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Potential transfer of aquatic organisms via ballast water with a particular focus on harmful and non-indigenous species: A survey from Adriatic ports

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ABSTRACT

Ballast water discharges may cause negative impacts to aquatic ecosystems, human health and economic activities by the introduction of potentially harmful species. Fifty untreated ballast water tanks, ten in each port, were sampled in four Adriatic Italian ports and one Slovenian port. Salinity, temperature and fluorescence were measured on board. Faecal indicator bacteria (FIB), phyto- and zooplankton were qualitatively and quantitatively determined to identify the species assemblage arriving in ballast water. FIB exceeded the convention standard limits in 12% of the sampled tanks. *Vibrio cholerae* was not detected. The number of viable organisms in the size groups (minimum dimension) < 50 and $\geq 10\,\mu m$ and $\geq 50\,\mu m$ resulted above the abundances required from the Ballast Water Management Convention in 55 and 86% of the samples, respectively. This is not surprising as unmanaged ballast waters were sampled. Some potentially toxic and non-indigenous species were observed in both phyto- and zooplankton assemblages.

1. Introduction

Ships' ballast water (BW) is recognized as one of the primary transport vectors for the global transfer of aquatic organisms including Harmful Aquatic Organisms and Pathogens (HAOP) (Carlton, 1985, 1999; Gollasch, 1996; Ruiz et al., 1997, 1999, 2000a; Hewitt et al., 1999; Hewitt, 2002; Brettar et al., 2007; David et al., 2007; Drake et al., 2007; Nellemann et al., 2008; Seiden and Rivkin, 2014). Although the first study suggesting ships as vectors of non-indigenous species (NIS) was already published in the early 1900s (Ostenfeld, 1908), it was only since the 1980s that the problem of species introductions gained a considerable interest, supported by several studies detailing organisms transfer through BW (Medcof, 1975; Carlton, 1985; Hallegraeff and Bolch, 1991; David et al., 2007; Gollasch et al., 2007). The long-distance transfer of marine organisms facilitates their dispersal across biogeographic barriers that would naturally prevent their spreading (Colautti and MacIsaac, 2004). Introduced organisms can survive in receiving ecosystems but the success of a species survival depends on favorable environmental conditions, appropriate food supply and lack of predation (Carlton, 1996). Economic and ecological impacts of introduced NIS were already observed in the Black Sea, in the Australian off shore waters and in the North American Great Lakes (Nellemann et al., 2008).

Concerning phytoplankton, communities in BW are generally dominated by diatoms and dinoflagellates, including potentially toxic taxa (Burkholder et al., 2007; Kang et al., 2010), which can adversely affect public health and local economies (Hallegraeff and Bolch, 1992). Microalgal vegetative stages have usually a limited survival rate in ballast tanks due to lack of light and nutrients; contrarily, resting stages such as dinoflagellate cysts and diatom spores are supposed to survive for very long time also in severe environmental conditions (Rigby and Hallegraeff, 1994). The zooplankton species abundance and diversity seem to decrease sharply during the first days of a voyage, and only few specimens are able to survive for longer (Gollasch et al., 2000b).

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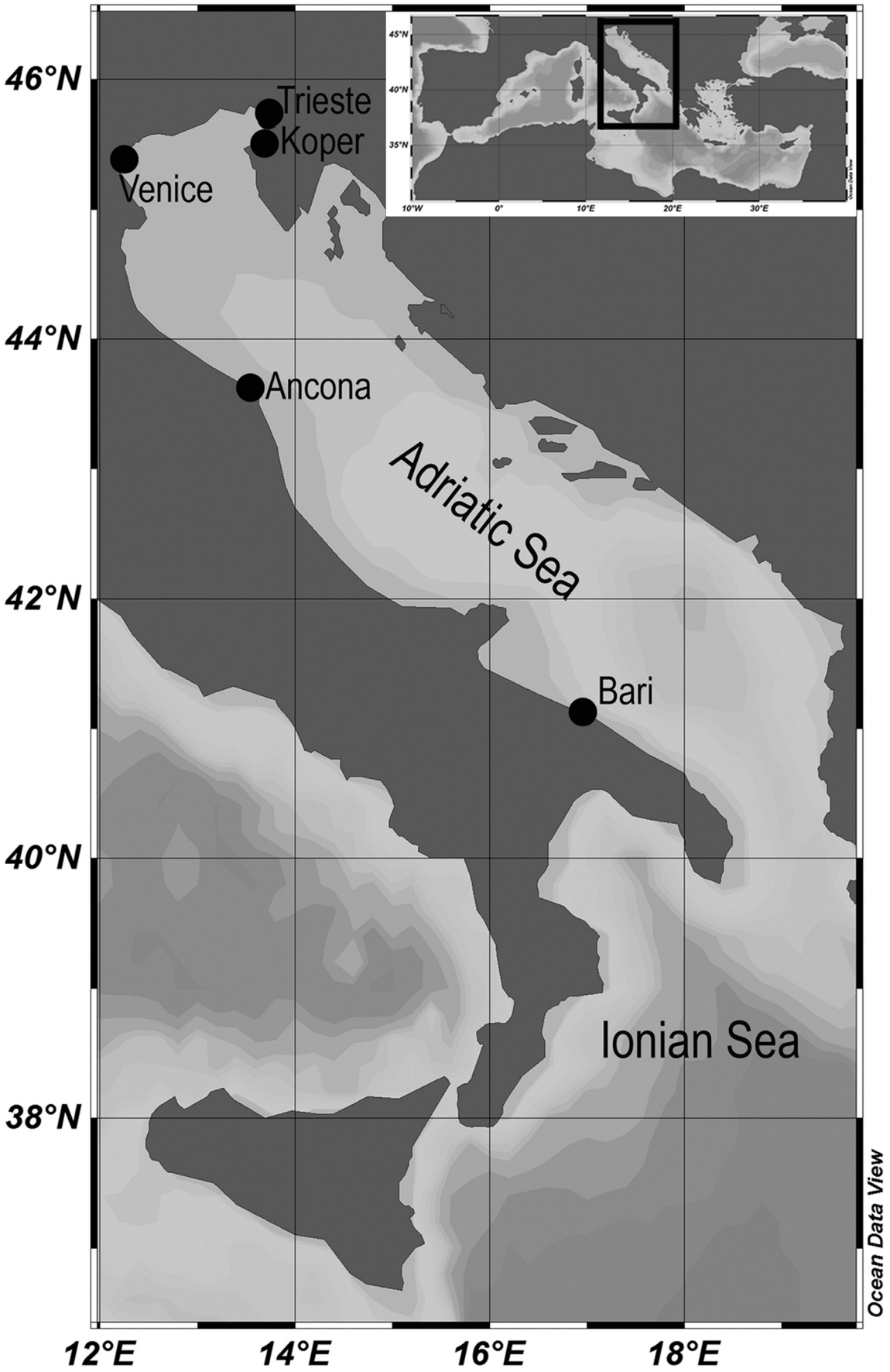

Fig. 1. Map of the study area in the Adriatic Sea (Mediterranean Sea) showing the location of the sampling ports.

Table 1
Details of sampling date, sampling port, ship type, donor port or area, BW donor sea area and days in tanks. IT: Italy; SI: Slovenia; GR: Greece; HR: Croatia; LY: Lybia; AL: Albania; UK: United Kingdom; IL: Israel; DZ: Algeria; nd: not documented.

Tank	Sampling date	Sampling port	Ship type	Donor port/area	BW donor sea area	BW days in tank
A1-EM	11/09/2015	Ancona (IT)	Passenger ship	Patras (GR)	Eastern Mediterranean (EM)	1
A2-MA	16/09/2015	Ancona (IT)	Passenger ship	Split (HR)	Middle Adriatic (MA)	1
A3-NAf	11/09/2015	Ancona (IT)	Container ship	Misrata (LY)	North Africa (NAf)	6
A4-AO	15/09/2015	Ancona (IT)	General cargo	Atlantic Ocean	Atlantic Ocean (AO)	29
A5-NA	14/09/2015	Ancona (IT)	Container ship	Trieste (IT)	North Adriatic (NA)	76
A6-NA	16/09/2015	Ancona (IT)	Passenger ship	Ancona (IT)	North Adriatic (NA)	nd
A7-NA	17/09/2015	Ancona (IT)	Passenger ship	Ancona (IT)	North Adriatic (NA)	nd
A8-NA	17/09/2015	Ancona (IT)	Passenger ship	Ancona (IT)	North Adriatic (NA)	nd
A9-NA	18/09/2015	Ancona (IT)	Container ship	Venice (IT)	North Adriatic (NA)	nd
A10-IS	18/09/2015	Ancona (IT)	Passenger ship	Ionian Sea	Ionian Sea (IS)	nd
B1-SA	30/07/2015	Bari (IT)	Bulk cargo	Durres (AL)	Southern Adriatic (SA)	2
B2-SA	23/07/2015	Bari (IT)	Bulk cargo	Durres (AL)	Southern Adriatic (SA)	3
B3-TS	23/07/2015	Bari (IT)	Container ship	Gioia Tauro (IT)	Tyrrhenian Sea (TS)	7
B4-AO	28/07/2015	Bari (IT)	Bulk cargo	Atlantic Ocean	Atlantic Ocean (AO)	8
B5-TS	30/07/2015	Bari (IT)	Container ship	Gioia Tauro (IT)	Tyrrhenian Sea (TS)	8
B6-NA	11/09/2015	Bari (IT)	Bulk cargo	Ravenna (IT)	North Adriatic (NA)	12
B7-SA	11/09/2015	Bari (IT)	Container ship	Bari (IT)	Southern Adriatic (SA)	18
B8-AO	30/09/2015	Bari (IT)	Bulk cargo	Swansea (UK)	Atlantic Ocean (AO)	20
B9-EM	10/09/2015	Bari (IT)	Container ship	Crete (GR)	Eastern Mediterranean (EM)	42
B10-EM	24/07/2015	Bari (IT)	Container ship	Piraeus (GR)	Eastern Mediterranean (EM)	50
K1-NA	09/12/2015	Koper (SI)	Bulk carrier	Marghera (IT)	North Adriatic (NA)	2
K2-NA	09/12/2015	Koper (SI)	Bulk carrier	Ravenna (IT)	North Adriatic (NA)	2
K3-EM	24/11/2015	Koper (SI)	Container ship	Haifa (IL)	Eastern Mediterranean (EM)	4
K4-NA	07/12/2015	Koper (SI)	Bulk carrier	Chioggia (IT)	North Adriatic (NA)	4
K5-NAf	24/11/2015	Koper (SI)	General cargo	Misurata (LY)	North Africa (NAf)	5
K6-NA	13/01/2016	Koper (SI)	Chemical tanker	Ravenna (IT)	North Adriatic (NA)	6
K7-NA	20/01/2016	Koper (SI)	General cargo	Rijeka (HR)	North Adriatic (NA)	6
K8-MA	03/02/2016	Koper (SI)	General cargo	Split (HR)	Middle Adriatic (MA)	6
K9-NAf	26/11/2015	Koper (SI)	General cargo	Bejaia (DZ)	North Africa (NAf)	7
K10-EM	03/02/2016	Koper (SI)	Container ship	Piraeus (GR)	Eastern Mediterranean (EM)	10
T1-MA	26/05/2015	Trieste (IT)	Ro ro cargo	Middle Adriatic	Middle Adriatic (MA)	1
T2-IS	13/06/2015	Trieste (IT)	Ro ro cargo	Ionian Sea	Ionian Sea (IS)	1
T3-IS	27/06/2015	Trieste (IT)	Ro ro cargo	Ionian Sea	Ionian Sea (IS)	1
T4-SA	09/06/2015	Trieste (IT)	Ro ro cargo	Southern Adriatic	Southern Adriatic (SA)	5
T5-IS	16/06/2015	Trieste (IT)	Ro ro cargo	Ionian Sea	Ionian Sea (IS)	12
T6-IS	06/06/2015	Trieste (IT)	Ro ro cargo	Ionian Sea	Ionian Sea (IS)	16
T7-IS	23/06/2015	Trieste (IT)	Ro ro cargo	Ionian Sea	Ionian Sea (IS)	20
T8-IS	30/06/2015	Trieste (IT)	Ro ro cargo	Ionian Sea	Ionian Sea (IS)	26
T9-IS	20/06/2015	Trieste (IT)	Ro ro cargo	Ionian Sea	Ionian Sea (IS)	28
T10-BS	23/04/2015	Trieste (IT)	Oil tanker	Black Sea	Black Sea (BS)	nd
V1-NA	14/07/2015	Venice (IT)	Container ship	Venice (IT)	North Adriatic (NA)	1
V2-NA	21/07/2015	Venice (IT)	Container ship	Venice (IT)	North Adriatic (NA)	1
V3-EM	28/07/2015	Venice (IT)	Container ship	Piraeus (GR)	Eastern Mediterranean (EM)	2
V4-NA	24/08/2015	Venice (IT)	Container ship	Trieste (IT)	North Adriatic (NA)	2
V5-EM	31/08/2015	Venice (IT)	Container ship	Piraeus (GR)	Eastern Mediterranean (EM)	2
V6-EM	07/07/2015	Venice (IT)	Container ship	Eastern Medit.	Eastern Mediterranean (EM)	11
V7-EM	07/07/2015	Venice (IT)	Container ship	Cyclades (GR)	Eastern Mediterranean (EM)	14
V8-NA	07/09/2015	Venice (IT)	Container ship	Venice (IT)	North Adriatic (NA)	36
V9-EM	07/09/2015	Venice (IT)	Container ship	Piraeus (GR)	Eastern Mediterranean (EM)	65
V10-NA	24/08/2015	Venice (IT)	Container ship	Rijeja (HR)	North Adriatic (NA)	nd

Opportunistic species are apparently able to thrive and propagate in BW tanks under certain conditions. BW tanks may thus serve as incubators for some species depending on their characteristics (Gollasch et al., 2000b).

The International Maritime Organization (IMO) has adopted in 2004 the “International Convention for the Control and Management of Ship's Ballast Water and Sediments” (BWM Convention), aimed to reduce the spread of HAOP among ports and areas, by establishing standards and procedures for the management and control of ships' BW and sediments. The BWM Convention defined HAOP as organisms which, if introduced into the sea including estuaries, or into fresh water courses, may create hazards to the environment, human health, property or resources, impair biological diversity or interfere with other legitimate uses of such areas (IMO, 2004). Accordingly, HAOP include potentially harmful NIS and cryptogenic species, harmful native species and pathogens (David et al., 2013; Gollasch et al., 2015).

As stated in Regulation D-2 of the BWM Convention, the performance standard includes viable organisms above 10 μm and faecal indicator bacteria (FIB) in the BW. In order to avoid the transfer of faecally contaminated water, *Escherichia coli* and intestinal enterococci have been included in the D-2 standard as indicators of microbiological pollution. Among human pathogens, only toxigenic *Vibrio cholerae* (O1 and O139) detection is required. This is likely because *V. cholerae* O1 was responsible for the cholera outbreak in Peru in 1991, which was thought to have occurred due to bacteria release with BW (Tomaru et al., 2014 and references therein). Additionally, according to the D-2 standard, ships should discharge BW containing a limited number of viable organisms in relevant size classes (organisms < 50 μm and greater than or equal to 10 μm in minimum dimension, hereafter 10–50 μm, and organisms greater than or equal to 50 μm in minimum dimension, hereafter > 50 μm) and not only HAOP (Gollasch et al., 2015).

BW exchange (BWE) is currently the measure most widely used for mitigating the transfer of coastal organisms from port to port and it is addressed in Regulation D-1 of the BWM Convention as an *interim* solution. BWE is the replacement of at least 95% (in volume) of the BW of

coastal origin with water from the open sea (i.e. waters with at least 200 m depth and at the distance from the coast of at least 200 or 50 nmi). The implementation of the BWM Convention has particular relevance in the Adriatic basin, where the volume of BWs discharged is estimated to be higher than 10 million tons per year. BWs originate from donor ports all around the world, with the majority being from the Mediterranean, which hosts one of the highest diversities of NIS that can potentially represent a high risk for the Adriatic (David and Gollasch, this issue). For intra-coastal voyages and routes of commercial ships through the central and the northern Adriatic Sea, BWE, in accordance to the provisions of the BWM Convention, is unfeasible due to the shallow basin and limited distances between the shorelines. The BW transferred in intra-Adriatic traffic should therefore be treated using approved ballast water management systems installed on-board. However, if the risk of introduction of HAOP is considered low for a specific voyage between specified ports, an exemption from ballast water management requirements may also be granted in accordance to the regulation A-4. Data on target species present in ports, in the sub-region and in ballast waters are therefore particularly needed for considering the risks posed by ballast water as a vector of HAOP introduction in the Adriatic Sea. This basin is particularly vulnerable to biological introductions because of its geographical conformation, heavy anthropogenic pressures, intense maritime traffic and wide variety of marine and brackish environments. Until now, the only study on biological BW sampling in the Adriatic Sea was performed on vessels in the port of Koper, Slovenia (David et al., 2007).

The present study was conducted to test BW sampling on board of 50 ships in five ports located in the Adriatic Sea, in order to document quantitatively viable organisms, including faecal indicator bacteria, in the BW being discharged in these ports. To support risk assessment studies (see e.g. David et al., this issue), the phyto- and zooplankton organisms were taxonomically identified to contribute further information on harmful and non-indigenous species.

2. Material and methods

2.1. Sampling activities

BW sampling was conducted in the ports of Trieste, Venice, Ancona and Bari in Italy, and in the port of Koper in Slovenia (Fig. 1). Ten ships were sampled in each port. In the Italian ports ships were mainly selected based on their availability to be sampled. In the port of Koper, BWs to sample were selected among those that would have been discharged, preferring the uploaded in the main Mediterranean ports. For each port, samples were labelled with the initial of the sampling port, an increasing number to identify the 10 sampled tanks ordinated according to increasing BW days in tank and the initial of BW donor sea area (Table 1). In each port, different types of ships were sampled, with the exception of the ports of Trieste and Venice where BW samples were only collected on ro ro vessels (and one oil tanker) and container ships, respectively.BW sampling was conducted in the tanks with the shortest in-tank holding time from the targeted donor port. Specifications for each sampled ballast tank are reported in Table 1. A total of 50 ballast tanks were sampled through manholes or sounding pipes, according to the “in-tank” method described in the BALMAS Ballast Water Sampling Protocol for Compliance Monitoring and Enforcement of the BWM Convention and Scientific Purposes (David and Gollasch, 2015).

In each ballast tank of the Italian ports, equal aliquots (2 l) were collected at surface, intermediate and bottom depths, lowering, via manhole, a hand pump with a 20 m hose on the suction side. Aliquots were mixed to obtain a composite sample (6 l, final volume). Temperature, salinity and fluorescence were determined. Here we report temperature and salinity data, obtained by a multiparameter probe (HQ14d, Hach Inc., Loveland, CO, USA). Two subsamples (500 ml) were collected, directly from the composite sample, and kept for viability analysis of 10–50 μm organisms and phytoplankton taxonomy. Samples for the bacteriological analysis were also collected directly from the 6 l representative sample and kept in 0.5–1 l polyethylene bottles (washed with 1 N HCl and rinsed three times with sterile MilliQ water), stored in cooling bags (6 ± 2 °C) and processed within few hours after sampling. For the viability of > 50 μm organisms and zooplankton taxonomic analysis, samples were collected with a plankton net (mesh size 50 μm in diameter) through manholes or by using a plastic hose with a suction hand pump mounted on the sounding pipe. In the latter case, 100 l of BW was pumped up, filtered through the 50 μm mesh plankton net and concentrated to a volume of 1 l. 500 ml were used for the viability of organisms > 50 μm and other 500 ml were fixed with neutralized formalin for the taxonomic analysis.

In the port of Koper, the hose of the hand-pump was gradually inserted into the sounding pipe and 100 l of BW was pumped up and filtered through the 50 μm mesh (diagonal dimension) plankton net. The zooplankton sample collected in to the net's cod-end was rinsed into a bucket with filtered BW and diluted to approximately 5 l with filtered BW to ensure better organisms survival. Every 10 l sampled, 300 ml of BW were collected to gather a time-integrated sample for phytoplankton and microbiological analyses. Temperature, salinity and fluorescence were determined on spot immediately after sampling. Here we only report temperature and salinity data, obtained by a multiparameter probe.

2.2. Bacteriological analyses

Abundances of *Escherichia coli* and enterococci were determined by the membrane filtration (MF) technique. Appropriate sample volumes (10, 50 and 100 ml) were filtered, in three replicates, to concentrate bacterial cells on sterile membrane filters (0.45 μm pore size, diameter 47 mm, purchased from Sartorius or Millipore).

The abundance of faecal coliforms was estimated after incubation of filters on m-FC agar (Merck) plates, at 44.5 °C for 24 h. The production of indole was further used to confirm the presumptive blue colonies to the species *E. coli*.

Abundances of enterococci in Italian samples were estimated using the MF technique described above and filters were incubated on Slanetz-Bartley agar (Merck) plates at 37 °C for 48 h. BW samples collected in the port of Koper were similarly processed, but filters placed onto Slanetz-Bartley plates were incubated first at 35 °C for 4 h and subsequently at 44 °C for 48 h (Oxoid Production).

Typical colonies (pink-reddish, dark red or maroon colonies with 0.5–2 mm in diameter) were counted to estimate presumptive enterococci .

All FIB abundances were reported as Colony Forming Units (CFU) $100\ ml^{-1}$ of water.

The presence of *Vibrio cholerae* in BW samples was investigated in Italian samples using cultivation assays. In contrast, the port of Koper samples were analysed using molecular methods. To perform cultivation assays, water (1 l) was filtered through 0.45 μm pore size acetate-cellulose membranes of Millipore or Sartorius, subsequently incubated in 250 ml of alkaline peptone water (APW) at 36 ± 1 °C. After 18 h (no longer than 24 h), 1 ml of enrichment broth, taken from the surface layer, was diluted in 9 ml of APW and incubated at 42 ± 1 °C for 18–24 h. In all samples, to isolate *Vibrio* strains, a spoonful of enrichment broth was streaked onto thiosulfate-citrate-bile-sucrose (TCBS, Merck) agar and maintained at 37 °C for 24 h. The preliminary selection of the strains has been performed on the basis of colony morphology and sucrose utilization on TCBS. Sucrose-positive (*sac+*) strains forming smoothly colonies with 2–3 mm of diameter, were purified and cultured on 1% NaCl Tryptone Soy Agar (TSA, Oxoid). The strains presumptively identified as *V. cholerae* were subjected to biochemical identification using commercially available miniaturized systems API 20E and API 20NE (bioMérieux). When isolated, presumptive toxigenic *V. cholerae* should be serologically characterized by certified laboratories through assays in compliance with official methods.

2.3. Detection of toxigenic Vibrio cholerae through molecular analyses

The toxigenic *Vibrio cholerae*, serotypes O1 and O139, were analysed in BW samples collected in the port of Koper using molecular methods. BW samples (1 l) were filtered onto 0.2 μm polyethersulfonic filters (47 mm diameter, PALL). Filters were stored at −80 °C, until total bacterial community DNA extraction was performed as described by Boström et al. (2004), with slight modifications: DNA was precipitated at −20 °C for 1 h, with 0.1 volume of sodium acetate (3 M NaAc, pH 5.2) and 0.6 volume of isopropanol, the pellet was washed with 70% ice-cold ethanol and dried in a vacuum concentrators. Precipitated DNA was re-suspended in 0.02 μm prefiltered 1× TE buffer and kept at −20 °C. The extracted DNA was measured using the Qubit® dsDNA HS Assay Kit and Qubit fluorometer, according to manufacturer's instructions. Multiplex polymerase chain reaction assays were performed using O1– and O139-*rfb* specific primer sets (Sigma-Aldrich). The *V. cholerae* O139– *rfb* specific primers used were O139F[5'-AAGCCTCTTTATTAC GGGTGG-3′] and O139R[5′-GTCAAACCCGATCGTAAAGG-3′]; the *V. cholerae* O1– *rfb* specific primers used were O1 F [5′-TGGTTTCACTGA ACAGATGGG-3′] and O1 R [5′-AGGTCATCTGTAAGTACAACATTC-3′] (Hoshino et al., 1998 with some modifications). The amplification with the two primer pairs was performed simultaneously in 0.2-ml microcentrifuge tubes. DNA samples (2 μl) were added to the PCR mixture in a 25-μl final mixture volume containing 1× Tris-KCl, 1.5 mM $MgCl_2$, 200 μM dNTP mixture, 0.28 μM *rfb*-O1 F, 0.28 μM *rfb*-O1 R, 0.28 μM *rfb*-O139 F, 0.28 μM *rfb*-O139 R and 1.25 U of Taq polymerase (Fermentas). The amplification condition used was 10 min at 94 °C for denaturation of DNA, followed by 35 cycles, each consisting of 30 s at 94 °C, 30 s at 57 °C, 30 s at 72 °C, with a final incubation at 72 °C for 10 min. The size and quality of the PCR products was confirmed by agarose gel electrophoresis performed in 2% agarose gel in 1× TAE buffer, running for 45 min at 60 V. The PCR products were compared to 100 bp ladder (Biotools). Bands were visualized with UV transillumination (BioDocAnalyze Gel documentation system, Biometra). The expected size was 192 bp for *rfb*-O1 amplicon and 449 bp for *rfb*-0139 amplicon. At the same time, the positive and negative control for *V. cholerae* serotypes O1 and O139 were analysed.

2.4. Viability analyses

For the viability analyses, living organisms were differently treated depending on their size (10–50 μm and > 50 μm organisms). Cells of 10–50 μm were stained with the viability fluorescein diacetate (FDA from Aldrich) stain following Adams et al. (2014). Only fluorescently green cells, indicating intact cell membrane and metabolic activity, were classified as living and counted (Gollasch et al., 2015 and references therein). From two to four replicates for each sample were observed with an epifluorescence microscope (blue filter, excitation at 450–490 nm, emission at N515 nm) using a Sedgewick-Rafter chamber and observing the half or whole chamber.

For > 50 μm organisms, subsamples of 500 ml were completely analysed in a Bogorov chamber using a stereomicroscope to count moving organisms (David and Gollasch, 2015).

2.5. Taxonomic analyses of phytoplankton and zooplankton

For phytoplankton taxonomic analyses, a subsample was immediately fixed with Lugol solution (1% final concentration) or with neutralized formaldehyde (2% final concentration). The analyses were carried out using the sedimentation method (Utermöhl, 1958; Zingone et al., 2010) using an inverted microscope equipped with phase contrast. A variable volume of seawater (25–50 ml) was settled depending on cell concentrations. Random fields or transects were analysed at a magnification of 400× for nanoplankton, while the half or whole chamber were examined at a magnification of 200× for less abundant microplankton. The identification was performed to the lowest possible taxonomic level and heterotrophic species and/or genera were also included.

For zooplankton taxonomic analysis, a subsample was concentrated on a mesh of 20 μm and fixed in ethanol > 70% (Trieste, Venice and Ancona samples) or formaldehyde at 4% (Bari samples) or 1.5% (Koper samples) final concentration. The identification of organisms was performed using a stereomicroscope at 100× magnifications to the lowest possible taxonomic level.

2.6. Data analysis

A data correlation analysis was performed to characterize relationships between viability, number of taxa and BW residence time in the tank. The same correlation was applied to FIB abundances and BW residence time.

To analyse the spatial pattern of the community structure, a non-metric Multi-Dimensional Scaling (nMDS) (Kruskal and Wish, 1978) ordination method was performed using phyto- and zooplankton group-samples matrix and Bray-Curtis similarity, after a square root or log (X + 1) transformation of the abundance data. In the nMDS plot, the normalized (z-standardization) environmental variables were fitted as supplementary variables (vectors) onto ordination spaces to investigate their effects on the community structure, using a Euclidean distance matrix for physical-chemical data.

3. Results

3.1. Ballast water origin

Most sampled ships were mainly ballasted with waters from Mediterranean areas (92%), while very few ships contained BW from outside the Mediterranean Sea (8%) (Table 1). In the ports of Ancona and Koper, half of the sampled ships contained BW originating from the northern Adriatic whereas the remaining came from the Ionian Sea, middle Adriatic and Atlantic Ocean as well as from the middle Adriatic, eastern Mediterranean and North Africa, respectively. In the port of Bari, the sampled BW had more diverse origin, from the northern and southern Adriatic, Tyrrhenian Sea, eastern Mediterranean Sea and Atlantic Ocean. Finally, BW sampled in the port of Trieste came predominantly from the Ionian Sea (and from the Black Sea, middle Adriatic and southern Adriatic), while in the port of Venice half was from the eastern Mediterranean Sea and the other half from the northern Adriatic. All ships had a Ballast Water Management Plan (BWMP), but no ship had a BW treatment system aboard. The holding time of BW in-tank varied between 1 and 76 days: 54% of BW was kept on board within tanks from 1 to 10 days, while only 10% of the BW had > 30 residence days (Table 1).

3.2. Physico-chemical parameters

The BW temperature ranged from 10.6 °C (K7-NA) to 30.4 °C (V2-NA) (Fig. 2, Table 2). The majority of ships' tanks (24) ranged from 20 to 25 °C (on average, 23.7 ± 1.5 °C), 15 had rather high BW temperatures (on average 26.8 ± 1.6 °C), while the last 11 (all in the port of Koper and one in the port of Trieste) showed the lowest tank water temperatures (on average, 12.7 ± 2.0 °C) (Table 2).

BW salinity ranged from 10.2 (A4-AO) to 40 (B7-SA) (Fig. 2, Table 2). For most ships (36) BW had a typical seawater salinity (on average, 36.9 ± 0.9), but 10 BW samples had salinity values lower than 35 (on average, 33.0 ± 1.4). Two samples, V6-EM and B7-SA, showed very high values (39.0 and 40.0) whereas other two samples, A4-AO and T10-BS, had a very low salinity (10.2 and 22.5, respectively).

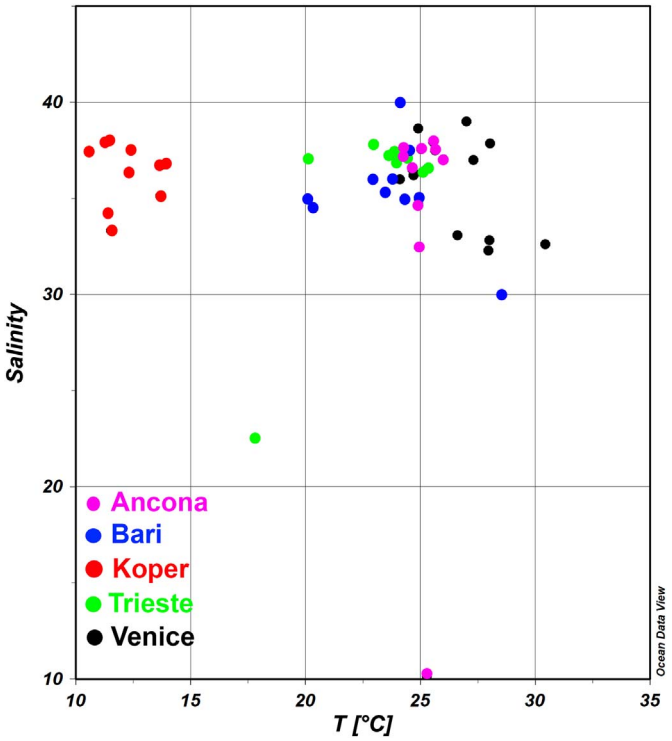

Fig. 2. Temperature and salinity measured in ten BW tanks in each port.

3.3. Faecal indicator bacteria and Vibrio cholerae

Escherichia coli was detected in 19 samples out of all, ranging between 1 and 330 CFU 100 ml^{-1} (Fig. 3 a). High *E. coli* abundance was only found in one BW sample (B3-TS) originated in the Tyrrhenian Sea and collected in the port of Bari (Table 1).

Presumptive enterococci were detected in 27 samples out of all, varying between 1 and 800 CFU 100 ml^{-1} (Fig. 3 b). High abundances were documented in six BW samples, coming from different coastal areas of the Mediterranean Sea, i.e., the Tyrrhenian Sea (B3-TS), northern and middle Adriatic (K2-NA and A2-MA, respectively), and Mediterranean coast of North Africa (K5-NAf), as well as from the Atlantic Ocean (A4-AO and B8-AO) (Table 1).

Toxigenic *Vibrio cholerae* was not detected, neither through cultivation nor by the use of multiplex PCR method.

3.4. Number of viable organisms

Viable organisms in the size range of 10–50 μm were recorded in all tanks (Fig. 4a). In 23 tanks the number was lower than the threshold (10 cells ml^{-1}) proposed in the D-2 standard, with a mean value of 4.7 viable cells ml^{-1}. In the remaining samples numbers ranged from 13 to 92.5 cells ml^{-1} (Fig. 4a).

Concerning organisms > 50 μm in size, only in 7 ballast tanks the numbers of viable organisms resulted below the D-2 standard threshold (10 ind. m^3) (Fig. 4b), with a mean value of 1.4 ind. m^{-3}. In most tanks (42) the values were higher than the standard threshold, ranging from 15 to 32,662 ind. m^{-3}.

3.5. Phytoplankton

For some tanks (A7-NA, A10-IS, T1-MA and T10-BS), phytoplankton analysis was biased by a large amount of organic and inorganic matter.

Phytoplankton abundance was highly variable ranging from 0.08×10^3 (B10-EM) to 12.4×10^6 (V2-NA) cells l^{-1} (Fig. 5a). Tanks with the highest phytoplankton abundance (on average, $1.7 \pm 3.6 \times 10^6$ cells l^{-1}) were sampled on ships docked in the ports of Trieste and Venice containing BW mainly originating from the northern Adriatic Sea, the eastern Mediterranean and the Ionian Sea. Tanks on ships docked in the port of Bari recorded the lowest phytoplankton abundance (on average, $3.8 \pm 3.7 \times 10^3$ cells l^{-1}) whereas in Ancona and Koper, intermediate values of phytoplankton abundances ($6.7 \pm 8.7 \times 10^4$ cells l^{-1}) were detected (Fig. 5a).

The phytoplankton assemblages were mainly dominated by diatoms and nanoflagellates (on average, 48.5 ± 37.6% and 39.4 ± 38.1%, respectively) (Fig. 5b). Dinoflagellates and coccolithophores accounted for 3.4 ± 7.0 and 5.7 ± 19%, respectively. A total of 141 taxa were recorded in ballast tanks, among these 63 diatoms (45 identified at the species level), 33 dinoflagellates (25 identified at the species level), 2 chlorophytes (1 at the species level), 1 chrysophyte at the species level, 3 coccolithophores (2 at the species level), 2 cryptophytes (1 at the species level), 1 dictyochophyte species, 1 euglenophyte species, 4 prasinophytes, 1 prymnesiophyte, 2 ebridian zooflagellates and 1 choanoflagellate, undetermined nanoflagellates and undetermined phytoplankton (Table 3).

Table 2
Temperature and salinity values in each tank.

Tank	Temperature (°C)	Salinity
A1-EM	24.4	37.2
A2-MA	24.3	37.5
A3-NAf	25.0	37.6
A4-AO	25.3	10.2
A5-NA	24.9	34.8
A6-NA	25.6	37.5
A7-NA	24.7	36.5
A8-NA	26.0	37.0
A9-NA	24.9	32.5
A10-IS	25.6	37.9
B1-SA	23.8	36.0
B2-SA	24.3	35.0
B3-TS	23.5	35.3
B4-AO	20.3	34.5
B5-TS	22.9	36.0
B6-NA	24.9	35.0
B7-SA	24.1	40.0
B8-AO	28.5	30.0
B9-EM	24.5	37.5
B10-EM	20.1	35.0
K1-NA	12.3	36.3
K2-NA	11.4	34.2
K3-EM	13.7	35.1
K4-NA	12.4	37.5
K5-NAf	13.9	36.8
K6-NA	11.5	33.3
K7-NA	10.6	37.4
K8-MA	14.4	38.0
K9-NAf	13.7	36.7
K10-EM	11.4	37.9
T1-MA	20.1	37.1
T2-IS	25.1	36.4
T3-IS	25.3	36.6
T4-SA	23.7	37.2
T5-IS	24.0	36.9
T6-IS	24.2	37.3
T7-IS	23.0	37.8
T8-IS	24.0	37.4
T9-IS	24.4	37.2
T10-BS	17.8	22.5
V1-NA	27.9	32.3
V2-NA	30.4	32.6
V3-EM	28.0	37.9
V4-NA	24.0	36.0
V5-EM	27.3	37.0
V6-EM	27.0	39.0
V7-EM	28.0	32.8
V8-NA	26.6	33.1
V9-EM	24.9	38.6
V10-NA	24.7	36.2

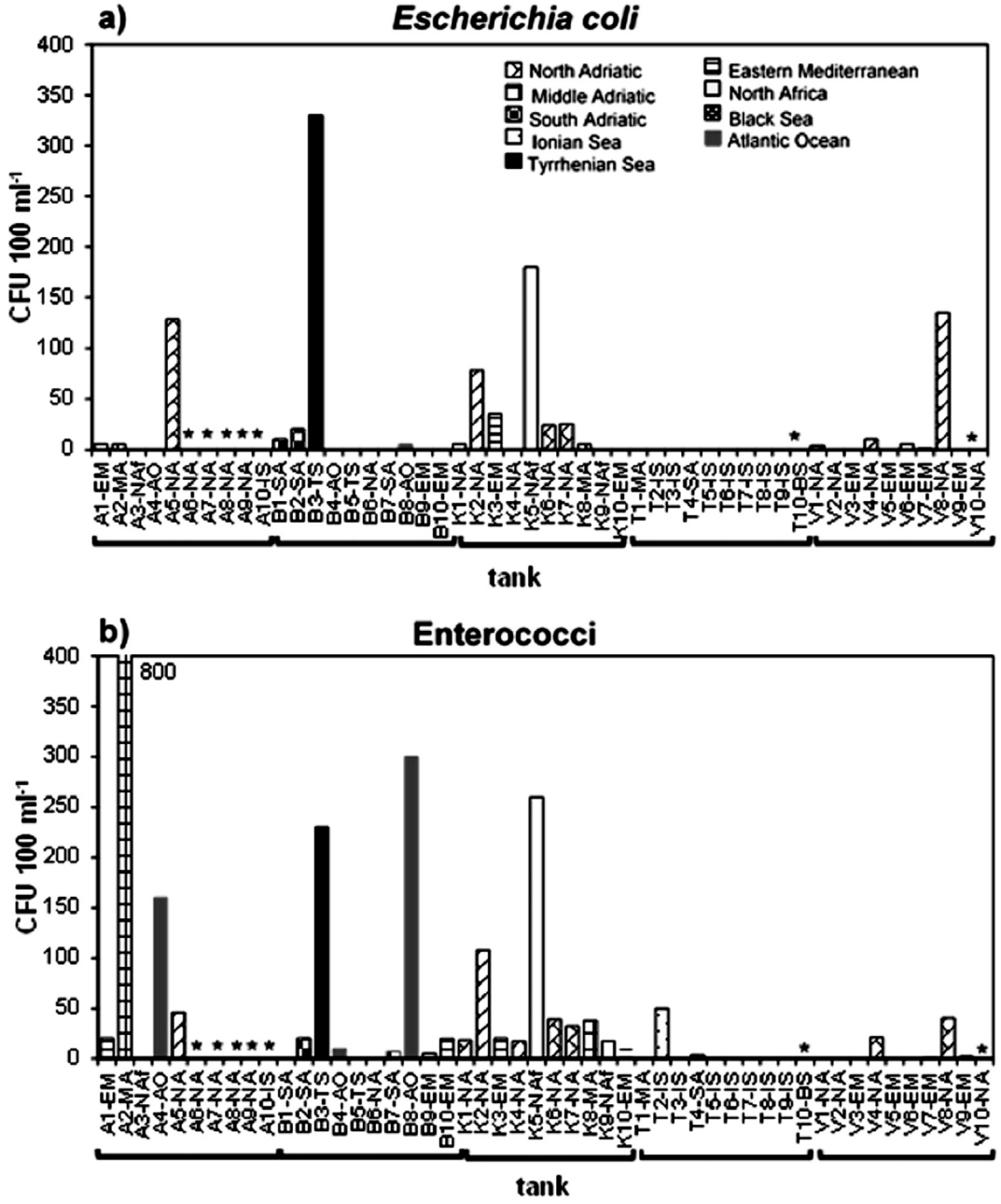

Fig. 3. Abundances of *Escherichia coli* (a) and enterococci (b) with the indication of BW donor sea area. The data are grouped for ports and arranged in ascending order of ballast water days in tank, proceeding from left to right within each port. Asterisks indicate the absence of information about the BW days in tank.

Among diatoms, the most abundant taxa, *Chaetoceros* spp., cf. *Leptocylindrus* sp., *Skeletonema* spp. and undetermined diatoms, were also the most frequently found in the samples with the exception of cf. *Leptocylindrus* sp., found in only 11% of samples. Other taxa, such as *Cerataulina pelagica*, *Cylindrotheca closterium*, *Pseudo-nitzschia* spp., *Thalassionema* spp. and *Thalassiosira* spp., were present in many tanks but in low abundances (Table 3).

Among nanoflagellates, the most abundant taxa were cryptophytes (up to 4.5×10^5 cells l^{-1}) and small (< 10 μm) unidentified forms (4.4×10^6 cells l^{-1}). All the other groups belonging to chlorophytes, chrysophytes, coccolithophores, dictyochophytes, euglenophytes, prasinophytes, prymnesiophytes, ebridian flagellates and choanoflagellates have been observed with low abundances (Table 3).

Dinoflagellates were mainly found in the BW sampled in the ports of Trieste and Koper. *Prorocentrum* and *Tripos* species along with undetermined forms were the dominant taxa in the port of Trieste, while *Protoperidinium* spp. and undetermined species were the most frequent in the port of Koper.

Coccolithophores, represented by *Calciosolenia murrayi*, *Emiliania huxleyi* and undetermined taxa, were only recorded in the BW sampled in the port of Koper.

Six potentially harmful taxa, *Pseudo-nitzschia* spp., *Alexandrium minutum*, *Dinophysis caudata*, *D. sacculus*, *Noctiluca scintillans* and *Prorocentrum* cf. *cordatum*, were identified in this study (Table 3). Among diatoms, different undetermined species belonging to the potentially toxic genus *Pseudo-nitzschia* were commonly present in all ballast tanks except those containing water from the Atlantic Ocean. Their maximum abundance was recorded in BW originating from the northern Adriatic (on average, 9.7×10^3 cells l^{-1} and up to 1.0×10^5 cells l^{-1} in A8-NA). One NIS, which is also potentially toxic, *P. multistriata*, was detected in four BW samples (V2-NA, K9-NAf, K2-NA and K10-EM) with a maximum abundance of 4.6×10^3 cells l^{-1} in V2-

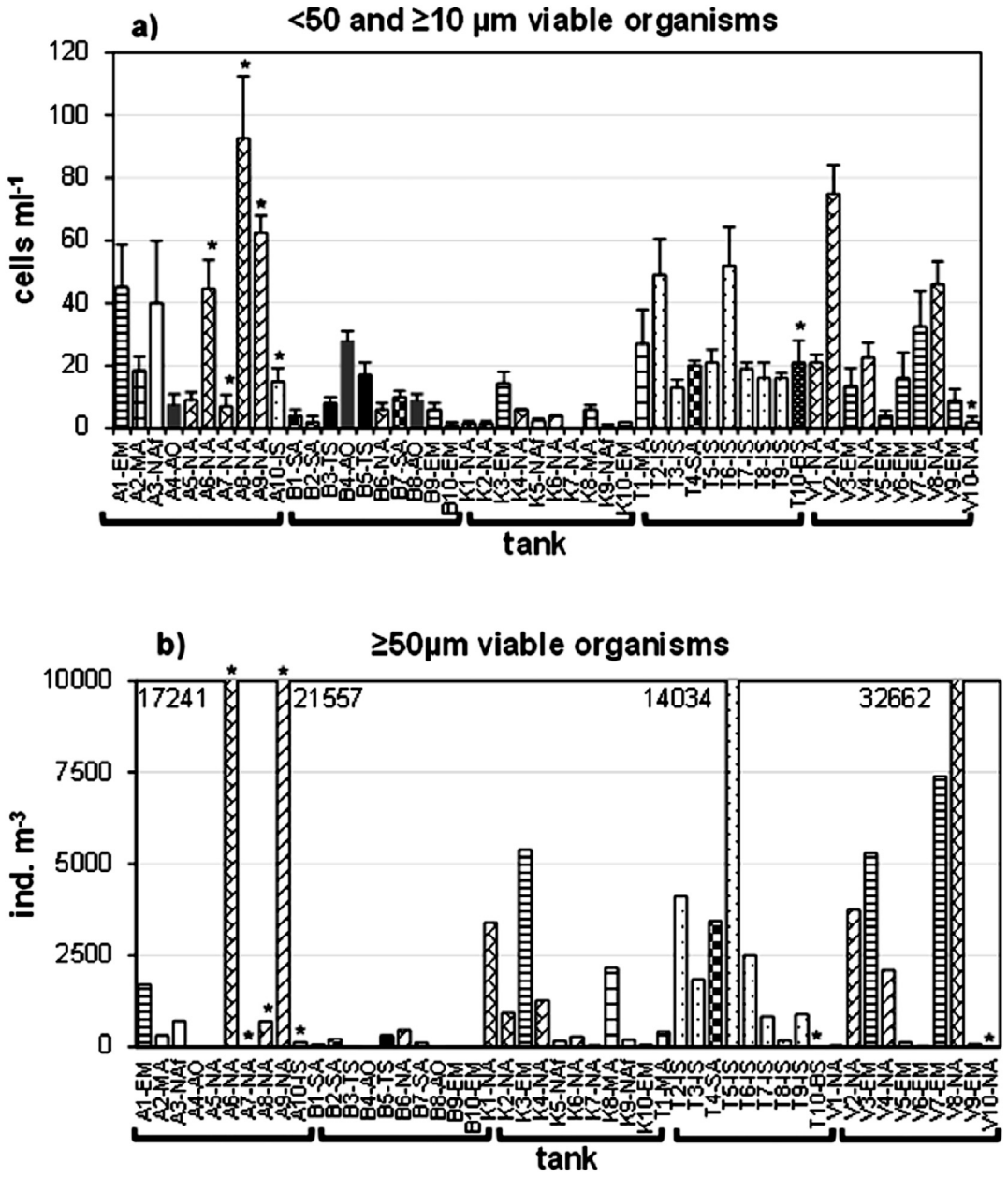

Fig. 4. Abundances of viable cells by size, < 50 and ≥10 µm (mean ± SD, $n = 4$ for Ancona, Trieste and Venice, $n = 2$ for Bari and Koper) (a) and ≥50 µm (b) organisms, with the indication of BW donor sea area, in each ballast tank. The data are grouped for ports and arranged in ascending order of ballast water days in tank proceeding from left to right within each port. Asterisks indicate the absence of information about BW days in tank.

NA. Among potentially toxic dinoflagellates, *P.* cf. *cordatum* was the most abundant species (up to 4.4×10^3 cells l^{-1} in T2-IS), found in the BW sampled in the ports of Trieste and Ancona with BW originating from the northern Adriatic and Ionian Sea, while the other potentially toxic dinoflagellates were only sporadically found in the ports of Ancona, Venice, Trieste, Bari and Koper as reported in the Table 3. None of the recorded species was in bloom conditions.

3.6. Zooplankton

Zooplankton was absent in 4 out of 50 tanks (A4-NA, A7-NA, B10-EM, V10-NA). Where present, abundances ranged from 5 ind. m^{-3} (B9-EM) to 2.0×10^5 ind. m^{-3} (V1-NA) (Fig. 6a). These abundances were very variable probably due to the condition and origin of ballast waters. Tanks with the highest zooplankton abundance were sampled on ships docked in the port of Venice (on average, $5.2 \pm 7.3 \times 10^4$ ind. m^{-3}). Intermediate values were detected in the BW sampled in ports of Ancona and Trieste (on average, $0.8 \pm 0.7 \times 10^4$ ind. m^{-3}), with ballast water that mainly arrived from the Ionian Sea, eastern Mediterranean Sea and northern Adriatic Sea. In ports of Bari and Koper, the lowest zooplankton abundance was documented (on average, $0.9 \pm 0.9 \times 10^3$ ind. m^{-3} and $0.5 \pm 0.6 \times 10^3$ ind. m^{-3}, respectively) (Fig. 6a).

The taxonomic analysis indicated the presence of typically neritic species that for the most part are already present in the Adriatic Sea according to the origin of ballast waters in which the organisms have survived at the adapt environmental conditions. Copepods dominated the community followed by other taxa, including Mollusca, Anellida, Echinodermata, Hydrozoa, Malacostraca, Chaetognata, Tunicata, Vertebrata and Cladocera (Fig. 6b). A total of 82 taxa were identified: among the Cladocera 3 species and 1 genus, 50 taxa of Copepoda (33 to species level) and 28 other taxa (Table 3). In terms of frequency, the

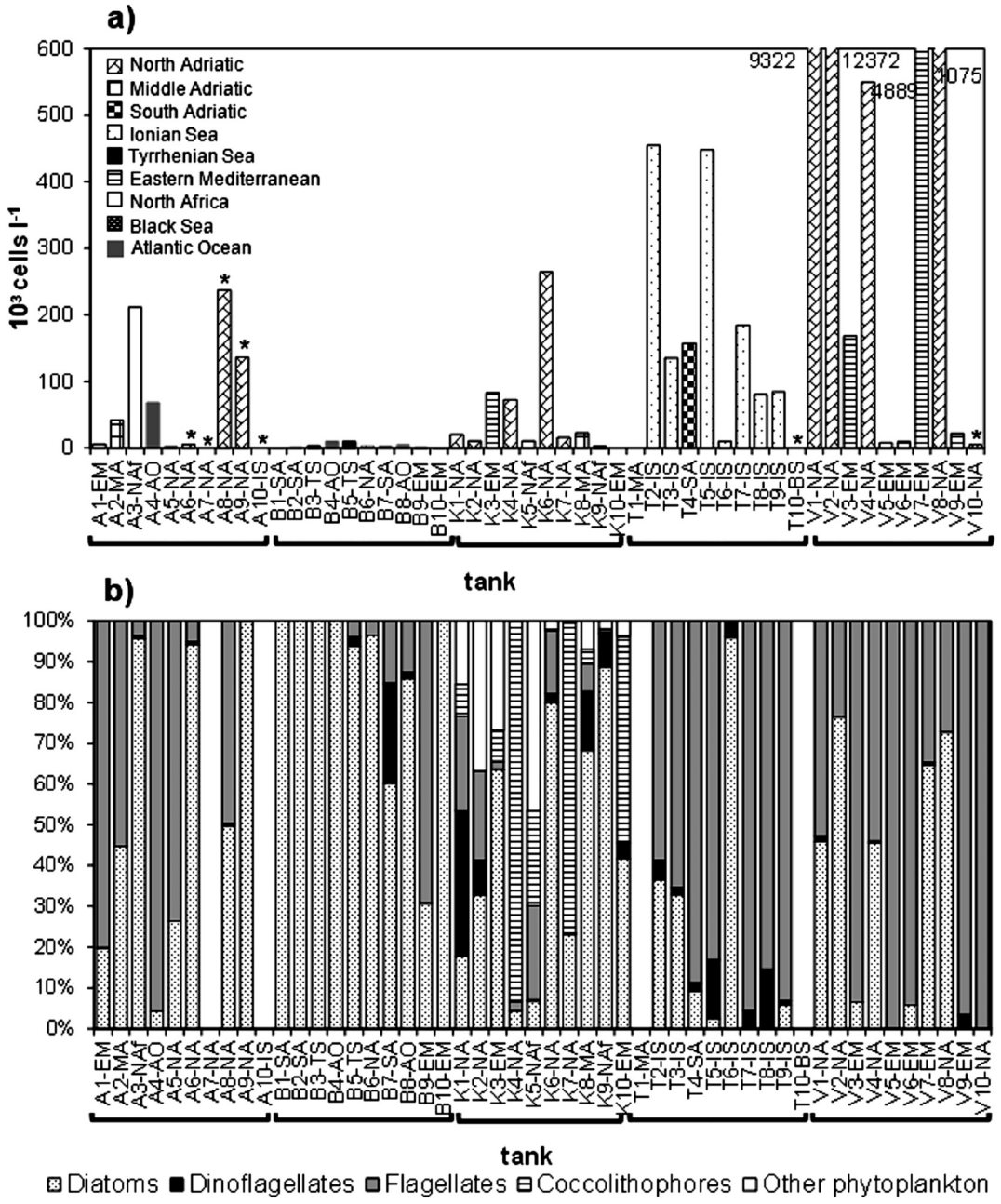

Fig. 5. Abundances of total phytoplankton with the indication of BW donor sea area (a) and percentage of main phytoplankton groups (b) in each ballast tank. The data are grouped for ports and arranged in ascending order of ballast water days in tank proceeding from left to right within each port. Asterisks indicate the absence of information about BW days in tank.

cyclopoid *Oithona nana* accounted for 80% of the samples, the Harpacticoida undetermined for 67%, *Euterpina acutifrons* for 50% and the copepod *Acartia clausi* for 33%, respectively (Table 3 and Fig. 6b). Bivalves, polychaete larvae and copepod nauplia were present in the 61%, 39% and 56% of tanks, respectively (Table 3). All these taxa were present in tanks of all analysed ports and in ships coming from several different areas. The harpacticoid copepod *Tisbe battagliai*, recorded in 15% of samples (maximum value 1.2×10^4 ind. m^{-3} in T4-SA) and its copepodite stages showed remarkably high values of abundance, particularly in tanks of the ships docked in the port of Trieste.

Six NIS were identified, three of which never observed in the Adriatic Sea. The copepod *Calanus euxinus* was observed only in the BW from the Black Sea (T10-BS), where reached the abundance of 78 ind. m^{-3}; the copepod *Oithona brevicornis* was detected in 28% of sampled BW, reaching the highest value (2.3×10^4 ind. m^{-3}) in V1-NA sample, originated in North Adriatic Sea; *Acartia* (*Odontoacartia*) *erythraea* was observed only in V41- KP1, originated in the port of Haifa (Israel), reaching an abundance of 145 ind. m^{-3}. NIS previously documented in the Adriatic Sea were *Paracartia grani*, *Acartia (Acanthacartia) tonsa* and *Pseudodiaptomus marinus*. *P. grani* was detected in 7% of the samples, reaching the highest abundance (159 ind. m^{-3}) in A6-NA sample. *A.* (*Acanthacartia*) *tonsa* was found in 17% of BW samples and the maximum value of abundance (5.9×10^4 ind. m^{-3}) characterized V2-NA sample. *P. marinus,* was detected in 28.3% of samples and the highest abundance (705 ind. m^{-3}) was observed in V1-NA sample.

3.7. Data analysis

The FIB abundance, viable 10–50 and > 50 μm organisms, and taxa number (both for phyto- and zooplankton) were regressed against BW age (Fig. 7).

Table 3
List of phytoplankton and zooplankton taxa identified in the BW with the indication of their maximum abundance (Max; expressed in cells l^{-1} and ind. m^{-3} for phyto and zooplankton, respectively) in all ships and frequency (Fr %; number of times the taxa occurred in samples). NA: north Adriatic; MA: middle Adriatic; SA: south Adriatic; IS: Ionian Sea; TS: Tyrrhenian Sea; EM: eastern Mediterranean; NAf: north Africa; BS: Black Sea; AO: Atlantic Ocean; A: Ancona; B: Bari; K: Koper; T: Trieste; V: Venice. In bold, the potentially toxic species. The asterisk indicates a non-indigenous species.

Taxon	BW donor sea area	Sampling port	Max (cells l^{-1}/ ind. m^{-3})	Fr (%)
Phytoplankton				
Diatoms				
Asterionellopsis glacialis (Castracane) Round, 1990	NA, MA	K	240	4.3
Bacteriastrum cf. *parallelum* Sarno, Zingone & Marino, 1997	IS	T	1484	2.2
Bacteriastrum spp. Shadbolt, 1854	MA, NA, EM, IS	A, K, T	742	8.7
Cerataulina pelagica (Cleve) Hendey, 1937	MA, NA, TS, EM, IS	A,B, K, T, V	1120	37.0
Chaetoceros affinis Lauder, 1864	TS, MA	B, K	700	4.3
Chaetoceros anastomosans Grunow, 1882	IS	T	480	2.2
Chaetoceros compressus Lauder, 1864	TS	B	400	2.2
Chaetoceros curvisetus Cleve, 1889	IS, NA	T, V	7000	4.3
Chaetoceros danicus Cleve, 1889	NA	V	80	2.2
Chaetoceros cf. *danicus* Cleve, 1889	NA	K	80	4.3
Chaetoceros decipiens Cleve, 1873	NA, NAf, MA	K	1000	8.7
Chaetoceros diversus Cleve, 1873	MA,	A	120	2.2
Chaetoceros didymus Ehrenberg, 1845	NA, EM	A,K	560	6.5
Chaetoceros peruvianus Brightwell, 1856	MA, NA, SA, NAf	A, B, K	80	10.9
Chaetoceros pseudocurvisetus Mangin, 1910	NA	K	120	2.2
Chaetoceros simplex Ostenfeld, 1902	SA,	B	240	2.2
Chaetoceros socialis Lauder, 1864	MA,	K	60	2.2
Chaetoceros spp. Ehrenberg, 1844	EM, MA, NA, Naf, IS, SA	A, K, T, V	2,406,328	52.2
Chaetoceros throndsenii (Marino, Montresor & Zingone) Marino, Montresor & Zingone, 1991	NA	V	1484	2.2
Cocconeis spp. Ehrenberg, 1837	NA	K	120	2.2
Coscinodiscus spp. Ehrenberg, 1839	AO, SA, NA	B, K	10,200	8.7
Cyclotella spp. (Kützing) de Brébisson, 1838	MA, NAf, IS, SA, EM	A, T, V	80,878	10.9
Cylindrotheca closterium (Ehrenberg) Reimann & Lewin, 1964	EM, NA, SA, TS, AO, NAf, MA, IS	A, B, K, T, V	3000	54.3
Dactyliosolen blavyanus (Peragallo) Hasle, 1975	MA, NA	A	40	4.3
Dactyliosolen fragilissimus (Bergon) Hasle, 1996	MA, NA, IS, SA, EM	A, K, T, V	11,520	23.9
Dactyliosolen phuketensis (Sundström) Hasle, 1996	MA, NA, NAf	A, K	9440	8.7
Diploneis spp. (Ehrenberg) Cleve, 1894	NA	K	120	10.9
Ditylum brightwellii (T.West) Grunow, 1885	NA	K	60	2.2
Entomoneis sp. Ehrenberg, 1845	EM	V	120	2.2
Eucampia spp. Ehrenberg, 1839	EM	K	40	2.2
Guinardia flaccida (Castracane) Peragallo, 1892	NA, MA, IS	A, K, T, V	440	8.7
Guinardia striata (Stolterfoth) Hasle, 1996	NA, MA, SA, Naf, IS, EM	A, K, T, V	1400	21.7
Hemiaulus hauckii Grunow ex Van Heurck, 1882	NA, MA, IS	A, K, T	4960	13.0
Hemiaulus sinensis Greville, 1865	SA, MA	B, K	80	4.3
Lauderia annulata Cleve, 1873	NA, MA	K	60	4.3
Leptocylindrus danicus Cleve, 1889	MA, NA, EM	A, K, V	1520	21.7
Leptocylindrus minimus Gran, 1915	SA, TS, NA, AO	B	800	13.0
cf. *Leptocylindrus* sp. Cleve in Petersen, 1889	NA, EM	A, V	2,141,247	10.9
Lioloma pacificum (Cupp) Hasle, 1996	NA, MA	A, K	880	4.3
Lithodesmium sp. Ehrenberg, 1839	NA	V	40	2.2
cf. *Minutocellus* sp. Hasle, von Stosch & Syvertsen, 1983	NA	V	14,844	4.3
Nitzschia longissima (Brébisson) Ralfs, 1861	NA	K	400	6.5
Paralia sulcata (Ehrenberg) Cleve, 1873	MA	K	120	2.2
Pleurosigma spp. Smith, 1852	TS, NA, EM	B, K	100	13.0
Proboscia alata (Brightwell) Sundström, 1986	MA, NA, NAf, EM, IS	A, K, T, V	2560	26.1
Proboscia indica (Peragallo) Hernández-Becerril, 1995	NA, NA	A, V	120	4.3
Pseudo-nitzschia multistriata* (Takano) Takano, 1995	NA, NAf, EM	K, V	4600	8.7
Pseudo-nitzschia spp. Peragallo in Peragallo & Peragallo, 1900	NA, SA, TS, EM, Naf, IS	A, B, K, T, V	102,224	52.2
Pseudosolenia calcar-avis (Schultze) Sundström, 1986	MA	K	20	2.2
Rhizosolenia cf. *setigera* Brightwell, 1858	EM, Naf	K	120	4.3
Rhizosolenia styliformis Brightwell, 1858	SA	B	80	2.2
Rhizosolenia spp. Ehrenberg, 1843	NA, SA	K, T	40	4.3
cf. *Skeletonema menzelii* Guillard, Carpenter & Reimann, 1974	NA, EM	V	259,770	4.3
Skeletonema spp. Greville, 1865	NA, AO, EM, NAf	A, B, K, V	5,737,660	32.6
cf. *Surirella smithii* Ralfs, 1861	NA	K	20	2.2
Tenuicylindrus belgicus (Meunier) Nanjappa & Zingone, 2013	NA, NAf	K	340	6.5
Thalassionema frauenfeldii (Grunow) Tempère & Peragallo, 1910	MA	K	1920	2.2
Thalassionema nitzschioides (Grunow) Mereschkowsky, 1902	NA, EM, MA	K	8480	8.7
Thalassionema spp. Grunow ex Mereschkowsky, 1902	MA, NA, SA, AO, EM, NAf, IS	A, B, K, T,V	125,499	37.0
Thalassiosira cf. *gravida* Cleve, 1896	EM	K	20	2.2
Thalassiosira spp. Cleve, 1873	NAf, SA, TS, AO, EM	A, B, K, T, V	3640	32.6
Trieres mobiliensis (Bailey) Ashworth & Theriot, 2013	NA	K	20	2.2
Und. Diatoms	EM, MA, NAf, AO, NA, SA, IS	A, B, K, T,V	1,269,162	65.2
Dinoflagellates				
Alexandrium minutum Halim, 1960	NA	K	20	2.2
Cochlodinium sp. Schütt, 1896	NA	K	20	2.2
Dinophysis caudata Saville-Kent, 1881	NA	A	40	2.2

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Table 3 (*continued*)

Taxon	BW donor sea area	Sampling port	Max (cells l^{-1}/ ind. m^{-3})	Fr (%)
Dinophysis sacculus Stein, 1883	IS	T	80	4.3
Diplopsalis group Bergh, 1881	NA, MA, IS	K, T	80	8.7
Gonyaulax cf. *fragilis* (Schütt) Kofoid, 1911	IS	T	40	2.2
Gymnodinium spp. Stein, 1878	SA, AO	B	400	4.3
Heterocapsa minima Pomroy, 1989	TS, SA	B	120	4.3
Heterocapsa niei (Loeblich III) Morrill & Loeblich III, 1981	SA	B	240	2.2
Tripos candelabrus (Ehrenberg) Gómez, 2013	IS	T	40	2.2
Tripos cf. *carriensis* (Gourret) Gómez, 2013	EM	V	40	2.2
Tripos furca (Ehrenberg) Gómez, 2013	NA, IS, EM	K, T, V	640	13.0
Tripos fusus (Ehrenberg) Gómez, 2013	NA, IS	A, T	80	6.5
Noctiluca scintillans (Macartney) Kofoid & Swezy, 1921	NA, IS	K, T	80	2.2
Oxytoxum caudatum Schiller, 1937	IS	T	40	2.2
Oxytoxum longiceps Schiller	IS	T	40	2.2
Oxytoxum viride Schiller, 1937	IS	T	40	2.2
Phalacroma oxytoxoides (Kofoid) Gomez, Lopez-Garcia & Moreira, 2011	NA	K	40	4.3
Prorocentrum dactylus (Stein) Dodge, 1975	NA	A	40	2.2
cf. *Prorocentrum* sp. Ehrenberg, 1834	IS	T	56,407	6.5
Prorocentrum* cf. *cordatum (Ostenfeld) Dodge, 1975	NA, IS	A, T	4452	8.7
Prorocentrum cf. *gracile* Schütt, 1895	NA, IS, SA	A, T	240	13.0
Prorocentrum compressum (Bailey) Abé ex Dodge	NA, IS	A, K, T	320	13.0
Prorocentrum micans Ehrenberg, 1834	NA, EM	K	40	4.3
Prorocentrum cf. *triestinum* Schiller, 1918	NAf, EM	K, V	120	4.3
Protoperidinium cf. *crassipes* (Kofoid, 1907) Balech, 1974	IS	T	40	2.2
Protoperidinium steinii (Jørgensen, 1899) Balech, 1974	IS, NA	T, V	120	4.3
Protoperidinium spp. Bergh, 1882	EM, NA,MA, NAf, IS	K, T	800	10.9
Pyrocystis lunula (Schütt) Schütt	IS	T	40	2.2
Scrippsiella trochoidea (Stein) Loeblich III, 1976	TS	B	100	2.2
cf. *Scrippsiella* spp. Balech ex Loeblich III, 1965	NA	K	40	2.2
Und. Dinoflagellates	NAf, NA, MA, IS, SA, EM	A, K, T, V	126,174	39.1
cf. und. Cysts	NA, NAf, EM, IS	K, T	7200	15.2
Chlorophytes				
Scenedesmus quadricauda (Turpin) Brébisson, 1835	NA	A, V	14,844	6.5
Und. Chlorophytes	AO, NA, SA, EM	A, B	37,842	10.9
Chrysophytes				
Dinobryon faculiferum (Willén) Willén, 1992	EM, NA	A, V	742	6.5
Coccolithophores				
Calciosolenia murrayi Gran, 1912	NA	K	320	4.3
Emiliania huxleyi (Lohmann) Hay & Mohler, 1967	NA, EM	K	67,200	15.2
Und. Coccolithophores	EM, NAf	K	4800	8.7
Cryptophytes				
Plagioselmis prolonga Butcher ex Novarino, Lucas & Morrall, 1994	TS, SA, AO, EM	B	600	8.7
Und. Cryptophytes	NA, IS, EM	K, T, V	452,742	23.9
Dictyochophytes				
Dictyocha fibula Ehrenberg, 1839	IS, NA	T, V	360	4.3
Ebriidae				
Ebria tripartita (Schumann) Lemmermann, 1899	NA, Naf, EM	K, V	4000	6.5
Hermesinum adriaticum Zacharias, 1906	NA		280	2.2
Euglenophytes				
Lepocinclis acus (Müller) Marin & Melkonian, 2003	TS, AO	B	100	4.3
Prasinophytes				
Pterosperma sp. Pouchet, 1893	NA, IS	A, T	742	4.3
Pyramimonas spp. Schmarda, 1849	IS, NA	T, V	37,110	8.7
Tetraselmis spp. Stein, 1878	IS	T, V	17,066	2.2
Und. Prasinophytes	NA	K	12,000	2.2
Prymnesiophytes				
Und. Prymnesiophytes	AO, NA	B, K	800	4.3
Choanoflagellates				
Und. Choanoflagellates	NAf, AO, IS, SA, NA	A, T	14,844	13.0
Und. nanoflagellates	EM, MA, NAF, NA, IS, SA, AO	A, K, T,V	4,430,934	63.0
Zooplankton				
Cladocera				
Evadne spinifera Müller,1867	MA, NA, IS	A, T, V	1212	13.3
Evadne spp. Lovén,1836	NA	V	31	2.2
Penilia avirostris Dana,1849	MA, NA, IS, EM	A, T, V	1015	13.3
Pseudevadne tergestina (Claus, 1877)	IS, NA	T, V	31	4.4
Copepoda Calanoida				
Acartia clausi Giesbrecht,1889	MA, TS, AO, NA, IS, BS, EM	A, B, K, T, V	10,527	33.3
Acartia copepodites Dana, 1849	EM, NA, SA, TS, AO, MA, IS, BS	A, B, T, V	22,756	40.0
Acartia (*Acartiura*) *marghalefi* Alcaraz,1976	TS	B	25	2.2

(*continued on next page*)

Table 3 (*continued*)

Taxon	BW donor sea area	Sampling port	Max (cells l^{-1}/ ind. m^{-3})	Fr (%)
Acartia (*Acanthacartia*) *tonsa** Dana, 1849	NA, TS, IS, EM	A, B, T, V	58,982	17.8
Acartia (*Odontacartia*) *erythraea** Giesbrecht,1889	EM	K	145	2.2
Anomalocera copepodites Templeton, 1837	IS	T	19	2.2
*Calanus euxinus** Hulsemann,1991	BS	T	78	2.2
Calanoida copepodites	MA, NA, IS, SA, TS, AO,EM, NAf, BS	A, B, K, T, V	1787	66.7
Centropages typicus Krøyer,1849	NA, MA, IS, SA, BS	A, T	257	15.6
Centropages kroyeri Giesbrecht,1893	NA, EM	A, V	610	6.7
Centropages copepodites Krøyer,1849	MA, NA, SA, IS, EM	A, B, T, V	1034	28.9
Centropages spp. Krøyer,1849	NA,TS	A, B	26	4.4
Isias clavipes Boeck,1865	NA, EM	A, V	2308	4.4
Clausocalanus arcuicornis (Dana,1849)	MA	T	23	2.2
Clausocalanus furcatus (Brady,1883)	MA, IS	A, T	269	8.9
Clausocalanus lividus Frost & Fleminger,1968	NA, MA, IS	A, T	692	8.9
Clausocalanus jobei Frost & Fleminger,1969	MA, IS	T	19	4.4
Clausocalanus parapergens Frost & Fleminger,1970	MA	T	8	2.2
Clausocalanus copepodites Giesbrecht,1888	MA, NA, AO, IS	A, B, T	492	13.3
Ctenocalanus vanus Giesbrecht,1888	MA	T	46	2.2
Nannocalanus minor (Claus,1863)	MA, IS	T	32	6.7
Paracalanus denudatus Sewell,1929	MA	T	23	2.2
Paracalanus copepodites Boeck,1865	NA, MA, IS,EM	A, T, V	2038	13.3
Paracalanus nanus Sars G.O.,1925	MA	T	62	2.2
Paracalanus parvus (Claus,1863)	NA, MA, IS, EM	K, T, V	938	20.0
Paracalanus spp. Boeck,1865	IS	T	477	2.2
Paraeuchaeta hebes (Giesbrecht,1888)	MA	T	8	2.2
Pseudocalanus elongatus (Boeck,1865)	BS	T	2171	2.2
*Paracartia grani** Sars G.O.,1904	EM, NA	A, V	159	6.7
Paracatia copepodites Sars G.O.,1905	NA	A	27	2.2
*Pseudodiaptomus marinus** Sato,1913	EM, NA, TS	A, B, K, V	705	28.9
Temora stylifera (Dana,1849)	MA, NA	A, K	269	6.7
Temora longicornis (Müller O.F.,1785)	EM	V	38	2.2
Temora copepodites Baird, 1850	MA, NA, IS, SA,EM	A, B, T, V	282	13.3
Copepoda Cyclopoida				
*Oithona brevicornis** Giesbrecht,1891	MA, NAf, NA, AO, BS, EM	A, B, T, V	26,667	28.9
Oithona nana Giesbrecht,1893	MA, NA, IS, SA, TS, AO, EM, NAf, BS	A, B, K, T, V	4487	82.2
Oithona similis Claus,1866	NA, MA, BS	K, T	100	13.3
Monothula subtilis Giesbrecht,1891	NA	V	185	2.2
Oithona copepodites Baird, 1843	EM, MA, NAf, NA, IS, SA,TS, AO	A, B, K, T, V	97,115	64.4
Copepoda-Poecilostomatoida				
Und. *Corycaeidae* Dana, 1852	MA, NA, IS, SA, AO	A, B, T, V	135	24.4
Oncae mediterranea (Claus,1863)	MA	T	38	2.2
Oncaea copepodites Philippi, 1843	MA, NA, IS, SA, TS, EM	A, B, T, V	885	35.6
Oncaea curta Sars G.O.,1916	SA, MA	B, T	15	4.4
Oncaea spp. Philippi, 1843	MA, NA, SA, EM, IS	A, B, K, T, V	400	24.4
Copepoda Harpacticoida				
Euterpina acutifrons (Dana,1847)	EM, MA, NA, IS, SA, TS, AO	A, B, K, T, V	16,731	51.1
Und. Harpacticoida	EM, NAf, NA, IS, SA, TS, AO	A, B, K, T, V	6090	68.9
Longipedia spp. Claus,1862	TS, NA	B, V	705	11.1
Microsetella spp. Brady & Robertson, 1873	NA, IS, AO,TS, MA, EM	A, B, T, V	154	26.7
Tisbe battagliai Volkmann-Rocco, 1972	SA, IS, EM	B, T, V	11,888	15.6
Tisbe spp. Lilljeborg, 1853	EM, AO, IS, TS, SA, MA	A, B, T	15,385	26.7
Copepoda				
Copepoda nauplii	EM, NA, MA, NAf, IS, SA, TS, AO	A, B, K, T, V	8145	57.8
Cirripedia				
Cirripedia nauplius	EM, NA, IS, SA, TS, AO, MA	A, B, T, V	3654	42.2
Ostracoda				
Und. Ostracoda	NA	A, V	64	4.4
Crustacea				
Malacostraca	MA, EM, IS, NA	A, K, T, V	38	15.6
Isopoda				
Und. Epicaridea	MA, NA	T, V	22	4.4
Amphipoda				
Und. Amphipoda	MA	T	23	2.2
Plathelminthes				
Müller's larva	NA	V	226	2.2
Hydrozoa				
Podocoronoyides minima (Trinci, 1903)	NA	K	5	2.2
Solamaris spp. Haeckel, 1879	NAf	K	5	2.2
Hydromedusae efira	NA	V	22	2.2
Mollusca				

(*continued on next page*)

Table 3 (*continued*)

Taxon	BW donor sea area	Sampling port	Max (cells l^{-1}/ ind. m^{-3})	Fr (%)
Bivalvia	EM, NAF, NA, IS, SA, TS, AO, MA	A, B, K, T, V	5588	62.2
Creseis spp. Rang, 1828	NAf, SA, TS, NA, MA, IS	A, B, K, T, V	231	15.6
Gastropoda	MA, IS, SA, NA, EM,	T, V	769	22.2
Anellida				
Polychaeta	NAF, NA, IS, SA, TS, AO, MA, BS, EM	A, B, T, V	17,949	40.0
Nematoda				
Und. Nematoda	MA	T	8	2.2
NEMERTEA				
Nemertea larvae	SA	B	47	2.2
CHAETOGNATHA				
Und. Sagittidae Claus & Grobben, 1905	IS	T	38	4.4
Echinodermata				
Echinodermata pluteus	IS, EM	T, V	262	4.4
TUNICATA				
Oikopleura spp. Mertens, 1830	IS	T	62	4.4
Ascidiacea	EM, NA	V	88	4.4
VERTEBRATA				
Pisces egg	IS, NA	T, V	44	6.7
Pisces larve	IS	T	31	2.2
TARDIGRADA				
Und. Tardigrada	IS	T	38	2.2
RADIOZOA				
Acantharea	IS	T	231	2.2

A lack of correlation between BW age and FIB abundances was evidenced ($r = 0.1331$ for *Escherichia coli* and $r = 0.0866$ for presumptive enterococci) (Fig. 7a), although FIB-positive samples had mostly < 7 days of retention time in tank (74% of *E. coli*-positives and 59% of enterococci -positives).

Even if a low viability occurred in older BW, no statistically significant relationship was discerned between BW age and viable organisms ($r = -0.1319$ and $r = 0.1131$ for cells 10–50 μm and organisms > 50 μm, respectively) (Fig. 7b). A slightly stronger negative relationship was discerned between taxa number and BW age ($r = -0.4365$ and -0.4280, for phyto- and zooplankton taxa, respectively) (Fig. 7c).

Non-metric multidimensional scaling based on transformed biological and normalized physical–chemical data distinguished the samples collected in the ports of Koper and Bari, while Ancona, Trieste and Venice grouped together. This separation was more evident for the phytoplankton than for zooplankton ordination (Fig. 8).

4. Discussion

This is the first study considering the presence and abundance of indicator microbes, the organisms' viability and the species-specific abundance of phytoplankton and zooplankton as well as environmental parameters in the ballast tanks of ships calling to five Adriatic ports. Species may survive the tank's environmental conditions during a vessel voyage, be introduced and become dominant. The environmental conditions in the tanks during a voyage are critical for their survival and transfer into the new environment. For this reason, environmental factors in tanks should be always measured and compared to those of the recipient habitat to assess the survival potential of the species. In this study, the abiotic factors measured show that the highest temperature (30.4 °C) was found in the port of Venice (V2-NA), with ballast water originating from North Adriatic. Slightly lower but still high temperature (28.5 °C) was documented in the port of Bari, in a tank containing Atlantic Ocean ballast water (B8-AO), having residence time of 20 days (Table 2). These environmental conditions have probably favoured the survival of heterotrophs, as enterococci (Fig. 3 b) and zooplankton organisms (Fig. 4b), but also the phytoplankton viability. As already reported in Burkholder et al. (2007), viable diatoms are common in BWs since they can survive extended periods in low light or darkness. Anyway, lower temperatures better support the survival of phyto- and zooplankton fractions (Fig. 4 a and b). The temperatures found in the previous study on BW sampling in the port of Koper showed a mean value of 27.6 °C (David et al., 2007). The lower mean value in our study is likely due to the sampling period from May to July, to a different source region of the BW sampled and to the residence time of the BW in the tank. In the vessels sampled in Koper during winter time, tank temperatures ranged from 10.8 °C to 14.4 °C. Such temperatures do not favour the viability of cells for both phytoplankton and zooplankton, while bacteria's concentration did not seem to be affected by low temperature values (Fig. 3).

There are different studies focusing on the survival of species within ballast tanks at different stages of the voyage, while only few assess the viability of organisms inside the tanks just before the discharge of BW (e.g., Rigby and Hallegraeff, 1994; Gollasch et al., 2000a, 2000b; Olenin et al., 2000; Steichen and Quigg, 2015; Desai et al., 2017). The species that survive in the ballast tanks not always can reproduce and become invasive after being discharged in a new ecosystem but, if this happens, the community structure may be impacted. Moreover, toxic microalgae and pathogenic bacteria have also been documented to survive during long voyages (e.g., Burkholder et al., 2007). For this reason, detailed investigations on the presence of NIS in particular, and HAOP in general, in BW are fundamental for any study on the risks posed by BW being discharged in the Adriatic basin. At the same time, a monitoring program on the presence and abundance of HAOP in ports and surrounding areas is necessary to complement data needed for risk assessment and the early warning system to be implemented at the national and international level. To this end, the BALMAS database with data on environmental, microbiological, and biological parameters in 12 Adriatic ports will greatly support any decision-making on BW management in the Adriatic area (Kraus et al., this issue; Luna et al., this issue; Mozetič et al., 2012, this issue; Vidjak et al., this issue).

Environmental conditions in the ballast tanks were strongly related to seasonality: all ships berthed in the Italian ports were sampled from

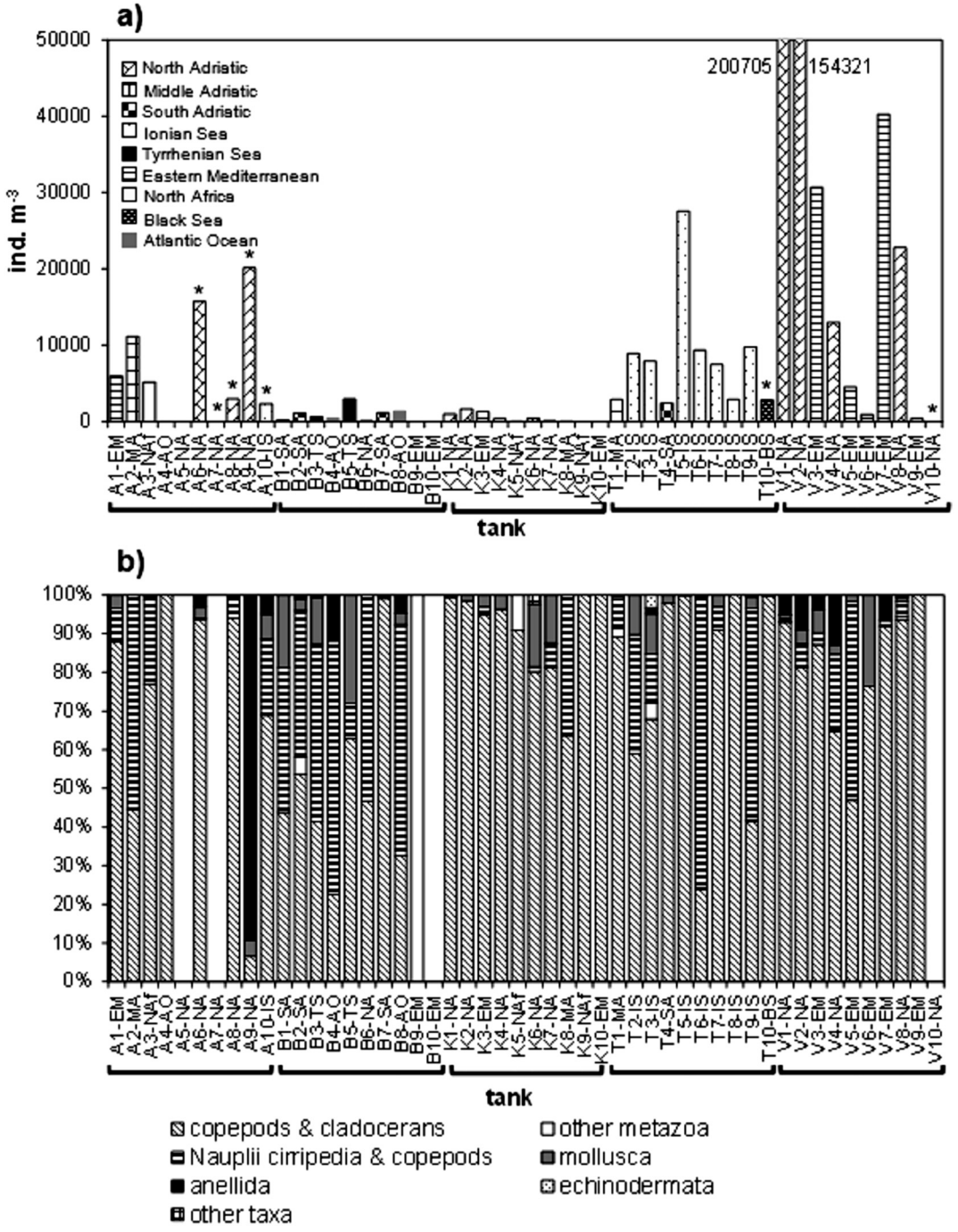


Fig. 6. Abundances of total zooplankton with the indication of BW donor sea area (a) and percentage of main zooplankton groups (b) in each ballast tank. The data are grouped for ports and arranged in ascending order of ballast water days in tank proceeding from left to right within each port. Asterisks indicate the absence of information about BW days in tank.

June to September 2015 while in the port of Koper BW sampling was carried out from November 2015 to February 2016. Consequently, the highest abundance of phytoplankton was recorded in the BW loaded from spring to autumn, with a short residence time in tank. On the other hand, lower phytoplankton abundances, observed in the port of Koper, reflected the winter conditions of marine ecosystems. Furthermore, phytoplankton abundances in BW confirmed seasonal occurrence of diatoms and dinoflagellates in natural environment (Cabrini et al., 2012; Mozetič et al., 2012). These observations highlight that abundances of organisms transported with ballast are strongly influenced by seasonality, in addition to voyage length and environmental conditions in the tank.

When considering the performance standard set out in the regulation D-2 of the BWM Convention, 96% of the investigated tanks were not compliant. This finding is not surprising because untreated ballast waters were sampled. According to Kang et al. (2010) and David et al. (2013), the salinity can be an appropriate parameter for estimating the possibility of future invasions of species into receiving waters.

4.1. Faecal indicator bacteria and Vibrio cholerae

Faecal indicator bacteria (FIB) were recovered from BW mostly loaded in coastal waters of the north and middle Adriatic, where BWE procedures are inapplicable, and sampled on ships docked in the ports of Bari, Ancona, Venice and Koper. Contrarily, BW sampled in the port

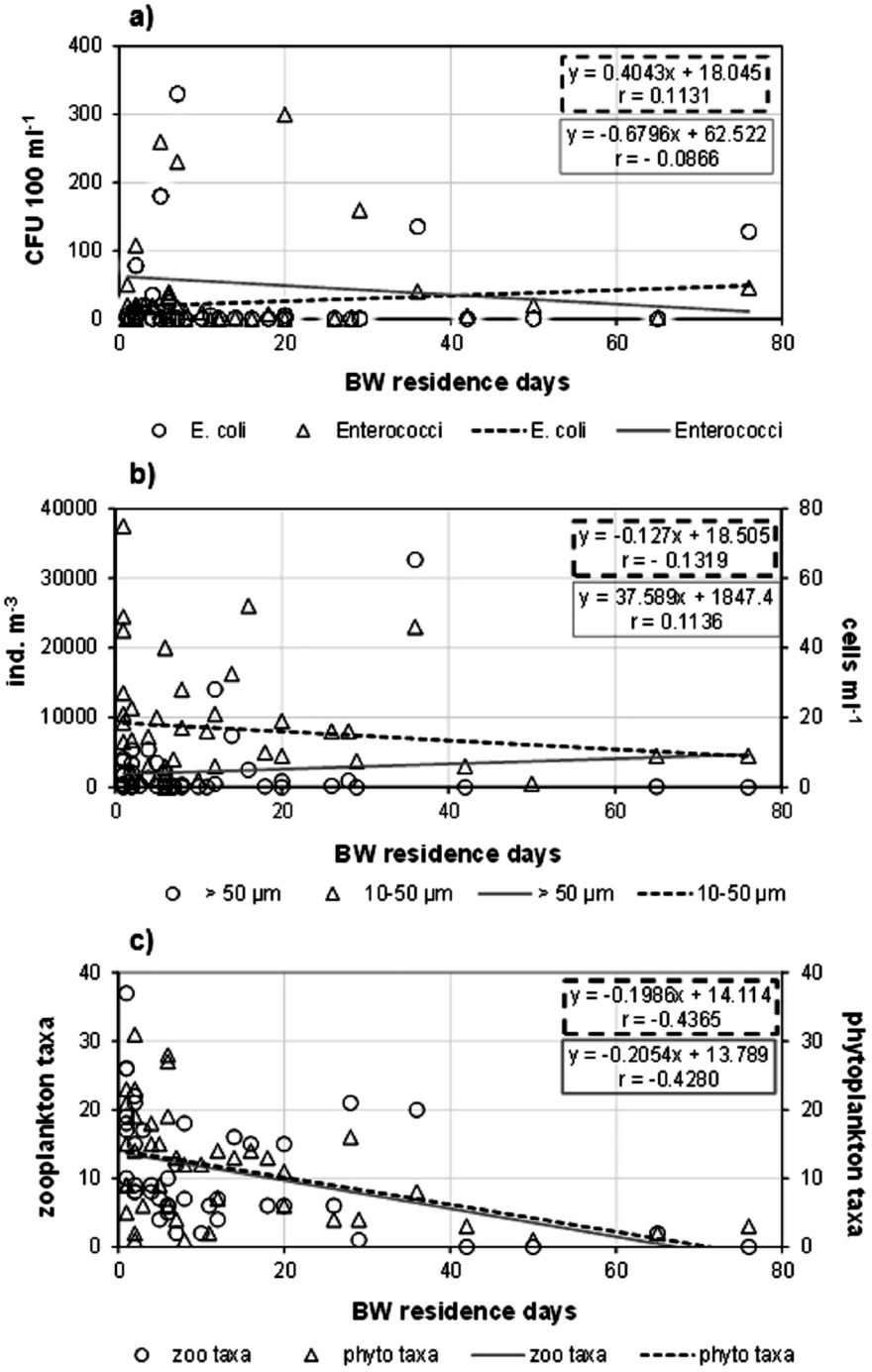


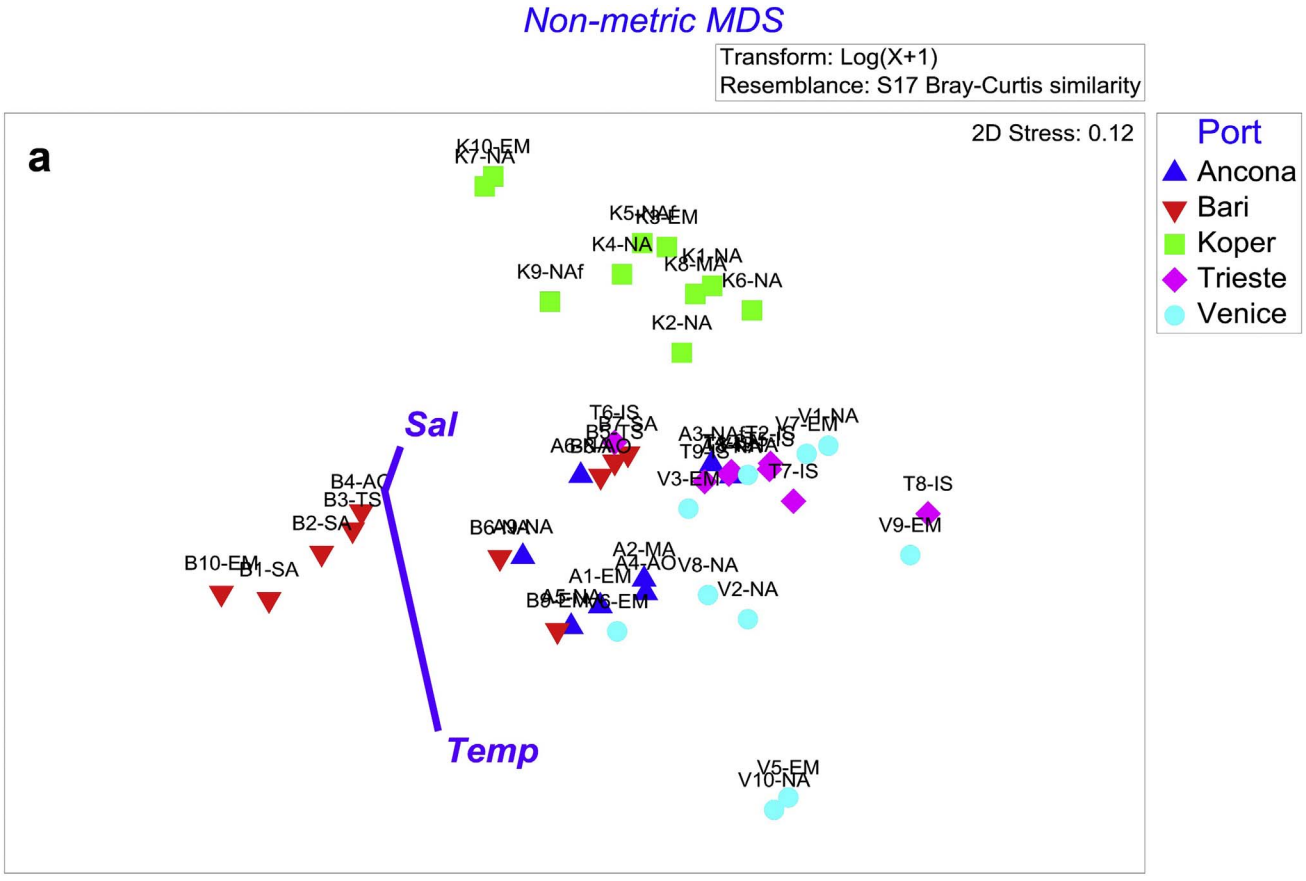
Fig. 7. Relationship between FIB (*Escherichia coli* and enterococci) (a), viable organism (10–50 µm and > 50 µm) abundances (b), number of taxa (phytoplankton and zooplankton) (c) and BW days in tank.

of Trieste, were not contaminated with FIB, with the exception of two presumptive enterococci positive samples (50 and 4 CFU 100 ml^{-1} estimates in T2 and T4, respectively).

Results evidenced that FIB may survive under the challenging environmental conditions inside BW tanks. FIB, as both *Escherichia coli* and presumptive enterococci, were recovered in 32% of the analysed BW; in particular, *E. coli*-positive samples resulted 38% while enterococci -positive ones were 54%. Presumptive enterococci were found in high abundances in BW with 20 and 29 days of retention time; furthermore, *E. coli* and presumptive enterococci were estimated in BW both freshwater influenced (salinity value = 10.2 and 30.0, in A4 and B6 samples, respectively) and with typical seawater salinity.

Among FIB, presumptive enterococci were recovered more frequently, possibly due to their ability to grow in the presence of salt (6.5% NaCl) which is a distinguishing feature of the genus *Enterococcus* (Byappanahalli et al., 2012). The greater salt tolerance of enterococci probably contributes to their better performance as indicators of human health risk in marine waters compared to members of the coliform group (Byappanahalli et al., 2012). Enterococci are currently the only FIB recommended by the U.S. Environmental Protection Agency (EPA) for brackish and marine waters, since they correlate with human health better than other FIB (Wade et al., 2003).

The data collected suggest that long BW retention times (more than seven days) and seawater salinity > 33 do not ensure a complete decay of FIB. Furthermore, FIB abundances in low concentrations are not reliable assurances that human pathogens are absent (Stewart et al.,



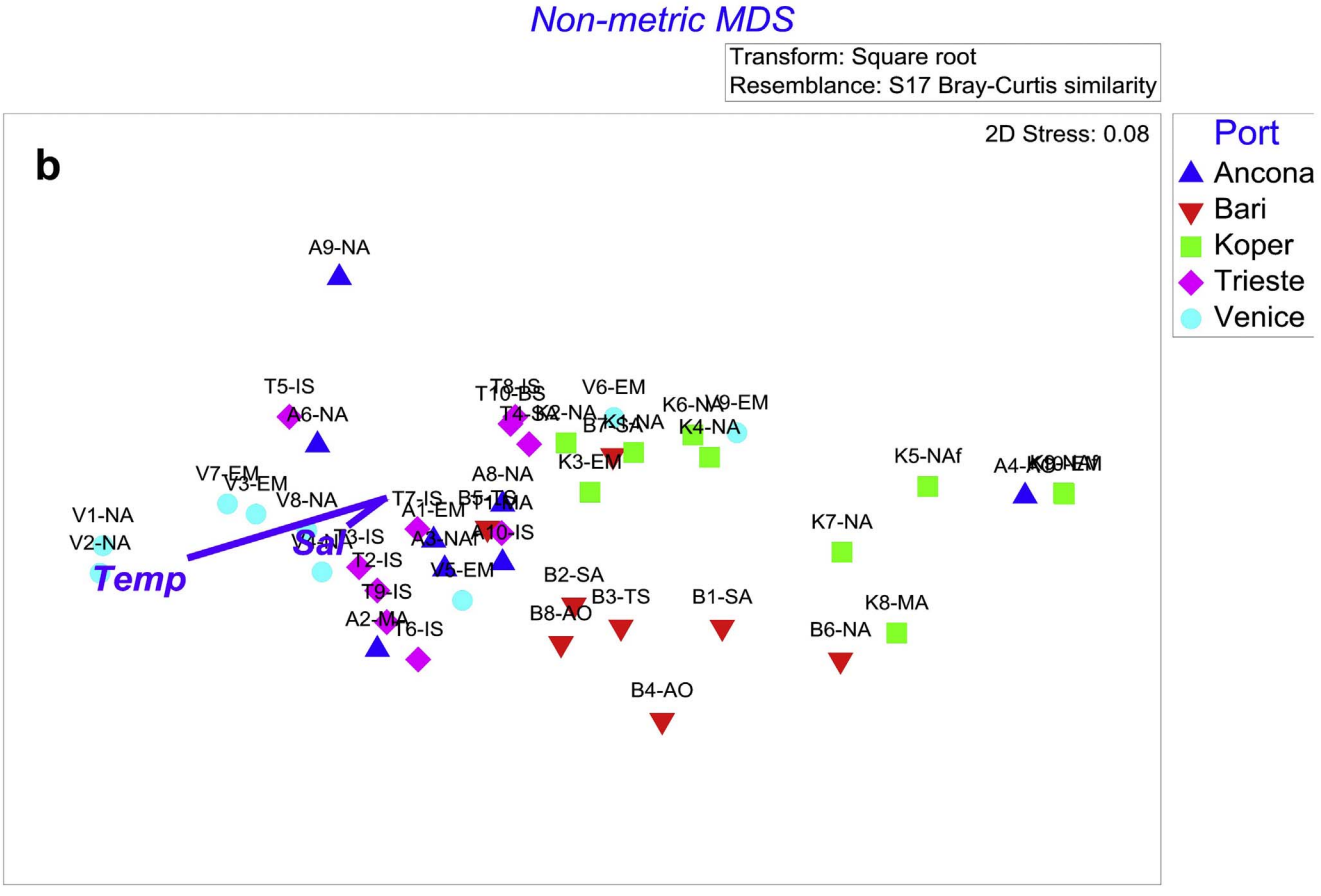


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Fig. 8. Non-metric multidimensional scaling (nMDS) ordination plots of the main phytoplankton (a) and zooplankton (b) groups. The length of arrows indicates the correlations between the environmental variables and the ordination axes. Temp: temperature, Sal: salinity.

2008). Several researchers reported the presence of pathogens, not listed as BW indicator bacteria, in ballast tanks environment (Aridgides et al., 2004; Altug et al., 2012; Brinkmeyer, 2016; Ruiz et al., 2000b) and supported the hypothesis that BW tanks may act as incubators of microbes (Tomaru et al., 2010, 2014).

4.2. Phytoplankton

Phytoplankton assemblages in ballast waters were mainly dominated by diatoms and nanoflagellates, whereas dinoflagellates and coccolithophores accounted for lower abundances, reflecting what commonly observed in water column. Similarly, David et al. (2007) found a dominance of diatoms in ballast water samples of the port of Koper, which was until now the only study on BW conducted in the Adriatic Sea. In a study on phytoplankton viability in ballast tanks aboard of 28 ships sampled in 13 ports on the U.S West and East Coast, Burkholder et al. (2007) reported a dominance of chain-forming diatoms and dinoflagellates while flagellated green algae were negligible. According to Kang et al. (2010), both pennate and centric diatoms such as *Chaetoceros debilis*, *Skeletonema costatum* and *Cylindrotheca closterium* were dominant in BW of international commercial ships in 2 Korean ports, while in our samples centric diatoms (*Chaetoceros* spp., cf. *Leptocylindrus* sp., *Skeletonema* spp. and undetermined diatoms) were the most abundant. The remarkable dominance of diatoms can likely be related to their tolerance to darkness (Peters and Thomas, 1996).

Some potentially toxic diatom and dinoflagellate species were identified. Different undetermined diatoms belonging to the potentially toxic genus *Pseudo-nitzschia* were present in all ballast tanks except in those containing water from the Atlantic Ocean. Additionally, the potentially toxic NIS *P. multistriata* was also identified. The genus *Pseudo-nitzschia*, which is frequently occurring in the Adriatic Sea from spring to autumn, includes species causing the ASP (Amnesic Shellfish Poisoning) syndrome. *P. multistriata* is considered a NIS for the Adriatic Sea (Corriero et al., 2016) and it was found in ballast tanks with waters from the northern Adriatic, North Africa and eastern Mediterranean in ships berthed in the ports of Koper and Venice. During the Port Baseline Survey performed to assess the presence and abundance of HAOP in 12 Adriatic ports, *P. multistriata* was also found in the ports of Venice, Trieste and Koper (Mozetič et al., this issue).

Among dinoflagellates, five potentially harmful taxa, *Alexandrium minutum*, *Dinophysis caudata*, *D. sacculus*, *Noctiluca scintillans* and *Prorocentrum* cf. *cordatum*, were recorded. *P. cordatum* is a small dinoflagellate that may produce toxins with hepatotoxic activity in mice (Lassus et al., 2016). Some intoxications due to this species have been reported in Japan, where it proliferates every year in winter or spring with 20 blooms recorded between 1974 and 1984. More recently, *P. cordatum* has become very abundant in Narragansett Bay and in the Black Sea, where is considered as a keystone species (Lassus et al., 2016). Along the Croatian coasts, in the northeastern part of the Adriatic Sea, *P. cordatum* is scarcely present (Marić et al., 2012) as well as in the Gulf of Trieste, where it occurs with a frequency of 0.3% (Cabrini et al., 2012). In the Venice lagoon the percentage of *P. cordatum* frequency is slightly higher (1%) (Bernardi Aubry et al., 2012).

N. scintillans was found in tanks sampled in the ports of Trieste and Koper with BW uploaded in the Ionian Sea and northern Adriatic. This heterotrophic dinoflagellate is neritic and cosmopolitan and can live well in both cold and warm waters. *N. scintillans* caused red tides in the north Adriatic Sea even at very low temperature (Malej, 1983; Fonda Umani et al., 2004). This species does not produce toxins but it is able to accumulate large volumes of ammonia in the vacuole, which can be released into the environment during blooms thus decreasing fish and invertebrates biomass (Fonda Umani et al., 2004).

The mixotrophic dinoflagellate *D. sacculus* was only found in tanks sampled in the port of Trieste containing waters originated from the Ionian Sea. It is typically present in the summer phytoplankton community, very common along the Atlantic and Mediterranean coasts and often observed in the Tyrrhenian and Adriatic Sea (Lassus et al., 2016). *D. sacculus* is a potential producer of okadaic acid, a toxin responsible of the DSP (Diarrhetic Shellfish Poisoning) syndrome. In Europe, DSP events were mainly recorded in Italy (Honsell et al., 1992), Slovenia (Francé and Mozetič, 2006), Spain (Delgado et al., 1996) and Portugal (Lassus et al., 2016). Another species belonging to the *Dinophysis* genus, *D. caudata*, was only found in a BW loaded in the northern Adriatic and sampled in the port of Ancona. *D. caudata* is also a DSP producer, responsible for the closure of mussel farms in the Gulf of Trieste (Cabrini et al., 1995) and in Catalonia (Spain) where a mixed bloom of *D. caudata* and *D. sacculus* caused closures of mussel and oyster farms for exceeded DSP sanitary threshold (Lassus et al., 2016).

Finally, *A. minutum*, potentially involved in the Paralytic Shellfish Poisoning (PSP), was observed with low abundances in BW from the northern Adriatic, inside a tank of a ship berthed in the port of Koper. *A. minutum* is widely distributed along the Italian coasts as well as in the other parts of the Mediterranean and all around the world, prevalently during late winter-late spring (Hallegraeff, 2003; Anderson et al., 2012; Lassus et al., 2016). In Thailand, it was detected in an estuary at a very low salinity (Hallegraeff, 2003). It seems that supply of nutrient-rich freshwater favours its bloom development. In Australia, PSP was unknown until the 1980s, when *A. minutum* was introduced into the port of Adelaide through BW (Hallegraeff, 2003). In Europe, *A. minutum* is distributed along the Catalan and Sicilian coasts (Giacobbe and Maimone, 1994; Franco et al., 1994; Hallegraeff, 2003) and is also reported in the northern Adriatic (Honsell et al., 1996).

4.3. Zooplankton

In this study, the frequent detection of many juvenile forms of Copepoda (e.g. *Acartia, Oithona, Oncaea* copepodites) and other meroplanktonic taxa in the BW is noteworthy; this suggests that ballast tanks could function as incubators for the dispersal of early stages. Due to the short residence time of the water in the ballast tanks, the maritime traffic between neighbouring areas allows certain taxa to survive from one harbour to another. Many NIS (Zenetos et al., 2012) that have been found in the BW were already recorded in the Adriatic or in the Mediterranean Sea, such as *A. tonsa* (Comaschi et al., 1999; Camatti et al., 2006), *P. grani* (de Olazabal et al., 2006; David et al., 2007) and *P. marinus* (de Olazabal and Tirelli, 2011; Sabia et al., 2012). The latter was recently observed for the first time in the port of Koper (Lučić et al., 2015). *C. euxinus* (Unual et al., 2006; Isinibilir et al., 2009) and *O. brevicornis* (Hure and Scotto di Carlo, 1969; Gubanova and Altauhov, 2007) not (yet) reported from the Adriatic Sea were detected in more restricted adjacent areas, like in the Black Sea.

The species *A.* (*Odontoacartia*) *erythraea*, native of the Red Sea (Razouls et al., 2005-2017), was never observed in the Adriatic area and in the western Mediterranean. The discovery of this species in the ballast tanks points out the crucial importance that BW has in spreading of NIS. However, it is important to note that the viability of the organisms in the tanks was good. Furthermore, it is of fundamental importance to know if *A.* (*Odontoacartia*) *erythraea* is present in the Levantine waters in order to understand if a natural spreading through the Suez Canal also occurred. However, clarity of this assumption may only be reached when sampling the Canal.

When the maritime traffic occurs within restricted and shallow areas, such as in the Adriatic basin, ships cannot perform BWE in accordance to the provisions of the BWM Convention in the absence of a

BWE designated area. Consequently, organisms with similar characteristics could be easily transported from one place to another within the northern Adriatic (Occhipinti-Ambrogi, 2000, Occhipinti-Ambrogi et al., 2010).

We documented that organisms may easily be transported from one place to another within the northern Adriatic, which was also observed by other studies (Occhipinti-Ambrogi, 2000, Occhipinti-Ambrogi et al., 2010). This highlights how the data gathered through this and similar studies are particularly relevant for assessing if and to what extent BW can be a mechanism of transfer and introduction of HAOP to/from this semi-enclosed basin. In consequence, ballast water management is an essential protection measure and it is hoped that the entry into force of the BWM Convention will eventually reduce the introduction rate of NIS.

5. Conclusions

This is the first study on BW in Adriatic ports after David et al. (2007) focusing on the species-specific abundance and composition of phyto- and zooplankton in BW tanks and pointing out the presence of HAOP.

Our results point out that in most samples BWs contained high viable organisms abundance.

According to Kang et al. (2010) as well as David et al. (2013) salinity can be an appropriate parameter for estimating the possibility of future invasions into receiving environments. In our study, organisms eventually discharged in the new environment could therefore survive, since most of sampled BWs had salinity comparable to that of recipient waters.

Faecal contamination, mostly evidenced by presumptive enterococci, was not completely reduced by the (sometimes) long residence time in ballast tanks. *Vibrio cholerae* was never detected neither through cultivation nor by multiplex PCR method. Viable organisms were found in most sampled tanks. The taxonomic analysis provided valuable data useful for the risk assessment linked to the BW discharge in the Adriatic ports, until the BWM Convention will be formally applied. Indeed, analyses of the phyto- and zooplankton showed the presence of harmful and potentially toxic species; moreover, several phyto- and zooplankton NIS were recorded. With the enlargement of the Suez Canal, maritime traffic in the Mediterranean Sea intensified and the consequent introduction of pathogens and invasive, harmful species may increase in this semi-enclosed, highly diverse and vulnerable marine ecosystem. Knowledge of the potential risk associated to presence and survival of HAOP in ballast waters is of paramount importance in order to carefully evaluate and apply the best BW management options for the Adriatic Sea and for the Mediterranean basin.

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