoete, brakke en zeewater milieus. In veel gevallen hadden deze onbedoelde introducties ernstige economische, ecologische of volksgezondheids gevolgen.

De Internationale Maritieme Organisatie werkt aan regelgeving om het ballastwaterprobleem aan te pakken. Om een Nederlands standpunt te bepalen, initieerde de Directie Noordzee van het Ministerie van Verkeer en Waterstaat een aantal ballastwaterstudies. De te beantwoorden vragen van deze ballastwaterstudie waren: 1) welke organismen worden geïmporteerd in ballastwater van schepen die Nederlandse havens aandoen? en 2) kunnen deze organismen overleven in Nederlands oppervlakte- en havenwater?

In de periode november 1998 - november 2000 werden ballastwatermonsters genomen aan boord van 30 schepen (containerschepen, multi purpose schepen, chemicaliëntankers en bulk carriers) in de havengebieden van Rotterdam, Amsterdam en Vlissingen. Het onderzochte ballastwater was in de meeste gevallen opgenomen in Europese havens of estuaria, maar ook cocktails van estuarien en oceanisch water werden bemonsterd. De temperatuur van het ballastwater verschilde altijd wel een paar graden met die van het havenwater. Het meeste bemonsterde ballastwater was afkomstig uit brak water- en zeewatergebieden; bijna alle havenwatermonsters werden geclassificeerd als brak water.

In de geanalyseerde ballastwatermonsters werd een groot aantal soorten plankton aangetroffen. Het aantal fytoplanktonsoorten en de celdichtheden nam significant toe bij een kortere verblijftijd van het ballastwater in de tanks. Bij de analyse van de ballastwatermonsters werd een stringent onderscheid gemaakt tussen soorten die tot op soorts-, geslachts- of groepsniveau gedetermineerd konden worden. Er werden 122 soorten fytoplankton (voornamelijk diatomeeën en autotrofe dinoflagellaten), 37 soorten microzoöplankton (voornamelijk heterotrofe dinoflagellaten en raderdieren) en 12 soorten mesozoöplankton (watervlooien en copepoden) tot op soortsniveau gedetermineerd. De meeste soorten waren al bekend uit het Nederlandse fytoplankton monitoringprogramma, andere programma's en uit de literatuur. Er werden slechts 3 uitheemse soorten dinoflagellaten gevonden in de ballastwatermonsters. Verder werden in 6 tot 19% (afhankelijk van de soort) van de onderzochte ballast tanks diatomeeën, blauwwieren en dinoflagellaten gevonden, waarvan toxische effecten op mens en dier bekend zijn.

In de geanalyseerde havenwatermonsters werden 72 soorten fytoplankton (voornamelijk diatomeeën en autotrofe dinoflagellaten) en 17 soorten microzoöplankton (voornamelijk heterotrofe dinoflagellaten) gedetermineerd tot op soortsniveau. Mesozoöplankton soorten werden niet aangetroffen als gevolg van het kleine monstervolume.

Incubatie van ballastwater bij temperaturen van 10 and 20 °C in verschillende media en in gefiltreerd havenwater met saliniteiten van 0.3 tot 30 psu, resulteerde altijd in groei van zeker 5 tot 20 fytoplankton soorten. Ook een paar potentieel toxische fytoplankton soorten, die werden waargenomen in het ballastwater, groeiden in de gebruikte media. Vanwege de grote saliniteitstolerantie van fytoplankton werd er geen significant verband gevonden tussen het aantal opgekomen soorten en het saliniteitsverschil (saliniteitsverschil van het gebruikte medium en het ballastwater).

Het blijkt dat tegelijk met ballastwater veel levend plankton in Nederlandse havens wordt aangevoerd, inclusief ongewenste uitheemse, toxische en potentieel toxische fytoplankton soorten.
Na lozing van ballastwater overleeft een deel van de aangevoerde organismen in het Nederlandse oppervlakte- en havenwater. Bij de bemonsteringen werd steeds slechts een kleine hoeveelheid van het aan boord aanwezige ballastwater bemonsterd. Wanneer we onze resultaten extrapoleren naar de schaal waarop ballastwater geloosd wordt in Nederland, dan kunnen we aannemen dat ongewenste soorten regelmatig in grote hoeveelheden worden aangevoerd in ons oppervlakte- en havenwater. Wanneer deze soorten met grote regelmaat en aantallen worden geloosd, dan verhoogt dit de kans dat lozing plaatsheeft onder specifieke abiotische omstandigheden, die gunstig zijn voor deze soorten, bijvoorbeeld een hoge rivierafvoer met grote hoeveelheden nutriënten. Samengevat betekent dit dat het in Nederlandse havens geloosde ballastwater zeker niet vrij is van risico's, zoals bijvoorbeeld groei van uitheemse, toxische of potentieel toxische soorten fytoplankton.

Summary

The world-wide transport of ballast water has been shown to be an effective distribution vector for numerous non-native organisms. Discharge of this ballast water may lead and has led to the introduction of these organisms into all kinds of fresh, brackish and seawater environments. In many cases, these unintended introductions had serious economic, ecological or public health consequences.

The International Maritime Organization is developing regulations to tackle the ballast water problem. To define a Dutch point of view, the North Sea Directorate of the Ministry of Transport, Public Works and Water Management, initiated a number of ballast water studies. The objectives of this ballast water study were to answer the following questions: 1) which organisms are imported with ballast water in ships arriving in Dutch ports? and 2) do these organisms survive in Dutch surface and port waters?

During the period November 1998 - November 2000 ballast water samples were taken on board of 30 ships (container ships, multi-purpose ships, chemical tankers and bulk carriers) in the port areas of Rotterdam, Amsterdam and Vlissingen. In most cases the investigated ballast water was taken up in European ports or estuaries, but also mixtures of estuarine and oceanic waters were sampled. The temperatures of ballast water almost always differed by a few degrees from those of port water. Most of the sampled ballast water originated from brackish and seawater environments; port water samples almost always could be classified as brackish.

A large number of plankton species was found in the analysed ballast water samples. The number of phytoplankton species and cell numbers increased significantly when the residence time of the ballast water in the tanks had been shorter. In the analysed ballast water samples a conservative distinction was made between species analysed to species, genus or group level. 122 phytoplankton species (mainly diatom and autotrophic dinoflagellate species), 37 microzooplankton species (mainly heterotrophic dinoflagellate and rotifer species) and 12 mesozooplankton species (cladoceran and copepod species) were determined to species level. Most species were known already from the Dutch phytoplankton monitoring programme, from other programs and from literature. Only 3 non-native dinoflagellate species were found in the ballast water samples. Furthermore, we found diatom, bluegreen and dinoflagellate species with recorded toxic effects on humans and animals, in 6 to 19% (depending on the species) of the investigated ballast tanks.

In the analysed port water samples, 72 phytoplankton species (mainly diatom and autotrophic dinoflagellate species) and 17 microzooplankton species (mainly heterotrophic dinoflagellate species) were determined to species level. Mesozooplankton species were not recorded because of the small sample volume.

Incubation of ballast water at temperatures of 10 and 20 °C in different media and in filtered port water with salinities of 0.3 to 30 psu, always resulted in growth of approximately 5 to 20 phytoplankton species.

Also a few potentially toxic phytoplankton species, that were observed in the ballast water samples, grew in the media used. A significant relation between the number of growing species and difference in salinity (salinity of the medium used minus the salinity of ballast water) was not found, very probably because of the large salinity tolerance range of phytoplankton.

Obviously, many living plankton species are imported with ballast water into Dutch ports, including unwanted non-native, toxic and potentially toxic phytoplankton species. After the ballast water is discharged, part of the imported organisms is able to survive in Dutch surface and port waters. We sampled only a very small fraction of ballast water on board of each ship. Extrapolating our results to the scale with which ballast water is discharged in Dutch surface and port waters, we may assume that unwanted species are released in large numbers into these waters. If these species are being released regularly and in large numbers, there is a great chance of interfacing with specific abiotic conditions, such as a great river run-off for example, that may favour these unwanted species. In summary, ballast water discharged into Dutch ports, is certainly not free from risks, such as, for example, the growth of non-native, toxic or potentially toxic phytoplankton species.

1 Introduction

Since the end of the nineteenth century ships switched from carrying rocks for ballast to carrying water (Carlton, 1985). This ballast water is needed for safety and stability at sea as well as during port operations. However, the ballast water transported world-wide has been shown to be an effective transport vector of numerous non-native organisms (Williams et al., 1988; Hallegraeff & Bolch, 1991; Carlton & Geller, 1993; Gollasch, 1996 and Galil & Hülsmann, 1997). Discharge then will lead to the introduction of these organisms into all kinds of receiving waters. Many introduced organisms will not survive upon discharge or already form part of the receiving ecosystem or survive without causing any harm. An example of the latest is the American razor clam *Ensis directus*, introduced as larvae in ballast water into German waters, and now an abundant species in the Dutch Wadden Sea and coastal waters (Essink, 1986). In many cases, however, these unintended introductions have had serious economical, ecological or human health consequences.

Toxic phytoplankton pose a serious threat to human health and natural and cultivated shellfish and fish resources. For that reason there is a lot of concern about the possibility of worldwide transport of toxic phytoplankton species with ballast water (and sediments) from one place to another. For example, transport of toxic dinoflagellates in ballast water from Asia to Australia has been reported by Hallegraeff & Bolch (1991). Another toxic dinoflagellate, *Karenia mikimotoi*, might have been brought with ballast water from North-America to Europe in the sixties. Since then it has regularly been the cause of mass mortality of caged fish and invertebrates (Gollasch et al., 1999). Also, other organisms are transported with ballast water and introduced elsewhere, for example bacteria, larvae from molluscs, zooplankton and jelly fish. Examples of the large economic and ecological consequences of these introductions have been described in Carlton (1996a), Ruiz et al. (1997), Cohen & Carlton (1998), Carlton (1999) and McCarthy & Khambaty (1994).

The International Maritime Organization (IMO) is developing regulations to tackle the ballast water problem. To define a Dutch point of view, the North Sea Directorate of the Ministry of Transport, Public Works and Water Management initiated a number of ballast water studies.

One of these studies (AquaSense, 1998) focussed on the amount and origin of ballast water in ships entering and leaving the ports of Rotterdam and Amsterdam and the risks of the introduction of non-native species into Dutch coastal waters. The main conclusions were:
The total amount of ballast water discharged into Dutch ports is estimated to be approximately 7.5 million tonnes per year (42% of all discharges in Europe) and the total amount of ballastwater loaded is estimated to be approximately 68 million tonnes (86% of ballast water loaded in Europe). With some assumptions it is estimated that approximately 70% of the ballast water discharged in Dutch ports was taken up in European ports.

Predictions about possible effects of introductions into The Netherlands were not possible because it is not known which and how many organisms are released in Dutch coastal and port waters. Even if we have knowledge about the species that are entering with ballast water (and sediments), and thus having the possibility of being discharged into Dutch waters, we should also have to know whether or not these organisms will survive in the receiving waters. Taking these conclusions into account, it was decided to start a pilot study with a sampling programme of ballast water in Dutch ports.

The objectives of this ballast water study were to answer the following questions: 1) which organisms are imported with ballast water in ships arriving in Dutch ports? and 2) do these organisms survive in Dutch surface and port waters?

2 Material and methods

2.1 Sample choice

In 1999 and 2000 it was planned to take ballast water (and if possible also sediment) samples on board 30 ships in the port areas of (mainly) Rotterdam and Amsterdam. These ships were chosen in a such a way as to reflect the types of ship and the possible origin of the ballast water as was reported in the desk study mentioned earlier (AquaSense, 1998), emphasizing container ships and multi-purpose ships with European ports as a possible origin of the ballast water.

2.2 Sampling

Samples were taken during the period November 1998 - May 2000. On every ship visited, 1-3 ballast tanks were sampled. The temperature of each ballast water sample was measured immediately. Salinity (psu) was always measured with a WTW conductivity meter in the laboratory after the sample had reached a temperature of 20 °C.

Sampling methods differed for each type of ship, depending on the accessibility of sampling points, which differ per ship type, on the willingness of the ships' officers and available time during cargo operations. On chemical tankers phytoplankton and zooplankton samples were taken with plankton nets through opened manholes. On multi-purpose ships sampling was done via a tap near one of the ballast water pumps or by hand pumping water through a sounding pipe (Figure 1); in one case, sampling was possible by pumping ballast water to the deck wash pump.

Figure 1.
Sampling of ballast water with a hand pump through a sounding pipe in the engine room.

Sampling on container ships was almost always done via a tap near one of the ballast water pumps or by removing the manometer from the ballast water pump; occasionally, sampling could be done through manholes in a heeling tank with a plankton net and in forepeaks with a bucket and on on occasion ballast water was sampled from a deck overflow by the crew. On bulk carriers without cargo, ballast water was sampled from deck overflows (Figure 2).

Figure 2.
Deck overflows on a bulk carrier.

Phytoplankton samples were taken through opened manholes by lowering a 20 µm plankton net once as deeply as possible. Water collected for the phytoplankton samples with the other methods mentioned, was passed through a 20 µm plankton filter. Together with each > 20 µm sample a 1 liter sample of the filtered water was taken for analysis of phytoplankton < 20 µm. For each phytoplankton sample > 20 µm, it was the ambition to filter at least 100 l. All phytoplankton samples were fixed with acid Lugol (Throndsen, 1978) to a final concentration of 0.4%.

Zooplankton samples obtained through manholes were taken by lowering a 55 µm plankton net one to three times as deeply as possible. It was the ambition to filter at least 300 l. Zooplankton samples were fixed with formalin to a final concentration of 4%. Only a few samples were preserved in this way. Later, if zooplankton was visually present in the > 20 µm phytoplankton samples, these samples were also used for zooplankton analyses after fixation with Lugol.

From each ballast tank sampled, a 1 litre live sample was taken, transported in grey polythene bottles, and used for the incubation experiments and the measurement of salinity.

If possible, sediment was scraped with a filling-knife from the bottom, walls and other places in empty and ventilated ballast tanks. Sediment samples were always stored in a refrigerator at 5 °C until use.

Starting with the November 1999 samples, the “receiving” port water beside the ship was also sampled. Sampling of surface water was done with a bucket. From this port water, a Lugol-fixed sample was taken for analysis of phytoplankton and the temperature was measured immediately.

A live sample of port water was taken for use in the incubation experiments (see later), to measure salinity (WTW conductivity meter) and to determine the nutrient concentrations (after filtration through Whatman GF/F filters).

From each ship sampled, general information on ship and ballast water data was gathered using forms in both Dutch and English (see Appendix 1 for the English version).

2.3 Incubation experiments

Incubation experiments were performed in 250 ml Erlenmeyer flasks. All the Erlenmeyer flasks used and also small petri dishes were thoroughly cleaned before use. Cleaning comprised rinsing with tap water, one night in detergent (Decon), rinsing with demineralized water, one night in 0.1% HCl and again rinsing with demineralized water. After cleaning, the Erlenmeyer flasks were closed with the small glass petri dishes or silicon caps and autoclaved in a high pressure cooker.

As a basis for the culture media water from the marine tidal basin Oosterschelde (salinity approximately 30 - 32 psu) was used. In this report the highest salinity used will be referred to as 30 psu. After pre-filtration through 20 µm the water was filtered through Whatmann GF/F filters. Dilution with demineralized water resulted in water with salinities of 15, 5, 1.3 and 0.3 psu, using 1 liter Duran borosilicate bottles with blue polypropylene screw-caps. All bottles containing 1 litre of the 0.1, 1.3, 5, 15 and 30 psu media then were autoclaved using a high pressure cooker. Dilution and subsequent autoclaving hardly influenced salinity and pH of the media. After cooling nutrients, trace metals and vitamins from sterile stock solutions were added to final solutions as described by Peperzak et al. (2000), supplemented with Si and Na-EDTA. This results in media with nutrient concentrations comparable with spring concentrations in Dutch coastal waters and sufficient trace metals and vitamins to sustain phytoplankton growth.

Also Whatman GF/F filtered port water was used as a culture medium. Nutrient concentrations in this filtered port water were also measured to get an insight into whether or not nutrients are limiting phytoplankton growth in the incubation experiments.

Incubation experiments with ballast water added to the 5, 15 and 30 psu media started with the November 1999 samples. Starting at August 2000, the 0.3 and 1.3 psu media were sometimes also used when fresh ballast water was sampled. As soon as possible 20 ml ballast water was inoculated with a sterile pipette into 200 ml of each of the media described. In addition, 220 ml unfiltered port water was also incubated. Incubations were done in two culture chambers (Sanyo) at 10 and 20 °C. In each culture chamber, two fluorescent lamps (Sanyo FL40SS.W/37) were used at a 16:8 hour light:dark cycle at a mean light intensity of approximately 115 $\mu E.m^{-2}s^{-1}$ (range 104-127, depending on the position in the culture chamber). If sufficient phytoplankton growth was visible (by eye), the Erlenmeyer content was fixed with acid Lugol (Throndsen, 1978) to a final concentration of 0.4%.

Incubation of ballast water in filtered port water samples started with the December 1999 samples. In a number of cases we also incubated port water and port water with ballast water to estimate the effect of the autochthonous plankton present on the ballast water plankton.

From the available sediment samples the 20-100 µm fraction was prepared using artificial seawater and plankton gauze. Small amounts of this fraction were incubated in glass petri dishes with the media described above (salinities 5, 15 and 30 psu), temperatures and light conditions. The petri dishes were inspected weekly with an inverted microscope to see if growth of organisms occurred.

2.4 Plankton analyses and frame of reference

All the Lugol and formalin samples obtained were stored at 12 °C in the dark until analysis. Phytoplankton was analysed using an Olympus inverted microscope and (larger) zooplankton by using a stereo-microscope.

Results from microscopic analyses had to be compared with knowledge about phytoplankton and zooplankton species already known to occur in Dutch fresh, brackish and marine waters.

As a frame of reference for freshwater phytoplankton species the species list, belonging to Anonymous (2000), was used; this list contains more than 1200 phytoplankton and epiphytic diatom species observed in fresh water from the provinces of Noord-Holland and Zuid-Holland. For brackish and marine phytoplankton an annotated species list (AquaSense, 2000a) was used, containing more than 400 phytoplankton species observed in Dutch brackish and marine waters within the phytoplankton monitoring program during the period 1990 – 1999. Added to this list were the (yet unpublished) observed species from the monitoring programme in 2000. These species lists also contain many freshwater phytoplankton species that were flushed into brackish and marine waters, including heterotrophic dinoflagellates. Also, other reports providing historical data were used: Kat (1977), reporting approximately 220 species (mainly diatoms and dinoflagellates) observed in the Dutch coastal area during 1973-1976; Leewis (1985) with approximately 350 species from the Dutch coastal zone during 1974-1975.

As a frame of reference for marine microzooplankton (20 – 200 µm) the list with approximately 100 observed species in Dutch marine waters from the microzooplankton monitoring program during the period 1994 – 1999 (AquaSense, 2000b) will be used. For freshwater microzooplankton, literature in which microzooplankton species observed in Dutch freshwater are mentioned, will be used.

Observed mesozooplankton (200 – 2000 µm) species will be compared with literature in which mesozooplankton species observed in Dutch fresh and marine waters are mentioned.

3 Results

3.1 Sampling

During the period November 1998 – November 2000, ballast water was sampled on board 30 ships in the port areas of Rotterdam (Rotterdam and Dordrecht, 20 ships), Amsterdam (Amsterdam and Ijmuiden, 6 ships) and Vlissingen (Vlissingen-Oost, 4 ships). An overview of the ports visited, the ships sampled and the types of ship (12 container ships, 9 multi-purpose ships, 5 chemical tankers and 4 bulk carriers) is given in Appendix 2.

In general, after explaining the objectives of this study, shipowners and crews co-operated constructively to get access to and to take ballast water samples on board their ships.

From each ship sampled, data on the year of delivery, tonnage as Gross Registered Tonnage (GRT) and Dead Weight Tonnage (DWT), number of ballast water tanks, ballast water capacity and the amount of ballast water on board at the time of sampling, are given in Appendix 3. Short sea container ships (2699 – 2906 GRT) were among the smallest of the ships sampled and transoceanic container ships (21586 – 91550 GRT) among the largest. From the ship types sampled, transoceanic container ships had the largest ballast water capacity (6511 – 35043 m^3) and also the largest amounts of ballast water (8172 – 11906 m^3) on board and, in many cases, a mix of ballast water from different origins. Also, the bulk carriers sampled had a large ballast water capacity (7857 – 13431 m^3). These bulk carriers arrived without cargo and thus carried a lot of ballast water (6532 – 11100 m^3). Almost all of the 30 ships reported to discharge some or all of their ballast water in port here.

37 ballast tanks were sampled on board the 30 ships. Table 1 lists the sampling methods used on board the different types of ship.

Table 1.
Sampling methods used.

Sampling method	Container ship	Multi-purpose	Chemical tanker	Bulk carrier	Total
Ballast water pump	11	7	-	1	**19**
Deck overflow	1	-	2	4	**7**
Manhole/plankton net	1	-	5	-	**6**
Manhole/bucket	2	1	-	-	**3**
Sounding pipe/hand pump	-	2	-	-	**2**
Total	**15**	**10**	**7**	**5**	**37**

On container ships and multi-purpose ships, most of the samples were taken at one of the ballast pumps, by removing the manometer or, if present, from a stopcock. The last possibility always resulted in a larger flow than was the case after removing the manometer. In only a few cases could samples be taken through an opened manhole on board container ships and multi-purpose ships, once with a plankton net in a side tank and twice in almost empty forepeak tanks with a bucket. Two samples on board multi-purpose ships were taken from double bottom tanks through one of the sounding pipes using a hand pump. In most cases it was possible to take a sample of 25 – 50 litres, but sometimes samples were smaller.

On board chemical tankers it was always possible to sample upper wing tanks on deck, either through opened manholes or by using a deck overflow and on bulk carriers samples were mostly taken from side tanks and aft peak tanks by using a deck overflow. On chemical tankers, as well as on bulk carriers, large samples of 100 litres or more could easily be obtained.

The filtered volumes of phytoplankton samples ranged from 1 to 172 litres (average 64 litres) and of zooplankton samples from 25 to 344 litres (average 112 litres). Figures 3 and 4 give more detailed information about the distribution of the filtered amounts of ballast water used for the phytoplankton and zooplankton samples.

Figure 3.
Number of samples versus filtered volume of phytoplankton samples (n = 37).

Figure 4.
Number of samples versus filtered volume of zooplankton samples (n = 17).

3.2 Origin of ballast water

The origin of the sampled ballast water is given in Appendix 4 and Figure 5. As explained in section 2.1, most of the sampled ballast water originated from European ports. 26 of the 37 samples were taken up at one discrete port or route, 9 samples were mixed samples with different origins of ballast water uptake and 2 samples had an unknown origin. Almost all mixed samples with different ballast water origins were taken on board container ships. The 26 samples from a discrete port or route originated from Europe (17), North-America (3), oceanic water (3), Asia (1), Australia (1) and New Zealand (1). The 9 mixed samples were combinations of water from European ports (4) and the other 5 samples were combinations of water from Europe, North-America and oceanic water.

Figure 5.
Origin of the sampled ballast water.

3.3 Temperature of ballast water and port water

Temperatures of ballast water and port water samples are presented in Figure 6. The measured ballast water temperatures upon arrival

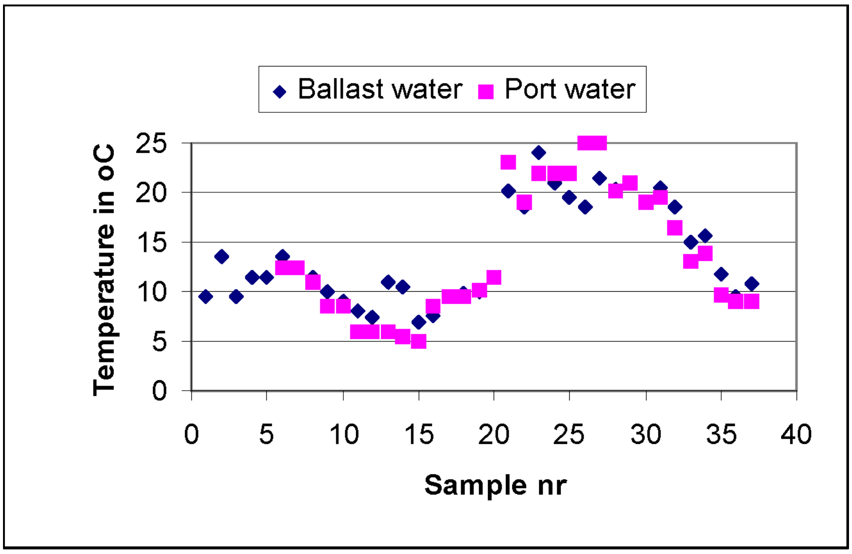


Figure 6.
Temperature of ballast water (n = 36) and port water samples (n = 26). Data from the port areas of Rotterdam, Amsterdam and Vlissingen.

ranged from 7.0 to 24.0 °C. Temperatures of port water, always taken after ballast water sampling, ranged from 5.0 to 25.0 °C. The difference between ballast water temperature and port water temperature varied between –6.5 and +5.0 °C (31 combinations). For 19 temperature combinations, the temperature of the ballast water was > the temperature of the port water, 8 combinations resulted in the same water temperature and in 4 combinations the temperature of the ballast water was < the temperature of the port water. However, within 24 of the 31 combinations the difference was within the range ±2 °C.

3.4 Salinity of ballast water and port water

The measured salinities of the ballast water samples are presented in Figure 7. A distinction was made between ballast water samples originating from one port (or route) (26) and samples with a mixed (9) and unknown (2) origin.

Figure 7.
Number of samples versus different salinity ranges of ballast water samples (n = 37) from one, mixed or unknown origin.

The measured salinities of the samples from one origin (or route) reflect the presence of fresh, brackish and seawater (including oceanic water) in the sampled ballast tanks. With respect to salinity the following classification (Kinne, 1971) will be followed: fresh (<0.5 psu), brackish (0.5 – 30 psu) and seawater (30 – 40 psu). Within all samples, the salinity ranged from 0.1 (Antwerp and Montreal, St. Lawrence River) to 37.2 (Piombino, Mediterranean) psu. 12% of the ballast water samples from one origin can be classified as fresh, 46% as brackish and 42% as seawater.

The measured salinities of the port water samples are given in Figure 8. A distinction was made between port water samples taken in the port areas of Rotterdam (18), Amsterdam (6) and Vlissingen (2).
Salinities in the port area of Rotterdam ranged from 0.3 to 28.6 psu, in the port area of Amsterdam from 3.5 to 21.0 psu and in the port area of Vlissingen from 26.3 to 26.8 psu. Salinity in the more than 70 km long port area of Rotterdam is influenced by river water on one side and seawater on the other. In the port area of Amsterdam, salinity in the ports of Amsterdam is much lower in Amsterdam itself than in IJmuiden, situated behind the sea-locks. The port area of Vlissingen is situated along the mouth of the Westerschelde and variation will not

be as large as in the port areas of Rotterdam and Amsterdam. 4% of the port water samples can be classified as fresh and 96% as brackish.

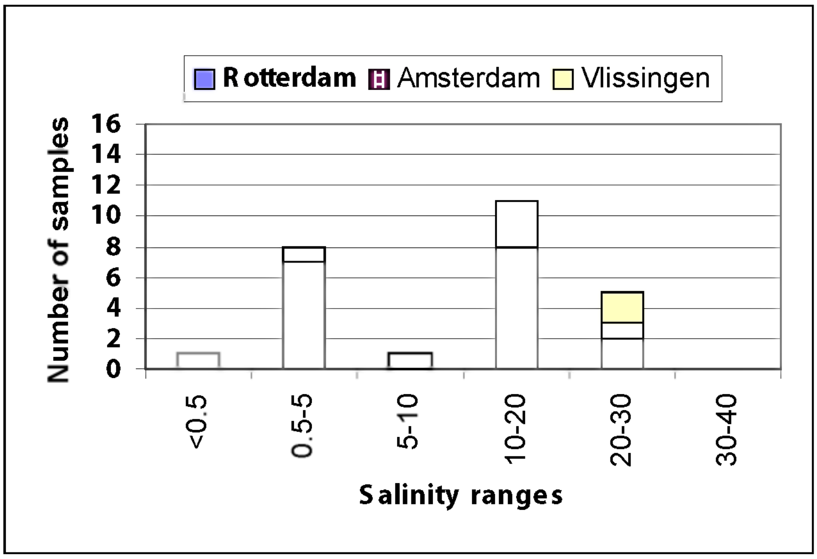


Figure 8.
Number of samples versus different salinity ranges of port water samples (n = 26) from the port areas of Rotterdam, Amsterdam and Vlissingen.

3.5 Plankton in ballast water

All observed plankton species in the ballast water samples analysed are listed in Appendix 5. In this species list, a distinction is made between phytoplankton, microzooplankton (20 - 200 µm) and mesozooplankton (200 – 2000 µm). In practice, some zooplankton groups like heterotrophic dinoflagellates, ciliates, tintinnids and ciliates are analysed together with the (autotrophic) phytoplankton, but these (and some other) groups were consequently categorized as microzooplankton. Determination to species level of plankton was not always possible in all the taxonomical groups distinguished. Very often, however, it was possible to determine a species to genus level. Phytoplankton and zooplankton that could not be categorized within the species and genus levels were classified as a group. Hence, a distinction was made between three different levels of determination: species level (for example *Ceratium furca*), genus level (for example *Gyrosigma* sp.) and group level (for example Cryptophyceae < 10 µm).

With respect to phytoplankton we found on average 28 (range 0 to 59) phytoplankton species, genera and groups per ballast water sample. Within the phytoplankton 122 species were determined to species level, 58 to genus level and 36 to group level (Table 2).

Table 2.
Number of observed phytoplankton species, genera and groups in ballast water samples (n = 37).

PHYTOPLANKTON	SPECIES	GENUS	GROUP
Diatoms	85	18	10
Dinoflagellates	28	6	11
Green algae	5	17	2
Blue green algae	1	7	2
Other groups	3	10	11
Total	**122**	**58**	**36**

Diatoms (Bacillariophyceae) and autotrophic dinoflagellates (Dinophyceae), almost all brackish and sea water species, were the most abundant classes with 85 and 28 species respectively determined to species level. Most of the green algae (Chlorophyceae), almost all fresh water species, could only be determined to genus level (17).

Sorting Appendix 5 in order of cell numbers, high values (criterion 10^5 or more cells/l) were found for the diatoms Centrales, diameter < 10 µm, *Skeletonema potamos*, *Skeletonema costatum*, *Paralia marina* and *Chaetoceros subtilis* and for the autotrophic dinoflagellates *Heterocapsa* sp., *Gymnodinium*, length 10-30 µm and *Prorocentrum minimum*. High cell numbers (criterion 10^5 or more cells/l) were also found for the green algae Chlorophyceae, *Monoraphidium* sp., *Scenedesmus* sp. and *Crucigenia* sp., the autotrophic micro-flagellates Chrysomonadales 2-10 and 0.2-2 µm, the chrysophycean *Pseudopedinella* sp., autotrophic cryptophyceans, the blue green algae Chroococcales, *Merismopedia* sp., *Planktothrix* sp. and *Microcystis* sp., prasinophyceans, the prymnesiophycean *Chrysochromulina* sp. and unidentifiable species. In most cases, phytoplankton concentrations were comparable to or lower than the concentrations known from Dutch coastal waters. However, when voyage time had been very long, concentrations were much lower. Some species occurred more frequently in the analysed ballast water samples. High frequencies of occurrence (criterion 50% or more, see also Appendix 1) were found for the diatoms Centrales, diameter < 10 and 10-30 µm, Pennales, width < 10 length < 50 µm and *Actinoptychus senarius*, cryptophyceans < 10 µm, and unidentifiable species with diameters < 3, 3-10 and < 10 µm. Comparison with the frame of reference (see section 2.4) showed that (almost) all observed species, genera and groups were already known from the Dutch phytoplankton monitoring programme. Almost all observed phytoplankton species are harmless. Only three thecate dinoflagellate species were not yet reported for Dutch waters and can be considered as non-native (see also section 4.2 and Appendix 8): *Corythodinium tesselatum* (9 cells/l), *Oxytoxum scolopax* (264 cells/l) and *Peridiniella catenata* (1170 cells/l), a chain forming dinoflagellate species (see Figure 9).

Figure 9.
The armoured dinoflagellate *Peridiniella catenata* found in a ballast water sample from Finland. Length of the upper specimen approximately 30 µm. Photo: Reinoud Koeman.

Also a rather large number of phytoplankton species with a recorded toxicity and groups that are known to contain toxic species or strains were found in the ballast water samples. The toxic and potentially toxic (see Appendix 8 for explanation) species found, belonged to the classes diatoms, bluegreens, dinoflagellates and prymnesians; they will be discussed in section 4.2 and Appendix 8. Their concentrations were comparable with or lower than concentrations known from Dutch coastal waters.

Within the microzooplankton 37 species were determined to species level, 22 to genus level and 30 to group level (Table 3). Heterotrophic dinoflagellates, almost all brackish and sea water species, and rotifers (Rotifera), almost all fresh water species, were the most abundant groups with 22, and 8 species respectively determined to species level.

Table 3.
Number of observed microzooplankton species, genera and groups in ballast water samples (n = 37).

MICROZOOPLANKTON	SPECIES	GENUS	GROUP
Dinoflagellates	22	2	6
Rotifers	8	12	1
Other groups	7	8	23
Total	**37**	**22**	**30**

With respect to cell numbers (see also Appendix 5), high cell values (criterion 10^4 or more cells/l) were found for the ciliates < 20 µm, choano-flagellates (Craspedomonadaceae), the heterotrophic cryptophyceans *Leucocryptos marina* and *Leucocryptos* sp., the heterotrophic dinoflagellate *Ebria tripartita*, heterotrophic micro-flagellates length < 10 and > 10 µm, the fresh water testacean *Paulinella* sp. and tintinnids with width < 20 µm. High frequencies of occurrence (criterion 25% or more, see also Appendix 5) were found for veliger larvae of bivalves, ciliates < 20 and 20-40 µm, choano-flagellates (Craspedomonadaceae), heterotrophic micro-flagellates (Protomonadales) length < 10 and > 10 µm, the rotifers *Keratella cochlearis* and *Keratella quadrata* (both fresh water species) and rotifers non det and the tintinnids with width < 20 and 20-40 µm and *Tintinnopsis lacustris*. Comparison with the frame of reference (see section 2.4) showed that all observed species, genera and groups were already known in Dutch waters.

Within the mesozooplankton 12 species were determined to species level, 7 to genus level and 11 to group level (Table 4). Cladocerans, calanoid copepods and cyclopoid copepods were the most important groups with 4, 4 and 4 species respectively determined to species level.

Table 4.
Number of observed mesozooplankton species, genera and groups in ballast water samples (n = 17).

MESOZOOPLANKTON	SPECIES	GENUS	GROUP
Cladocerans	4	2	3
Copepods	8	5	7
Other groups	0	0	1
Total	**12**	**7**	**11**

Concentrations of cladocerans and calanoid and cyclopoid copepods were always low. The highest observed densities were recorded for copepod nauplii. All the cladocerans were fresh water species, the calanoid copepods brackish and seawater species and the cyclopoid copepods fresh and seawater species. High frequencies of occurrence (criterion 25%, see also Appendix 5) were found for copepod nauplii, calanoid copepodites, the calanoid copepods *Acartia* sp. (Figure 10) and *Eurytemora affinis*, cyclopoid copepodites, the cyclopoid copepod *Oithona* sp. and harpacticoid copepods. Comparison with the frame of reference (see section 2.4) showed that all observed species, genera and groups were already known in Dutch waters.

Figure 10.
A calanoid copepod (*Acartia* sp.) from a ballast water sample.

From the ballast water data sheets (see Appendix 1) and information received from the ships' crews it was possible to make a good estimate of the age of the ballast water of the tanks sampled. Combined with the results of the phytoplankton analyses the survival of phytoplankton as a function of the age of the sampled ballast water was estimated. After excluding the samples with a mixed or unknown origin and samples with inaccurate information about age of the ballast water, 22 samples could be used for this purpose. By using these 22 samples, a significant (exponential) relation ($y = 32.197 * e^{-0.016x}$, $R^2 = 0.49$, $F = 18.994$, $p = 0.000$) between the number of surviving phytoplankton species (y) and the age of ballast water (x, in days), was found (Figure 11). Using only the 'winter' data (October - March, n = 11), no significant relationship was found between the number of surviving phytoplankton species and the age of ballast water ($R^2 = 0.02$, $F = 0.168$, $p = 0.691$). However, using the 'summer' data (April - September), a significant relationship was found again ($y = 38.034 * e^{-0.0189x}$, $R^2 = 0.68$, $F = 19.302$, $p = 0.002$).
The same regression model can be used for the number of surviving phytoplankton cells instead of species. Using all the 22 samples no significant (exponential) relationship was found for the number of surviving cells (y, in cells/l) and the age of ballast water (x, in days) ($y = 856177 * e^{-0.0216x}$, $R^2 = 0.08$, $F = 1.802$, $p = 0.195$). Using the 'winter' data (October - March) also resulted in an insignificant relationship. However, again using the 'summer' data (April - September), a significant relationship was again found ($y = 3 * 10^6 e^{-0.0392x}$, $R^2 = 0.69$, $F = 20.190$, $p = 0.002$) (Figure 12). According to the statistical program SYSTAT, these data contain 2 serious outliers. Removing these outliers resulted in a more significant relationship ($y = 4 * 10^6 e^{-0.0686x}$, $R^2 = 0.93$, $F = 95.635$, $p = 0.000$).

Figure 11.
Relationship between the number (y, cells/l) of phytoplankton species found and the age (x, in days) of the ballast water:
$y = 32.197*e^{-0.016x}$, $R^2 = 0.4871$, $F = 18.994$, $p = 0.000$, $n = 22$.

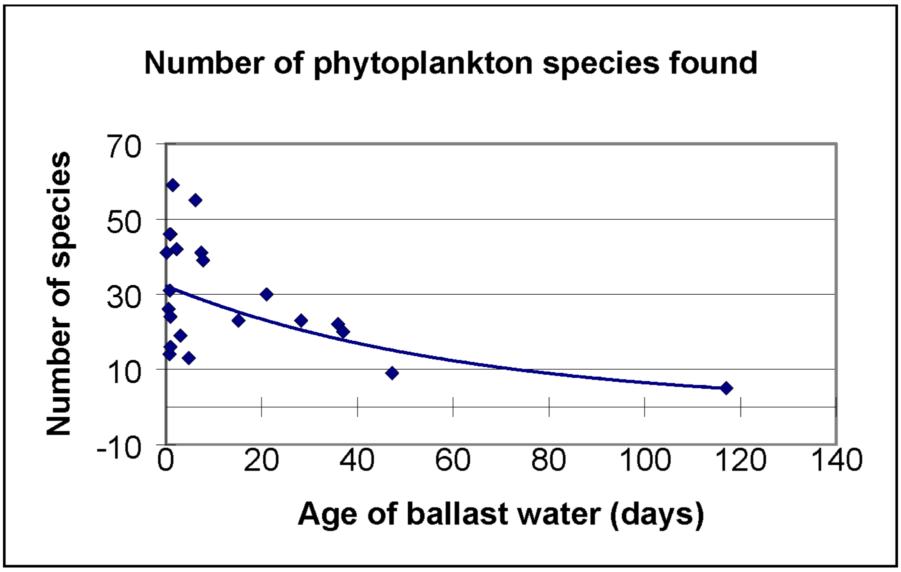
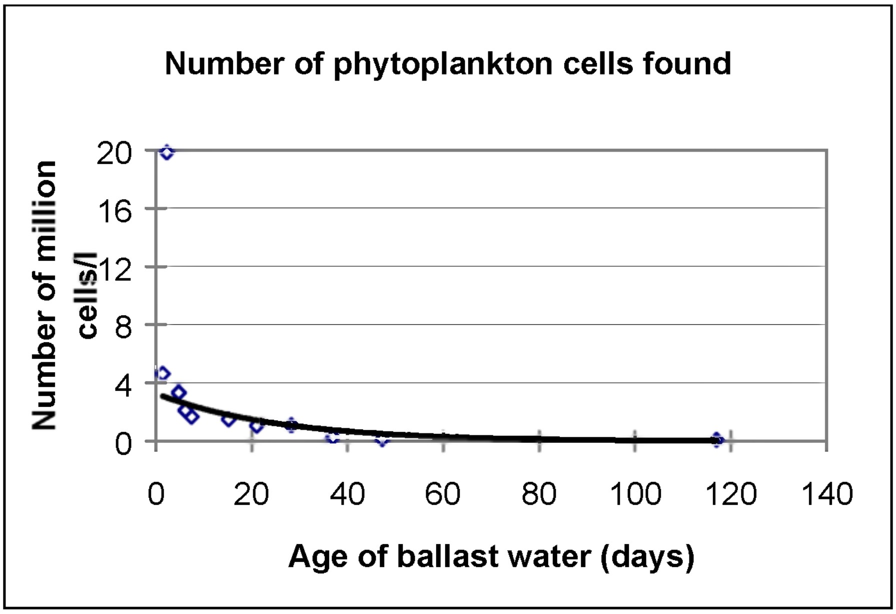


Figure 12.
Relationship between the number (y, million cells/l) of phytoplankton cells found and the age (x, in days) of the ballast water:
$y = 3.2649*e^{-0.0392x}$, $R^2 = 0.6917$, $F = 20.190$, $p = 0.002$, $n = 11$.



3.6 Plankton in port water

All the plankton species observed in port water samples are listed in Appendix 6. Also, in this species list a distinction is made between phytoplankton and microzooplankton. Mesozooplankton species were not found in the port water samples, very probably because of the small sampled volume (1 litre). As with the ballast water samples, a distinction was made in three different levels of determination: species level, genus level and group level.

With respect to phytoplankton, we found on average 30 (range 12 to 54) phytoplankton species, genera and groups per port water sample. From the phytoplankton in all port water samples, 72 species were determined to species level, 42 to genus level and 34 to group level (Table 5). Diatoms (Bacillariophyceae) and autotrophic dinoflagellates (Dinophyceae), almost all brackish and sea water species, were the most abundant classes with 56 and 13 species respectively determined to species level. Most of the green algae (Chlorophyceae), almost all fresh water species, could only be determined to genus level (14).

Table 5.
Number of observed phytoplankton species, genera and groups in port water samples (n = 23).

PHYTOPLANKTON	SPECIES	GENUS	GROUP
Diatoms	56	10	8
Dinoflagellates	13	2	11
Green algae	0	14	1
Blue green algae	1	9	3
Other groups	2	7	11
Total	**72**	**42**	**34**

High cell numbers (criterion 10^5 or more cells/l) were found for the diatoms Centrales, diameter < 10 µm, *Chaetoceros socialis*, *Leptocylindrus minimus*, Pennales, width <10 length < 50 µm, *Skeletonema potamos*, *Skeletonema costatum*, *Skeletonema subsalsum*, *Stephanodiscus hantzschii*, *Thalassiosira* sp. <30 µm and *Chaetoceros debilis* and the autotrophic dinoflagellates *Heterocapsa minima* cf, Peridiniaceae, diameter 10-30 µm, Dinophyceae and *Gymnodinium,* length 10-30 µm. In the remaining classes/groups (see Appendix 2), many species, genera and groups also occurred with cell densities > 10^5 cells/l and, in many cases, even much higher densities were recorded. High frequencies of occurrence (criterion 50% or more, see also Appendix 2) were found for the diatoms Centrales, diameter < 10 µm and Pennales, width < 10 length < 50 µm and the autotrophic dinoflagellate *Gymnodinium*, length 10-30 µm. Within the remaining classes/groups, high frequencies of occurrence (50% or more) were found for the green algae Chlorophyceae, *Monoraphidium* sp. and *Scenedesmus* sp., Chrysomonadales 2-10 µm, Cryptophyceae < 10 and > 10 µm, the blue green algae Chroococcales, the euglenophycean *Eutreptiella* sp., prasinophyceans and unidentifiable species < 3 and 3-10 µm. Also a few toxic and potentially toxic phytoplankton species were found in port water samples (see Appendix 8).

With respect to microzooplankton, 17 species were determined to species level, 3 to genus level and 12 to group level (Table 6).

Table 6.
Number of observed microzooplankton species, genera and groups in port water samples (n = 23).

MICROZOOPLANKTON	SPECIES	GENUS	GROUP
Dinoflagellates	15	1	1
Ciliates	1	0	8
Other groups	1	2	3
Total	**17**	**3**	**12**

Heterotrophic dinoflagellates, almost all brackish and seawater species, formed the most abundant group, with 15 species determined to species level. Ciliates (naked ciliates and tintinnids) were only determined to group level. High cell numbers (criterion 10^4 or more cells/l, see also Appendix 6) were found for the choano-flagellates (Craspedomonadaceae), the heterotrophic cryptophycean *Leucocryptos* sp., the heterotrophic dinoflagellates *Ebria tripartita* and *Protoperidinium* sp. 30-50 µm, the ciliate *Mesodinium rubrum*, heterotrophic micro-flagellates (Protomonadales) < 10 and > 10 µm, the testacean *Paulinella* sp. and tintinnids < 20 µm. High frequencies of occurrence (criterion 25% or more, see also Appendix 2) were found for ciliates < 20 and 20-40 µm, the heterotrophic dinoflagellate

Katodinium glaucum, heterotrophic micro-flagellates (Protomonadales) < 10 and > 10 µm and the tintinnids < 20 and 20-40 µm.

3.7 Incubation of ballast water

Almost all incubations of ballast water in the different media used, including filtered port water, resulted in moderate to good growth of phytoplankton. In only 3 culture flasks did no growth occur. In general, and for all salinities used, growth of phytoplankton was faster at 20 °C than at 10 °C. Cultures were always fixed at a moment that they still looked visually healthy. At 10 °C the cultures were fixed between 11 and 22 days and at 20 °C between 7 and 12 days. In general, all phytoplankton species that were cultured at 10 °C and a given salinity, also occurred in the culture flasks at 20 °C at the same salinity. This was the case for all salinities used. Differences between the salinities used were much more pronounced. Because of the very large similarity in temperature data with respect to the cultured species, a distinction will only be made between the different salinities used.

All plankton species that were cultured in all the media used, including filtered port water, are listed in Appendix 7. Also, in this species list a distinction was made between phytoplankton and microzooplankton. Mesozooplankton did not occur in the culture flasks. Again, three different levels of determination were used: species level, genus level and group level. All cultured species reached concentrations that never occur in field situations (note that the concentrations in Appendix 7 are given in cells/l or organisms/l.

An overview of cultured phytoplankton species is given in Table 7. Within the phytoplankton, 48 species, 34 genera and 31 groups were distinguished. Diatoms (Bacillariophyceae) were the most abundant group with at least 40 species. Also a number of dinoflagellate species and green algae genera was found in the culture flasks.

Table 7.
Number of cultured phytoplankton species, genera and groups in all media used, including port water (n = 220 culture flasks).

PHYTOPLANKTON	SPECIES	GENUS	GROUP
Diatoms	40	15	10
Dinoflagellates	6	1	5
Green algae	0	12	2
Blue green algae	0	1	3
Other groups	2	6	11
Total	**48**	**35**	**31**

Concentrations of more than one million cells/l were reached by the diatoms Centrales, diameter < 10 µm, *Skeletonema costatum* and *Skeletonema potamos* and unidentifiable species < 3 µm; concentrations between a half and one million cells/l were reached by the diatoms *Chaetoceros* sp., *Delphineus minutissima*, Pennales with width < 10 and length < 50 µm, *Skeletonema* sp., *Thalassiosira* sp. < 30 µm and the green algae *Monoraphidium* sp.

From the microzooplankton, mainly ciliates < 20 µm and heterotrophic flagellates were cultured (see Table 8).

Also a number of potentially toxic species were cultured from the ballastwater: the diatoms *Pseudo-nitzschia* sp., *Pseudo-nitzschia delicatissima* and *Pseudo-nitzschia delicatissima* cf, the blue green algae *Planktothrix* sp., the dinoflagellate *Prorocentrum minimum* and the prymnesian *Chrysochromulina* sp. In Appendix 8 more information on potentially toxic species is given.

Table 8.
Number of cultured microzooplankton species, genera and groups in all media used, including port water (n = 220 culture flasks).

MICROZOOPLANKTON	SPECIES	GENUS	GROUP
Ciliates	0	0	2
Dinoflagellates	1	0	0
Other groups	0	0	3
Total	**1**	**0**	**5**

Appendix 7 also contains information about the cultured phytoplankton species at different salinities. According to the frequency figures, a number of phytoplankton species (or better: groups) occur within the entire salinity range used. Examples are Centrales with diameter < 10 µm, *Chaetoceros* sp., *Cylindrotheca closterium*, Pennales with width < 10 µm, *Skeletonema costatum*, *Thalassiosira* sp. < 30 µm, Chrysomonadales 2-10 µm and Cryptophyceae. Most of the cultured microzooplankton that occurred within the entire salinity range was formed by heterotrophic flagellates. A number of cultured phytoplankton species had a preference for lower salinities and did not or almost not occur at the highest salinities: *Skeletonema potamos*, *Chlamydomonas* sp., *Kirchneriella* sp., *Monoraphidium* sp. and *Scenedesmus* sp. Not surprisingly, these are fresh and brackish water species. On the other hand, a number of cultured phytoplankton species were not or almost not observed at the lower salinities, but became more abundant at the higher salinities: *Asterionella glacialis*, *Dytilum brightwellii*, *Odontella aurita*, *Rhizosolenia* species and *Thalassionema nitzschioides*, all brackish and seawater species.

To test the influence of salinity on the growth of the ballast water species, the number of cultured phytoplankton species (+ genera + groups) was plotted against the salinity difference experienced in the different culture media. Salinity difference is defined as the salinity of the medium used minus the salinity of the incubated ballast water.

At any salinity difference a number of phytoplankton species was growing. Incubation always led to growth of approximately 5 – 15 phytoplankton species in each culture flask. On average we found 8.4 species per culture flask. Using all culture results from the media with salinities from 0.3 to 30 psu and the port water (salinity range 0.3 – 28.6 psu) incubation data results, a fit of the second order polynomal relation $y = -0.0059x^2 - 0.0464x + 10.074$, $R^2 = 0.1728$, n = 220 was found. In this relation, y is the number of cultured phytoplankton species and x is the salinity difference as defined above. The salinity media differ from the port water media in a way that within the salinity media the only different variable is salinity, which is not the case with the port water media. By not using the port water incubation data, a better fit (Figure 13) was found: $y = -0.0067x^2 - 0.0429x + 10.247$, $R^2 = 0.2191$, n = 192. Initially we also regressed the growing phytoplankton species against salinity difference as a percentage of the species originally present in the ballast tanks. On average 30% (range 0 to 90%) of these species were growing in the cultures. Also this fit was very poor ($y = -0.0185x^2 - 0.3146x + 34.301$, $R^2 = 0.1078$, n = 192). Because the statistical program Systat does not generate a probability for a polynomal relation, we have no information on probability. In the model used, salinity difference only explains approximately 20% of the variation found in the number of cultured phytoplankton species.

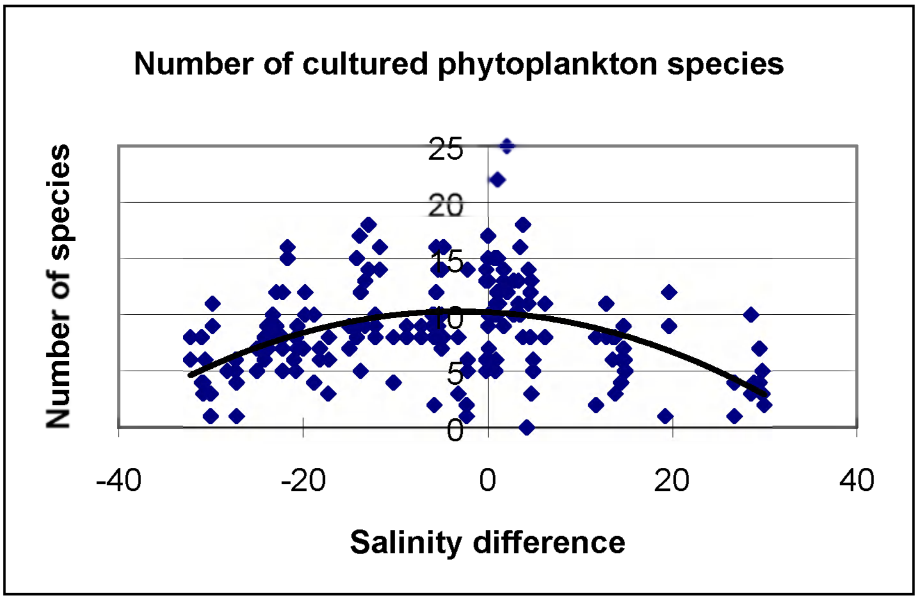


Figure 13.
Number of cultured phytoplankton species as a function of salinity difference (defined as the salinity of the medium used minus salinity of ballast water):
y = -0.0067x2 -0.0429x +
+ 10.247, R^2 = 0.2191, n = 192.

Growth was also always found in the cultured port water and port water incubated with ballast water. However, not all species found in the port water samples, also grew in the cultures. Most growth, in terms of species number and cell densities, occurred when salinities of the ballast water and the port water were comparable. Also, in these cases, a number of ballast water species that were not present in the port water sample, were growing between the port water species. When fresh ballast water (salinity 0.3) was added to port water with a much higher salinity (21.0), all fresh water species like *Monoraphidium* sp. and *Scenedesmus* sp. were not found to be present in the cultures. On the contrarary, when seawater was incubated in fresh port water, most of the seawater species did not grow.

Growth of phytoplankton even occurred after a less favourable prehistory for the phytoplankton. In one such case, a sample was taken in a heeling tank of a three months old container ship, still strongly smelling of its wall protective coating. Nevertheless, 7 living phytoplankton species, although with low concentrations, were found in and cultured from the ballast water of this tank. On another occasion, ballast water was sampled from the aftpeak of a bulk carrier. This water was taken up in the Mississippi and the age of the ballast water was approximately 30 days. It was used as cooling-water for the propeller shaft. Nevertheless, the ballast water contained 30 phytoplankton species and also ciliates and many of the phytoplankton species also occurred in the cultures, especially fresh water species, for example green algae like *Scenedesmus* species (Figure 14).

3.8 Incubation of sediments

Most growth of the two incubated sediments occurred at a salinity level of 15 psu. At all salinities, especially small solitary diatoms (probably *Thalassiosira* sp., see Figure 15) were found. At a salinity level of 15 psu, many black thread-like structures that looked like fungi

Figure 14.
Green algae (*Scenedesmus* species), cultured from ballast water that was taken up in the Mississippi river and used as cooling-water for the propeller shaft.

also grew. At a salinity level of 5 psu, the cryptophycean *Cryptomonas* sp., the green algae *Scenedesmus* sp. and the diatom *Navicula* sp. were also observed. At least one nematode and a number of ciliates were found after incubation of the sediments. The small solitary diatoms and the ciliates were not seen in the sediments before incubation.

Figure 15.
Small solitary diatoms (probably *Thalassiosira* sp.), cultured from a sediment sample.

4 Discussion

4.1 Sampling, representativeness of ballast water samples

In general, sampling on board ships is not as easy as it is for example on the open sea. The main reason for this is that the ballast water in the tanks is not directly within reach and one has to sample from the ballast pump, through narrow sounding pipes or through manholes, after a member of the ships' crew has removed 20 – 30 bolts securing a manhole cover.

Currently there is no standard method for sampling ballast tanks using plankton nets, pumps or other devices (Hay et al., 1997). Furthermore, a weak point of all sampling methods on board ships is that there is no method that will sample each plankton taxon in a representative way, in terms of sample size, species composition and numbers of organisms. Besides that, it is not always possible to use each method on each type of ship.

The best results are obtained when sampling with a plankton net through opened manholes is possible. The messy task of removing the cover of a manhole is not always possible because of overlying cargo, strict interpretation of safety regulations or lack of personnel during busy port schedules (Gollasch, 1996). Depending on the type of ballast tank, consolidation structures between the walls of the tank and the amount of ballast water in the tank, it is usually possible to sample part of the water column. In this way a large volume of water will be filtered. However, in most cases it is only possible to sample the upper part of the water column and part of the larger phytoplankton might have been precipitated. Mesozooplankton (cladocerans and copepods), on the other hand, tend to concentrate in this upper layer of the ballast tank and are also attracted by light when a manhole cover is removed. Sampling via deck overflows has the same advantage that large amounts of water can be filtered. This water originates from the upper part of the ballast tank from which part of the phytoplankton might have been precipitated, while the mesozooplankton will be more concentrated. Both methods have the advantage that the fast swimming mesozooplankton will not escape when the plankton net is moved fast enough nor from the very fast water flow through a deck overflow. Thus, sampling with a plankton net through a manhole and sampling via deck overflows will result in a large sample volume, an underestimation of larger phytoplankton and an overestimation of the mesozooplankton abundance.

Sampling at one of the ballast pumps resulted in half of the samples (Table 1). In many cases, it was possible to collect water from a tap in line with the ballast pump, which resulted in a flow that was large enough to fill two or more 25 litre drums. On the other hand, when sampling was done after removing the manometer it was much more time consuming to fill the drums. In general, the water flow is too small to sample the fast swimming mesozooplankton in a quantitative way. Because in both cases the sampled water originates from the lower part of the ballast tank, there is a real possibility that larger phytoplankton cells will be overestimated and that mesozooplankton will be underestimated for the same reasons as mentioned above. However, in all ballast pump samples small phytoplankton species were also found.

Sampling through sounding pipes also will result in an overestimation of the larger precipitated phytoplankton and an underestimation of mesozooplankton. Moreover, the small water flow when sampling through sounding pipes will result in a relative small amount of water.

Half of our samples were ballast pump samples, in which the larger phytoplankton might be overestimated and mesozooplankton might be underestimated. One third of our samples came from opened manholes and deck overflows with possible opposite effects. We did not use more than one method in a ballast tank, so it is speculative to try to quantify the representativeness of our samples. Given the large number of observed phytoplankton species (see section 4.2) and the rather high concentrations, collected with all methods, it seems likely that the sampled phytoplankton reasonably reflect the composition and concentrations in the sampled ballast tanks. With respect to the larger zooplankton, the number of species in the sampled tank will also be sampled but the number of organisms/l will probably be overestimated.

4.2 Plankton organisms in ballast water and port water

Before looking at the observed plankton species in the sampled ballast water, it is good to realize that for many species it is uncertain whether or not they are native, because of a lack of historical documentation. These are the so-called cryptogenic species. Carlton (1996b) defined a cryptogenic species as “a species that is not demonstrably native or introduced”. An introduced species then is defined as "any species intentionally or accidentally transported and released by humans into an environment outside its present range (From: Code of Practice of the International Council for the Exploration of the Sea (ICES), Working Group on Introductions and Transfers of Marine Organisms WGITMO). In this report, species for which historical evidence exists from the references mentioned in section 2.4, will be considered as native. Species not recorded in these references very probably are introduced or overlooked species.

A large number of plankton species was found in the analysed ballast water samples. Confining ourselves to the species that could be determined to species level, we found 122 phytoplankton species (a.o. 85 diatom and 28 autotrophic dinoflagellate species), 37 microzooplankton species (mainly heterotrophic dinoflagellates and rotifers) and 12 mesozooplankton species (mainly cladocerans and copepods), see Tables 2, 3 and 4 and Appendix 5. Our figures are conservative estimates, because only the real species were counted. Should we also count the species that were determined to genus level and of which genus no other species are determined to species level than the number of phytoplankton, microzooplankton and mesozooplankton species would end up being 162, 53 and 16 species respectively. Ultimately, the number would be a little higher if we were also to take into account the information of the recognized phytoplankton and zooplankton groups. All observed species (with the exception of three marine dinoflagellate species, see later) are known to occur in Dutch fresh, brackish or marine waters. Also in other shipping studies, for example Carlton & Geller (1993) and Gollasch et al. (2001) a predominance of diatoms and dinoflagellates within the phytoplankton was found. Cohen (1998, Table 5) summarized the literature regarding the number of organisms (also conservatively counted) collected in ballast tanks and found a range of 18 to 136 phytoplankton species (data from 5 to 159 sampled ships), probably also including heterotrophic dinoflagellates, which we have placed in the category microzooplankton. On average, we found 28 phytoplankton species, genera and groups per tank with a range from 0 to 59 (note that we excluded the species rich group of heterotrophic dinoflagellates from these counts). In a recent study even 145 different phytoplankton morphospecies, 132 of which were diatom species, were found in one ship (McCarthy & Crowder, 2000). In the above mentioned overview, the number of microzooplankton species ranges from 3 to 55 species, but the number of mesozooplankton species is larger (because of summarized data, a precise range estimate cannot be given). The reason for the large number of phytoplankton and microzooplankton species we found is that most of our sampled ballast water originated from nearby locations around the North Sea, leading to a short residence time in the ballast tanks and thus contributing to a high rate of survival. This is clearly shown in Figure 11, illustrating that the number of organisms found in ballast tanks is decreases with increasing age of the ballast water.

The phytoplankton and microzooplankton concentrations we found in the ballast water samples are comparable with concentrations known from the Dutch plankton monitoring programme. Moreover, with respect to diatoms and dinoflagellates we found higher concentrations than in the summarized data reported by Cohen (1998, Table 6). Again, the rather short age of the sampled ballast water, resulting in a high survival rate, must have contributed to high concentrations of surviving phytoplankton and microzooplankton. This is illustrated in Figure 12.

The potential of ballast water to introduce phytoplankton species outside their native range was firstly suggested by Ostenfeld (1908) after a phytoplankton bloom of *Odontella sinensis* was found in the Danish part of the North Sea in 1903. In 1905 *Odontella sinensis* also occurred in samples from Dutch marine waters (Ostenfeld, 1908). Nowadays, *Odontella sinensis* is a common diatom species in the North Sea and also in Dutch coastal waters. We found only three non-native (armoured) dinoflagellate species in the ballast water samples we analysed:

Corythodinium tesselatum

This dinoflagellate arrived in ballast water (9 cells/l) taken on in Piombino, Italy. *Corythodinium tesselatum* is a species from warm temperate to tropical waters and most records are from the Atlantic Ocean (Steidinger & Tangen, 1997).

Oxytoxum scolopax

This dinoflagellate was found in a tank (264 cells/l), taken on between the Azores and the Channel. *Oxytoxum scolopax* is a dinoflagellate from warm temperate to tropical waters, but more typical of the Atlantic Ocean (Steidinger & Tangen, 1997).

Peridiniella catenata

This chain forming dinoflagellate was found in ballast water (1170 cells/l) taken on in Vaasa, Finland. *Peridiniella catenata* is a brackish cold water species that can form blooms (Steidinger & Tangen, 1997).

Toxic phytoplankton pose a serious threat to human health and natural and cultivated shellfish and fish resources. For that reason there is a lot of concern about the possibility of the world-wide transport of toxic phytoplankton species in ballast water (and sediments) from one place to another. Indeed, the transport of toxic dinoflagellates from Asia to Australia has been reported by Hallegraeff & Bolch (1991). In this shipping study a number of toxic and potentially toxic phytoplankton species were found in the ballast water samples. An overview of and information on these species is given in Appendix 8. All the observed toxic and potentially toxic species are known to occur in Dutch fresh, brackish and marine waters. Depending on the species, the frequency of occurrence varied between 3 and 19% of the analysed ballast water samples (Appendix 8). Toxic and potentially toxic species were found in all types of ship and in ballast water from all continents. Of these species, the marine dinoflagellate *Dinophysis acuminata* causes Diarrheic Shellfish Poisoning in humans and resulted in the closure of the shellfish fisheries in the Wadden Sea and Oosterschelde (Kat, 1983). A number of observed potentially toxic blue greens (*Aphanizomenon* sp. and *Microcystis* sp.) are responsible almost annually for closure of small fresh water bodies to recreation.

In the port water samples analysed, we found 72 phytoplankton species (of which 56 were diatom and 13 were autotrophic dinoflagellate species) and 17 microzooplankton species (of which 15 were heterotrophic dinoflagellate species) (see Tables 5 and 6 and Appendix 6). As with the ballast water samples, the species were counted in a conservative way. On average we found 30 phytoplankton species, genera and groups per port sample with a range from 12 to 54 (note that we excluded the species rich group of heterotrophic dinoflagellates from these counts), very often with high densities. This high species richness and the high cell densities were not expected and were contrary to what is mostly believed to be the case for Dutch port water. Also very striking is the great similarity in ballast water and port water species with a frequency of occurrence > 40%. Also a number of toxic and potentially toxic phytoplankton species were found in the port water samples; these are described in Appendix 8.

4.3 Survival of plankton organisms

Several studies have reported dramatic declines in the diversity and number of plankton organisms at increasing duration of the voyage, i.e. with the age of ballast water. The results have been summarized by Cohen (1998, Table 7), leading to the conclusion that, even with large declines, considerable diversity and substantial numbers of living organisms may remain in ballast tanks after voyages of 10-20 days. Similar results were also reported by Gollasch et al. (2000). Our results, shown in Figures 11 and 12, are in line with these conclusions. Because most of our ballast water samples originated from European ports, leading to short voyage times, this also explains the large number of plankton species and cell numbers found in these samples.

A limited number of studies on the survival of organisms after transport in ballast tanks has been carried out. The highest survival probability is expected to occur after the transport of organisms if there are comparable circumstances with respect to the origin and discharge areas. Important factors that determine the chance of survival are climate (Gollasch, 1996) and salinity (Carlton, 1985). Because the majority of our samples originated from European ports, the climate factor will not be present in our results to any great extent. Salinity, therefore, is then of more importance.

The survival probability of organisms with respect to the salinities of the uptake and discharge areas is depicted in Table 9. Because most of our port water is brackish (see Figure 8), there is medium risk with respect to organisms from discharged fresh water and high risks for discharged brackish and seawater.

Table 9.
Survival probability of imported
species by comparison of the salinities of the uptake region and the receiving region (after Gollasch, 1996).

Discharge region	Uptake region Fresh water	Uptake region Brackish water	Uptake region Seawater
Fresh water	high	medium	low
Brackish water	medium	high	high
Seawater	low	high	high

Remarkably little research has been done to test the survival of ballast water organisms in receiving waters of other salinities. In most cases, research was done to the survival of discharged zooplankton and zoobenthos, for example settling of bivalve, crustacean, polychaete and ascidian larvae by Chu et al. (1997) and survival in port sediments of bivalves, crustaceans and polychaetes by Smith et al. (1999).

We tested the survival of plankton by culturing ballast water in media with different temperatures (10 and 20 °C) and salinities (range 0.3 to 30 psu, including filtered port water). In all culture flasks, there was a good phytoplankton growth, also in the filtered port water, at least indicating that the port water samples contained enough nutrients to support phytoplankton growth. This is confirmed by the high nutrient concentrations found in the port water samples (Appendix 9). All these concentrations do not limit phytoplankton growth when compared with the half-saturation constants for nutrient uptake by natural phytoplankton populations as cited from literature by Fisher et al. (1988).

Temperature only led to a difference in growth rate of the phytoplankton, not in species diversity. After all, this is not all to surprising because many phytoplankton have a large temperature tolerance range, especially diatoms (Baars, 1979) and the majority of the ballast water (Table 2) and cultured (Table 7) species were diatoms.

The culturing of ballastwater showed that many phytoplankton species also have a large salinity tolerance range, growing at salinities between 5 and 30 psu. Besides that, there were also species found that restrict their salinity tolerance range to fresh and brackish water values or to brackish and seawater values. In any case, a large number of species and cells survived in each culture flask. In only a few cases were potentially toxic phytoplankton species cultured that were also present in the ballast water samples. We did not manage to culture the toxic dinoflagellates that were found in the ballast water samples, but it is known that culturing of dinoflagellates is difficult. The majority of cultured species were harmless species already present in the ballast water. The difference in salinity between ballast water and culture medium could only explain 20% of the variation found in the number of cultured phytoplankton species.

From each ballast tank investigated, we analysed only a small fraction of the water in that tank. Each ship has approximately 10 to 40 ballast tanks (Appendix 3), which are partly or completely filled. Because we sampled 1 to 3 tanks per ship, we sampled only a small fraction of the ballast water on board that ship. The number of ships boarded was 30. The total number of ships entering the ports of Rotterdam and Amsterdam each year is approximately 27000, and 9000 respectively (AquaSense, 1998). The conclusion can only be that enormous amounts of plankton are continuously being introduced when ballast water is discharged into Dutch surface and port waters. Probably this is also the reason that we found a great similarity in phytoplankton species in ballast water and port water samples. In any case, the number of phytoplankton species found in Dutch ports was unexpectedly high.

Non-native, toxic and potentially toxic species are a potential threat to existing ecosystems. For the Dutch situation, the threat seems the greatest when non-native or toxic species are released in, for example, the port of Rotterdam and are then transported by the river flow, residual currents or secondary uptake, to areas of shellfish culture. The chance that non-native species will survive seems smaller than the chance of survival of toxic species. All toxic species that we found in the ballast water samples, are known from Dutch waters. Because the amount of water analysed was small, we might have missed non-native, toxic or potentially toxic species.

The risks of these introduced non-native, toxic and potentially toxic phytoplankton species arise especially from the regularity and large amounts with which ballast water is discharged. Unwanted phytoplankton species thus discharged, then have a great chance of interfacing with specific abiotic conditions (Carlton, 1996c) such as, for example, a great river run-off with large amounts of nutrients, that may favour these unwanted species. Thus, ballast water discharged in Dutch ports, is certainly not free from risks, such as, for example, the growth of non-native, toxic or potentially toxic phytoplankton species.

5 Conclusions

Most of the ballast water in ships arriving in Dutch ports was taken up in ports around the North Sea. As a consequence of the short voyage time of these ships, a large number of plankton species and cells survived in the ballast tanks, and were found in the ballast water samples analysed. Only three non-native phytoplankton species, armoured dinoflagellates, were found in the ballast water samples analysed. All other observed plankton species are known to occur in Dutch fresh, brackish and seawater. Also, a number of toxic and potentially toxic phytoplankton species were found in all ship types, not only in ballast water taken up in North Sea ports and estuaries, but also in ballast water from other continents, in fresh as well as in brackish and seawater.

The survival of ballast water species after discharge was tested at two temperatures in media with different salinities and in port water. At both temperatures used, the results were very similar. A significant relationship between the number of species growing and the difference in salinity (the salinity of the medium used minus the salinity of the ballast water) was not found, very probably because of the large salinity tolerance range of phytoplankton. Nevertheless, 5 to 20 species, including a few toxic and potentially toxic species, were always growing in the culture media and the port water media used. This means that at least a part of the plankton will grow further in surface and port waters after being discharged. This might also be the explanation for the large similarity between ballast water species and port water species.

In the relatively small amount of ballast water investigated, we found a large number of living plankton species, in terms of species diversity and cell numbers, including a few non-native and a number of toxic and potentially toxic species. We sampled only a very small fraction of ballast water on board each ship. Extrapolating our results to the scale with which ballast water is discharged in Dutch surface and port waters, we may assume that unwanted non-native, toxic and potentially toxic species are released in large numbers into these waters. The risks associated with the introduction of non-native, toxic and potentially toxic phytoplankton species arise especially from the regularity and large amounts with which ballast water is discharged. Unwanted phytoplankton species thus discharged, then have a great chance of interfacing with specific abiotic conditions such as, for example, a great river run-off with high levels of nutrients, that may favour these unwanted species. Thus, ballast water discharged in Dutch ports, is certainly not free from risks, such as, for example, the growth of non-native, toxic or potentially toxic phytoplankton species.

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Appendix 1.
Sample data sheet.

BALLAST WATER DATA SHEET

Date	....../....../20...

Vessel information

Vessel name	
Vesseltype	
Tonnage	BRT / Dead Weight/...
Year of delivery	
Arrival port	Amsterdam/Rotterdam/..........
Arrival date/time	
Departure date/time	
Last visited port	Departure date/time:
Last port but one	Departure date/time:
Next port:	

NOTES:

Ballast water information

Number of BW tanks		
Total BW on board		m3 / ton
Total BW capacity		m3 / ton
Last port BW uptake		
Last port but one BW uptake		
Deballasting here:	Y / N	
earlier:	Y / N	where:
later:	Y / N	where:

NOTES:

Appendix 2.
Overview of ports visited and ships sampled.

SHIP NR	DATE	PORT	SHIP TYPE
1	03-11-98	Rotterdam, YVC-werf/Bolnes	Chemical tanker
2	17-03-99	Vlissingen-Oost, Bijleveldhaven	Multi-purpose
3	29-03-99	Vlissingen-Oost, Bijleveldhaven	Multi-purpose
4	28-04-99	Rotterdam, Botlekhaven	Chemical tanker
5	01-11-99	Rotterdam, Beatrixhaven	Container ship(1)
6	01-11-99	Rotterdam, Beatrixhaven	Container ship(1)
7	12-11-99	Rotterdam, Beatrixhaven	Container ship(1)
8	09-12-99	Rotterdam, Europahaven	Container ship(2)
9	23-12-99	Rotterdam, Europahaven	Container ship(2)
10	07-01-00	Rotterdam, Europahaven	Container ship(2)
11	19-01-00	Rotterdam, Europahaven	Container ship(2)
12	28-01-00	Rotterdam, Europahaven	Container ship(2)
13	13-04-00	Rotterdam, Europahaven	Container ship(2)
14	14-04-00	Rotterdam, Europahaven	Container ship(2)
15	19-04-00	Rotterdam, Europahaven	Container ship(2)
16	19-04-00	Rotterdam, Europahaven	Container ship(2)
17	16-05-00	Amsterdam, Suezhaven	Multi-purpose
18	31-07-00	Vlissingen-Oost, Handelskade	Multi-purpose
19	02-08-00	IJmuiden, Buitenkade3	Bulk carrier
20	07-08-00	IJmuiden, Buitenkade3	Bulk carrier
21	29-08-00	Rotterdam, Eerste Petroleumhaven	Chemical tanker
22	30-08-00	IJmuiden, Buitenkade3	Bulk carrier
23	11-09-00	IJmuiden, Buitenkade3	Bulk carrier
24	13-09-00	Rotterdam, Waalhaven	Multi-purpose
25	21-09-00	Rotterdam, Brittanniëhaven	Multi-purpose
26	06-10-00	Rotterdam, Waalhaven/Werf v/d Brink	Chemical tanker
27	17-10-00	Vlissingen-Oost, Handelskade	Multi-purpose
28	24-10-00	Rotterdam, Waalhaven	Multi-purpose
29	11-11-00	Dordrecht, Julianahaven	Chemical tanker
30	16-11-00	Amsterdam, Shipdock	Multi-purpose

(1) Short sea.
(2) Transoceanic.

Appendix 3.
Overview of ship and ballast water (BW) data. Ship types arranged according to increasing Gross Registered Tonnage. YEAR = year of delivery.

SHIP NR	SHIP TYPE	YEAR	TONNAGE (BRT)(3)	TONNAGE (DWT)(4)	NUMBER OF BW TANKS	BW CAPACITY (m3)(5)	BW ON BOARD (m3)(5)
6	Container ship(1)	1995	2699	3950	10	*2121*	*647*
7	Container ship(1)	1995	2699	3950	10	*2121*	Unknown
5	Container ship(1)	1996	2906	3500	10	*2144*	*1292*
15	Container ship(2)	1982	21586	21370	21	*6511*	Unknown
13	Container ship(2)	1995	45648	47171	19	18695	8172
16	Container ship(2)	1984	45648	58869	41	*19150*	*11906*
8	Container ship(2)	1990	52181	60350	29	22125	8313
9	Container ship(2)	1991	52181	60350	28	21586	*10000*
12	Container ship(2)	1989	52191	60639	43	*22178*	10052
14	Container ship(2)	1989	52191	60639	43	*22178*	Unknown
10	Container ship(2)	1997	81488	90456	20	29738	*10180*
11	Container ship(2)	1999	91550	104750	24	35043	*8354*
17	Multi-purpose	1995	2561	3326	18	1489	871
28	Multi-purpose	1981	4281	7436	12	1530	260
18	Multi-purpose	1984	4983	8038	14	1897	*650*
3	Multi-purpose	1990	7949	12239	16	*3918*	*1500*
30	Multi-purpose	1989	7949	12239	16	3918	*920*
2	Multi-purpose	1994	8448	12730	16	*4141*	*3110*
27	Multi-purpose	1994	8448	12730	16	4141	1600
24	Multi-purpose	1997	10990	15634	23	6045	3250
25	Multi-purpose	1997	10990	15634	23	6700	200
1	Chemical tanker	1996	3419	4442	8	2443	538
4	Chemical tanker	1989	3693	5098	10	2450	400
26	Chemical tanker	1989	4297	6259	13	2999	*2999*
29	Chemical tanker	1991	4297	6258	13	2999	1481
21	Chemical tanker	2000	4670	6414	11	3242	*500*
22	Bulk carrier	1983	16694	26605	16	7857	*6532*
20	Bulk carrier	1985	16697	26696	17	13431	*7800*
19	Bulk carrier	1985	16705	26678	24	13426	*7670*
23	Bulk carrier	2000	21387	34947	14	11087	*11100*

(1) Short sea.
(2) Transoceanic.
(3) GRT (Gross Registered Tonnage) is the estimated maximum ship's carrying capacity, as it is derived from the total volume of enclosed spaces which are available for cargo, stores, crew, passengers etc. within the hull and superstructure.
(4) DWT (Dead Weight Tonnage, at summer draught) is the weight in metric tonnes (1000 kg) of cargo, stores, fuel, crew and passengers carried by a ship when loaded to the maximum level.
(5) Figures in Italics: values originally given as tonnes and transformed to m^3 by assuming a density of 1.025.

Appendix 4.
Sampled ballast tanks and origin of the sampled ballast water.

SAMPLE NR	VESSEL NR	TANK NR	ORIGIN1	ORIGIN2	ORIGIN3	ORIGIN4
1	1	WB-1C	Schelde(Doel)			
2	2	DB6SB	Tauranga			
3	3	DB5SB	Melbourne			
4	4	WBSB2A	Antwerpen(80%)	Tees(20%)		
5	4	PS2A	Antwerpen(80%)	Tees(20%)		
6	5	Tank20+21	Humber(Hull)			
7	6	Tank10+11	Theems(Tilbury)			
8	7	Unknown	Theems(Tilbury)			
9	8	Unknown1	Charleston(300t)	Le Havre(200t)		
10	8	Unknown2	Charleston(300t)	New York(200t)		
11	9	WB3-4P	Bremerhaven			
12	10	WBTK2-3P	Felixstowe			
13	10	WBTU12-13P	Algeciras(700t)	Felixstowe(50t)		
14	11	WT14-15P	Unknown			
15	12	DBTK5-6P	N. Pacific(200m3)	New York(20m3)	Felixstowe(147m3)	
16	13	Forepeak	Jacksonville			
17	14	DBTK1-2	Oceanic1(312m3)	Oceanic2(187m3)	Le Havre(500m3)	Bremerhaven(140m3)
18	14	DBTK5-6	New York(200m3)	Le Havre(167m3)		
19	15	Forepeak	Unknown			
20	16	WT7PS	Le Havre			
21	17	DB	Vaasa			
22	18	DB2B	Kaap Verdische Eilanden			
23	19	Tank4	Esbjerg			
24	20	Tank4+5	Liverpool(?m3)	Kanaal(?m3)		
25	20	Aftpeak	Mississippi(Baton Rouge)			
26	21	WB2S	Huelva			
27	21	WB1	Kanaal			
28	22	Tank3PS	Bremen			
29	23	Aftpeak	St. Lawrence(Montreal)			
30	24	DB3S	Oceaan(Brazilie)			
31	25	WTTS2SB	Oceaan(Azoren-Kanaal)			
32	26	Tank9C	Antwerpen			
33	27	WBST2BB	Baai van Tokyo(Funabashi)			
34	28	Tank4	Kanaal, western approach			
35	29	Tank6	Botlek(Rotterdam)			
36	30	Forepeak	Gent			
37	30	WBT5	Piombino			

Appendix 5.
Phytoplankton and zooplankton species observed in ballast water samples, arranged alphabetically; phytoplankton samples: n = 37, zooplankton samples: n = 19. MIN, MAX = minimum, maximum observed cells/l or organisms/l; FREQUENCY = frequency of occurrence. Non-native species are marked yellow; toxic and potentially toxic species are marked grey.

PHYTOPLANKTON IN BALLAST WATER SAMPLES		MIN	MAX	FREQUENCY
		cells/l	cells/l	%
Bacillariophyceae	Actinocyclus normanii	6	1073	22
Bacillariophyceae	Actinocyclus octonarius	10	1124	8
Bacillariophyceae	Actinocyclus sp	9	9	3
Bacillariophyceae	Actinoptychus senarius	1	3754	53
Bacillariophyceae	Actinoptychus splendens	9	9	3
Bacillariophyceae	Amphora sp	6	9	6
Bacillariophyceae	Asterionella formosa	3	3131	19
Bacillariophyceae	Asterionella glacialis	1687	1687	3
Bacillariophyceae	Asterionella kariana	42	63	6
Bacillariophyceae	Aulacoseira granulata	29	60	11
Bacillariophyceae	Aulacoseira muzzanensis	16310	16310	3
Bacillariophyceae	Aulacoseira sp	31	32620	19
Bacillariophyceae	Bacillaria paxillifer	9	9	3
Bacillariophyceae	Bellerochea malleus	51	51	3
Bacillariophyceae	Brockmanniella brockmannii	77	37990	11
Bacillariophyceae	Campylodiscus sp	6	6	3
Bacillariophyceae	Centrales, diameter < 10 µm	12	5308189	78
Bacillariophyceae	Centrales, diameter 10-30 µm	6	32619	78
Bacillariophyceae	Centrales, diameter 30-50 µm	10	160	22
Bacillariophyceae	Centrales, diameter > 50 µm	5	5	3
Bacillariophyceae	Cerataulina pelagica	14	296	6
Bacillariophyceae	Chaetoceros affinis	26	26	3
Bacillariophyceae	Chaetoceros danicus	5	40	6
Bacillariophyceae	Chaetoceros debilis	18	3161	19
Bacillariophyceae	Chaetoceros decipiens	125	125	3
Bacillariophyceae	Chaetoceros didymus	3	133	8
Bacillariophyceae	Chaetoceros similis	6332	6332	3
Bacillariophyceae	Chaetoceros socialis	8	4197	17
Bacillariophyceae	Chaetoceros sp	2	47797	39
Bacillariophyceae	Chaetoceros subtilis	20	101972	14
Bacillariophyceae	Chaetoceros teres	16	16	3
Bacillariophyceae	Coscinodiscus commutatus	2	115	6
Bacillariophyceae	Coscinodiscus radiatus	5	281	8
Bacillariophyceae	Cyclostephanos dubius	1856	1856	3
Bacillariophyceae	Cyclotella meneghiniana	6	1623	14
Bacillariophyceae	Cyclotella sp	3	9297	17
Bacillariophyceae	Cylindrotheca closterium	2	1858	47
Bacillariophyceae	Cymatopleura solea	5	5	6
Bacillariophyceae	Cymatosira belgica	4641	4641	3
Bacillariophyceae	Delphineis minutissima	83	18995	17
Bacillariophyceae	Delphineis surirella	166	166	3
Bacillariophyceae	Detonula pumila	10	34	6
Bacillariophyceae	Diatoma tenuis	8	1547	19
Bacillariophyceae	Ditylum brightwellii	2	562	42
Bacillariophyceae	Eucampia zodiacus	97	763	8
Bacillariophyceae	Eunotogramma dubium	9	9282	11
Bacillariophyceae	Fragilaria crotonensis	54	54	3
Bacillariophyceae	Fragilaria sp	836	72226	8
Bacillariophyceae	Fragilaria ulna	5	12	6

Appendix 5.
Continued.

PHYTOPLANKTON IN BALLAST WATER SAMPLES		MIN	MAX	FREQUENCY
		cells/l	cells/l	%
Bacillariophyceae	Fragilaria ulna var acus	29	147	6
Bacillariophyceae	Guinardia flaccida	1	1	3
Bacillariophyceae	Gyrosigma sp	2	281	8
Bacillariophyceae	Haslea sp 5640	208	276	6
Bacillariophyceae	Leptocylindrus danicus	370	3361	8
Bacillariophyceae	Leptocylindrus mediterraneus	6332	6332	3
Bacillariophyceae	Leptocylindrus minimus	3	9295	11
Bacillariophyceae	Lithodesmium undulatum	4	567	11
Bacillariophyceae	Melosira nummuloides	4	556	6
Bacillariophyceae	Melosira sp	2	300	8
Bacillariophyceae	Melosira varians	20	40	8
Bacillariophyceae	Minutocellus scriptus	15240	15240	3
Bacillariophyceae	Navicula sp	56	4951	17
Bacillariophyceae	Nitzschia coarctata	248	3218	6
Bacillariophyceae	Nitzschia sigma cf	13707	13707	3
Bacillariophyceae	Nitzschia sp	102	13707	19
Bacillariophyceae	Odontella aurita	34	1439	25
Bacillariophyceae	Odontella aurita var. minima	562	586	6
Bacillariophyceae	Odontella mobiliensis	277	277	3
Bacillariophyceae	Odontella regia	7	281	6
Bacillariophyceae	Odontella rhombus	10	281	6
Bacillariophyceae	Odontella sinensis	4	25	17
Bacillariophyceae	Paralia marina	7	193568	39
Bacillariophyceae	Pennales	1535	12718	6
Bacillariophyceae	Pennales, width < 10 length < 50 µm	44	50530	64
Bacillariophyceae	Pennales, width < 10 length > 50 µm	3	6435	31
Bacillariophyceae	Pennales, width > 10 length > 50 µm	5	50	11
Bacillariophyceae	Plagiogrammopsis vanheurckii	126	3105	14
Bacillariophyceae	Pleurosigma sp	1	254	14
Bacillariophyceae	Podosira stelliger	5	65	11
Bacillariophyceae	Pseudo-nitzschia sp	60	3218	14
Bacillariophyceae	Pseudo-nitzschia delicatissima	2	2	3
Bacillariophyceae	Pseudo-nitzschia delicatissima cf	45	6310	8
Bacillariophyceae	Pseudo-nitzschia fraudulenta	41	41	3
Bacillariophyceae	Pseudo-nitzschia pungens cf	3	166	14
Bacillariophyceae	Rhaphoneis amphiceros	4	4498	33
Bacillariophyceae	Rhizosolenia alata	1	6	6
Bacillariophyceae	Rhizosolenia delicatula	2	7278	28
Bacillariophyceae	Rhizosolenia fragilissima	12580	12580	3
Bacillariophyceae	Rhizosolenia pungens	10	10	3
Bacillariophyceae	Rhizosolenia setigera	5	843	22
Bacillariophyceae	Rhizosolenia shrubsolei	1	13	17
Bacillariophyceae	Rhizosolenia sp 6989	3144	3144	3
Bacillariophyceae	Rhizosolenia stolterfothii	6	15	6
Bacillariophyceae	Roperia tesselata	45	260	6
Bacillariophyceae	Skeletonema costatum	25	203905	44
Bacillariophyceae	Skeletonema potamos	990	2694745	11
Bacillariophyceae	Skeletonema subsalsum	18127	32619	6
Bacillariophyceae	Stauroneis membranacea	5	5	3
Bacillariophyceae	Stephanodiscus hantzschii	3218	6188	6
Bacillariophyceae	Stephanodiscus sp	237	18641	11
Bacillariophyceae	Streptotheca tamensis	1	281	11

Appendix 5.
Continued.

PHYTOPLANKTON IN BALLAST WATER SAMPLES		MIN	MAX	FREQUENCY
		cells/l	cells/l	%
Bacillariophyceae	Surirella sp	3	152	14
Bacillariophyceae	Thalassionema nitzschioides	8	5169	42
Bacillariophyceae	Thalassiosira eccentrica	2	440	17
Bacillariophyceae	Thalassiosira hendeyi	154	154	3
Bacillariophyceae	Thalassiosira lacustris	114	114	3
Bacillariophyceae	Thalassiosira nordenskioeldii	5	3116	8
Bacillariophyceae	Thalassiosira punctigera	8	147	17
Bacillariophyceae	Thalassiosira rotula	25	843	14
Bacillariophyceae	Thalassiosira sp	40	40	3
Bacillariophyceae	Thalassiosira sp < 30 µm	3	72917	39
Bacillariophyceae	Thalassiosira sp 30-80 µm	8	7028	36
Bacillariophyceae	Thalassiosira sp > 80 µm	38	281	6
Bacillariophyceae	Trachyneis aspera	52	52	3
Bacillariophyceae	Triceratium alternans	4	80	17
Chlorophyceae	Chlamydomonas sp	3105	35940	11
Chlorophyceae	Chlorococcales < 20 µm	present	281	5
Chlorophyceae	Chlorophyceae	3393	2503677	36
Chlorophyceae	Closterium sp	6	311	6
Chlorophyceae	Coelastrum sp	120	97937	8
Chlorophyceae	Crucigenia sp	17021	112401	11
Chlorophyceae	Franceia sp	9028	9028	3
Chlorophyceae	Gloeotila pelagica	27706	27706	3
Chlorophyceae	Gloeotila sp	221	221	3
Chlorophyceae	Kirchneriella sp	578	14038	11
Chlorophyceae	Koliella sp	10	12870	14
Chlorophyceae	Lagerheimia sp	152	9028	6
Chlorophyceae	Monoraphidium contortum	1407	2392	6
Chlorophyceae	Monoraphidium sp	88	497102	36
Chlorophyceae	Nephrocytium sp	36113	36113	3
Chlorophyceae	Oocystis sp	2128	33430	6
Chlorophyceae	Pediastrum sp	4	14736	28
Chlorophyceae	Planctonema sp	104	281	6
Chlorophyceae	Scenedesmus acuminatus	6	6	3
Chlorophyceae	Scenedesmus sp	6	295615	43
Chlorophyceae	Selenastrum capricornutum	8865	14353	6
Chlorophyceae	Tetraedron sp	8155	8155	3
Chlorophyceae	Tetrastrum sp	608	16626	17
Chlorophyceae	Tetrastrum staurogeniaeforme	141	141	3
Chrysomonadales	Chrysomonadales 0.2-2 µm	162602	162602	3
Chrysomonadales	Chrysomonadales 2-10 µm	1376	256040	47
Chrysomonadales	Chrysomonadales > 10 µm	152	154	6
Chrysophyceae	Apedinella spinifera	48151	48151	3
Chrysophyceae	Dinobryon sp	40	9466	8
Chrysophyceae	Pseudopedinella sp	103973	103973	3
Cryptophyceae	Cryptomonas sp	present	present	3
Cryptophyceae	Cryptophyceae	35	228916	30
Cryptophyceae	Cryptophyceae < 10 µm	243	585119	56
Cryptophyceae	Cryptophyceae > 10 µm	309	66171	39
Cryptophyceae	Cyclostephanos dubius	3105	3105	3
Cyanophyceae	Aphanizomenon sp	1151	1151	3
Cyanophyceae	Chroococcales	21115	330919	14
Cyanophyceae	Hormogonales	8155	8155	3

Appendix 5.
Continued.

PHYTOPLANKTON IN BALLAST WATER SAMPLES		MIN	MAX	FREQUENCY
		cells/l	cells/l	%
Cyanophyceae	Limnothrix sp	2130	2130	3
Cyanophyceae	Merismopedia sp	2972	326192	6
Cyanophyceae	Microcystis sp	2600	107958	8
Cyanophyceae	Planktothrix agardhii	2	2073	16
Cyanophyceae	Planktothrix sp	52	257317	19
Cyanophyceae	Pseudanabaena sp	7143	7143	3
Cyanophyceae	Snowella sp	6100	13298	6
Dinophyceae	Alexandrium ostenfeldii	106	106	3
Dinophyceae	Ceratium furca	5	69	6
Dinophyceae	Ceratium fusus	1	64	11
Dinophyceae	Ceratium horridum	27	27	3
Dinophyceae	Ceratium lineatum	1	33	6
Dinophyceae	Ceratium longipes	11	11	3
Dinophyceae	Ceratium macroceros	1	1	3
Dinophyceae	Corythodinium tesselatum	9	9	3
Dinophyceae	Dinophyceae	1399	1399	3
Dinophyceae	Dinophyceae cyste	1	1862	25
Dinophyceae	Dinophysis acuminata	1	67	11
Dinophyceae	Dinophysis sp	9	9	3
Dinophyceae	Dissodinium pseudolunula	5	562	6
Dinophyceae	Fragilidium subglobosum	1	1	3
Dinophyceae	Gonyaulax sp	2	32	8
Dinophyceae	Gonyaulax spinifera	16	32	6
Dinophyceae	Gonyaulax verior	48	48	3
Dinophyceae	Gymnodiniaceae, diameter < 10 µm	932	3105	11
Dinophyceae	Gymnodiniaceae, diameter 10-30 µm	767	8985	14
Dinophyceae	Gymnodinium gracile	6287	6287	3
Dinophyceae	Gymnodinium simplex	291	31268	11
Dinophyceae	Gymnodinium, length < 10 µm	33	62713	11
Dinophyceae	Gymnodinium, length 10-30 µm	79	127123	31
Dinophyceae	Gymnodinium, length 30-50 µm	6	6	3
Dinophyceae	Heterocapsa minima cf	248	32057	19
Dinophyceae	Heterocapsa niei	44	44	3
Dinophyceae	Heterocapsa rotundata	1535	12718	6
Dinophyceae	Heterocapsa sp	79	548199	8
Dinophyceae	Karenia mikimotoi	1056	3042	8
Dinophyceae	Mesoporos perforatus	705	705	3
Dinophyceae	Oxytoxum scolopax	264	264	3
Dinophyceae	Oxytoxum sp	9	752	11
Dinophyceae	Peridiniaceae, diameter 10-30 µm	1	9519	42
Dinophyceae	Peridiniaceae, diameter 30-50 µm	5	798	11
Dinophyceae	Peridiniales, diameter 10-30 µm	2	15	14
Dinophyceae	Peridiniales, diameter 30-50 µm	2	15	6
Dinophyceae	Peridiniella catenata	1170	1170	3
Dinophyceae	Prorocentrum micans	1	3209	33
Dinophyceae	Prorocentrum minimum	5	104139	14
Dinophyceae	Prorocentrum sp	3	3	3
Dinophyceae	Prorocentrum triestinum	796	2507	8
Dinophyceae	Pyrophacus horologicum	5	5	3
Dinophyceae	Scripsiella sp	9	3148	8
Dinophyceae	Stephanopyxis turris	32	32	3
Dinophyceae	Torodinium robustum	704	704	3

Appendix 5.
Continued.

PHYTOPLANKTON IN BALLAST WATER SAMPLES		MIN	MAX	FREQUENCY
		cells/l	cells/l	%
Euglenophyceae	Euglena sp	3	7455	11
Euglenophyceae	Euglenophyceae	2013	2569	8
Euglenophyceae	Eutreptiella sp	42	19150	22
Euglenophyceae	Phacus sp	5	271	8
Euglenophyceae	Trachelomonas sp	253	311	6
Prasinophyceae	Halosphaeraceae	1	1	3
Prasinophyceae	Prasinophyceae	158	650407	36
Prasinophyceae	Pterosperma sp	10	352	6
Prasinophyceae	Pyramimonas sp	1013	16345	11
Prasinophyceae	Pyramimonas sp, length < 10 µm	3105	50834	22
Prymnesiophyceae	Chrysochromulina sp	146	219383	17
Prymnesiophyceae	Phaeocystis cell	9315	9315	3
Prymnesiophyceae	Phaeocystis flagellate	37943	37943	3
Unidentifiable	Unidentifiable, diameter < 3 µm	12138	8376986	64
Unidentifiable	Unidentifiable, diameter 3-10 µm	2748	402655	64
Unidentifiable	Unidentifiable, diameter < 10 µm	258	153370	22
Unidentifiable	Unidentifiable, diameter 10-30 µm	16	15623	42
Xanthophyceae	Goniochloris sp	348	1547	8
Xanthophyceae	Trachydiscus sp	66512	66512	3
MICROZOOPLANKTON IN BALLAST WATER SAMPLES		**MIN**	**MAX**	**FREQUENCY**
		organisms/l	**organisms/l**	**%**
Bivalvia	Veliger non det	<0,1	5,8	47
Choreotrichia	Ciliata < 20 µm	1,0	22277	47
Choreotrichia	Ciliata 20-40 µm	9,0	2294	47
Choreotrichia	Ciliata 40-60 µm	3,0	292	18
Choreotrichia	Ciliata 60-80 µm	76	76	6
Choreotrichia	Ciliate non det	present	present	6
Ciliata	Ciliate non det	0,3	0,3	6
Cirripedia	Balanidae nauplius	0,1	1,3	12
Cirripedia	Semibalanus balanoides	<0,1	1,1	24
Craspedomonadaceae	Craspedomonadaceae	3345	569940	28
Cryptophyceae	Leucocryptos marina	42	31290	17
Cryptophyceae	Leucocryptos sp	3930	25435	6
Dinophyceae	Dinophyceae heterotrophic	42	9902	6
Dinophyceae	Dinophysis rotundata	6,0	21	6
Dinophyceae	Diplopsalis sp gr	1,0	43	11
Dinophyceae	Ebria tripartita	83	35940	11
Dinophyceae	Glenodinium danicum	53	53	3
Dinophyceae	Gyrodinium sp, length 10-30 µm	541	3906	6
Dinophyceae	Gyrodinium sp, length 30-50 µm	752	752	3
Dinophyceae	Gyrodinium spirale	5,0	1254	8
Dinophyceae	Katodinium glaucum	1755	1755	3
Dinophyceae	Noctiluca scintillans	<0,1	<0,1	6
Dinophyceae	Oblea rotundata	2,0	251	17
Dinophyceae	Oxyphysis oxytoxoides	6,0	6,0	3
Dinophyceae	Oxyrrhis marina	1160	1160	3
Dinophyceae	Preperidinium meunierii	5,0	5,0	6
Dinophyceae	Protoperidinium achromaticum	213	213	3
Dinophyceae	Protoperidinium bipes	67	67	3
Dinophyceae	Protoperidinium brevipes	59	61	6
Dinophyceae	Protoperidinium conicum	5,0	5,0	3

Appendix 5.
Continued.

MICROZOOPLANKTON IN BALLAST WATER SAMPLES		MIN	MAX	FREQUENCY
		organisms/l	organisms/l	%
Dinophyceae	Protoperidinium excentricum	5,0	11	6
Dinophyceae	Protoperidinium leonis	6,0	16	6
Dinophyceae	Protoperidinium marielebourae	108	108	3
Dinophyceae	Protoperidinium minutum	57	133	8
Dinophyceae	Protoperidinium mite	6,0	37	6
Dinophyceae	Protoperidinium punctulatum	5,0	27	6
Dinophyceae	Protoperidinium sp, length 10-30 µm	1,0	266	11
Dinophyceae	Protoperidinium sp, length 30-50 µm	21	798	8
Dinophyceae	Protoperidinium sp, length > 50 µm	5,0	5,0	3
Dinophyceae	Protoperidinium steinii	5,0	19	6
Dinophyceae	Protoperidinium subinerme	3,0	100	6
Dinophyceae	Warnowia sp	147	147	3
Echinodermata	Echinoid larvae	<0,1	<0,1	6
Haptoria	Mesodinium rubrum	18	251	6
Mysidacea	Mysidacea non det	<0,1	<0,1	6
Nematoda	Nematode	0,1	18	18
Oligochaeta	Oligochaeta non det	2,9	2,9	6
Oligochaeta	Oligotrichida	<0,1	<0,1	6
Peritrichia	Vorticella cf	4,5	902	12
Peritrichia	Vorticellidae	present	present	6
Polychaeta	Nereidae non det	0,5	0,5	6
Polychaeta	Polychaeta larvae	0,1	0,1	6
Polychaeta	Spionida	1,3	1,3	6
Polychaeta	Spionidae non det	0,1	12,8	18
Protomonadales	Heterotrophic flagellate	63	5240	14
Protomonadales	Heterotrophic flagellate, length < 10 µm	2653	336854	61
Protomonadales	Heterotrophic flagellate, length > 10 µm	197	136578	50
Rotatoria	Asplanchna sp	<0,1	<0,1	6
Rotatoria	Brachionus angularis	<0,1	<0,1	6
Rotatoria	Brachionus calyciflorus	7,1	7,1	6
Rotatoria	Cephalodella sp	0,2	0,7	12
Rotatoria	Colurella sp	<0,1	0,7	18
Rotatoria	Epiphanes sp	0,1	0,1	6
Rotatoria	Euchlanis sp	0,2	0,2	6
Rotatoria	Keratella cochlearis	<0,1	14,89	41
Rotatoria	Keratella quadrata	<0,1	6,5	35
Rotatoria	Lecane bulla	0,2	0,2	6
Rotatoria	Lecane sp	<0,1	0,7	12
Rotatoria	Lepadella ovalis	0,2	0,2	6
Rotatoria	Lepadella sp	0,3	0,3	6
Rotatoria	Mytilina sp	0,7	0,7	6
Rotatoria	Polyarthra sp	0,3	2,6	18
Rotatoria	Pompholyx sp	0,7	0,7	6
Rotatoria	Rotaria neptunia	0,7	0,7	6
Rotatoria	Rotifer non det	<0,1	4,3	47
Rotatoria	Synchaeta sp	0,2	1,0	35
Rotatoria	Synchaeta sp cf	0,1	0,1	6
Rotatoria	Trichocerca capucina	<0,1	<0,1	6
Rotatoria	Trichocerca sp	<0,1	<0,1	6
Testacea	Arcella hemisphaerica	0,1	1,3	12
Testacea	Arcella sp	1,3	1,3	6
Testacea	Arcella vulgaris	<0,1	<0,1	6

Appendix 5.
Continued.

MICROZOOPLANKTON IN BALLAST WATER SAMPLES		MIN	MAX	FREQUENCY
		organisms/l	organisms/l	%
Testacea	Centropyxis sp	0,3	0,3	6
Testacea	Difflugia sp	0,1	0,1	6
Testacea	Euglypha sp	0,2	0,2	6
Testacea	Paulinella sp	535824	535824	6
Tintinnidae	Parafavella sp	0,5	0,5	6
Tintinnidae	Tintinnida, width < 20 µm	546	15679	53
Tintinnidae	Tintinnida, width 20-40 µm	5,0	9720	53
Tintinnidae	Tintinnida, width 40-60 µm	292	292	6
Tintinnidae	Tintinnidium fluviatile	1,9	1,9	6
Tintinnidae	Tintinnopsis lacustris	0,3	14	29
Turbellaria	Flatworm non det	0,1	0,1	6
MESOZOOPLANKTON IN BALLAST WATER SAMPLES		**MIN**	**MAX**	**FREQUENCY**
		organisms/l	**organisms/l**	**%**
Arachnida	Hydracarine	<0,1	<0,1	6
Cladocera	Bosmina longirostris	<0,1	<0,1	6
Cladocera	Ceriodaphnia sp	present	present	6
Cladocera	Cladocera juvenile	0,3	0,3	6
Cladocera	Cladocera non det	<0,1	<0,1	6
Cladocera	Daphnia cf ambigua	present	present	6
Cladocera	Daphnia galeata	present	present	6
Cladocera	Daphnia juvenile	<0,1	0,6	12
Cladocera	Daphnia pulex	present	present	6
Cladocera	Daphnia sp	<0,1	<0,1	6
Copepoda	Nauplius	0,3	193	82
Copepoda, Calanoida	Acartia discaudata	0,1	0,1	6
Copepoda, Calanoida	Acartia sp	0,1	13	29
Copepoda, Calanoida	Calanoid non det	1,3	1,3	6
Copepoda, Calanoida	Copepodite	<0,1	28	82
Copepoda, Calanoida	Eurytemora affinis	<0,1	4,7	29
Copepoda, Calanoida	Eurytemora hirundoides	0,2	0,2	6
Copepoda, Calanoida	Eurytemora lacustris	14	16	12
Copepoda, Calanoida	Eurytemora sp	<0,1	4,1	12
Copepoda, Calanoida	Nauplius	13	36	12
Copepoda, Cyclopoida	Acanthocyclops robustus	0,2	0,2	6
Copepoda, Cyclopoida	Copepodite	<0,1	23	65
Copepoda, Cyclopoida	Cyclopidae non det	<0,1	<0,1	6
Copepoda, Cyclopoida	Cyclopoid non det	0,2	1,1	24
Copepoda, Cyclopoida	Cyclops vicinus	0,2	0,2	6
Copepoda, Cyclopoida	Oithona sp	0,1	4,9	47
Copepoda, Cyclopoida	Thermocyclops crassus	0,1	0,1	6
Copepoda, Cyclopoida	Thermocyclops crassus cf	present	present	6
Copepoda, Cyclopoida	Thermocyclops dybowskii	1,0	1,0	6
Copepoda, Harpacticoida	Harpacticoid non det	<0,1	1,9	59
Copepoda, Harpacticoida	Laophonte sp	0,1	0,1	12
Copepoda, Harpacticoida	Zaus sp	<0,1	4,4	24

Appendix 6.
Phytoplankton and microzooplankton species observed in port water samples (n = 23), arranged alphabetically. MIN, MAX = minimum, maximum observed cells/l or organisms/l; FREQUENCY = frequency of occurrence. Toxic and potentially toxic species are marked grey.

PHYTOPLANKTON IN PORT WATER SAMPLES		MIN	MAX	FREQUENCY
		cells/l	cells/l	%
Bacillariophyceae	Actinocyclus normanii	259	1306	22
Bacillariophyceae	Actinoptychus senarius	88	743	9
Bacillariophyceae	Asterionella formosa	913	5447	35
Bacillariophyceae	Asterionella glacialis	537	537	4
Bacillariophyceae	Aulacoseira sp	175	34740	22
Bacillariophyceae	Bacillaria paxillifer	2586	2586	4
Bacillariophyceae	Brockmanniella brockmannii	1342	17780	9
Bacillariophyceae	Campylosira cymbelliformis	1610	1610	4
Bacillariophyceae	Centrales	6511	6511	4
Bacillariophyceae	Centrales, diameter < 10 µm	3853	5335205	96
Bacillariophyceae	Centrales, diameter 10-30 µm	304	74504	48
Bacillariophyceae	Cerataulina pelagica	271	9097	13
Bacillariophyceae	Chaetoceros ceratosporus cf	7861	7861	4
Bacillariophyceae	Chaetoceros costatus	27003	27003	4
Bacillariophyceae	Chaetoceros danicus	3233	3233	4
Bacillariophyceae	Chaetoceros debilis	1152	100000	17
Bacillariophyceae	Chaetoceros decipiens	2683	2683	4
Bacillariophyceae	Chaetoceros didymus	3259	3259	4
Bacillariophyceae	Chaetoceros socialis	2167	2257297	22
Bacillariophyceae	Chaetoceros sp	1084	23582	9
Bacillariophyceae	Chaetoceros subtilis	2586	3387	9
Bacillariophyceae	Coscinodiscus commutatus	273	542	9
Bacillariophyceae	Cyclostephanos dubius	1622	15874	13
Bacillariophyceae	Cyclotella atomus cf	39841	39841	4
Bacillariophyceae	Cyclotella sp	1632	67764	30
Bacillariophyceae	Cylindrotheca closterium	1004	13553	26
Bacillariophyceae	Delphineis minutissima	17459	17459	4
Bacillariophyceae	Detonula pumila	6984	6984	4
Bacillariophyceae	Diatoma tenuis	202	1622	30
Bacillariophyceae	Ditylum brightwellii	2000	2000	4
Bacillariophyceae	Eucampia zodiacus	1344	5121	9
Bacillariophyceae	Eunotogramma dubium	257	1857	9
Bacillariophyceae	Fragilaria sp	248	6839	9
Bacillariophyceae	Fragilaria ulna	1616	1616	4
Bacillariophyceae	Fragilaria ulna var acus	88	1038	26
Bacillariophyceae	Guinardia flaccida	259	259	4
Bacillariophyceae	Lauderia annulata	2167	13501	13
Bacillariophyceae	Leptocylindrus danicus	2333	21875	13
Bacillariophyceae	Leptocylindrus minimus	1034	845034	26
Bacillariophyceae	Melosira sp	406	440	9
Bacillariophyceae	Melosira nummuloides	124	23475	13
Bacillariophyceae	Melosira varians	203	203	4
Bacillariophyceae	Navicula sp	271	4328	26
Bacillariophyceae	Nitzschia levidensis	1000	1000	4
Bacillariophyceae	Nitzschia reversa	1780	1780	4
Bacillariophyceae	Nitzschia sp	285	61218	39
Bacillariophyceae	Odontella aurita	4849	4849	4
Bacillariophyceae	Odontella mobiliensis	1293	1293	4
Bacillariophyceae	Paralia marina	438	25101	30
Bacillariophyceae	Pennales, width < 10 length < 50 µm	1693	744285	74

Appendix 6.
Continued.

PHYTOPLANKTON IN PORT WATER SAMPLES		MIN	MAX	FREQUENCY
		cells/l	cells/l	%
Bacillariophyceae	Pennales, width < 10 length > 50 µm	304	5494	13
Bacillariophyceae	Pennales, width > 10 length > 50 µm	542	542	4
Bacillariophyceae	Plagiogrammopsis vanheurckii	1000	7861	9
Bacillariophyceae	Pleurosigma normanii	1056	9777	9
Bacillariophyceae	Pleurosigma sp	259	259	4
Bacillariophyceae	Podosira stelliger	129	129	4
Bacillariophyceae	Pseudo-nitzschia delicatissima	3714	3714	4
Bacillariophyceae	Pseudo-nitzschia delicatissima cf	2183	2183	4
Bacillariophyceae	Pseudo-nitzschia fraudulenta	517	517	4
Bacillariophyceae	Pseudo-nitzschia pungens cf	1626	63690	17
Bacillariophyceae	Pseudo-nitzschia turgidula	11639	11639	4
Bacillariophyceae	Rhaphoneis amphiceros	268	4328	17
Bacillariophyceae	Rhizosolenia delicatula	776	6052	22
Bacillariophyceae	Rhizosolenia setigera	96	3259	39
Bacillariophyceae	Rhizosolenia shrubsolei	819	819	4
Bacillariophyceae	Skeletonema costatum	1173	361152	48
Bacillariophyceae	Skeletonema potamos	8109	523831	35
Bacillariophyceae	Skeletonema subsalsum	11395	230396	35
Bacillariophyceae	Stephanodiscus hantzschii	13853	125522	22
Bacillariophyceae	Stephanodiscus sp	675	92961	30
Bacillariophyceae	Surirella sp	88	4328	26
Bacillariophyceae	Thalassionema nitzschioides	202	35141	35
Bacillariophyceae	Thalassiosira eccentrica	248	6640	9
Bacillariophyceae	Thalassiosira sp < 30 µm	225	102191	39
Bacillariophyceae	Thalassiosira sp 30-80 µm	371	1362	22
Chlorophyceae	Chlamydomonas sp	8659	2667603	30
Chlorophyceae	Chlorophyceae	25976	124488124	70
Chlorophyceae	Coelastrum sp	1406	3114	13
Chlorophyceae	Crucigenia sp	23160	46950	17
Chlorophyceae	Gloeotila sp	16470	16470	4
Chlorophyceae	Kirchneriella sp	16470	91185	17
Chlorophyceae	Koliella sp	271	82698	30
Chlorophyceae	Lagerheimia sp	4328	4328	4
Chlorophyceae	Monoraphidium sp	268	4001404	70
Chlorophyceae	Oocystis sp	259	115098	13
Chlorophyceae	Pediastrum sp	2595	4294	9
Chlorophyceae	Scenedesmus sp	1004	252383	70
Chlorophyceae	Spermatozopsis sp	30395	316511	17
Chlorophyceae	Tetrastrum sp	13678	189287	13
Chlorophyceae	Treubaria sp	8659	8659	4
Chrysomonadales	Chrysomonadales 2-10 µm	17313	757576	57
Chrysomonadales	Chrysomonadales > 10 µm	1819	1819	4
Chrysophyceae	Apedinella spinifera	28774	204381	9
Chrysophyceae	Mallomonas sp	225	261	9
Chrysophyceae	Pseudopedinella sp	15305	151515	17
Cryptophyceae	Cryptophyceae	10276	203481	22
Cryptophyceae	Cryptophyceae < 10 µm	17313	14227214	74
Cryptophyceae	Cryptophyceae > 10 µm	4328	1969697	78
Cyanophyceae	Anabaena sp	960	30640	9
Cyanophyceae	Aphanizomenon sp	77844	117375	9
Cyanophyceae	Aphanothece sp	157739	157739	4
Cyanophyceae	Chroococcales	15305	89809290	57

Appendix 6.
Continued.

PHYTOPLANKTON IN PORT WATER SAMPLES		MIN	MAX	FREQUENCY
		cells/l	cells/l	%
Cyanophyceae	Cyanophyta	257	257	4
Cyanophyceae	Hormogonales	2747	649254	13
Cyanophyceae	Limnothrix sp	876	481105	9
Cyanophyceae	Merismopedia sp	173699	2646347	9
Cyanophyceae	Microcystis sp	8278	32512	13
Cyanophyceae	Planktothrix agardhii	440	2008	22
Cyanophyceae	Planktothrix sp	2020	227046	48
Cyanophyceae	Pseudanabaena sp	93900	153046	9
Cyanophyceae	Snowella sp	97305	97305	4
Dinophyceae	Alexandrium tamarense	96	96	4
Dinophyceae	Ceratium fusus	192	1034	13
Dinophyceae	Dinophyceae	2223002	2223002	4
Dinophyceae	Dinophyceae cyste	4328	4328	4
Dinophyceae	Gonyaulax spinifera	259	12105	9
Dinophyceae	Gymnodiniaceae	9767	9767	4
Dinophyceae	Gymnodiniaceae, diameter < 10 µm	147	10988	13
Dinophyceae	Gymnodiniaceae, diameter 10-30 µm	101	1004	13
Dinophyceae	Gymnodinium simplex	1616	6250	9
Dinophyceae	Gymnodinium, length < 10 µm	6250	24705	17
Dinophyceae	Gymnodinium, length 10-30 µm	1702	172646	65
Dinophyceae	Gymnodinium, length 30-50 µm	268	931	22
Dinophyceae	Heterocapsa minima cf	3251	18673219	43
Dinophyceae	Heterocapsa rotundata	1004	13735	22
Dinophyceae	Heterocapsa sp	1297	69480	13
Dinophyceae	Heterocapsa triquetra	271	14547	17
Dinophyceae	Mesoporos perforatus	271	1500	9
Dinophyceae	Peridiniaceae sp 3	1862	11580	9
Dinophyceae	Peridiniaceae, diameter 10-30 µm	268	6669007	39
Dinophyceae	Peridiniaceae, diameter 30-50 µm	124	8803	13
Dinophyceae	Prorocentrum compressum	466	466	4
Dinophyceae	Prorocentrum micans	96	1552	13
Dinophyceae	Prorocentrum minimum	466	3125	17
Dinophyceae	Prorocentrum triestinum	517	2000	13
Dinophyceae	Scripsiella sp	271	9332	26
Dinophyceae	Torodinium robustum	202	268	9
Euglenophyceae	Euglena sp	124	4865	9
Euglenophyceae	Euglenophyceae	203	293	9
Euglenophyceae	Eutreptiella sp	1355	444600	52
Prasinophyceae	Prasinophyceae	6640	5335205	52
Prasinophyceae	Pyramimonas sp	12718	43952	17
Prasinophyceae	Pyramimonas sp, length < 10 µm	6250	2272727	35
Prasinophyceae	Pyramimonas sp, length > 10 µm	500	8467	26
Prymnesiophyceae	Chrysochromulina sp	8657	454545	43
Prymnesiophyceae	Phaeocystis cell	185279	185279	4
Prymnesiophyceae	Phaeocystis colony (<100) cell	81657	81657	4
Raphidophyceae	Raphidophyceae	192	2910	9
Unidentifiable	Unidentifiable, diameter < 3 µm	40658	22163152	78
Unidentifiable	Unidentifiable, diameter 3-10 µm	51952	1333801	74
Unidentifiable	Unidentifiable, diameter < 10 µm	27851	50870	13
Unidentifiable	Unidentifiable, diameter 10-30 µm	268	57549	17
Xanthophyceae	Trachydiscus sp	24705	141081	9

Appendix 6.
Continued.

MICROZOOPLANKTON IN PORT WATER SAMPLES		MIN	MAX	FREQUENCY
		organisms/l	organisms/l	%
Choreotrichia	Ciliata < 20 µm	1000	9375	30
Choreotrichia	Ciliata 20-40 µm	271	3125	26
Choreotrichia	Ciliata 60-80 µm	96	96	4
Choreotrichia	Ciliata > 80 µm	466	466	4
Craspedomonadaceae	Craspedomonadaceae	4328	31443	9
Cryptophyceae	Leucocryptos sp	1857	12718	9
Cryptophyceae	Leucocryptos marina	259	6640	9
Dinophyceae	Diplopsalopsis orbicularis	259	2000	9
Dinophyceae	Ebria tripartita	268	180576	17
Dinophyceae	Glenodinium danicum	817	817	4
Dinophyceae	Glenodinium foliaceum	192	192	4
Dinophyceae	Gyrodinium spirale	466	1293	22
Dinophyceae	Katodinium glaucum	268	6250	26
Dinophyceae	Micracanthodinium sp	3500	3500	4
Dinophyceae	Noctiluca scintillans	288	466	9
Dinophyceae	Oblea rotundata	96	259	9
Dinophyceae	Preperidinium meunierii	259	259	4
Dinophyceae	Protoperidinium achromaticum	776	5869	9
Dinophyceae	Protoperidinium bipes	259	931	13
Dinophyceae	Protoperidinium marielebourae	466	466	4
Dinophyceae	Protoperidinium minutum	259	466	9
Dinophyceae	Protoperidinium punctulatum	96	96	4
Dinophyceae	Protoperidinium sp, length 30-50 µm	35212	35212	4
Dinophyceae	Protoperidinium subinerme	96	259	9
Haptoria	Mesodinium rubrum	1167	21875	17
Protomonadales	Heterotrophic flagellate	12718	31565	9
Protomonadales	Heterotrophic flagellate, length < 10 µm	14151	7558208	78
Protomonadales	Heterotrophic flagellate, length > 10 µm	1819	91827	70
Testacea	Paulinella sp	7937	49411	9
Tintinnidae	Tintinnida, width < 20 µm	202	40625	61
Tintinnidae	Tintinnida, width 20-40 µm	202	6999	65
Tintinnidae	Tintinnida, width 40-60 µm	124	537	22
Tintinnidae	Tintinnida, width > 80 µm	259	259	4

Appendix 7.
Cultured phytoplankton and microzooplankton species from ballast water in culture media and filtered port water (n = 220 culture flasks), arranged alphabetically. MIN, MAX = minimum, maximum observed cells/ml or organisms/ml; FREQUENCY = frequency of occurrence. Toxic and potentially toxic species are marked grey.

									PORT
SALINITIES		ALL	ALL	ALL	0.3-1.3	5	15	30	0.3-28.6
CULTURED PHYTOPLANKTON		MIN	MAX	FREQ	FREQ	FREQ	FREQ	FREQ	FREQ
		n=220	n=220	n=220	n=12	n=60	n=60	n=60	n=28
		cells/ml	cells/ml	%	%	%	%	%	%
Bacillariophyceae	Actinocyclus normanii	93	466	1	0	0	0	2	4
Bacillariophyceae	Actinocyclus sp	559	559	0	0	0	2	0	0
Bacillariophyceae	Asterionella glacialis	1	10708	9	0	0	3	28	4
Bacillariophyceae	Asterionella kariana	93	686	2	0	0	3	3	0
Bacillariophyceae	Attheya sp	93	93	0	0	2	0	0	0
Bacillariophyceae	Aulacoseira granulata	20202	20202	0	0	2	0	0	0
Bacillariophyceae	Centrales, diameter < 5 µm	3980	350196	12	0	15	17	12	4
Bacillariophyceae	Centrales, diameter < 10 µm	101	1979798	70	58	69	71	73	71
Bacillariophyceae	Centrales, diameter 10-30 µm	1	101010	19	8	12	20	23	25
Bacillariophyceae	Centrales, diameter 30-50 µm	6	811	3	0	0	2	5	11
Bacillariophyceae	Cerataulina pelagica	466	5354	2	0	0	3	0	7
Bacillariophyceae	Chaetoceros affinis	5051	224242	2	0	0	3	3	0
Bacillariophyceae	Chaetoceros ceratosporus	1210	7449	1	0	2	2	0	0
Bacillariophyceae	Chaetoceros ceratosporus cf	10152	111111	1	0	0	2	3	0
Bacillariophyceae	Chaetoceros costatus	279	279	0	0	2	0	0	0
Bacillariophyceae	Chaetoceros debilis	5584	11639	1	0	0	0	3	0
Bacillariophyceae	Chaetoceros similis	17766	17766	0	0	0	2	0	0
Bacillariophyceae	Chaetoceros simplex var calcitrans	2133	2133	0	0	0	0	0	4
Bacillariophyceae	Chaetoceros socialis	85859	85859	0	0	0	0	2	0
Bacillariophyceae	Chaetoceros sp	98	878788	37	0	42	39	38	39
Bacillariophyceae	Chaetoceros subtilis	244	5587	2	0	2	3	0	4
Bacillariophyceae	Chaetoceros wighamii	126263	126263	0	0	0	0	2	0
Bacillariophyceae	Coscinodiscus sp	308	308	0	0	2	0	0	0
Bacillariophyceae	Cyclotella sp	186	15152	5	17	3	3	3	14
Bacillariophyceae	Cyclotella striata	85	85	0	0	0	0	0	4
Bacillariophyceae	Cylindrotheca closterium	1	71631	30	17	20	27	47	21
Bacillariophyceae	Delphineis minutissima	205	904040	8	0	12	7	8	7
Bacillariophyceae	Diatoma tenuis	1	1451	2	17	2	0	0	7
Bacillariophyceae	Ditylum brightwellii	2	1397	6	0	0	8	10	7
Bacillariophyceae	Entomoneis alata	28	28	0	0	0	0	0	4
Bacillariophyceae	Entomoneis sp	466	466	0	0	0	0	2	0
Bacillariophyceae	Eunotogramma dubium	265	1006	1	0	0	3	2	0
Bacillariophyceae	Fragilaria sp	1	1	0	8	0	0	0	0
Bacillariophyceae	Leptocylindrus danicus	1140	1280	1	0	0	2	2	0
Bacillariophyceae	Lithodesmium undulatum	49	4190	1	0	0	0	3	0
Bacillariophyceae	Melosira varians	6599	6599	0	0	0	0	0	4
Bacillariophyceae	Minutocellus polymorphus	15152	60606	1	0	0	0	3	0
Bacillariophyceae	Navicula sp	49	1010	3	0	3	2	2	7
Bacillariophyceae	Nitzschia fruticosa	13636	15152	1	17	0	0	0	0
Bacillariophyceae	Nitzschia longissima	186	186	0	0	0	0	0	4
Bacillariophyceae	Nitzschia sp	1	111111	26	75	22	20	28	25
Bacillariophyceae	Odontella aurita	2	559	5	0	0	5	15	0
Bacillariophyceae	Odontella aurita var. minima	9	427	1	0	0	2	0	4
Bacillariophyceae	Odontella mobiliensis	19	931	1	0	0	0	3	4
Bacillariophyceae	Odontella sinensis	31	62	1	0	0	2	2	0
Bacillariophyceae	Pennales	31	265	1	0	0	5	0	0
Bacillariophyceae	Pennales, width < 10 length < 50 µm	53	538200	38	33	39	41	42	29
Bacillariophyceae	Pennales, width < 10 length > 50 µm	7	54121	12	0	5	10	18	18
Bacillariophyceae	Pennales, width > 10 length < 50 µm	94	1794	1	0	2	0	2	0
Bacillariophyceae	Pennales, width > 10 length > 50 µm	62	62	1	0	0	0	2	4
Bacillariophyceae	Pseudo-nitzschia delicatissima	125	125	0	0	0	0	2	0
Bacillariophyceae	Pseudo-nitzschia delicatissima cf	5587	14141	1	0	0	0	3	0
Bacillariophyceae	Pseudo-nitzschia sp	93	15152	1	8	0	0	2	4
Bacillariophyceae	Rhizosolenia delicatula	2	2793	5	0	0	8	7	4
Bacillariophyceae	Rhizosolenia setigera	31	427	1	0	0	3	0	0
Bacillariophyceae	Rhizosolenia sp	1010	1010	0	0	0	2	0	0
Bacillariophyceae	Skeletonema costatum	62	2787879	66	67	58	75	73	54
Bacillariophyceae	Skeletonema potamos	1010	1095960	12	25	24	12	3	0
Bacillariophyceae	Skeletonema sp	125	639860	12	0	10	8	18	11
Bacillariophyceae	Stephanodiscus sp	30303	30303	0	0	0	2	0	0
Bacillariophyceae	Surirella sp	93	2700	3	0	5	2	0	11
Bacillariophyceae	Thalassionema nitzschioides	1	3259	6	0	2	3	15	7
Bacillariophyceae	Thalassiosira decipiens	23350	23350	0	0	0	0	2	0
Bacillariophyceae	Thalassiosira nordenskioeldii	499	499	0	0	0	0	0	0
Bacillariophyceae	Thalassiosira rotula	2	2	0	0	0	0	2	0
Bacillariophyceae	Thalassiosira sp	1010	16162	1	0	0	2	2	4
Bacillariophyceae	Thalassiosira sp < 30 µm	1	696970	37	58	32	37	45	25
Bacillariophyceae	Thalassiosira sp 30-80 µm	466	739	1	0	0	0	3	0
Chlorophyceae	Actinastrum sp	1	37590	4	42	3	0	0	4
Chlorophyceae	Chlamydomonas sp	10	10152	8	17	15	2	3	14
Chlorophyceae	Chlorophyceae	1	489899	17	83	19	8	8	25

Appendix 7.
Continued.

									PORT
SALINITIES		ALL	ALL	ALL	0.3-1.3	5	15	30	0.3-28.6
CULTURED PHYTOPLANKTON		MIN	MAX	FREQ	FREQ	FREQ	FREQ	FREQ	FREQ
		n=220	n=220	n=220	n=12	n=60	n=60	n=60	n=28
		cells/ml	cells/ml	%	%	%	%	%	%
Chlorophyceae	Chlorophyceae 5-10 µm	51	5051	3	0	2	2	2	11
Chlorophyceae	Crucigenia sp	1616	2030	1	0	0	0	0	7
Chlorophyceae	Gloeotila sp	404	404	0	0	0	0	0	4
Chlorophyceae	Kirchneriella sp	19	20202	2	0	8	0	0	0
Chlorophyceae	Koliella sp	101	30303	4	33	2	0	0	11
Chlorophyceae	Lagerheimia sp	5051	131313	2	17	2	0	0	4
Chlorophyceae	Monoraphidium sp	10	595960	21	92	39	12	2	18
Chlorophyceae	Pediastrum sp	1	1	0	8	0	0	0	0
Chlorophyceae	Scenedesmus sp	1	477273	8	67	10	2	0	11
Chlorophyceae	Tetraedron sp	466	931	1	17	0	0	0	0
Chlorophyceae	Tetrastrum sp	112	34959	2	25	2	0	0	4
Chrysomonadales	Chrysomonadales 0.2-2 µm	101	106061	9	8	8	8	7	18
Chrysomonadales	Chrysomonadales 2-10 µm	1	161616	29	25	19	36	35	25
Chrysophyceae	Apedinella spinifera	133	9286	5	0	3	10	3	0
Cryptophyceae	Cryptophyceae	51	162717	20	0	22	22	13	36
Cryptophyceae	Cryptophyceae < 10 µm	505	119192	11	25	7	10	12	18
Cryptophyceae	Cryptophyceae > 10 µm	1	10101	8	17	7	7	7	11
Cyanophyceae	Chroococcales	20202	20202	0	0	2	0	0	0
Cyanophyceae	Cyanophyceae	1	459596	5	17	8	0	2	11
Cyanophyceae	Cyanophyta	12408	29469	1	0	2	2	0	0
Cyanophyceae	Hormogonales	265	265	0	0	2	0	0	0
Cyanophyceae	Planktothrix sp	3259	3259	0	0	0	2	0	0
Dinophyceae	Gymnodinium, length 10-30 µm	47	47	0	0	0	0	0	4
Dinophyceae	Heterocapsa minima cf	5	50	1	0	0	2	0	4
Dinophyceae	Heterocapsa rotundata	31	1592	2	0	0	0	2	11
Dinophyceae	Heterocapsa triquetra	50	50	0	0	0	2	0	0
Dinophyceae	Peridiniaceae, diameter < 10 µm	1	663	1	0	0	2	2	4
Dinophyceae	Peridiniaceae, diameter 10-30 µm	186	931	1	0	0	0	5	0
Dinophyceae	Peridiniales, diameter < 10 µm	531	796	1	0	0	3	2	0
Dinophyceae	Peridiniales, diameter 10-30 µm	531	749	1	0	0	3	0	0
Dinophyceae	Prorocentrum micans	466	466	0	0	0	2	0	0
Dinophyceae	Prorocentrum minimum	1	2020	3	0	0	3	3	7
Dinophyceae	Prorocentrum triestinum	1	931	1	0	0	2	2	4
Dinophyceae	Scripsiella sp	93	1397	1	0	0	2	0	4
Euglenophyceae	Euglena sp	1	863	2	0	2	3	0	4
Euglenophyceae	Euglenophyceae	476	17864	1	0	0	0	0	7
Euglenophyceae	Eutreptiella sp	205	2538	3	0	0	3	3	11
Euglenophyceae	Trachelomonas sp	1010	1010	0	0	0	2	0	0
Prasinophyceae	Prasinocladus sp	606	606	0	0	0	0	0	4
Prasinophyceae	Prasinophyceae	293	171717	18	8	19	14	15	36
Prasinophyceae	Pyramimonas sp	265	45101	12	0	10	17	17	0
Prasinophyceae	Pyramimonas sp, length < 10 µm	1	30303	16	0	14	22	22	4
Prasinophyceae	Pyramimonas sp, length > 10 µm	853	5051	2	0	2	2	2	4
Prymnesiophycea	Chrysochromulina sp	1	24242	11	17	7	3	15	29
Prymnesiophycea	Prymnesiaceae	51	3030	1	0	2	0	2	0
Unidentifiable	Unidentifiable	113	25913	2	0	0	0	0	18
Unidentifiable	Unidentifiable, diameter < 3 µm	152	1896465	48	92	49	41	40	61
Unidentifiable	Unidentifiable, diameter < 10 µm	265	143262	5	0	7	3	8	0
Unidentifiable	Unidentifiable, diameter 3-10 µm	61	191919	25	83	24	27	13	25
Unidentifiable	Unidentifiable, diameter 10-30 µm	2020	15152	1	0	2	2	0	0
Unidentifiable	Unidentifiable, diameter 30-50 µm	1010	1010	0	0	0	2	0	0
Xanthophyceae	Pseudogoniochloris tripus	186	186	0	0	0	0	0	4

									PORT
SALINITIES		ALL	ALL	ALL	0.3-1.3	5	15	30	0.3-28.6
CULTURED MICROZOOPLANKTON		MIN	MAX	FREQ	FREQ	FREQ	FREQ	FREQ	FREQ
		n=220	n=220	n=220	n=12	n=60	n=60	n=60	n=28
		organisms/ml	organisms/ml	%	%	%	%	%	%
Choreotrichia	Ciliata < 20 µm	9	36364	8	17	10	3	5	18
Choreotrichia	Ciliata 20-40 µm	1	3259	3	0	2	2	3	7
Craspedomonada	Craspedomonadaceae	1	48485	15	17	7	15	12	39
Dinophyceae	Glenodinium danicum	1	1	0	0	0	0	0	4
Protomonadales	Heterotrophic flagellate	133	121507	10	0	8	8	12	18
Protomonadales	Heterotrophic flagellate, length < 10 µ	1	212121	30	33	32	22	25	54
Protomonadales	Heterotrophic flagellate, length > 10 µ	1	60606	20	33	15	14	17	43

Appendix 8.
Information on observed toxic and potentially toxic phytoplankton species.

In this appendix some information is given on the toxic and potentially toxic phytoplankton species found in the ballast water and port water samples and in the ballast water cultures in different media. For each species, observations in ballast water are indicated with (B), in port water with (P) and in cultures with (C). Information on concentrations and frequency of occurrence can be found in the Appendices 5-7.

Toxic phytoplankton species are species with recorded toxic events from field situations. Potentially toxic phytoplankton species are species of which toxic and non-toxic strains are known or species with a variable toxicity. The toxic and potentially toxic phytoplankton species found in the above mentioned samples belonged to the classes Bacillariophyceae (diatoms), Cyanophyceae (bluegreens or cyanobacteria), Dinophyceae (dinoflagellates), Prymnesiophyceae (prymnesiophyceans) and Raphidophyceae (raphidophyceans).

Bacillariophyceae (diatoms)
All planktonic diatoms that have been confirmed to be toxic are marine and pennate and belong to the genus *Pseudo-nitzschia* (Skov et al., 1999). Toxic events associated with diatoms are relatively recent and have appeared since 1987. The causative agent was identified as the neurotoxin domoic acid, causing Amnesic Shellfish Poisoning (ASP). Within 24 hours of eating poisoned mussels, victims develop gastro-intestinal symptoms such as vomiting, diarrhoea, abdominal cramps and gastric bleeding and this may be followed by neurological symptoms such as confusion, loss of memory, coma and even death (cited literature in Skov et al., 1999). The main persistent symptom is loss of memory.
Pseudo-nitzschia sp. (B)(C)
Potentially toxic. *Pseudo-nitzschia* sp. can be *Pseudo-nitzschia australis* or *Pseudo-nitzschia pungens* cf or *Pseudo-nitzschia fraudulenta*. *Pseudo-nitzschia australis* causes (ASP, see above) (Skov et al., 1999), but have never been observed in Dutch waters. *Pseudo-nitzschia pungens* cf: see below. *Pseudo-nitzschia fraudulenta* is not toxic and known from Dutch marine waters.
Pseudo-nitzschia delicatissima (B)(P)(C)
Potentially toxic. Possible presence of toxic strains. Cultured strains of *Pseudo-nitzschia delicatissima* were able to produce the neurotoxin domoic acid, causing ASP (see above) (Skov et al., 1999). Regularly found in Dutch marine waters with concentrations of sometimes more than 100000 cells/l.
Pseudo-nitzschia delicatissima cf (B)(P)(C)
Potentially toxic. *Pseudo-nitzschia delicatissima* cf can be *Pseudo-nitzschia pseudodelicatissima* or *Pseudo-nitzschia delicatissima*. From both species, domoic acid producing strains are known (Skov et al., 1999).
Pseudo-nitzschia pungens cf (B)(P)
Potentially toxic. *Pseudo-nitzschia pungens* cf can be *Pseudo-nitzschia pungens* or *Pseudo-nitzschia multiseries*.
Pseudo-nitzschia pungens has been found to produce domoic acid in culture (Subba Rao et al., 1988; Skov et al., 1999). *Pseudo-nitzschia pungens* occurs regularly in Dutch marine waters. *Pseudo-nitzschia multiseries* is, very rare, known from the Dutch Wadden Sea and an isolated sample was able to produce domoic acid (Vrieling et al., 1996).

Cyanophyceae (bluegreens or cyanobacteria)
Anabaena sp. (P)
Potentially toxic. Possible presence of toxic strains. Regularly occurring in Dutch fresh waters.
Aphanizomenon sp. (B)(P)
Potentially toxic. Possible presence of toxic strains. Regularly occurring in Dutch fresh waters.
Microcystis sp. (B)(P)
Potentially toxic. Possible presence of toxic strains. Regularly occurring in Dutch fresh waters.
Planktothrix agardhii (B)(P)
Potentially toxic. Possible presence of toxic strains. Regularly occurring in Dutch fresh waters.
Planktothrix sp. (B)(P)(C)
Potentially toxic. Possible presence of toxic strains. Regularly occurring in Dutch fresh waters.
Pseudanabaena sp. (B)(P)
Potentially toxic. Possible presence of toxic strains. Regularly occurring in Dutch fresh waters.

Appendix 8.
Continued.

Dinophyceae (dinoflagellates)
Alexandrium ostenfeldii (B)
Toxic. *Alexandrium ostenfeldii* may possibly cause Paralytic Shellfish Poisoning (PSP) in humans. Typical symptoms of PSP are headache, nausea, vomiting, respiratory difficulties and in extreme cases death through respiratory paralysis (Hallegraeff, 1993). Samples isolated from Danish waters appeared to be toxic for tintinnid ciliates Hansen et al. (1992); toxic isolates were also found in New Zealand waters (Mackenzie et al., 1996). In culture experiments, *Alexandrium ostenfeldii* grew at temperatures between 11.3 and 23.7 °C and at salinities between 10 and 40 psu (Østergaard Jensen & Moestrup, 1997). This dinoflagellate is known to occur in Dutch marine waters, but is rare.
Alexandrium tamarense (P)
Toxic. *Alexandrium tamarense* produces PSP (see above) toxins and is toxic to humans, birds and fish (Larsen & Moestrup, 1989). This dinoflagellate causes many problems with respect to mussel culture (Nuzzi & Waters, 1993) in countries all over the world. It is known to occur with low concentrations in Dutch marine waters.
Dinophysis acuminata (B)
Toxic. *Dinophysis acuminata* causes Diarrheic Shellfish Poisoning (DSP) after eating poisoned mussels that consumed this dinoflagellate. The most characteristic symptoms are diarrhoea, nausea, vomiting and abdominal pain (Hallegraeff, 1993). Since 1961, several cases of DSP with gastro-intestinal complaints from consumers were observed in the Oosterschelde and since 1976 also in the Dutch Wadden Sea (Kat, 1983). This dinoflagellate is known from Dutch brackish and marine waters, mostly with low concentrations, and in some years it is even absent. The last problems with *Dinophysis acuminata* in the Dutch Wadden Sea date from 1987 (pers. comm. L. Peperzak).
Dinophysis rotundata (B)
Toxic. From this heterotrophic member of the genus *Dinophysis* toxic Japanese strains are known. Occurs regularly in Dutch brackish and marine waters.
Dinophysis sp. (B)
Toxic. Small *Dinophysis* species that are difficult to determine. Almost certainly contain toxic species. Occurring in Dutch brackish and marine waters.
Karenia mikimotoi (synonyms: *Gyrodinium aureolum*, *Gymnodinium mikimotoi*) (B)
Toxic. This species produces a chemical which attacks cell membranes, especially gill membranes and was originally described in north-eastern USA. This dinoflagellate was observed for the first time in European waters in 1966 with linked mortalities of aquatic organisms (Braarud & Heimdal, 1970). Since then, several cases of mass occurrence have been reported from north European waters, sometimes with mortality of various marine fish and invertebrates (Fig. 3 and Table 1 in Tangen, 1977). The first observations of *Gyrodinium* cf. *aureolum* in the stratified Dutch part of the North Sea date from 1989 and were reported by Peperzak (1990). Shortly after this report *Gyrodinium* cf. *aureolum* was also reported in the Dutch phytoplankton monitoring programme (Rademaker, 1990). During the early nineties, high concentrations were found in the thermocline at offshore stations at the Oystergrounds. After the early nineties, it has occurred now and then with low concentrations in monitoring samples from Dutch marine waters. Recently, this species was renamed *Karenia mikimotoi* (Daugbjerg et al., 2000).
Prorocentrum minimum (B)(C)
Potentially toxic. In the North Sea region, this dinoflagellate was first recorded in The Netherlands in 1976 by Kat (1979). Its appearance in the Dutch Wadden Sea coincided with gastro-intestinal illness in 25 consumers of cooked mussels from the Dutch Wadden Sea. However, a causal relationship between this mussel poisoning and the presence of *Prorocentrum minimum*, could not be confirmed. In 1989 the toxicity of *Prorocentrum minimum* was described as 'Some clones toxic to mice' (Larsen & Moestrup, 1989). In European waters *Prorocentrum minimum* has been associated with some cases of venerupin shellfish poisoning (VSP) (Tangen, 1983; Silva, 1985). A recent study has shown that senescent cultures of *Prorocentrum minimum* can produce toxins (Grzebyk et al., 1997). These authors also discussed the potential risks of human poisoning from the consumption of shellfish harvested during or after toxic blooms of *Prorocentrum minimum*. They conclude that several additional, complex conditions are needed for the development of toxicity in *Prorocentrum minimum* blooms. During the summer months, *Prorocentrum minimum* occurs with densities > 10000 cells/l in Dutch coastal waters.

Appendix 8.
Continued.

Prymnesiophyceae (prymnesiophyceans)
Chrysochromulina sp.(B)(P)(C)
Potentially toxic. May contain toxic species. A massive bloom of *Chrysochromulina polylepis* occurred in 1988 in Danish, Swedish and Norwegian coastal waters and caused the extensive mortality of caged fish and zoobenthos (Kaas et al., 1991). Another *Chrysochromulina* bloom, this time of the species *Chrysochromulina leadbeateri*, occurred in 1991 in Norway and killed 700 tonnes of Atlantic salmon (Tangen, 1991). Regularly found with high densities in Dutch coastal and offshore waters.

Raphidophyceae (raphidophyceans)
Raphidophyceae (P)
Toxic to fish. A number of raphidophycean species has been found regularly in Dutch coastal and offshore waters.

Appendix 9.
Nutrient concentrations in port water samples.

PORT	DATE	TIME	NO3NO2N	NO2N	NH4N	PO4P	SILI	NO3N
			μm	μm	μm	μm	μm	μm
Rotterdam, YVC-werf/Bolnes	03-11-98	no sample						
Vlissingen-Oost, Bijleveldhaven	17-03-99	no sample						
Vlissingen-Oost, Bijleveldhaven	29-03-99	no sample						
Rotterdam, Botlekhaven	28-04-99	no sample						
Rotterdam, Beatrixhaven	01-11-99	no sample						
Rotterdam, Beatrixhaven	01-11-99	no sample						
Rotterdam, Beatrixhaven	12-11-99	no sample						
Rotterdam, Europahaven	09-12-99	1225	92,8	1,8	7,4	2,3	44,5	90,7
Rotterdam, Europahaven	23-12-99	1700	143,5	1,4	7,1	2,4	64,1	142,1
Rotterdam, Europahaven	07-01-00	1000	124,2	1,2	6,9	2,2	59,1	122,8
Rotterdam, Europahaven	19-01-00	1045	183,5	2,8	9,1	2,6	87,2	180,6
Rotterdam, Europahaven	28-01-00	0940	129,2	1,6	8,4	2,1	60,9	127,1
Rotterdam, Europahaven	13-04-00	1400	129,9	1,1	6,2	1,7	47,4	128,5
Rotterdam, Europahaven	14-04-00	1450	129,2	1,1	5,3	1,5	46,3	127,8
Rotterdam, Europahaven	19-04-00	1135	124,9	1,0	5,4	1,5	40,9	124,2
Rotterdam, Europahaven	19-04-00	1400	134,2	1,0	4,9	1,5	43,1	133,5
Amsterdam, Suezhaven	16-05-00	1310	71,3	3,7	6,9	0,2	20,3	67,6
Vlissingen-Oost, Handelskade	31-07-00	1550	85,7	2,1	10,6	3,1	23,0	83,5
IJmuiden, Buitenkade3	02-08-00	1325	59,8	2,6	21,7	2,5	26,5	57,3
IJmuiden, Buitenkade3	07-08-00	1535	49,3	1,6	17,7	1,8	18,8	47,6
Rotterdam, Eerste Petroleumhaven	29-08-00	2100	145,6	1,0	8,2	3,2	68,4	144,9
IJmuiden, Buitenkade3	30-08-00	1055	61,2	5,2	25,3	2,6	33,4	56,0
IJmuiden, Buitenkade3	11-09-00	1440	61,2	4,4	20,6	2,9	39,9	56,8
Rotterdam, Waalhaven	13-09-00	1020	175,6	0,7	5,1	3,3	80,8	174,9
Rotterdam, Brittanniëhaven	21-09-00	1625	75,7	1,4	8,4	2,3	36,7	74,3
Rotterdam, Waalhaven/Werf v/d Brink	06-10-00	1335	183,5	0,9	7,0	3,4	90,4	182,8
Vlissingen-Oost, Handelskade	17-10-00	0920	82,1	1,3	4,2	3,8	34,1	80,7
Rotterdam, Waalhaven	24-10-00	1245	199,2	1,1	5,8	3,1	107,5	198,5
Dordrecht, Julianahaven	11-11-00	1625	194,2	1,6	9,3	3,3	116,8	192,8
Amsterdam, Shipdock	16-11-00	1400	194,2	5,4	22,0	5,7	142,1	188,5