Baltic Marine Environment Protection Commission

Testing monitoring methods for Non-indigenous species in Baltic ports

Photo: Galina Ganmaga

BALSAM

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Authors grouped by countries in alphabetical order

Muuga Harbour, Port of Tallinn (Estonia): Ojaveer H., Põllumäe A., Jaanus A., Kotta I.

Monitoring needs of non-indigenous (Finland): Lehtiniemi M.

Port of Liepaja and port of Riga (Latvia): Strake S, Alberte M., Barda I., Labucis A., Labuce A., Perkons V.

Port of Gdynia (Poland): Normant M., Bielecka L., Dmochowska B., Dumnicka E., Dziubińska A., Jakubowska M., Kobos J., Łądkowska H., Marszewska L., Zgrundo A, with Port of Gdynia Authority S.A., Poland as cooperative partner.

Coordinators in alphabetical order

Lehtiniemi M., Normant M., Ojaveer H.

Editors:
Marta Ruiz, Johanna Karhu and Hermanni Backer

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Galina Garnag

TESTING MONITORING METHODS FOR NON-INDIGENOUS SPECIES IN PORTS (BALSAM PROJECT - WORK PACKAGE 4)

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1 Introducing the BALSAM project and its WP 4

Baltic Sea Pilot Project: Testing new concepts for integrated environmental monitoring of the Baltic Sea (BALSAM) is a project to enhance the capacity of the Baltic Sea member states to develop their marine environmental monitoring programmes. BALSAM (October 2013 – May 2015) is one of the three Pilot Projects called "New Knowledge Projects" co-financed by the European Commission DG Environment. Sharing results and best practices with the projects in the North Sea (JMP NS/CS) and the Mediterranean (IRIS-SES) will support inter-regional coherence when developing proposals for integrated monitoring.

The activities of the project are organized in six work packages (WP) (**Figure 1**) conducted by the 19 partners forming the project consortium (**Table 1**). The project, coordinated by the HELCOM Secretariat, counts with the participation of partners from all Baltic Sea coastal states.

WP1 Project Coordination and cooperation for integrated monitoring
WP lead: HELCOM Secretariat, Project Coordinator
Partners: - Aquabiota (Sweden), Baltic Environmental Forum Latvia (BEF LV), Finnish Environment Institute (SYKE), Estonian Marine Institute (EMI), Klaipeda University (KUCORPI, Lithuania), Latvian Institute of Aquatic Ecology (LIAE), Leibniz Institute for Baltic Sea Research Warnemünde (IOW, Germany), Polish Institute of Meteorology and Water Management from Gdynia (IMGW), Swedish Meteorological and Hydrological Institute (SMHI), Tallinn University of Technology (TUT, Estonia), Aarhus University (AU, Denmark)
Stakeholders: Roshydromet, State Oceanographic Institute (SOI)

WP2 Promoting resource-efficiency in national monitoring strategies	WP3 Regional coordination of monitoring of marine mammals and seabirds	WP4 Non-indigenous species – multi-disciplinary monitoring schemes to gain synergies for ballast water risk-management and environmental monitoring	WP5 Tools for optimizing coordinated international use of research vessels for monitoring activities	WP6 A common standard for mapping and monitoring of Baltic Sea benthic biotopes and habitats, and format for data exchange
WP lead: HELCOM Secretariat **Partners:** Finnish Game and Fisheries Research Institute (FGFRI), Institute of Meteorology and Water Management from Gdynia (IMGW), SMHI, SYKE **Stakeholders:** Thünen Institute of Baltic Sea Fisheries (TI-OF) **External assistance:** ICES	**WP Lead:** Aarhus University (AU) **Partners:** Latvian Fund for Nature (LFN), Swedish Museum of National History (SMNH), Finnish Game and Fisheries Research Institute (FGFRI), WWF Finland, Estonian Fund for Nature (EFN), **External assistance:** not identified	**WP Lead:** HELCOM Secretariat **Partners:** Estonian Marine Institute (EMI), University of Gdansk (UG), Federal Maritime and Hydrographic Agency (BSH), Latvian Institute of Aquatic Ecology (LIAE), SYKE **External assistance:** BSH, HELCOM, UG	**WP Lead:** Tallinn University of Technology (TUT) **Partners:** SYKE, IMGW, Latvian Institute of Aquatic Ecology (LIAE), SMHI, Leibniz Institute for Baltic Sea Research Warnemünde (IOW)	**WP Lead:** Baltic Environmental Forum Latvia (BEF LV) **Partners:** Latvian Institute of Aquatic Ecology (LIAE), AquaBiota Water Research ABWR AB (AquaBiota), Estonian Marine Institute (EMI), Klaipeda University (KUCORPI) **External assistance:** not identified

Figure 1 - Organogram of the project structure.

HELCOM Secretariat (Coordinator)	Latvian Fund for Nature (LFN), Latvia
Aquabiota, Sweden	Latvian Institute of Aquatic Ecology (LIAE), Latvia
Baltic Environment Forum Latvia (BEF LV), Latvia	Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Germany
Estonian Fund for Nature (ELF), Estonia	Sveriges Meteorologiska och Hydrologiska Istitut (SMHI), Sweden
Estonian Marine Institute (EMI), Estonia	Swedish Museum of Natural History (SMNH), Sweden
Bundesamt für Seeschiffahrt und Hydrographie (BSH), Germany	Tallinn University of Technology (TUT), Estonia
Finnish Environment Institute (SYKE), Finland	University of Gdansk (UG), Poland
Finnish Game and Fisheries Research Institute (FGFRI), Finland	WWF Suomi, Finland
Instytut Meteorologii I Gospodarki Wodnej – Państwowy Instytut Badawczy (IMGW-PIB), Poland	Aarhus University (AU), Denmark
Klaipeda University (KUCORPI), Lithuania	

Table 1 - Project consortium.

Focusing on gaps, BALSAM provides recommendations for marine monitoring in the Baltic, especially for mammals and seabirds, non-indigenous species and benthic habitats. The project is also providing recommendations for coordinated use of research vessels and improved data management and infrastructure in the Baltic. Harmonizing and coordinating monitoring efforts will lead to better integration and cost-effectiveness of monitoring to support the needs of the Baltic Sea Action Plan (BSAP, 2007) and the Marine Strategy Framework Directive (MSFD, 2008).

Alongside migration via inland waterways, vessels are the most common pathway for non-indigenous species (NIS) introductions in the Baltic Sea (via ballast waters and as ships' biofouling). Thus to observe new introductions and spread of NIS from this source, routine biological monitoring should be complemented with monitoring in port areas. This has been the focus of BALSAM work in NIS monitoring conducted under its WP4, which aims to provide recommendations to harmonize the monitoring and sampling methods for these species to meet the needs of the MSFD as well as the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention, 2004) of the International Maritime Organization (IMO).

WP4 experts also tested monitoring methods for alien species distribution by conducting sampling in ports using the Joint HELCOM/OSPAR Harmonized Procedure on the granting of exemptions under the International Convention for the Control and Management of Ships' Ballast Water and Sediments, Regulation A-4 (HELCOM, 2013a). This activity started with a workshop held in October 2013, where the sampling methodology was introduced to the participants. The joint port sampling protocol included in the Joint HELCOM/OSPAR Harmonized Procedure was applied in four ports where it had not been tested before - Muuga (Estonia), Liepaja (Latvia), Riga (Latvia) and Gdynia (Poland) ports. Sampling conducted during these port surveys was analysed and fed to the Risk Assessment Tool. Further, as part of the project the web based Risk Assessment Tool which enables running the risk assessments agreed with the Joint HELCOM/OSPAR Harmonized Procedure was updated and improved.

The WP, led by the HELCOM Secretariat, consists of five partners: Estonian Marine Institute (EMI), University of Gdansk (UG), Federal Maritime and Hydrographic Agency (BSH), Latvian Institute of Aquatic Ecology (LIAE) and the Finnish Environment Institute (SYKE). The main results and conclusions achieved within this WP 4 are gathered in this publication.

2 Monitoring needs of non-indigenous species

There are several international legal instruments requiring monitoring of NIS or invasive alien species (IAS), which are NIS having a potential to cause harm and spread widely. The MSFD is one them placing emphasis on the "trends in abundance, temporal occurrence and spatial distribution in the wild of non-indigenous species notably in risk areas, in relation to the main vectors and pathways…". The MSFD also states that NIS should be monitored in relation to the main vectors. The most common pathway for NIS introductions globally and in the Baltic Sea are ships (via ballast waters and as ships' biofouling). To prevent further introductions via ballast water transfer the IMO adopted the BWM Convention in 2004. The BWM Convention calls on Parties to individually or jointly monitor the effects of ballast water management in their waters. According to the Convention ships will be required, when doing international journeys, to implement ballast water management measures specified in the Convention unless an exemption is granted. The Convention states that vessels on certain routes can be exempted from the application of BWM requirements based on a risk assessment according to the IMO G7 Guidelines (IMO, 2007) requiring reliable data on Harmful Aquatic Organisms and Pathogens, HAOP, in related ports. To ensure harmonised regional implementation of the IMO G7 Guidelines and to ensure that risk assessments are based on reliable information the HELCOM/OSPAR Joint Harmonized Procedure to BWM Convention risk assessments was developed and agreed for the Baltic (HELCOM) and north-East Atlantic (OSPAR) areas in 2013.

A third and the most recent of the legal acts is the EU regulation on the prevention and management of the introduction and spread of IAS (EU, 2014) that entered into force 1 January 2015. The regulation calls member states to monitor (assess the distribution and abundance) of IAS included in the target species list that is at present been made based on risk assessments in the EU Commission. Thus, there are three legal acts calling for the monitoring of NIS or IAS in the Baltic Sea at present.

Most of the information concerning NIS in the Baltic Sea is currently obtained through routine biological monitoring programs. These include the traditional regionally coordinated HELCOM COMBINE program taking place offshore and the coastal fish monitoring under HELCOM FISH-PRO. COMBINE program covers phytoplankton, zooplankton and benthic animals on soft bottoms. However, these monitoring programs do not target NIS and thus do not cover all habitats, seasons and areas that NIS may occupy. There is a large variation between countries in both areal coverage of sampling stations as well as in temporal resolution ranging from annual samples to bi-weekly or even weekly monitoring. The coordinated monitoring at present does not cover littoral shallow water environment and taxa well enough leaving huge gaps in the observations of several crustacean, bivalve, gastropod and small fish taxa inhabiting hard bottom environments. Thus, it would be important to develop harmonized and coordinated hard bottom monitoring under HELCOM to better cover all habitats and taxa to be able to record also NIS. Among the tested methods are different traps for mobile and sessile fauna, which work well. One of the well-working options already tested and used in Finland, Estonia and Poland are the habitat traps, which are deployed in spring-early summer in shallow water on mud, sand bottoms and in algal habitats and taken up in late summer-early autumn. During the deployment period these traps act as a habitat for both mobile and sessile species, which can be identified and counted in the laboratory afterwards. The traps are rather small and light, and thus easy to transport and deploy. They are made of plastic crates and are filled with e.g. pieces of flower pots and pieces of hose as hiding places (**Figure 2**).

Figure 2 - Habitat traps for monitoring shallow water mobile and sessile epifauna. Photo: Maiju Lehtiniemi.

As most of the new introduced species are transported via shipping, routine biological monitoring should be complemented with the monitoring in port areas. Ports with the most intensive international ship traffic should be taken under regular monitoring. This monitoring should be conducted following the Joint HELCOM/OSPAR Harmonized Procedure tailored for the Baltic Sea conditions to fullfill the data requirements of the BWM Convention (for exemption procedures). As the protocol includes sampling of phytoplankton, zooplankton, soft bottom benthos and mobile and sessile epifauna on hard surfaces as well as on soft bottoms it covers all taxonomic groups of NIS and thus gives valuable information needed not only for the BWM Convention for granting exemptions but also for the MSFD and EU IAS regulation purposes.

3 Overall results of port sampling

Three sampling sites were studied in the Port of Muuga Harbour, Port of Tallinn (Estonia) (**Figure 3** and **Table 2**). Five non-indigenous taxa were recorded, excluding phytoplankton species: *Acartia tonsa, Marenzelleria neglecta, Gammarus tigrinus, Neogobius melanostomus* and *Dreissena polymorpha,* whereas *Amphibalanus improvisus* was the cryptogenic species recorded.

Port survey monitoring methods were tested in the Latvian ports of Liepaja and Riga. Three sampling sites were selected at each port. Eleven non-indigenous taxa were recorded in the Port of Liepaja: *Prorocentrum minimum, Acartia tonsa, Evadne anonyx, Amphibalanus sp. nauplii (*most probably the cryptogenic species *A. improvisus), Palaemon elegans, Cordylophora caspia*[1]*, Dreissena polymorpha, Potamopyrgus antipodarum, Marenzelleria sp., Rhithropanopeus harrisii* and *Neogobius melanostomus,* whereas *Mya arenaria* was the cryptogenic species recorded. Four non-indigenous taxa were recorded in the Port of Riga: *Acartia tonsa*, *Dreissena polymorpha, Potamopyrgus antipodaru* and *Marenzelleria* sp., whereas *Amphibalanus improvisus* and *Mya arenaria* were the cryptogenic species recorded.

Three sampling sites were also studied in the Port of Gdynia (Poland), where all together 264 taxa were identified within all groups studied. Taxa composition, their abundance and biomass varied at the three sampling sites. Six non-indigenous taxa were recorded: *Acartia tonsa*, *Evadne anonyx*, *Marenzelleria* spp., *Cordylophora caspia, Palaemon elegans*, *Rhithropanopeus harrisii* and *Neogobius melanostomus,* whereas *Amphibalanus improvisus, Chaetoceros* cf. *lorenzianus* and *Mya arenaria* were the cryptogenic species recorded.

[1] There is an ongoing discussion on whether this species is a cryptogenic species or not.

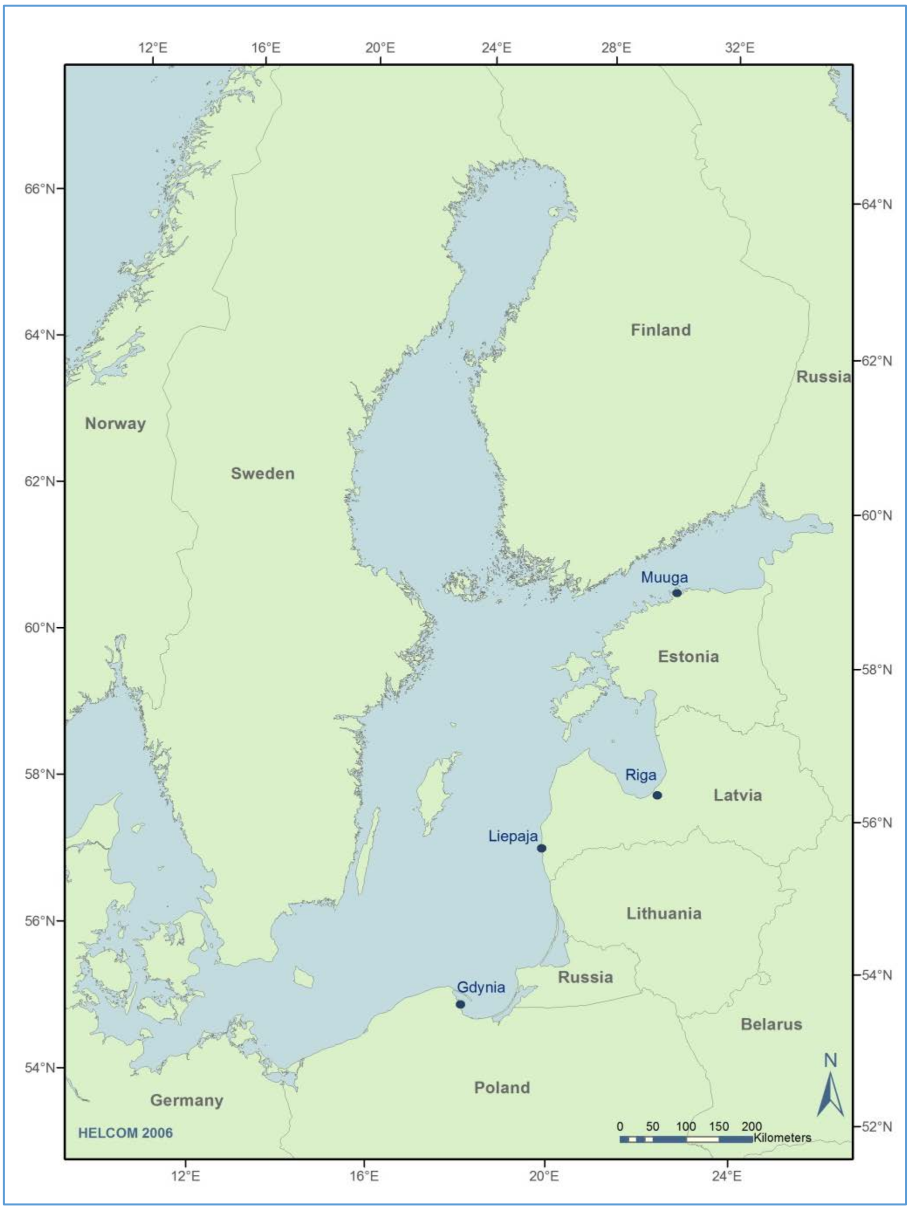

Figure 3- Location of the ports sampled.

Group	Taxa	Species	Non-indigenous taxa	Cryptogenic
		Port of Muuga Harbour, Port of Tallin (Estonia)		
Phytoplankton	80	_	**	**
Zooplankton	11	23	3	2
Mobile fauna	_	3	1	_
Fouling communities	1	17	2	1
Benthic infauna	2	17	1	1
		Port of Liepaja and Port of Riga (Latvia)		
Phytoplankton	85	_	_	_
Zooplankton	44	33	4	_
Mobile fauna	9	9	2	_
Fouling communities	21*	17*	3*	2*
Benthic infauna	31	31	4	2
		Port of Gdynia (Poland)		
Phytoplankton	188	145	_	1
Zooplankton	35	19	4	1
Mobile fauna	9	8	3	_
Fouling communities	31	20	4	2
Benthic infauna	15	13	2	_
ALL	**264**	**209**	**6**	**4**

Table 2 - Number of identified taxa and species number within each group studied in the ports sampled. (*Analysis of the samples is on-going; ** not possible to determine).

4 Detailed sampling results of the Port of Muuga Harbour, Port of Tallinn (Estonia)

Muuga Harbour is the biggest cargo harbour in Estonia and is specialized on handling transit origin goods. It is the main cargo harbour for the Port of Tallinn and locates ca 17 km east of Tallinn. The cargo volume handled accounts for around 80% of the total cargo volume of the Port of Tallinn and approximately 90% of the transit cargo volume passing through Estonia. Nearly 3/4 of cargo loaded in Muuga Harbour includes crude oil and oil products, but the harbour also serves dry bulk (mostly fertilizers, grain and coal) and other types of cargo. Muuga Harbour is among the deepest (up to 18 m) and most modern ports in the Baltic Sea region with aquatory of 752 ha, total length of berths of 6.4 km, and maximum vessel length/width of 300 m/48 m (http://www.portoftallinn.com/muuga-harbour).

4.1 Sampling site location and environmental parameters

Muuga harbour was sampled in three locations – container terminal, grain terminal and oil terminal – three times a year (spring – 28 April, summer – 8 June, and autumn – 1 October) – in 2014 (**Figure 4**).

Sampling was performed according to port biological sampling guidelines (HELCOM, 2013a). In total, 9 CTD measurements were performed. Of biota, 9 phytoplantkon, 19 zooplantkon samples with Juday net (100 µm), and a net of mesh size of 400 µm, 27 zoobenthos samples with Ekman sampler (triplicate at each sampling event), 27 mobile epifauna samples with crab trap (triplicate at each sampling event) and 27 fish samples with gee minnow trap (triplicate at each sampling event). In addition, fouling community was sampled by a scraping tool from various substrates (natural rock, rubber, metal and concrete) and investigated via installing settlement PVC plates (15x15cm) to the sea in April and collecting them in October.

All plantkton samples and benthos samples collected by Ekman grab were analysed according to HELCOM COMBINE manuals (HELCOM, 2013b).

Figure 4- Sampling locations in Muuga harbour (from right to left): general cargo terminal, grain terminal and oil terminal. Muuga harbour map taken from: http://www.panoramio.com/user/280236.

Detailed results on the key environmental variables affecting distribution and abundance of organisms – temperature, salinity and oxygen – are presented by 1 m depth intervals for all locations sampled in all occasions. The data are given in **Table 3** below.

Station	Depth	Salinity	T^a (^oC)	O_2 (%)	O_2 (mg/l)
			April		
Container terminal	0.5	5.5	6.6	118.8	14.1
Container terminal	1	5.5	6.6	117.1	14.0
Container terminal	2	5.6	5.7	114.8	14.0
Container terminal	3	5.6	5.6	113.7	13.9
Container terminal	4	5.6	5.5	113.6	13.9
Container terminal	5	5.6	5.4	113.1	13.9
Container terminal	6	5.6	5.3	112.7	13.8
Container terminal	7	5.7	5.2	111.2	13.7
Container terminal	8	5.7	5.0	110.4	13.7
Container terminal	9	5.7	4.9	108.7	13.5
Container terminal	10	5.8	4.8	107.0	13.3
Container terminal	11	5.8	4.7	105.3	13.2
Grain terminal	0.5	5.5	7.6	117.7	13.7

Table 3 - Abiotic conditions (temperature, salinity and oxygen) in three terminals in Muuga harbour (by 1 meter depth intervals) in 2014.

Grain terminal	1	5.5	7.1	118.1	13.9
Grain terminal	2	5.5	6.7	116.9	13.9
Grain terminal	3	5.6	6.6	117.0	13.9
Grain terminal	4	5.6	6.3	116.6	14.0
Grain terminal	5	5.6	5.7	116.6	14.2
Grain terminal	6	5.6	5.6	115.3	14.1
Grain terminal	7	5.7	5.2	114.6	14.1
Grain terminal	8	5.7	5.1	112.4	13.9
Grain terminal	9	5.7	5.0	111.2	13.8
Grain terminal	10	5.7	4.9	109.8	13.6
Grain terminal	11	5.8	4.6	108.5	13.6
Grain terminal	12	5.9	3.5	105.7	13.6
Oil terminal	0.5	5.6	7.1	118.0	13.8
Oil terminal	1	5.6	6.9	116.8	13.8
Oil terminal	2	5.6	6.2	117.0	14.0
Oil terminal	3	5.6	6.0	117.5	14.2
Oil terminal	4	5.6	5.9	117.2	14.2
Oil terminal	5	5.6	5.8	116.7	14.2
Oil terminal	6	5.7	5.7	115.3	14.0
			June		
Container terminal	0.5	4.5	17.1	96.3	9.1
Container terminal	1	4.5	16.9	96.4	9.1
Container terminal	2	4.5	16.6	97.0	9.2
Container terminal	3	4.5	16.3	97.0	9.3
Container terminal	4	4.6	15.4	94.8	9.2
Container terminal	5	4.7	14.1	92.9	9.3
Container terminal	6	4.8	13.8	90.8	9.1
Container terminal	7	5.0	11.1	87.8	9.4
Container terminal	8	5.2	9.6	86.4	9.5
Container terminal	9	5.7	6.7	82.9	9.8
Container terminal	10	5.8	6.1	81.7	9.8
Container terminal	11	5.9	5.9	80.1	9.6
Grain terminal	0.5	4.6	17.6	100.0	9.3
Grain terminal	1	4.6	17.5	100.4	9.3
Grain terminal	2	4.6	17.3	99.7	9.3
Grain terminal	3	4.6	17.1	99.5	9.3
Grain terminal	4	4.6	16.0	95.2	9.1
Grain terminal	5	4.6	15.5	94.3	9.2
Grain terminal	6	4.9	12.6	90.5	9.3
Grain terminal	7	5.2	10.0	86.7	9.5
Grain terminal	8	5.3	9.6	85.3	9.4
Grain terminal	9	5.3	9.4	84.7	9.4
Grain terminal	10	5.3	9.0	83.8	9.4
Grain terminal	11	5.4	8.7	84.0	9.4
Grain terminal	12	5.4	8.6	84.0	9.5
Oil terminal	0.5	4.5	19.7	105.2	9.4
Oil terminal	1	4.5	19.5	104.8	9.4

Table 3 – Continuation.

Oil terminal	**2**	**4.5**	**19.1**	**104.5**	**9.4**
Oil terminal	3	4.5	18.5	104.4	9.5
Oil terminal	4	4.5	18.2	103.4	9.5
Oil terminal	5	4.7	16.5	100.6	9.6
Oil terminal	6	4.8	13.7	95.4	9.6
			October		
Container terminal	0.5	5.2	12.4	92.1	9.6
Container terminal	1	5.2	12.4	94.6	9.9
Container terminal	2	5.2	12.4	95.7	10.0
Container terminal	3	5.2	12.3	96.3	10.1
Container terminal	4	5.2	12.3	96.6	10.1
Container terminal	5	5.2	12.3	96.7	10.1
Container terminal	6	5.2	12.3	97.4	10.2
Container terminal	7	5.2	12.3	97.9	10.2
Container terminal	8	5.2	12.3	98.1	10.3
Container terminal	9	5.2	12.3	98.2	10.3
Container terminal	10	5.2	12.3	98.4	10.3
Container terminal	11	5.2	12.3	98.9	10.4
Grain terminal	0.5	5.2	12.5	90.4	9.4
Grain terminal	1	5.2	12.4	91.4	9.5
Grain terminal	2	5.2	12.4	92.0	9.6
Grain terminal	3	5.2	12.4	92.3	9.6
Grain terminal	4	5.2	12.4	92.8	9.7
Grain terminal	5	5.2	12.4	93.3	9.8
Grain terminal	6	5.2	12.4	93.6	9.8
Grain terminal	7	5.2	12.4	94.5	9.9
Grain terminal	8	5.2	12.4	94.9	9.9
Grain terminal	9	5.2	12.4	95.3	10.0
Grain terminal	10	5.2	12.3	96.1	10.1
Grain terminal	11	5.2	12.3	96.4	10.1
Grain terminal	12	5.2	12.3	96.7	10.1
Oil terminal	0.5	5.2	12.5	92.6	9.6
Oil terminal	1	5.2	12.5	93.4	9.7
Oil terminal	2	5.2	12.4	94.5	9.9
Oil terminal	3	5.2	12.4	94.5	9.9
Oil terminal	4	5.2	12.4	95.5	10.0
Oil terminal	5	5.2	12.4	96.3	10.0

Table 3 –Continuation.

4.2 Biological survey

4.2.1 Phytoplankton

Phytoplankton dominant species were similar to those found in the middle part of Muuga Bay. In April, the autotrophic ciliate *Mesodinum rubrum* constituted up to 53 % of total biomass. In August and in October, the euglenophyte *Eutreptiella gymnastica* and the diatom *Coscinodiscus granii* dominated, respectively. There is no confident information on the occurrence of non-indigenous or cryptogenic phytoplankton species in Muuga harbour area in 2014.

A significant difference in total biomass was noted in April and October, probably caused by more turbid environment and poorer light conditions in harbour area. The total biomass values of phytoplankton varied between 1.32 and 2.26 mg l^{-1} in April (7.07 mg l^{-1} in station 3 in the middle part of Muuga Bay), 0.21−0.56 (0.49) mg l^{-1} in August and 0.20−0.41 (0.88) mg l^{-1} in October.

4.2.2 Zooplankton

Synchaeta spp. (*S. monopus*, *S. baltica* and *S. curvata*) was far the most abundant taxon (average >17 300 ind/m^3), followed by copepod nauplii (>10700 ind/m^3), *Acartia* spp. (>4300) and *Keratella* spp. (>2700 ind/m^3). Of biomass, Synchaeta spp. still dominated (average >84.5 mg/m^3), followed by Acartia spp. (>44.5 mg/m^3) and copepod nauplii (>32.1mg/m^3), see **Table 4**. The cirriped *Amphibalanus improvisus* is cryptogenic species and *Acartia* spp. and copepod nauplii most likely contain the non-indigenous *Acartia tonsa*.

Month	Location	*Synchaeta*	*Keratella*	*Acartia*	*Eurytemora*	*Cyclopidae*	Cop. nauplii	*Eubosmina*	*Pleopis*	*Amphibalanus*
ABUNDANCE (ind./m^3)										
April	Container terminal	4680	104	924	672	21	1040	21		
	Oil terminal	20086	664	1980	1089	33	2158	33	33	
	Grain terminal	2820	45	1455	585	15	885	15		
August	Container terminal	476	969	1666	1139	306	6647	34	85	1989
	Oil terminal	603	2680	988	247	156	5561	611	13	1206
	Grain terminal	594	660	1683	646	204	7095	527	51	594
October	Container terminal	44080	3360	6240	4080		22240	80	320	320
	Oil terminal	40504	10126	8241	3484	67	22078	67	67	830
	Grain terminal	42344	6164	16524	3591		28810	81	270	268
BIOMASS (mg/m^3)										
April	Container terminal	23.4	0.1	12.9	8.1	0.3	3.1	0.3		
	Oil terminal	100.4	0.7	28.6	11.9	0.5	6.5	0.3	0.3	
	Grain terminal	8.5	0	17.4	6	0.2	2.7	0.1		
August	Container terminal	2.3	1	28.1	9.5	4.7	19.9	0.4	0.8	9.9
	Oil terminal	2.7	2.7	12.9	2	1.2	16.7	7.2	0.1	6
	Grain terminal	2.9	0.7	21.3	4.6	1.9	21.3	6	0.5	3
October	Container terminal	217.2	3.4	48	31.3		66.7	0.8	3.2	1.6
	Oil terminal	196.3	10.2	79.9	27.9	0.5	66.2	0.7	0.7	5
	Grain terminal	206.9	6.2	152.2	26.1		86.4	0.9	3.3	1.3

Table 4 - Abundance (ind./m^3) and biomass (mg/m^3) of more abundant zooplantkon taxa in the three terminals sampled in Muuga harbour in 2014.

4.2.3 Mobile epifauna

In addition to the round goby *Neogobius melanostomus*, which strongly dominated in both fish and crab traps, two native fish species were found: the eelpout *Zoarces viviparus* (in total 6 specimen) and the pipefish *Nerophis ophidion* (18 individuals). An average, *N. melanostomus* was six times more abundantly present in the crab than in the gee minnow traps (see also **Table 5**).

	April	August	October
Container terminal	5.7	2.7	0.7
Grain terminal	0.0	2.0	0.0
Oil terminal	0.3	0.3	0.3

Table 5 - Catch per unit effort (CPUE, number of individuals) of the non-indigenous round goby *Neogobius melanostomus* in the gee minnow traps in the three locations in Muuga harbour in 2014.

4.2.4 Benthic infauna

In total, close to 19 taxa were found in the Ekman samples. The cryptogenic cirriped *Amphibalanus improvisus* strongly dominated amongst benthic invertebrates with a dry weight biomass of up to 22 g/m^2. However, algae – *Fucus vesiculosus* and *Ulva intestinalis* – dominated in some instances, as well as the bivalve *Macoma balthica* (**Table 6**).

Location	Taxon	Dry weight (g/m^2)
	April	
Container terminal	*Fucus vesiculosus*	52.7438
Container terminal	*Amphibalanus improvisus*	15.5154
Container terminal	*Pilayella littoralis*	7.9378
Container terminal	*Macoma balthica*	1.8662
Container terminal	*Hediste diversicolor*	0.0473
Container terminal	*Gammarus* juv.	0.0129
Container terminal	*Leptocheirus pilosus*	0.0086
Grain terminal	*Amphibalanus improvisus*	22.1364
Grain terminal	*Macoma balthica*	7.6884
Grain terminal	*Battersia arctica*	5.9383
Grain terminal	*Zostera marina*	0.4214
Grain terminal	*Polysiphonia fucoides*	0.3956
Grain terminal	*Hediste diversicolor*	0.2795
Grain terminal	*Pilayella littoralis*	0.1548
Grain terminal	*Chironomidae* larvae	0.0516
Grain terminal	*Zannichellia palustris*	0.0473
Grain terminal	*Corophium volutator*	0.043
Grain terminal	***Marenzelleria neglecta***	0.0258
Oil terminal	*Macoma balthica*	1.7974
Oil terminal	*Chironomidae l.*	0.0817
Oil terminal	*Hediste diversicolor*	0.0172

Table 6 - Taxonomic composition of zoobenthos samples (taken by Ekman sampler), ordered by maximum biomass observed, in three terminals in Muuga harbour in 2014. The cirriped *Amphibalanus improvisus* (marked in grey and underlined) is a cryptogenic species and *Marenzelleria neglecta* (marked in grey and bold) non-indigenous species. *Gammarus* juv. may contain the non-indigenous *Gammarus tigrinus*.

	June	
Container terminal	*Amphibalanus improvisus*	15.7079
Container terminal	*Battersia arctica*	4.6526
Container terminal	*Fucus vesiculosus*	2.9799
Container terminal	*Myriophyllum spicatum*	0.7525
Container terminal	*Cladophora glomerata*	0.301
Container terminal	*Ulva intestinalis*	0.2451
Container terminal	*Zostera marina*	0.1548
Container terminal	*Gammarus* juv.	0.0344
Grain terminal	*Macoma balthica*	11.6272
Grain terminal	*Pilayella littoralis*	0.4128
Grain terminal	*Monostroma balticum*	0.3569
Grain terminal	*Ulva intestinalis*	0.0731
Grain terminal	*Chironomidae* larvae	0.0731
Grain terminal	***Marenzelleria neglecta***	0.0258
Oil terminal	*Ulva intestinalis*	9.3181
Oil terminal	*Gammarus* juv.	0.1376
Oil terminal	*Hediste diversicolor*	0.1118
Oil terminal	*Chironomidae* larvae	0.086
	October	
Container terminal	*Hediste diversicolor*	0.3698
Container terminal	*Chironomidae* larvae	0.0215
Grain terminal	*Macoma balthica*	4.1065
Grain terminal	*Battersia arctica*	0.3483
Grain terminal	*Stuckenia pectinata*	0.473
Grain terminal	*Zostera marina*	0.2451
Grain terminal	*Battersia arctica*	0.2408
Grain terminal	Oligochaeta	0.0086
Oil terminal	*Chironomidae* larvae	0.0645

Table 6 - Continuation.

4.2.5 Fouling organisms

Settling plates

A total of 13 taxa were found to colonise the settling plates. The cryptogenic cirriped *A. improvisus* (marked in grey and underlined) dominated on the settling plates, both in terms of the presence of occurrence and maximum biomass observed, followed by *Cladophora glomerata*, *Ulva intestinalis* and *Jaera albifrons* in terms of the presence of occurrence and *Pilayella littoralis* in terms of the maximum biomass observed (**Table 7**). The zebra mussel *Dreissena polymorpha* (marked in grey and in bold) is the only non-indigenous species observed.

Taxon	Occurrence (No. of plates)	Dry wt (g/m^2)
Amphibalanus improvisus	5	27.9886
Cladophora glomerata	5	2.1269
Ulva intestinalis	5	0.9002
Jaera albifrons	5	0.0023
Heterotanais oerstedii	4	0.0048
Leptocheirus pilosus	4	0.0032
Pilayella littoralis	3	2.2317
Cerastoderma glaucum	3	0.566
Chironomidae larvae	2	0.0029
Mytilus trossulus	1	0.0108
Dreissena polymorpha	1	0.0033
Alderia modesta	1	0.0003
Gammarus zaddachi	1	0.0016

Table 7 - Taxonomic composition of settling plates with the number of occurrence and maximum biomass observed. In total, 5 settling plates were deployed from April to October, 2014.

Scraping samples

In total, 12 taxe were found on various substrata (natural rock, metal, rubber and concrete). In addition to four algal species (*Pilayella littoralis*, *Ulva intestinalis*, *Cladophora glomerata* and *Polysiphonia fucoides*), the cryptogenic cirriped *Amphibalanus improvisus*, the isopod *Idothea chelipes*, several gammarids – *Gammarus salinus*, *G*. *tigrinus*, *G*. *zaddachi*, *Gammarus* juv., the blue mussel *Mytilus trossulus* and larvae of Chironomidae were found. The gammarid *G. tigrinus* is the only non-indigenous species found in scraping samples.

5 Detailed sampling results of the Port of Liepaja and Port of Riga (Latvia)

In this section all the relevant information related to the sampling conducted in the Port of Liepaja and Port of Riga is compiled, together with the results of the identification of taxa conducted in the samples taken.

5.1 Sampling site location and environmental parameters

The Port of Liepaja provides a solid base for logistics connections with the rest of Europe. Latvia's third largest port in terms of cargo throughput, Liepaja is truly multifunctional, as a port service provider, dealing with most types of cargo. The port infrastructure – access canals, berths and cargo-handling equipment – allows for vessels with a maximum draught of 10.8 m and length of 235 m to call at the port. In total, there are 16 cargo handling terminals for various types of cargo, equipped with appropriate cargo handling and storage facilities – open-air and warehouses, silos, tanks and refrigerated space. Liepaja is one of the few nonfreezing ports in the region, providing continuous navigation at any weather conditions.

Since the beginning of 1990s Liepaja has rapidly developed as a multifunctional port, reaching fastest increase of cargo turnover among the Eastern Baltic's ports. Today Liepaja is an important international transportation hub, which provides cargo exchange between Eastern and Western European markets. Port companies depending on their specialization provide handling of dry bulk, liquid bulk and general cargoes.

In 2013 the Port of Riga was the biggest port of the Baltic States by cargo turnover that reached 35.5 million tons. The throughput capacity of the Port of Riga equals 47 million tonnes per year, and taking into account the rapid development of the Port, the capacity is expected to exceed 50 million tonnes per year within the near future. Number of vessels accomodated in 2013 amounted to 3850.

The Port of Riga lies on both banks of the River Daugava covering 15 km in length. Total port area is 6248 ha, total length of berths – 14.3 km, maximum draft of vessels – 14.5 m. Main types of cargo handled at the Port of Riga are containers, various metals, timber, coal, mineral fertilizers, chemical cargo and oil products.

Port survey monitoring methods were tested in the port of Liepaja in September 2013 as well as May and October 2014 and in the port of Riga in September 2014. Three sampling sites were selected at each port (**Figure 5** and **Figure 6**).

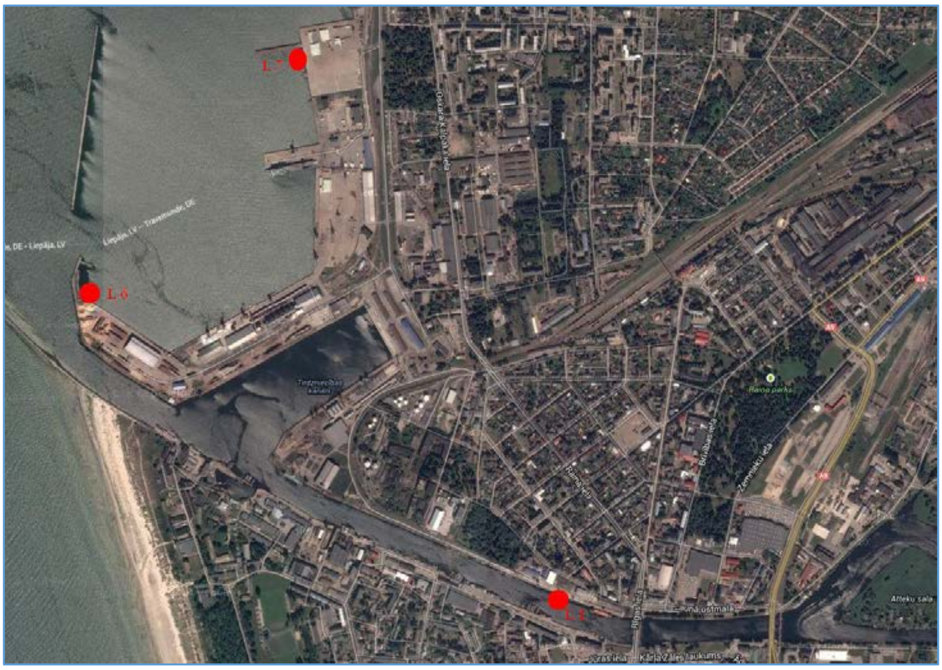

Figure 5 - Map of the Port of Liepaja with the three sampling sites L1, L6 and L7.

Figure 6 - Map of the Port of Riga with the three sampling sites R1, R2 and R3.

Information on the geographic location of the sampling sites is given in **Table 8**.

Sampling site	Name	Latitude (N)	Longitude (E)
Port of Liepaja	L1	56°30´974´´	21°00´016´´
Port of Liepaja	L6	56°31´395´´	20°58´992´´
Port of Liepaja	L7	56°31´876´´	20°59´702´´
Port of Riga	R1	57°03´144´´	24°01´801´´
Port of Riga	R2	57°01´144´´	24°05´801´´
Port of Riga	R3	57°01´302´´	24°05´161´´

Table 8 - Geographic location of the studied sampling points.

At each sampling site temperature, salinity, dissolved oxygen as well as transparency (Secchi disk), Chlorophyll *a* and chemical parameters were measured. Results are partly presented in **Table 9** for September 2013 and May and October 2014 at the Port of Liepaja and in **Table 10** for September 2014 at the Port of Riga.

		L1			L6			L7		
		2013	2014		2013	2014		2013	2014	
Parameter	Depth (m)	Sep.	May	Oct.	Sep.	May	Oct.	Sep.	May	Oct.
T^a (°C)	1	17.1	16.1	10.9	17.4	16.2	11.1	17.5	15.7	11.2
	2	17.6	16.1	11.0	17.4	16.2	11.1	17.4	15.8	11.2
	3	17.6	15.8	11.0	17.3	16.2	11.1	17.4	15.8	11.2
	4	17.5	15.7	11.0	17.3	16.2	11.2	17.4	15.8	11.2
	5		15.5	11.0	17.3	16.2	11.2	17.4	15.8	
	6		15.2	11.0	17.4	16.2	11.2	17.4		
	7				17.4		11.2			
Salinity (psu)	1	3.5	6.5	0.8	6.5	6.6	5.3	6.6	6.7	5.9
	2	5.5	6.5	0.8	6.6	6.6	5.4	6.6	6.7	5.9
	3	6.3	6.5	0.8	6.6	6.6	5.7	6.6	6.7	5.9
	4	6.5	6.5	1.2	6.6	6.6	6.0	6.6	6.7	5.9
	5		6.5	1.0	6.6	6.6	6.2	6.6	6.7	
	6		6.5	1.9	6.6	6.6	6.3	6.6	6.7	
	7				6.7		6.3			
O_2 (mg/l)	1	6.4	6.6	7.0	4.0	6.3	7.3	5.6	7.3	7.0
	2	5.9	6.5	6.8	4.4	6.4	7.2	5.5	7.3	7.1
	3	5.4	6.3	6.8	5.5	6.5	6.9	5.6	7.3	7.1
	4	4.6	6.1	6.6	5.8	6.5	6.9	5.5	7.3	7.1
	5		6.0	6.7	5.7	6.5	6.6	5.3	7.3	
	6		5.8	6.5	5.5	6.5	6.6	5.3	7.3	
	7				5.6		6.6			

Table 9 - Environmental parameters measured in September 2013 and May and October 2014 at the Port of Liepaja.

		R1	R2	R3
Parameter	**Depth (m)**	**Sept. 2014**		
T^a (°C)	1	17.9	18.3	18.0
	2	17.1	17.5	18.2
	3	16.5	16.8	18.0
	4	16.3	16.6	16.7
	5	16.0	16.2	15.8
	6	15.7	15.7	15.5
	7	15.3	15.6	15.0
	8	15.3		14.7
	9	15.2		
	10	15.1		
	11	15.1		
	12	15.0		
Salinity (psu)	1	1.5	0.7	0.4
	2	1.8	1.1	1.0
	3	2.2	1.4	1.1
	4	2.7	1.6	2.0
	5	3.2	2.3	3.0
	6	3.8	3.1	3.4
	7	4.6	3.3	4.2
	8	4.9		4.4
	9	5.0		
	10	5.0		
	11	5.1		
	12	5.1		
O_2 (mg/l)	1	8.0	6.1	7.2
	2	9.1	5.6	6.0
	3	7.0	5.7	5.6
	4	6.1	5.7	5.7
	5	5.9	5.5	5.4
	6	5.6	5.3	5.3
	7	5.7	5.2	5.2
	8	5.9		5.2
	9	5.9		
	10	5.9		
	11	5.9		
	12	5.8		

Table 10 - Environmental parameters measured in September 2014 at the Port of Riga.

Chlorophyll *a* samples were collected in the Port of Liepaja and the Port of Riga. In the Port of Liepaja samples were taken from three stations (L1, L6 and L7) in September 2013, as well as in May and October 2014, while in the Port of Riga harbor samples were taken from three stations (R1, R2 and R3) in September 2014. In each station samples were collected from two different – 1 m and 5 m – depths.

Chlorophyll *a* concentration showed no seasonal trend and ranged between 3.79 and 11.88, except for one extreme value in the sample collected from the Port of Riga (Station R2) in September 2014 (**Figure 7**).

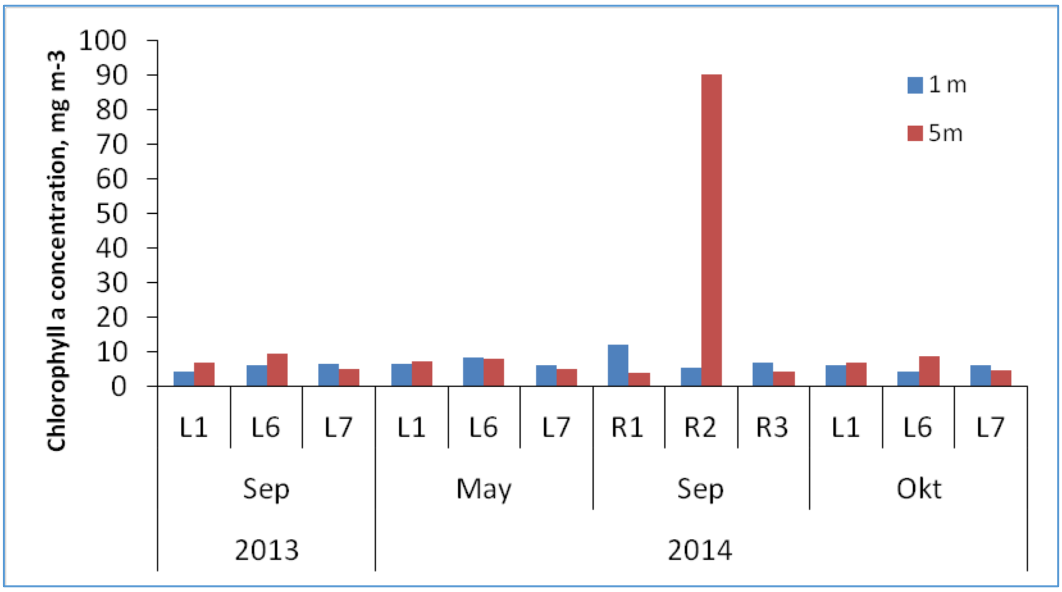

Figure 7 - Chlorophyll *a* concentration in Latvian harbors (L – Port of Liepaja; R – Port of Riga) at 1 m and 5 m depth.

5.2 Biological survey

5.2.1 Phytoplankton

Port of Liepaja

In the Port of Liepaja phytoplankton was collected in September 2013, May 2014 and October 2014 in three stations (L1, L6 and L7). Samples were obtained at 1 m and 5 m depth by Horizontal water sampler (4.2 l) and with the hand hauled Apstein type 53 µm net, preserved with acid Lugol solution and analysed in laboratory regarding to taxa composition and abundance (HELCOM, 2013b).

In September 2013, the highest total phytoplankton biomass was found in station L6 in both depths, while in stations L1 and L7 higher biomass was found in the surface (1 m) samples (**Figure 8**). However phytoplankton community showed clear differences between stations L6, L7 and station L1 in all sampling periods due to the location of the sampling points.

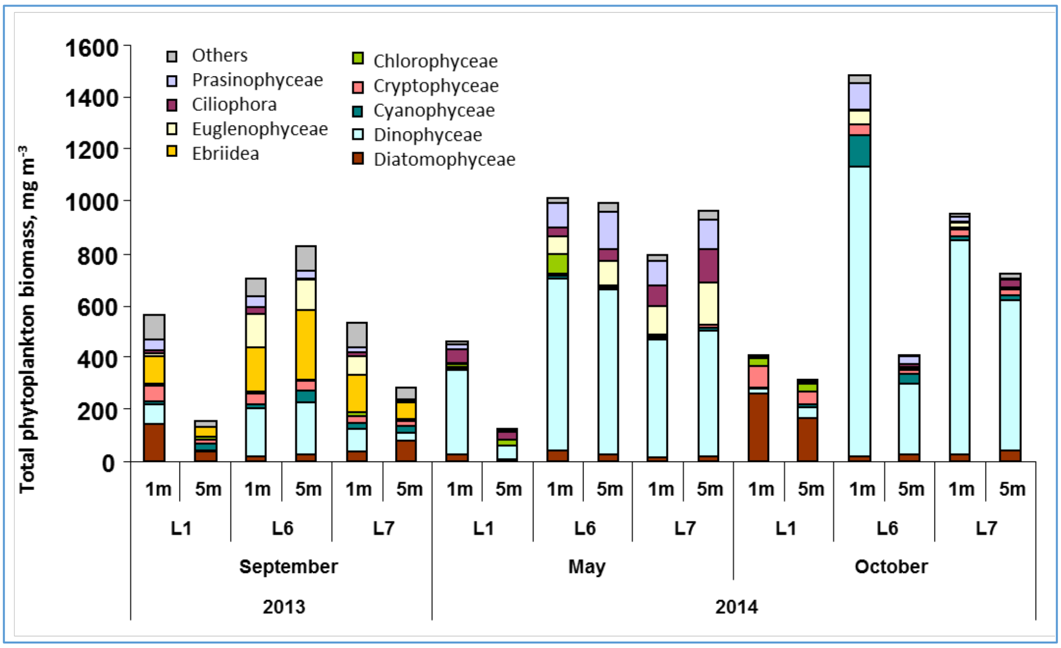

Figure 8 - Total phytoplankton biomass (mg m^{-3}) in the Port of Liepaja.

Prorocentrum minimum was the only identified non-indigenous species with very low biomass in September 2013 (**Figure 9**). Higher *Prorocentrum minimum* biomasses were found in the upper layer (1 m) samples in stations L1 and L6. However, results from the net samples showed twice higher *Prorocentrum minimum* abundance in the station located closer to Baltic Sea coast (L6).

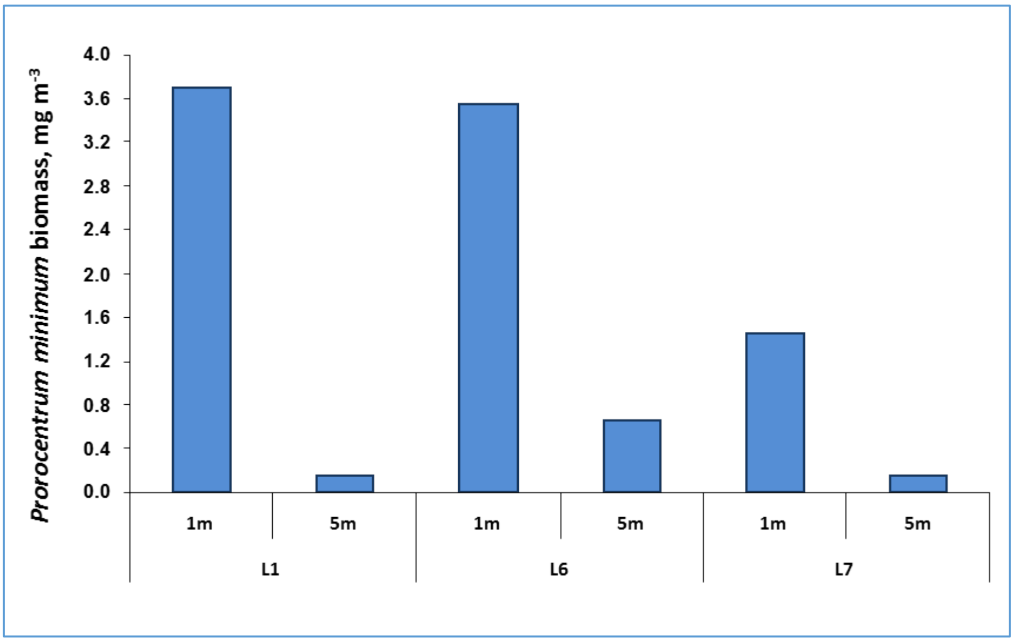

Figure 9 - *Prorocentrum minimum* biomass (mg m^{-3}) in the Port of Liepaja, September 2013.

In May 2014 dinoflagellates (Dinophyceae) composed the main proportion of the total phytoplankton in all stations (**Figure 8**), dominated by specie *Oblea rotundata*. Twice higher biomass was found in stations located closer to Baltic Sea (L6 and L7), compare to station L1. None invasive species were detected.

In October 2014 phytoplankton biomass mostly consisted of diatoms (Diatomophyceae) in station L1, while in L6 and L7 the dominant class was dinoflagellates. *Prorocentrum minimum* was the only invasive species detected and was found in all samples. In stations L6 and L7 it was the dominant species and composed 70% (L6) to 84% (L7) of the total phytoplankton biomass, furthermore high biomass was found in the upper water layer (1 m) (**Figure 10**).

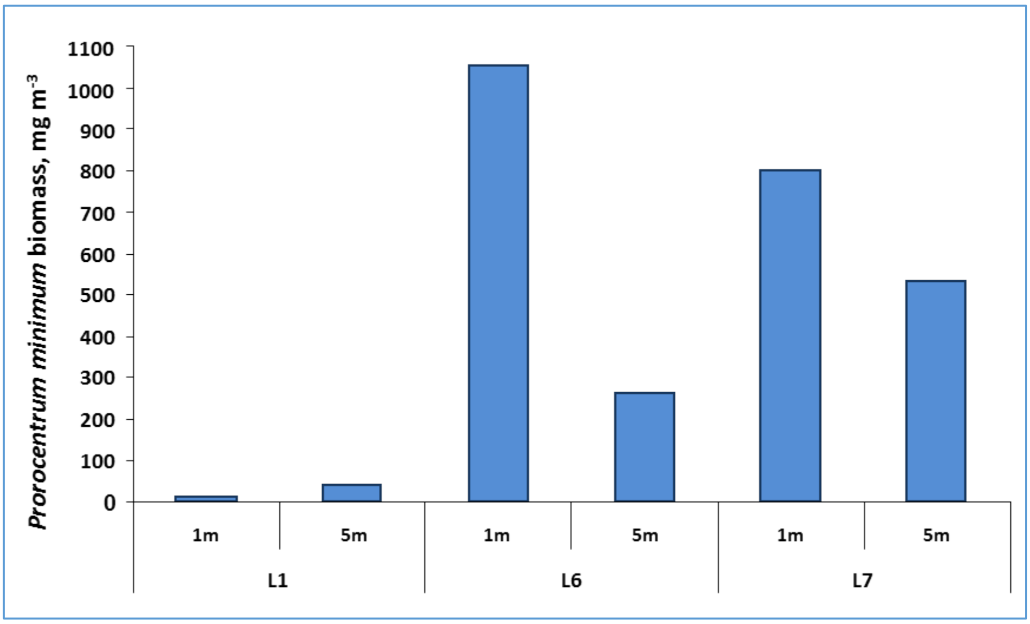

Figure 10 - *Prorocentrum minimum* biomass (mg m^{-3}) in the Port of Liepaja, October 2014.

Port of Riga

In the Port of Riga phytoplankton was collected in September 2014 in three stations (R1, R2 and R3). Samples were obtained at 1 m and 5 m depth by Horizontal water sampler (4.2 l) and with the hand hauled Apstein type 53 µm net, preserved with acid Lugol solution and analysed in laboratory regarding to taxa composition and abundance (HELCOM, 2013b).

The highest biomass was detected in station R1, located closest to Gulf of Riga (**Figure 11**), moreover phytoplankton community structure was much more diverse than in the other two stations (R2 and R3), located further in River Daugava. In the stations R2 and R3 the dominant classes were diatoms and cryptophytes, while in station R1 small autotrophic flagellates and dinoflagellates were also found.

No non indigenous species were detected in the phytoplankton samples from the port of Riga.

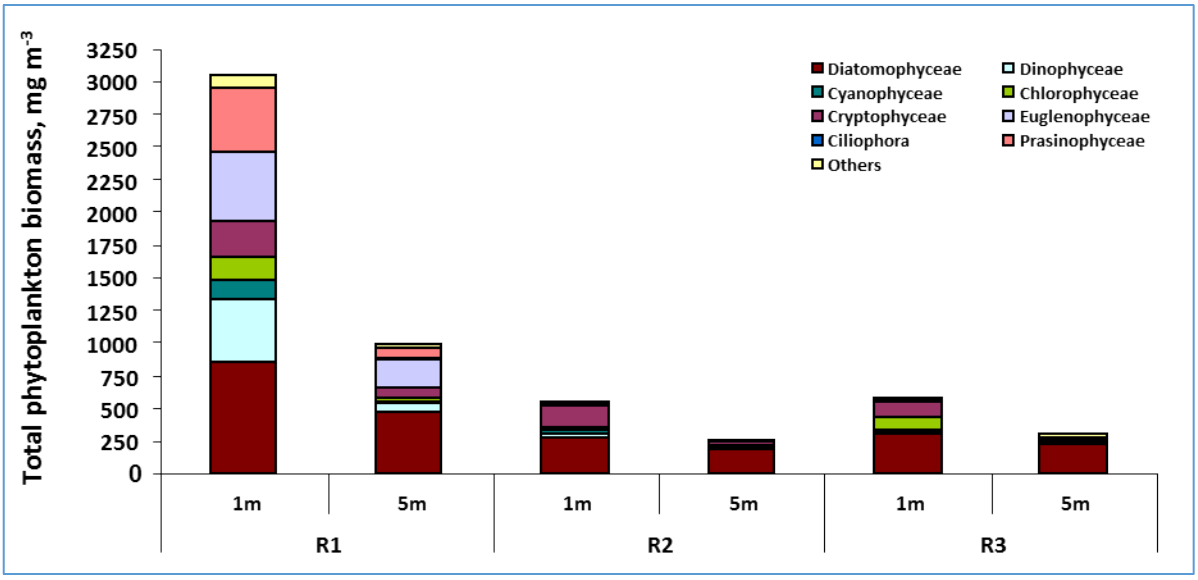

Figure 11 - Total phytoplankton biomass (mg m^{-3}) in the Port of Riga, September 2013.

5.2.2 Zooplankton

Mesozooplankton samples were collected from the bottom to the upper layer by means of vertical hauls, using Apstein plankton net with mesh size 53 µm. In the Port of Liepaja samples were taken from three stations (L1, L6 and L7) in September 2013, and in May and October 2014, while in the Port of Riga they were taken from three stations (R1, R2 and R3) in September 2014. In each sampling two samples per station were collected.

Port of Liepaja

Eight different copepods, five different cladocerans and 15 taxa of Rotifera, as well as six taxa, mainly larvae of benthic organisms, combined in group Varia, were found in samples collected in the Port of Liepaja. Four target species were among them – *Acartia tonsa*, *Evadne anonyx*, *Amphibalanus* sp. nauplii (most probably the cryptogenic species *A.improvisus*) and one adult *Palaemon elegans* (**Table 11**).

The highest zooplankton abundance and biomass was in May 2014 due to large quantity of rotiferan *Synchaeta* spp., while copepods, especially *Acartia* spp. and *Eurytemora* sp. were dominant in September 2013. Unfortunately, zooplankton abundance and biomass in samples collected in

October 2014 is too low to make any conclusions, obviously the sampling was done after the productive season (**Figure 12A**).

Port of Riga

Nine species of Copepoda, five different cladocerans, 15 different rotiferans, and three taxa of group Varia were found in waters of the Port of Riga. More freshwater cladocerans and rotiferans were found compared to the Port of Liepaja, and only two target species – *Acartia tonsa* and *Amphibalanus* sp. nauplii (most probably the cryptogenic species *A. improvisus*) (**Table 10**).

Rotifera, mainly *Synchaeta* spp. and *Keratella* spp., and copepods *Acartia* spp., *Eurytemora* sp. and Cyclopoida were the most abundant in September 2014. Zooplankton abundance as well as biomass did not differ considerably among stations (**Figure 12B**).

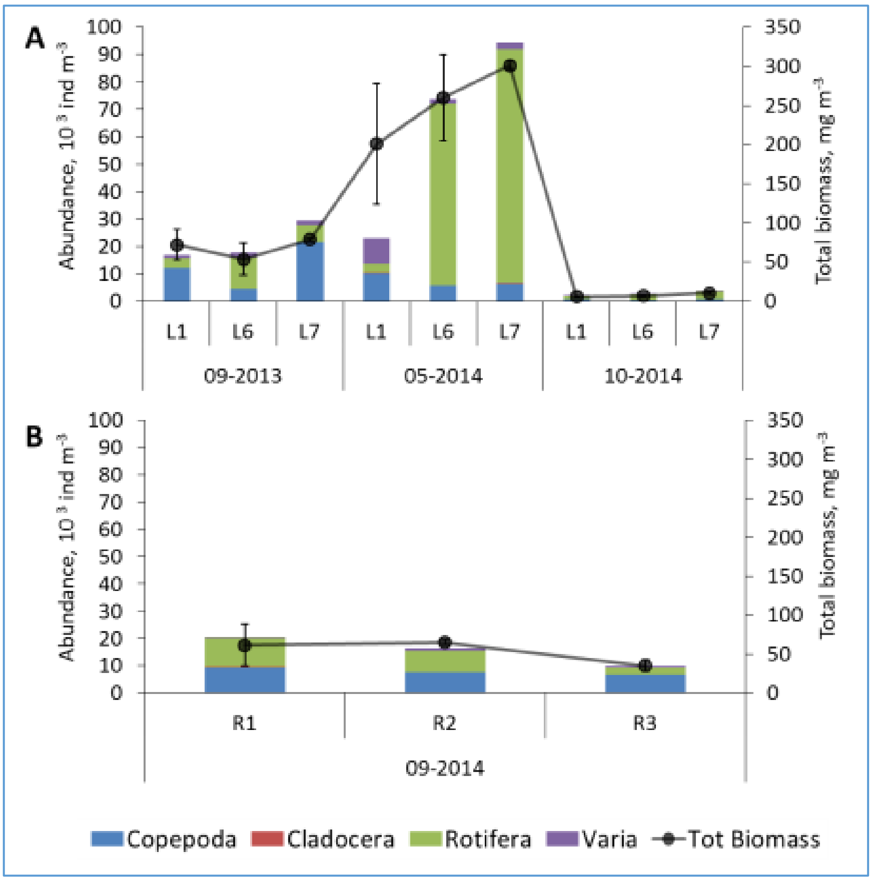

Figure 12 - Mean mesozooplankton abundance (stacked columns) and mean total biomass (line) in A) Port of Liepaja and B) Port of Riga during the research period. (Error bars show standard deviation).

		L1			L6			L7			R1	R2	R3
		2013	2014		2013	2014		2013	2014		2014		
Taxon	**Stage**	**Sep.**	**May**	**Oct.**	**Sep.**	**May**	**Oct.**	**Sep.**	**May**	**Oct.**	**Sep.**		
Copepoda													
***Acartia* sp.**	Nauplius	X	X	X	X	X	X	X	X	X	X	X	X
	C I-III	X	X		X	X		X	X	X	X	X	X
	C IV-V	X	X		X			X	X		X	X	X
	Male		X										
Acartia bifilosa	C IV-V					X							
	Female	X			X			X	X				
	Male	X	X					X	X		X		
Acartia longiremis	Male										X		
Acartia tonsa	Female	27.8			38.6								
	Male	83.3						19.8					28.9
***Centropages* sp.**	C I-III				X			X					
	C V								X				
Cyclopoida	Nauplius		X	X		X			X		X	X	X
	C I-III		X	X		X			X		X	X	X
	C IV-V		X			X			X		X	X	X
	Female										X	X	X
	Male									X	X	X	X
Diaptomidae	Nauplius	X		X	X		X			X		X	X
	C I-III					X					X		X
	C IV-V					X	X				X	X	X
	Female		X									X	X
	Male										X	X	X
***Eurytemora* sp.**	Nauplius	X	X	X		X	X		X	X	X	X	X
	C I-III	X	X			X			X		X	X	X
	C IV-V	X	X			X			X	X	X	X	X
	Female		X	X		X							
	Male		X	X		X					X		

Table 11 - Zooplankton species list in study area (L1, L6, L7 - Port of Liepaja; R1, R2, R3 - Port of Riga) and mean abundance (ind m^{-3}) of non-indigenous species (marked in grey) (* - Amount of individuals in the sample).

Limnocalanus macrurus	Nauplius										X	X	X
***Pseudocalanus* sp.**	Nauplius	X			X								
	C I-III				X	X					X	X	
Temora longicornis	Nauplius	X	X	X	X	X	X	X	X	X			
	C I-III	X	X		X			X	X		X		
	C IV-V	X											
Cladocera													
***Bosmina* spp.**			X				X	X		X	X	X	
***Chydorus* sp.**				X			X						X
Daphnia cucullata											X	X	X
***Diaphanosoma* sp.**											X	X	X
Evadne anonyx									31.7				
Evadne nordmanni			X			X		X	X				
***Podon* sp.**			X				X		X	X	X		
Rotifera													
Asplanchna priodonta												X	
Bdelloid rotifer							X			X	X		
***Brachionus* sp.**											X		X
Keratella cochlearis		X	X	X	X		X	X		X	X	X	X
Keratella cruciformis		X		X	X		X	X			X	X	X
Keratella quadrata		X		X	X	X	X	X	X	X	X	X	X
***Lecane* sp.**											X	X	
***Monostyla* sp.**				X									
Notholca acuminata		X		X						X			
Notholca squamula				X			X						
Notholca marina				X									
***Polyarthra* spp.**				X							X	X	X
Synchaeta baltica		X	X	X	X	X	X	X	X	X	X	X	X
Synchaeta fennica			X			X	X		X	X	X		
Synchaeta monopus			X		X	X	X	X	X		X	X	X
Synchaeta oblonga													X

Table 11 – Continuation.

Synchaeta pectinata													**X**
Synchaeta triophthalma							X			X			
Trichotria pocillum				X									
***Trichocerca* sp.**						X	X			X	X		X
Varia													
***Amphibalanus* sp.**	Nauplius	375.0	9467.6	41.7	1134.7	1504.6	161.5	585.3	2343.3	203.0	78.1	57.9	
Bivalvia	Veliger		X			X					X	X	X
Harpacticoida	Nauplius		X	X		X							
	Copepodite		X	X		X	X		X	X	X		
Oligohaeta	Larvae					X							
Palaemon elegans	Adult						1*						
Polychaeta	Larvae	X	X		X	X		X		X			

Table 11 - Continuation.

5.2.3 Mobile epifauna

Mobile fauna was collected using Chinese crab traps with the following dimensions – 60 cm x 40 cm x 20 cm and with 10 mm mesh size net. Ten traps were placed in different areas of the Port of Liepaja (**Figure 13**) from 11 to 13 September 2013, from 27 to 29 May 2014 and from 21 to 22 October 2014. Dead fish (bream, herring, cod and silver bream) were used as baits. Samples were collected every 24 h, placed seperately in ziplock bags and transported to laboratory.

Figure 13 - Location of the crab traps in the Port of Liepaja.

The following organisms were identified caught in crab traps: one non-indigenous crab species – green crab Harris mud crab (*Rhithropanopeus harrisii tridentatus*), green crab (*Carcinus maenas*), six fish species– burbot (*Lota lota*), perch (*Perca fluviatilis*), non-indigenous round goby (*Neogobius melanostomus*), straightnose pipefish (*Nerophis ophidian*), three-spined stickleback (*Gasterosteus aculeatus*) and white bream (*Blicca bjoerkna*) and one lamprey species – river lamprey (*Lampetra fluviatilis*). Non-indigenous round goby was the absolute dominating fish species caught in the crab traps. The largest number – 30 specimens of round goby was captured in October 2014. The other fish species were caught only in few specimen numbers.

Non-indigenous Harris mud crab was the most frequently trapped crab species (17 specimens), while the other crab species – the green crab - was detected only once (**Table 12**).

Species	Sampling date					
	Sept. 2013		May 2014		Oct. 2014	
	11-12	12-13	27-28	28-29	21-22	Total
Lota lota					2	2
Carcinus maenas	1					1
Rhithropanopeus harrisii tridentatus	6	5	2		4	17
Perca fluviatilis			1	2		3
Lampetra fluviatilis					1	1
Neogobius melanostomus	4		10	16	30	60
Nerophis ophidian					1	1
Gasterosteus aculeatus	2				3	5
Blicca bjoerkna				1		1
Total	13	5	13	20	41	**91**

Table 12 -Number of mobile fauna in the Port of Liepaja. Non-indigenous species are marked in grey.

5.2.4 Benthic infauna

Macrozoobenthos was collected in September 2013 (Liepaja), May 2014 (Liepaja), September 2014 (Riga) and October 2014 (Liepaja) by using a Ponar grab sampler (area: 43.283 m^2). In each station three replicates were taken. Samples were sieved through a sieve with 0.5 mm mesh size and then preserved in a 4% formaldehide solution. In laboratory macrozoobenthos was analysed regarding to taxonomical composition, abundance and biomass.

At this moment nine (with replicates: 27) samples have been analyzed, collected in the Port of Liepaja in September 2013 and October 2014 and in the Port of Riga in September 2014.

Altogether 31 macrozoobenthos taxa were identified. Among them, four non-indigenous species were found: *Cordylophora caspia, Dreissena polymorpha, Potamopyrgus antipodarum, Marenzelleria* sp. and, whereas *Mya arenaria* and *Amphibalanus improvisus* were the cryptogenic species recorded. The most abundant non-indigenous species found in all stations in each sampling time was *Marenzelleria* sp., followed by *P. antipodarum* and the cryptogenic species *A. improvisus.*

The highest macrozoobenthos biomasses were recorded in stations R2 (67.89 g/m^2) and L6 (>44 g/m^2, both years). In station L6, October 2014, also the highest macrozoobenthos abundance was found (13028 ind./m^2) – this was due to disproportionately large quantities of oligochaetes (**Figure 14**). In terms of abundance similar significant dominance by Oligochaeta was recorded in stations L1, October 2014, and R3, September 2014. Knowing the high tolerance of Oligochaeta to low oxygen conditions, this could indicate a high level of organic pollution in these stations.

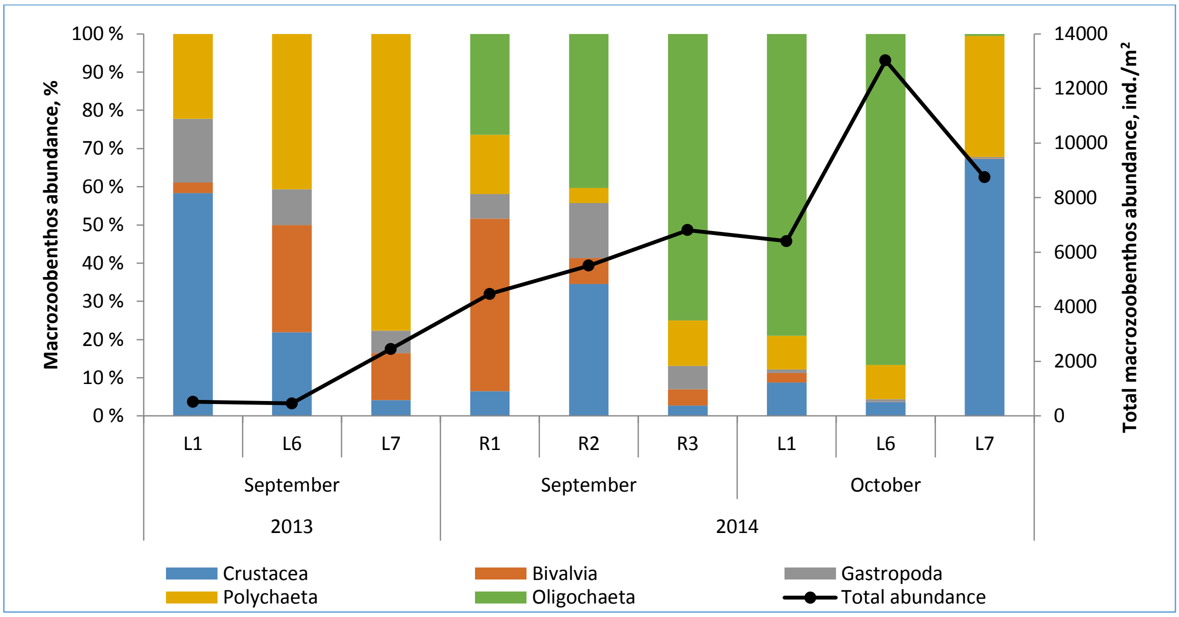

Figure 14 - Relative abundance of macrozoobenthos groups in the Port of Liepaja, September 2013 and October 2014, and the Port of Riga, September 2014. Average values.

5.2.5 Fouling organisms

Settling plates

The fouling plates were deployed for 12 weeks in summer/autumn 2013 and for 13 weeks in summer/autumn 2014 at the Port of Liepaja. All together 21 taxa were identified on plates and ropes. Three non-indigenous taxa were found: *Cordylophora caspia*, *Dreissena polymorpha*, and *Potamopyrgus antipodarum,* whereas *Amphibalanus improvisus* and *Mya arenaria* were the cryptogenic species identified.

Scraping samples

In the Port of Liepaja scrape samples were taken from two stations (L1 and L6) in September 2013 (**Figure 15**), as well as in May and October 2014, while in the Port of Riga they were taken from three stations (R1, R2 and R3) in September 2014.

Figure 15 - Collection of scraping samples in the Port of Liepaja, station L6, in September 2013.

6 Detailed sampling results of the Port of Gdynia (Poland)

In this section all the relevant information related to the sampling conducted in the Port of Gydnia is compiled, together with the results of the identification of taxa conducted in the samples taken.

6.1 Sampling site location and environmental parameters

Monitoring methods were tested at three sampling sites A, B and C located in the Port of Gdynia (**Figure 16**) in autumn 2013, as well as in spring and summer 2014.

Sampling Site A was located at the Szwedzkie Quay in the Maritime Bulk Terminal Gdynia Ltd which is located near the main entrance to the Port of Gdynia. It is a universal terminal that renders the services of reloading, warehousing, big-bagging and sorting all kinds of bulk cargoes in port and maritime turnover.

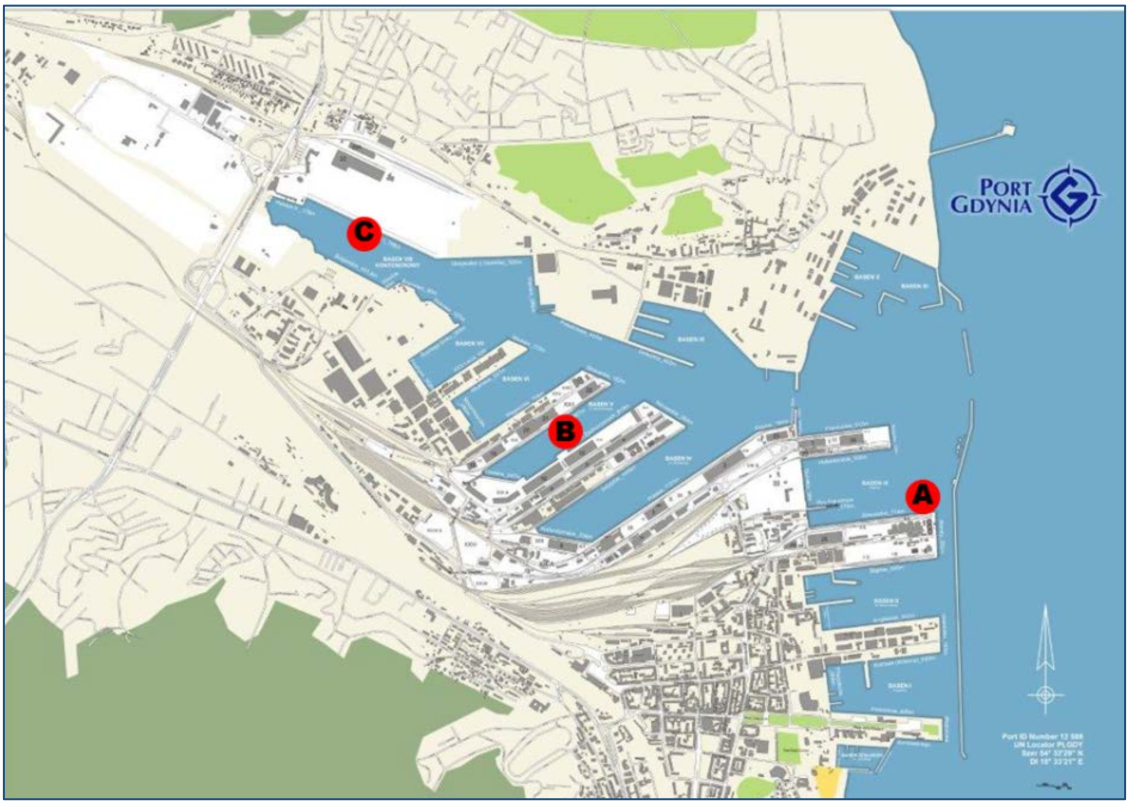

Figure 16 - Map of the Port of Gdynia with the three sampling sites A, B and C.

Sampling Site B was located at the Rumuńskie Quay in the Baltic General Cargo Terminal Ltd. which offers handling, stowing, warehousing and value added services to all kinds of: (1) conventional general cargoes in seaborn trade, such as steel products, fertilizers, chemicals, as well as heavy and oversize loads, project cargoes and containers and (2) bulk cargo, such as: coke, coal, soybean meal, chemicals, biomass. Site C was located at the Helskie Quay in the Baltic Container Terminal which specializes in handling and storage containers in different transportation modes. Detailed information on geographic location is given in **Table 13**.

Sampling site	Name	Latitude (N)	Longitude (E)
A	Szwedzkie Quay	54°31'708''	18°33'644''
B	Rumuńskie Quay	54°32'107''	18°31'781''
C	Helskie Quay	54°32'657''	18°30'600''

Table 13 - Names and coordinates of the sampling sites studied.

Depths at the sampling sites varied slightly between sampling periods (**Table 14**). The basic weather information during sampling is given in **Table 14**.

Parameter	September 2013			April 2014			July 2014		
	A	B	C	A	B	C	A	B	C
Depth (m)	12.0	12.0	13.0	14.5	12.0	14.0	7.0	12.3	12.0
Air temperature (°C)	17	19	19	7.7	7.7	7.7	21	21	21
Cloud cover (%)	0	0	0	10	10	10	50	50	50
Sea state (m)	0	0	2	0.5	0.5	0.5	0	0	0
Wind speed (m/s)	0.7	0	3.6	13	13	13	2	2	2
Wind direction (grad)	45	45	45	360	360	360	240	240	240

Table 14 - Depth of each sampling site as well as basic weather parameters during the sampling time.

Basic water parameters, i.e. temperature, salinity, dissolved oxygen were measured with Multi 340i meter (WTW, Germany, **Figure 17a**) at each sampling site, at the depths of 1, 3 and 7 m as well as at the bottom. Additionally, turbidity was determined with the aid of a Secchi disk (**Figure17b**). Obtained results are presented in **Table 15**, **Table 16** and **Table 17** for September 2013, April 2014 and July 2014, respectively.

Figure 17 - Measurements of basic water parameters with Multi 340i meter (a) and turbidity using a Secchi disk (b). Photos: M. Normant.

Depth (m)	Temperature (° C)	Salinity	Oxygen (mg l^{-1})	Turbidity (m)
Site A				
1	19.6	6.7	8.50	2.3
3	19.4	6.7	8.30	
7	18.9	6.8	7.92	
Bottom	18.9	6.8	7.46	
Site B				
1	19.0	6.7	8.88	2.2
3	19.0	6.7	8.25	
7	18.9	6.7	7.65	
Bottom	18.5	6.7	7.55	
Site C				
1	19.3	6.6	7.20	2.5
3	19.2	6.6	7.20	
7	19.0	6.6	7.12	
Bottom	18.8	6.7	6.89	

Table 15 - Environmental parameters measured in September 2013 at the three sampling sites A, B and C.

Depth (m)	Temperature (° C)	Salinity	Oxygen (mg l^{-1})	Turbidity (m)
Site A				
1	7.3	6.8	12.12	2.3
3	7.1	6.8	12.20	
7	6.9	6.9	12.42	
Bottom	6.7	7.1	11.10	
Site B				
1	6.8	6.9	11.71	2.5
3	6.8	6.9	12.15	
7	7.1	6.9	12.40	
Bottom	6.6	7.0	12.02	
Site C				
1	6.6	6.6	12.20	2.3
3	6.5	6.6	11.83	
7	6.6	6.6	11.27	
bottom	6.4	6.7	10.56	

Table 16 - Environmental parameters measured in April 2014 at the three sampling sites A, B and C.

Depth (m)	Temperature (° C)	Salinity	Oxygen (mg l^{-1})	Turbidity (m)
Site A				
1	21.4	7.0	10.30	1.8
3	20.4	7.0	9.90	
7	19.5	7.0	8.20	
bottom	19.5	7.0	8.20	
Site B				
1	20.2	7.1	10.10	1.4
3	19.4	7.0	9.40	
7	17.7	7.0	7.40	
bottom	16.1	7.0	6.21	
Site C				
1	18.9	7.0	9.54	1.5
3	18.7	7.0	8.69	
7	18.1	7.1	8.33	
bottom	15.9	7.0	6.46	

Table 17 - Environmental parameters measured in July 2014 at three sampling sites A, B and C.

Additionally, temperature changes were monitored by data logger with sensors (Hobo Data Loggers, Onset, USA) attached to the rope during six (2013) and twelve (2014) weeks of settlement of the fouling plates. In the studied period of 2013 temperature decreased from 21.47 (04.09.2013) to 12.59 ^{o}C (18.10.2013) at site A, from 21.44 (04.09.2013) to 12.88 ^{o}C (18.10.2013) at site B and from 21.47 (05.09.2013) to 12.98 ^{o}C (18.10.2013) at site C.

In the studied period of 2014 temperature ranged from 9.28 (13.05.2014) to 22.72 ^{o}C (22.07.2014) at site A, from 9.87 (14.05.2014) to 22.81 ^{o}C (22.07.2014) at site B and from 9.37 (16.05.2013) to 22.91 ^{o}C (22.07.2014) at site C. **Figure 18** shows changes in temperature at sampling site A in 2013 and 2014.

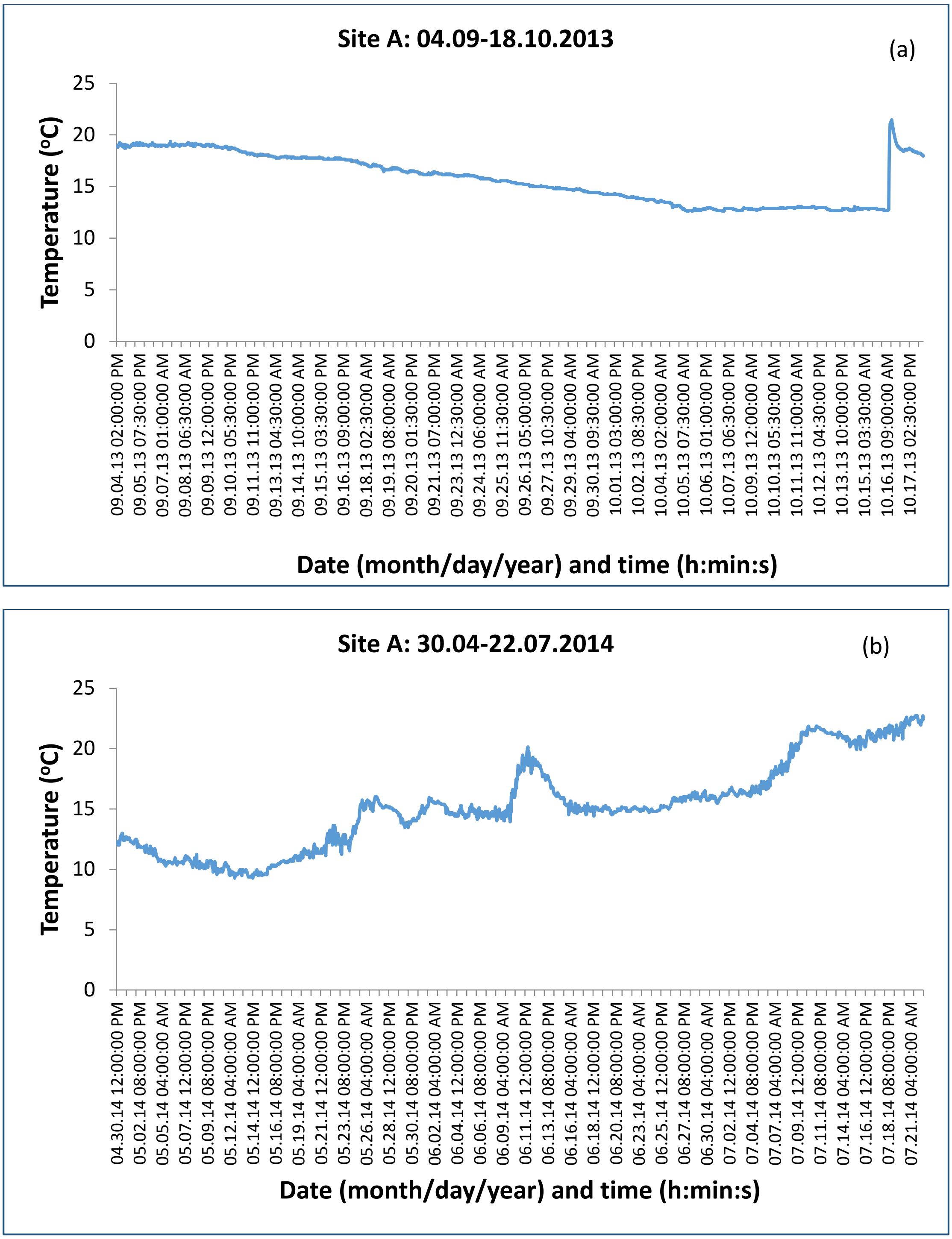

Figure 18 – An example of temperature changes during the fouling plates settlement at sampling site A in 2013 (a) and 2014 (b).

6.2 Biological survey

The biological survey was designed based on the port survey protocols developed under HELCOM ALIENS 2 (HELCOM, 2013c).

6.2.1 Phytoplankton

Phytoplankton was collected on 5 September 2013, 30 April 2014 and 17 July 2014 by obtaining water sample pooled from surface (1 m) and from 5 m depth by water sampler Niskin Type 5.0 L (**Figure 19a**) and with the hand hauled 10 µm net of the 25 cm ring diameter and 60 cm length (**Figure 19b**). An integrated water samples (1 and 5 m) were then placed in 0.5 L dark glass bottles and preserved in acid Lugol solution. They were analyzed in laboratory in regard to taxa composition and abundance (HELCOM, 2013b). Additionally, diatoms were extracted from the water sample based on digestive method in order to determine taxa composition and percentage contribution.

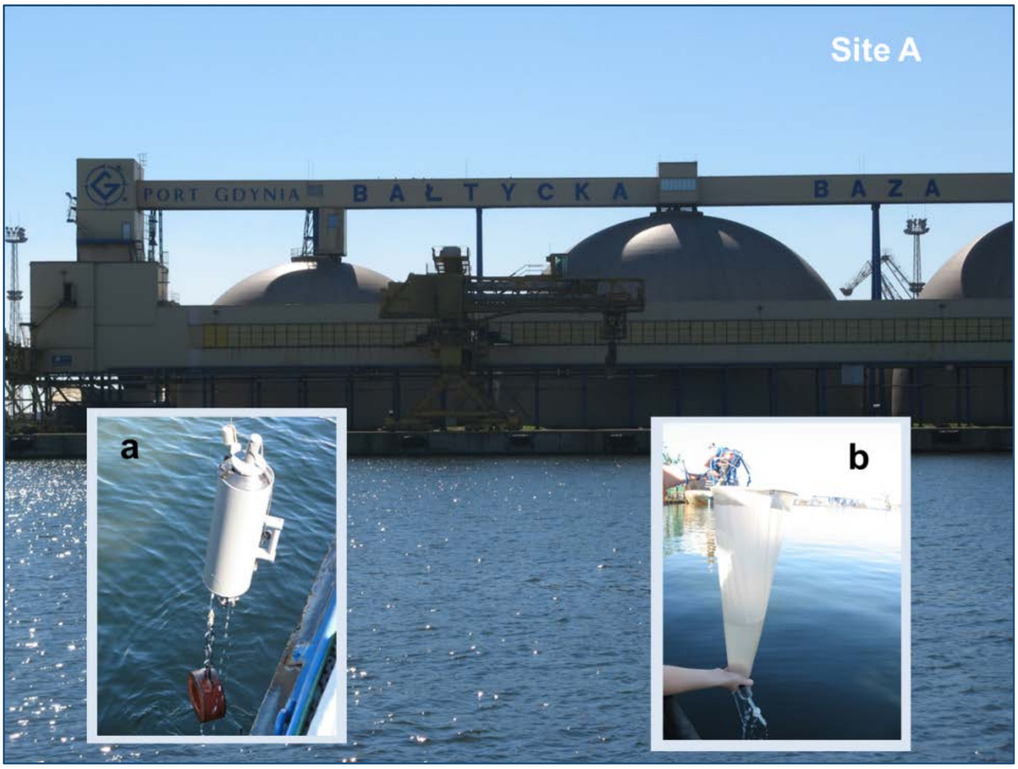

Figure 19 - Water sampler (a) and hand hauled phytoplankton net (b). Photos: M. Normant.

All together 188 taxa were found in the phytoplankton samples (**Tables 18** and **19**). Among them were Cyanophyceae (30 taxa), Cryptophyceae (4 taxa), Dinophyceae (21 taxa), Prymnesiophyceae (1 taxon), Chrysophyceae (1 taxon), Bacillariophycae (100 taxa), Euglenophyceae (1 species), Micromonadophyceae (1 taxon), Chlorophyceae (13 taxa), Trebouxiophyceae (9 taxa), Ebriophyceae (3 taxa), Ciliatea (1 species) and others (3 taxa). The most numerous one was class Bacillariophycae. *Chaetoceros cf. lorenzianus* was the only cryptogenic species identified.

In September 2013 the dominating group in phytoplankton biomass were Bacillariophycae (**Figure 20a**), in April 2014 Dinophycae and Ciliatea (**Figure 20b**) and in July 2014 Cyanobacteria (**Figure 20c**). Contribution of particular groups in the biomass as well as the total phytoplankton biomass varied also between sampling sites.

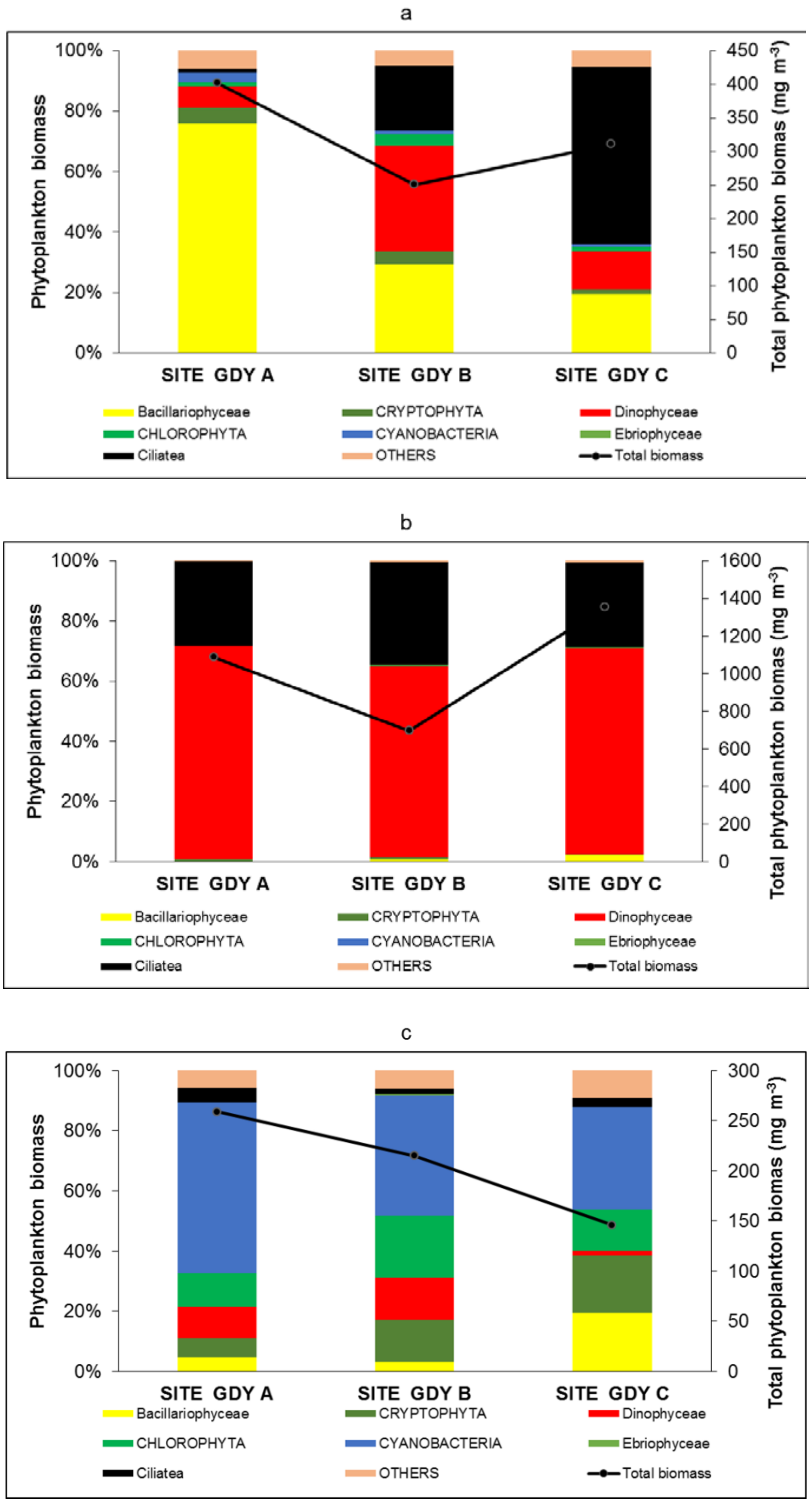

Figure 20 - Contribution of particular phytoplankton groups in biomass and the total phytoplankton biomass in three sampling sites of the Port of Gdynia in September 2013 (a), April 2014 (b) and July 2014 (c).

Taxon	Site A	Site B	Site C
Cyanophyceae			
Aphanizomenon flos-aquae	+	+	+
***Anabaenopsis* sp.**		+	
Dolichospermum* cf. *circinalis	+		
Dolichospermum* cf. *lemmermannii	+	+	
Dolichospermum* cf. *spiroides	+		
Dolichospermum* cf. *affine	+		
***Dolichospermum* sp.**	+		+
Nodularia spumigena	+	+	+
***Nodularia* sp.**			+
Spirulina subsalsa		+	
Pseudanabena galeata	+		
***Limnothrix* sp.**	+		
***Leptolyngbya* sp.**	+		
***Planctolyngbya* sp.**		+	
***Oscillatoriales* (LPP)**	+		
Aphanocapsa incerta	+		
Aphanocapsa delicatissima	+	+	+
***Anathece* sp.**	+	+	+
***Cyanodictyon* sp.**	+	+	+
Lemmermanniella pallida	+	+	+
Merismopedia punctata	+	+	
Merismopedia tenuissima	+	+	+
Merismopedia warmingiana	+	+	+
Snowella lacustris	+	+	+
Snowella litoralis	+	+	+
Snowella septentionalis	+	+	+
Snowella sp.	+	+	+
***Woronichinia* sp.**		+	
***Chroococcus* sp.**		+	
Chrococcales	+		
Cryptophyceae			
Hemiselmis		+	+
***Cryptomonas* sp.**	+	+	+
Plagioselmis prolonga	+	+	+
Teleaulax acuta	+	+	+
Dinophyceae			
Dinophysis norvegica	+		
Dinophysis acuminata	+		
Amphidinium crassum	+	+	+
Biecheleria baltica	+	+	

Table 18 - Presence (+) of phytoplankton taxa collected in three studied sampling sites of the Port of Gdynia during studied period (September 2013, April and July 2014). Cryptogenic species are marked in grey and underlined.

Taxon	Site A	Site B	Site C
Dinophyceae			
Gymnodinium simplex	+	+	+
Gymnodinium sp.	+	+	+
Gymnodiniales (sphere-10%)	+	+	+
Gymnodiniales (2 cones)	+		+
Gyrodinium spirale		+	+
Gyrodinium sp.			+
Katodinium sp.	+		
Heterocapsa rotundata	+	+	+
Heterocapsa triquetra	+	+	+
Oblea rotunda	+	+	+
Amylax triacantha		+	
Protoperidinium brevipes	+	+	+
Protoperidinium sp.	+		+
Peridiniella catenata	+	+	+
Scrippsiella hangoei		+	
Peridiniales (sphere-20%)		+	
Pseudopediniella sp.	+	+	+
Prymnesiophyceae			
Chrysochromulina sp.		+	+
Chrysophyceae			
Pseudopediniella sp.		+	+
Bacillariophyceae			
Chaetoceros cf. *lorenzianus*	+	+	+
Coscinodiscus cf. *granii*			+
Coscinodiscus sp.	+	+	+
Cyclotella meneghiniana	+		+
Cyclotella cf. *choctawhatcheeana*	+	+	+
Cyclotella cf. *atomus*		+	
Cyclotella sp.			+
Thalassiosira sp.			+
Skeletonema marinoi	+	+	+
Ceratoneis closterium		+	
Centrales		+	+
Melosira varians			+
Synedra ulna v. ulna			+
Gyrosigma sp.		+	+
Diploneis didyma	+	+	+
Navicula sp.			+
Nitzschia longissima	+		
Nitzschia sp.	+	+	
Tabularia fasciculata		+	
Achnanthes taeniata		+	
Pennales	+		+
Euglenophyceae			
Eutreptiella gymnastica	+	+	+
Micromonadophyceae			
Pyramimonas sp.	+	+	+

Table 18 – Continuation.

Taxon	Site A	Site B	Site C
Chlorophyceae			
Desmodesmus cf. armatus	+	+	
Desmodesmus maximus	+	+	
Monoraphidium arcuatum	+	+	+
Monoraphidium contortum	+	+	+
Monoraphidium komarkovae	+	+	+
Monoraphidium minutum		+	+
Tetrastrum elegans	+	+	
Tetrastrum staurogeniaeforme	+		
Pediastrum duplex	+		
Acutodesmus obliquus	+	+	
Scenedesmus ellipticus		+	
Scenedesmus sp.	+	+	
Chlorococcales undef.	+		
Trebouxiophyceae			
Crucigenia tetrapedia	+		
Crucigeniella cf. *rectangularis*	+		
Mucidosphaerium pulchellum	+		
Oocystis borgei	+	+	+
Oocystis lacustris		+	
Oocystis cf. *solitaria*	+	+	
Oocystis sp.			+
Planctonema lauterbornii	+	+	+
Pseudokirchineriella contorta	+	+	+
Ebriophyceae			
Ebria tripartita	+	+	+
Katablepharis sp.	+		
Leucocryptos marina	+	+	+
Ciliatea			
Mesodinium rubrum	+	+	+
Others			
Flagellates (rotational ellipsoid)	+	+	+
Flagellates (sphere)	+	+	+
Unicell	+	+	+

Table 18 – Continuation.

Taxon	Site A	Site B	Site C
Achnanthes lemmermannii	+	+	+
Actinocyclus normanii fo. subsalsa	+		+
Actinocyclus octonarius			+
Amicula specululum	+		+
Amphora coffeaeformis	+		
Amphora inariensis	+		
Amphora pediculus	+	+	+
Amphora tenerrima	+		
Asterionella formosa	+		
Aulacoseira granulata	+		+
Bacillaria paxilifer		+	+
Chaetoceros cf. lorenzianus/salsugineus		+	+
Chaetoceros sp.	+		+
Cocconeis hauniensis		+	
Cocconeis neothumensis			+
Cocconeis pediculus		+	
Cocconeis placentula	+		
Cocconeis sp.	+		
Coscinodiscus granii			+
Cyclostephanus dubius	+		
Cyclotella atomus	+	+	+
Cyclotella choctawhatcheeana	+	+	+
Cyclotella meneghiniana	+	+	+
Cyclotella radiosa	+		+
Denticula tenuis			+
Diatoma moniliformis	+		+
Diatoma tenuis		+	+
Diploneis didyma		+	+
Diploneis sp.	+		
Fallacia cassubiae			+
Fallacia florinae	+	+	
Fallacia tenera			+
Fragilaria atomus	+	+	
Fragilaria gedanensis	+		+
Gomphonema olivaceum var baltica	+	+	
Grammatophora marina	+		+
Hippodonta arctica			+
Hyalosira delicatula	+		
Karayevia clevei		+	+
Mastogloia smithii		+	

Table 19 - Presence (+) of particular Bacillariophyceae taxa (determined based on digestive method) found in September 2013 and April and July 2014 in the three studied sampling sites of the Port of Gdynia. Cryptogenic species are marked in grey and underlined.

Taxon	Site A	Site B	Site C
Navicula bipustulata		+	
Navicula bozenae	+		+
Navicula gregaria	+	+	+
Navicula lanceolata			+
Navicula meniscus			+
Navicula paul-schulzii			+
Navicula perminuta	+	+	+
Navicula prolifera			+
Navicula ramosissima	+		
Navicula reichardtiana	+		
Navicula sp.	+	+	
Navicula viminoides var cosmomarina		+	
Nitzschia aurariae	+	+	+
Nitzschia capitellata			+
Nitzschia cf thermaloides	+		
Nitzschia constricta	+		
Nitzschia frustulum	+		
Nitzschia microcephala	+	+	+
Nitzschia sigma	+		
Opephora guenter-grassii	+	+	+
Opephora krumbeinii	+	+	+
Opephora mutabilis	+	+	+
Parlibellus sp.	+		
Pauliella taeniata	+	+	+
Pennales			+
Planothidium delicatulum	+	+	+
Planothidium hackianum	+	+	+
Planothidium lanceolatum	+		+
Pseudostaurosira brevistriata		+	+
Pteroncola inane			+
Rabdonema arcuatum		+	
Rhoicosphenia abbreviata	+		+
Skeletonema marinoi	+	+	+
Stauroneis cf gracilima	+	+	
Staurosira construens var. venter			+
Stephanodiscus hantzschii	+	+	
Stephanodiscus parvus	+	+	+
Tabularia fasciculata	+	+	+
Tabularia tabulata		+	+
Tabularia waernii	+		
Thalassiosira baltica			+
Thalassiosira decipiens	+	+	
Thalassiosira levanderii	+	+	+
Thalassiosira oestrupii		+	+
Thalassiosira proschkinae	+	+	+
Thalassiosira weissflogii		+	
Ulnaria ulna var acus	+		

Table 19 - Continuation.

6.2.2 Zooplankton

Zooplankton was collected on 5 September 2013 (2 samples), 30 April 2014 (2 samples) and 17 July 2014 (1 sample) with a standard 100 µm mesh free-fall dropnet (ring diameter 57 cm, net length 2.6 m; **Figure 21a**) equipped with a flow meter. Additionally, in July 2014 one sample at each sampling point was collected with a standard 100 µm mesh free-fall dropnet of bigger ring diameter (75.4 cm, net length ca. 2.6 m) equipped with a flow meter. At each sampling site vertical zooplankton sample (stopped 1 m before the bottom) was collected. The volume of water filtrated varied from 1.148 to 3.442 m^3 in the case of the smaller free-fall dropnet and from 4.906 to 6.021 m^3 in the case of the bigger free-fall dropnet. Samples were placed in 0.25 L bottles and preserved in 4% formalin solution. In laboratory they were analyzed in regard to taxa composition, abundance, sex (Diplostraca, Calanoida) and stage of development (Calanoida, meroplankton) based on Mańkowski (1955), Żmudziński (1974) and Conway (2006).

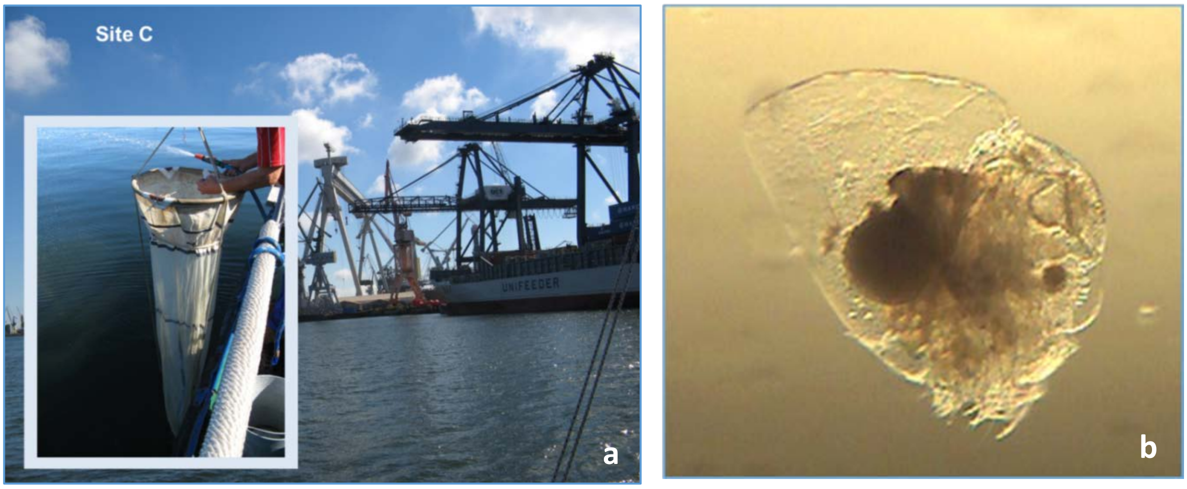

Figure 21 - Sampling of zooplankton with WP2 Net (a) (photos: M. Normant); Ponto-Caspian cladoceran Evadne anonyx (b) (photo: L. Bielecka).

All together 35 different taxa were identified in the zooplankton samples (**Table 20**). Among them were Rotifera (5 taxa), Cladocera (6 taxa), Calanoida (8 taxa), Cyclopoida (2 taxa), Apendiculariae (1 species), Harpacticoida, Oligochaeta, Isopoda, Insecta, Nematoda, Turbellaria and larvae of *Marenzelleria* spp. and *Hediste diversicolor*, *Palaemon* spp., *Rhithropanopeus harrisii* and *Amphibalanus improvisus*. Zooplankton abundance varied according to season and sampling site (**Figure 22**). Moreover, there were also differences between two repetitions, e.g. between B1 and B2 in September 2013 and April 2014 (**Figure 22a** and **22b**) or between C1 and C2 in July 2014 (**Figure 22c**). In September 2013 the more abundant groups were Rotifera and Calanoida (**Figure 22a**), whereas in April 2014 pelagic larvae of benthic taxa (Polychaeta and Malacostraca) dominated in zooplankton (**Figure 22b**). In July 2014 the most abundant was order Calanoida (**Figure 22c**).

Four non-indigenous taxa were found: *Acartia tonsa*, *Evadne anonyx* (**Figure 21b**) and larvae of *Marenzelleria* spp. and *Rhithropanopeus harrisii*. Larvae of cryptogenic species *Amphibalanus improvisus* were also found.

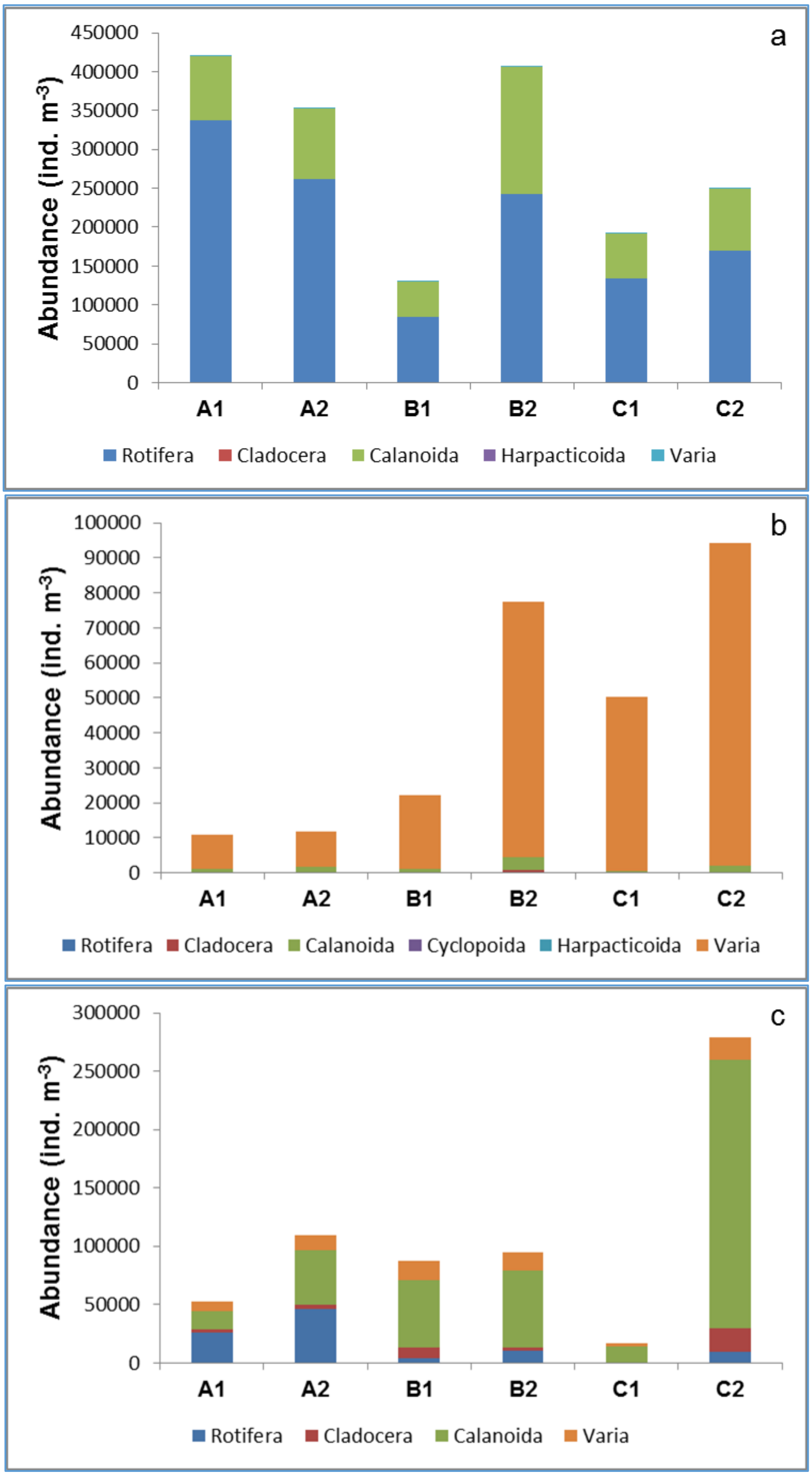

Figure 22 - Abundance of particular zooplankton groups at three sampling sites (two repetitions each) in the Port of Gdynia: September 2013 (a), April 2014 (b) and July 2014 (c).

Taxon	Stage/sex	Site A	Site B	Site C
Rotifera				
Keratella quadrata		+	+	+
Keratella cochlearis		+	+	+
Keratella cruciformis		+	+	+
***Synchaeta* spp.**		+	+	+
Trichocerca marina		+		
Cladocera				
***Bosmina/Eubosmina* spp.**	female/male	+	+	+
Pleopsis polyphemoides	female/male	+	+	+
Podon intermedius	female/male	+	+	
Evadne nordmanni	female/male	+	+	+
Evadne anonyx	female/male	+		
Calanoida				
***Acartia* spp.**	nauplius	+	+	+
	C I	+	+	+
	C II	+	+	+
	C III	+	+	+
	C IV	+	+	+
	C V	+	+	+
Acartia tonsa	female	+	+	+
	male	+	+	+
Acartia bifilosa	female	+	+	+
	male	+	+	+
Acartia longiremis	female		+	+
	male		+	+
Centropages hamatus	nauplius	+	+	+
	C I	+	+	+
	C II	+	+	+
	C III	+	+	+
	C IV	+	+	
	C V	+	+	+
	female	+	+	+
	male	+	+	+
***Pseudocalanus* sp.**	nauplius	+	+	+
	C I	+	+	+
	C II	+	+	+
	C III	+	+	+
	C IV			+
Eurytemora affinis	nauplius			+
	C I	+		+
	C II	+	+	+
	C III	+	+	+
	C IV	+	+	+
	C V	+	+	+
	female	+	+	+
	male	+	+	+

Table 20 - Occurrence (+) of zooplankton taxa in the three studied sampling sites of the Port of Gdynia during September 2013, April and July 2014. Non-indigenous species are marked in grey and bold, whereas cryptogenic species are marked in grey and underlined.

Taxon	Stage/sex	Site A	Site B	Site C
Temora longicornis	nauplius	+	+	+
	C I	+	+	+
	C II	+	+	+
	C III	+	+	+
	C IV	+	+	+
	C V	+	+	+
	female	+	+	+
	male	+	+	+
Cyclopoida				
Oithona similis	female			+
***Cyclopoida* sp.**	copepodit	+	+	+
Appendiculariae				
Fritillaria borealis		+	+	+
Harpacticoida	copepodit	+	+	+
Oligochaeta			+	
Hediste diversicolor	larvae	+	+	+
***Marrenzelleria* spp.**	larvae	+	+	+
Amphibalanus improvisus	nauplius	+	+	+
	cypris	+	+	+
Isopoda			+	
***Palaemon* spp.**	zoëa	+		
Rhithropanopeus harrisii	zoëa	+	+	+
Insecta		+	+	+
Gastropoda	veliger	+	+	+
Bivalvia	veliger	+	+	+
Nematoda				+
Turbellaria			+	
Cnidaria	planula	+	+	+

Table 20 – Continuation.

6.2.3 Mobile epifauna

Mobile fauna was collected using two different traps: Fukui designed box trap 60 cm x 45 cm x 20 cm, with 1.3 cm mesh netting (**Figure 23a**) and Gee-minnow trap 42 cm long and 23 cm wide with 1.0 x 0.8 cm netting and 2.5 cm mouth (**Figure 23b**). Dead European flounder *Platichthys flesus* and round goby *Neogobius melanostomus* were used as baits. Three traps of each type were deployed from 4 till 10 September 2013 and from 9 till 11 July 2014. After this time traps were removed out of the water. All collected organisms from each trap were placed separately in ziplock bag filled with water and then transported to laboratory, where they were identified to the lowest possible taxonomic level.

All together 9 different taxa (**Tables 21** and **22**) have been identified among organisms found in both trap types. Among them were 8 species and one specimen belonging to genera Idotea, which was not able to identify due to lack of telson. Three non-indigenous species were found: *Rhithropanopeus harrisii, Palaemon elegans* and *Neogobius melanostomus*.

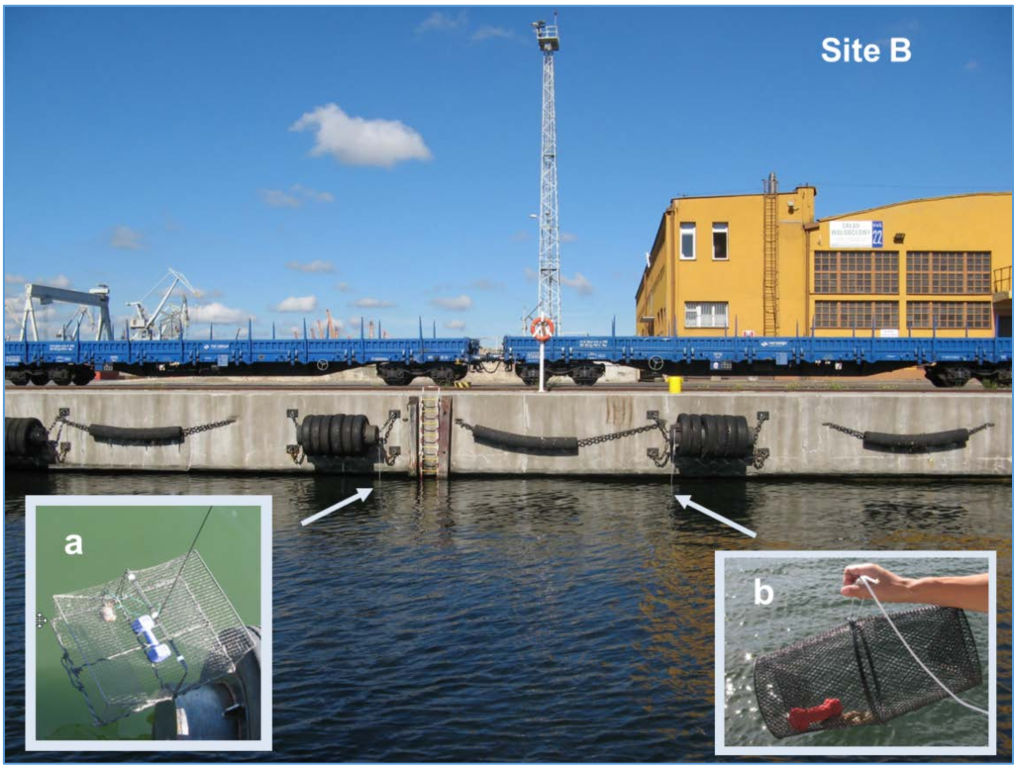

Figure 23 - Mobile fauna was collected using two different traps: Fukui designed box (a) trap and Gee-minnow trap (b). Photos: M. Normant.

Taxon	Site A		Site B		Site C	
	Fukui	Gee minnow	Fukui	Gee minnow	Fukui	Gee minnow
Aurelia aurita*	< 5	< 5	< 5	< 5	< 5	< 5
Rhithropanopeus harrisii			1	1		
Palaemon elegans				2		
Gammarus zaddachi			2			
Neogobius melanostomus	2		6			
Nerophis ophidion		3				

*damaged specimens were on the trap.

Table 21 - Number of mobile fauna found in Fukui (n=3) and Gee-minnow (n=3) traps in the three studied sampling sites of the Port of Gdynia during September 2013. Non-indigenous species are marked in grey.

Taxon	Site A		Site B		Site C	
	Fukui	Gee minnow	Fukui	Gee minnow	Fukui	Gee minnow
Rhithropanopeus harrisii				1		
Palaemon elegans		1		1		
Idotea chelipes			7	2	3	
***Idotea* spp.**				1		
Gammarus salinus				1	9	3
Neogobius melanostomus		3				
Nerophis ophidion		1	8	1		

Table 22 - Number of mobile fauna found in Fukui (n=3) and Gee-minnow traps (n=3) in the three studied sampling sites of the Port of Gdynia during July 2014. Non-indigenous species are marked in grey.

6.2.4 Benthic infauna

Benthic infauna was collected on 5 September 2013, 30 April 2014 and 17 July 2014 using an Ekman grab sampler (area 0.0225 m^2; **Figure 24a**) and a Van Veen grab (area 0.1 m^2; **Figure 24b**). At each sampling site three grab samples were collected. Sediment was then sieved with a 0.5 mm sieve (**Figure 24c**). Collected organisms were placed in 0.5 L bottles and preserved in 4% formalin solution. In laboratory they were identified to species level. Oligochaeta were selected form samples, placed in Amman's lactophenol to display chitinous structure of chaetae and penial sheaths and then they were identified to species level based on features given by Kasprzak (1981) and Timm (2009). Due to the fact that the Ekman sampler was too light to grasp a proper sediment layer only samples collected with the second sampler were analyzed.

Organisms representing 4 classes (Clitellata, Polychaeta, Malacostraca and Bivalvia) were identified among infauna in the Port of Gdynia in September 2013 and July 2014. Polychaeta consisted of two taxa (*Hediste diversicolor* and *Marenzelleria* spp.) accounted for 59.6% of all collected specimens (n = 1621). Less abundant (27.9%), but more diverse, was class Clitellata which consisted of 6 oligochaetes species (*Limnodrilus hoffmeisteri*, *Limnodrilus profundicola*, *Paranais littoralis*, *Psammoryctides barbatus*, *Tubifex blanchardi*, *Tubificoides heterochaetus*) and a group of juvenile individuals defined as *Tubificinae* gen. spp. juv. Contribution of Bivalvia which consisted of three species (*Cerastoderma glaucum*, *Macoma balthica*, *Mytilus edulis trossulus*) was 12.1%. The proportion of Malacostraca with three species: *Corophium multisetosum*, *Cyathura carinata*, and *Rhithropanopeus harrisii* was only 0.4%. The occurrence of an opossum shrimp *Neomysis integer* and round goby *Neogobius melanostomus* in the Van Veen grab samples was considered as accidental and not included in calculations. Both, the abundance and taxa diversity differed between the seasons studied as well as between sampling sites. In September 2013 abundance was more than 3 times lower compared to July 2014. Moreover, in September 2013 the most numerous among infauna (58.4%) were Clitellata with subclass Oligochaeta, whereas in July 2014 Polychaeta dominated (67.7%). In September 2013 the highest and lowest abundances of infauna were noticed at the sampling sites B and C, whereas in July 2014 it was the opposite (**Figure 25**).

Two non-indigenous taxa were found: *Marenzelleria* spp. and *Rhithropanopeus harrisii*. According to World Register of Marine Species (WoRMS) and online information system on the aquatic Non-Indigenous Species (AquaNIS) it is not clear whether oligochaete worm *Tubificoides heterochaetus* is non-native in the Baltic Sea.

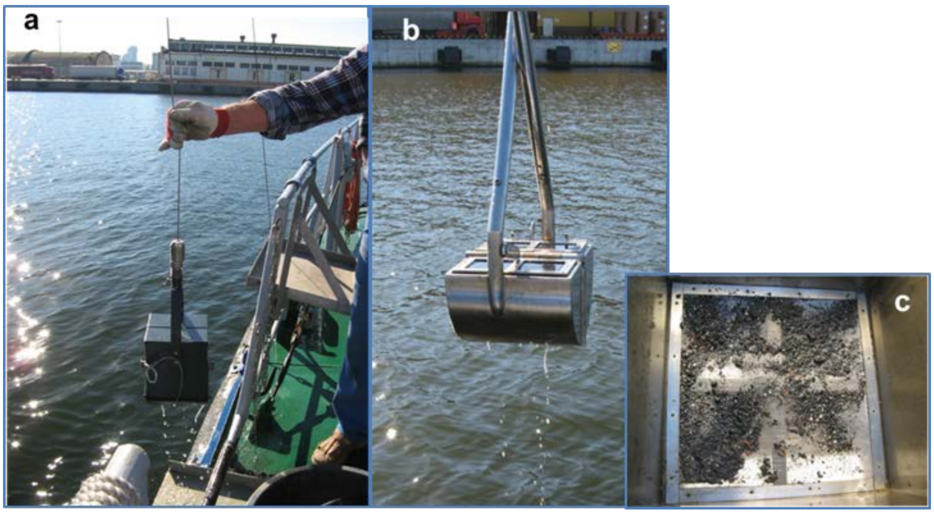

Figure 24 - Benthic infauna was collected using (a) Ekman grab sampler and (b) Van Veen grab; (c) samples were sieved with a 0.5 mm sieve (photos: M. Normant).

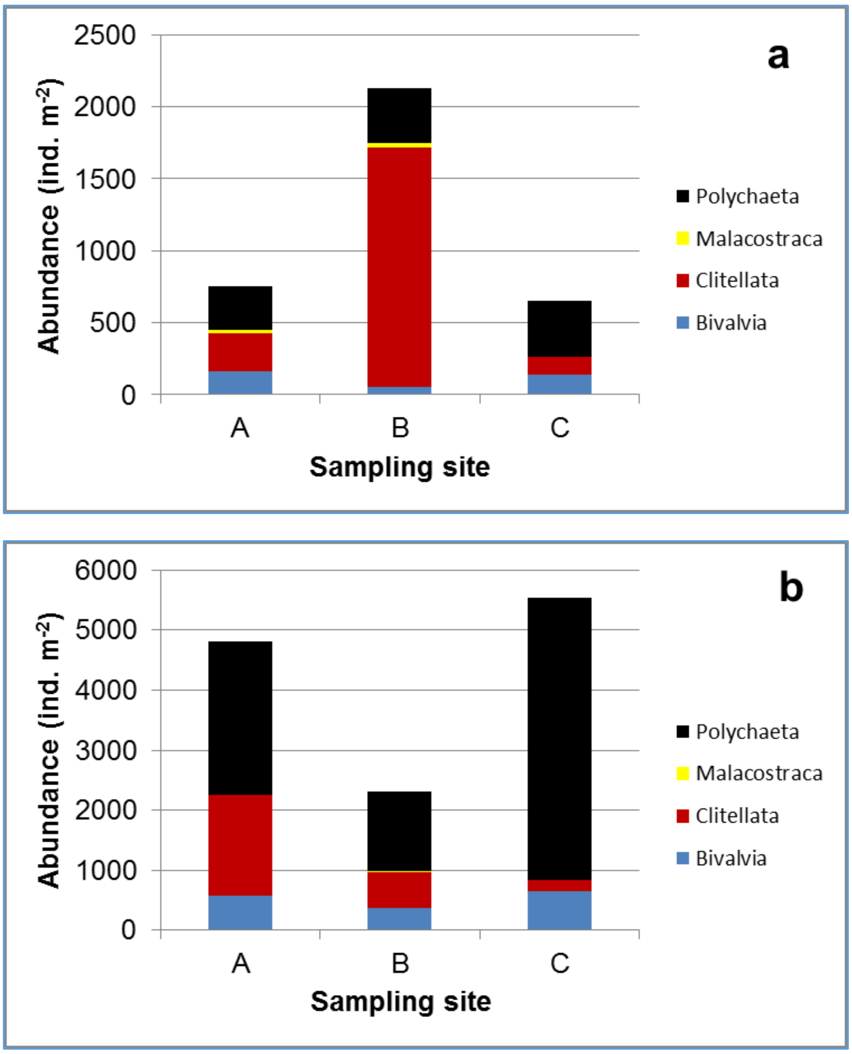

Figure 25 - Abundance of the four classes of infauna identified in September 2013 (a) and July 2014 (b) at the three sampling sites located in the Port of Gdynia.

6.2.5 Fouling organisms

Due to the fact that obtaining scrape samples as well as samples by snorkeling was impossible, the fouling plates were deployed to collect fouling organisms and associated fauna. A single set-up consisted of three grey PVC sanded plates (15 x 15 cm) with the hole drilled at the center of the plates for the rope. Plates were placed on the rope at the depths of 1, 3 and 7 m (**Figure 26a**). They were deployed for 6 weeks in autumn 2013 and for 13 (sampling site A), 11 (sampling site B) and 12 (sampling site C) weeks in spring/summer 2014. After removing plates from the water they were separated from the rope and placed in labeled plastic boxes filled with habitat water (**Figure 26b**). All detached organisms were collected and placed into a separate labeled ziplock bag. Similarly, the rope was placed in a separate labeled bag. In the laboratory plates were photographed and then analyzed for fouling community composition as well as for the percentage coverage by each taxa. Associated taxa and taxa found on the rope were also determined and counted. According to Dziubińska and Janas (2007) a margin of 1cm around the panel was ignored to prevent the sampling of edge effects. To this end, analyzed surface equaled 169 cm^2 (13 x 13 cm). It is supposed that two species, *Marenzelleria neglecta* and *M. viridis*, may occur in Polish waters. Since the species identity in the present material was not confirmed by molecular methods *Marenzelleria* individuals were classified as one taxon *Marenzelleria* spp.

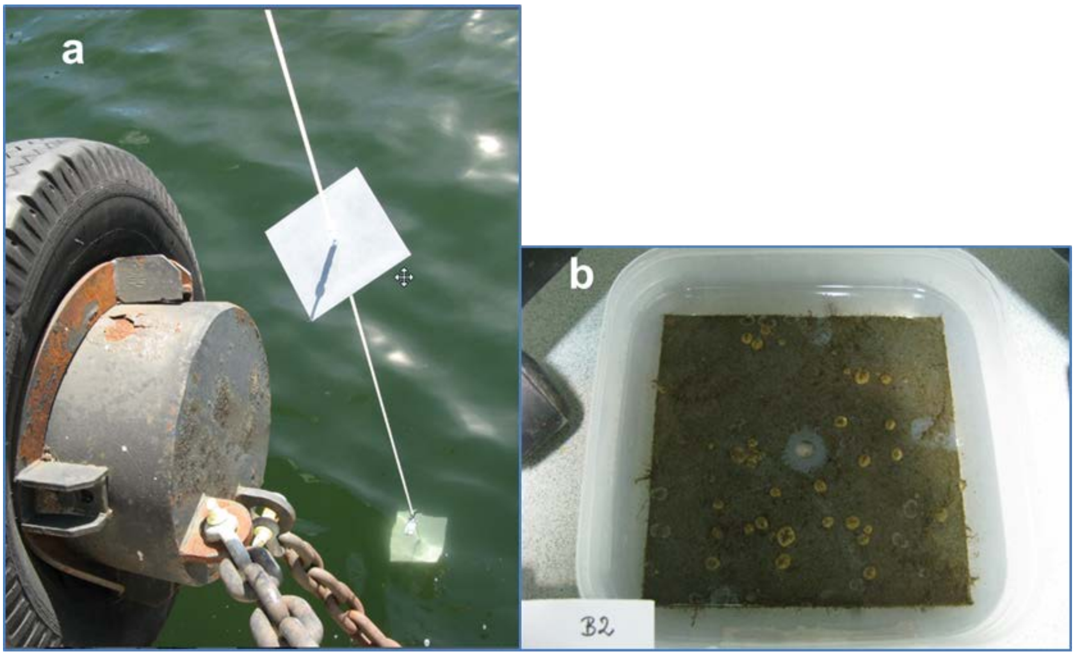

Figure 26 - Settlement PVC plates (a) and plate were removed from water in October 2013 after 6 weeks of exposure (b). Photos: M. Normant (a) and A. Dziubińska (b).

All together 11 sessile taxa were identified on the fouling plates, representing flora (n = 6) and fauna (n = 5). One non-indigenous species the hydroid *Cordylophora caspia* and the cryptogenic barnacle *Amphibalanus improvisus* were found (**Figure 27**, **Table 23**).

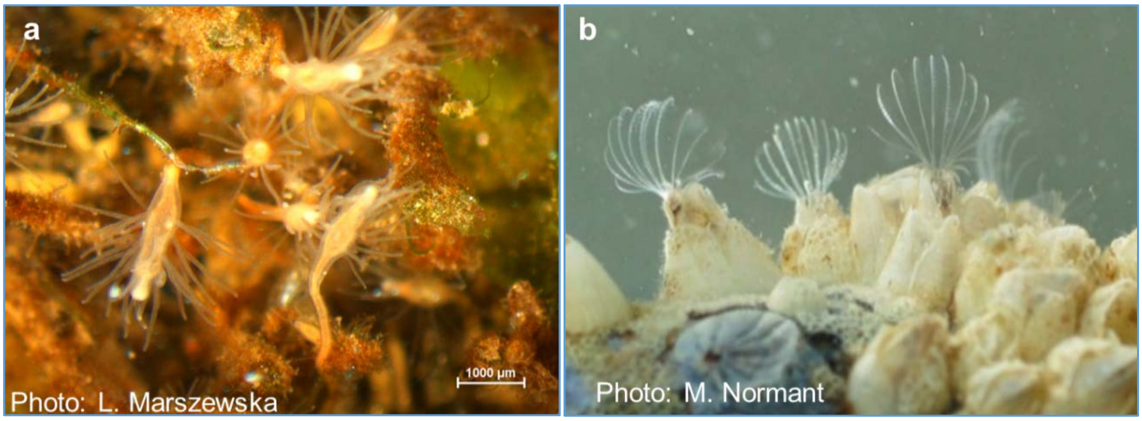

Figure 27 - The hydroid *Cordylophora caspia* (a) and the barnacle *Amphibalanus improvisus* (b).

Taxon/depth	Site A		Site B		Site C	
	S-O	A-J	S-O	A-J	S-O	A-J
1 m						
Pylaiella littoralis	10		80		50	
Ulva linza*			5		40	
Ulva* **spp.**	20					
Cladophora glomerata	70				5	
***Cladophora* sp.**		85		90		80
Gonothyraea loveni	50	1	5	1		
Cordylophora caspia		1				50
Amphibalanus improvisus	1	55	1	50		30
Einhornia crustulenta	1	1		1		
Mytilus edulis trossulus	1	1				
biofilm	90	100	90	100	70	100
3 m						
Spirulina subsalsa		1				
Pylaiella littoralis	10		80		50	
Ulva linza*			5		40	
Ulva** **spp.**		1				
Cladophora glomerata	70				5	
***Cladophora* sp.**		10		60		45
Gonothyraea loveni	50		5			1
Cordylophora caspia						25
Amphibalanus improvisus	1	65	1	70		35
Einhornia crustulenta	1	15		5		
Mytilus edulis trossulus	1	1				
biofilm	90	100	90	100	70	100
7 m						
Pylaiella littoralis	10		80		50	
Ulva linza*			5		40	
Ulva* **spp.**		1				
Cladophora glomerata	70				5	
***Cladophora* sp.**		1		1		
Gonothyraea loveni	50	5	5	1		1
Cordylophora caspia					10	
Amphibalanus improvisus	1	65	1	75		60
Einhornia crustulenta	1	45		10		1
Mytilus edulis trossulus	1	1				
biofilm	90	95	90		70	100

*old name *Enteromorpha ahlneriana*, ** old name *Enteromorpha*.

Table 23 - Composition and coverage (%) of the fouling organisms found in the three sampling sites studied of the Port of Gdynia during September-October 2013 (S-O) and April-July (A-J) 2014. Non-indigenous species are marked in grey and bold, whereas cryptogenic species are marked in grey and underlined.

All together 3782 mobile organisms representing 6 classes (Clitellata, Polychaeta, Malacostraca, Bivalvia, Gastropoda and Turbelaria) and one phylum (Nematoda) were associated with sessile organisms found on the fouling plates in 2014. The most numerous were Malacostraca which accounted for 71.5% of all collected specimens. They included 8 taxa: Gammaroidea, *Bathyporeia pilosa*, *Apocorophium lacustre*, *Idotea chelipes*, *Jaera albifrons* agg., *Heterotanais oerstedii*, *Rhithropanopeus harrisii* and *Palaemon elegans*. Clitellata which included only subclass Oligochaeta accounted for 15.2% of all collected specimens, whereas contribution of Bivalvia consisted of three species: *Cerastoderma glaucum*, *Mya arenaria*, *Macoma balthica,* and Gastropoda consisted of one order, Nudibranchia accounting for 7.3 and 4.9% of all collected specimens, respectively. Polychaeta with three taxa (*Hediste diversicolor*, *Fabricia stellaris*, *Marenzelleria* spp.) as well as Turbellaria and Nematoda were less abundant among fauna associated with fouling organisms.

The highest and lowest numbers of mobile fauna associated with fouling organisms were found in 2014 at the sampling sites A (**Figure 28a**) and C (**Figure 28c**), respectively. At the sampling sites A and C 12 associated taxa were recorded, whereas at the sampling site B there were 13 taxa.

Among mobile fauna associated with fouling organisms three non-indigenous taxa were found: *Marenzelleria* spp., *Palaemon elegans*, *Rhithropanopeus harrisii* (megalopa and adults) and one cryptogenic species *Mya arenaria*.

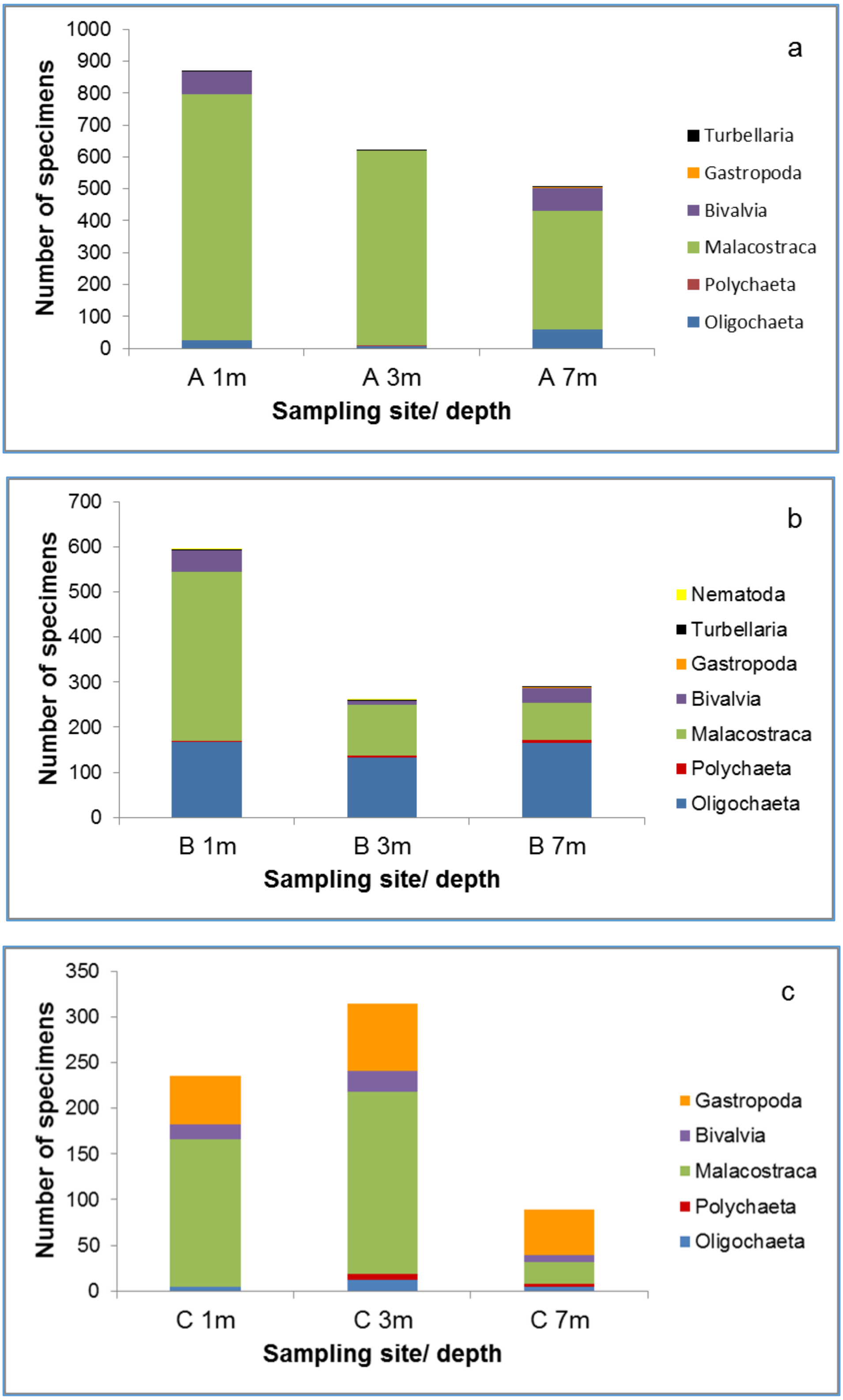

Figure 28 - Number of specimens identified within each taxonomic group of associated fauna found in 2014 on the fouling plates placed at 1, 3 and 7 m depth at the three sites studied A (a), B (b) and C (c) in the Port of Gdynia.

All together 21 taxa were found on the rope from the fouling set-ups developed in the three sampling sites studied of the Port of Gdynia (**Table 24**). Two non-indigenous species *Cordylophora caspia* and *Rhithropanopeus harrisii,* as well as one cryptogenic *Amphibalanus improvisus* were found.

Taxon	Site A		Site B		Site C	
	S-O	A-J	S-O	A-J	S-O	A-J
Cladophora glomerata	+		+		+	
Cladophora sp.		+		+		+
Pylaiella littoralis			+			
*Ulva linza**	+		+		+	
*Ulva*** spp.		+				
Amphibalanus improvisus		+		+		+
Cerastoderma glaucum		+		+		+
Chironomidae (larvae)		+				
Cordylophora caspia				+		+
Corophium spp.		+		+		
Einhornia crustulenta		+		+		+
Gammaroidea		+		+		+
Gonothyraea loveni	+	+	+	+	+	+
Hediste diversicolor						+
Heterotanais oerstedii		+		+		
Idotea spp.				+		
Mya arenaria		+				
Mytilus edulis trossulus		+				+
Rhithropanopeus harrisii		+				+
Nudibranchia				+		+
Turbellaria						+
biofilm	+	+	+	+	+	+

* old name *Enteromorpha ahlneriana*, ** old name *Enteromorpha.*

Table 24 - Taxa found on the rope (+) from fouling setups in three studied sampling sites of the Port of Gdynia during September-October 2013 (S-O) and April-July 2014 (A-J). Non-indigenous species are marked in grey and bold, whereas cryptogenic species are marked in grey and underlined.

All together 31 taxa (**Table 25**) were identified among organisms found on the fouling setups (sessile and mobile organisms on plates and ropes). The highest diversity was at the sampling site A (24 taxa) and the lowest at the site C (19 taxa).

Four non-indigenous taxa: *Marenzelleria* spp., *Palaemon elegans*, *Rhithropanopeus harrisii*, and *Cordylophora caspia,* as well as two cryptogenic species *Amphibalanus improvisus*, and *Mya arenaria* were found.

Class/Phylum	Taxon/Species	FP	R
Cyanophyceae	*Spirulina subsalsa*	+	+
Ulvophyceae	*Cladophora glomerata*	+	+
	Cladophora sp.	+	+
	*Ulva linza**	+	+
	Ulva** spp.	+	+
Ochrophyta	*Pylaiella littoralis*	+	+
Clitellata	Oligochaeta	+	
Polychaeta	*Hediste diverscicolor*	+	+
	Fabricia stellaris	+	
	***Marenzelleria* spp.**	+	
Maxillipoda	<u>*Amphibalanus improvisus*</u>	+	+
Malacostraca	*Apocorophium lacustre*	+	
	Corophium spp.		+
	Gammaroidea	+	+
	Heterotanais oerstedii	+	+
	Idotea chelipes	+	
	Idotea spp.		+
	Jaera albifrons agg.	+	
	Palaemon elegans	+	
	Rhithropanopeus harrisii	+	+
Insecta	Chironomidae		+
Gymnolaemata	*Einhornia crustulenta*	+	+
Hydrozoa	***Cordylophora caspia***	+	+
	Gonothyraea loveni	+	+
Bivalvia	*Cerastoderma glaucum*	+	+
	Macoma balthica	+	
	<u>*Mya arenaria*</u>	+	+
	Mytilus edulis trossulus	+	+
Gastropoda	Nudibranchia	+	+
Turbellaria		+	+
Nematoda		+	

* old name *Enteromorpha ahlneriana*, ** old name *Enteromorpha.*

Table 25 - Occurrence (+) of different classes/phyla with identified taxa/species found on the fouling plates (FP) and ropes (R) developed in the Port of Gdynia in 2013 and 2014. Non-indigenous species are marked in grey and bold, whereas cryptogenic species are marked in grey and underlined.

7 Conclusions

The present coordinated COMBINE and coastal fish monitoring should be complemented with a regular shallow hard bottom fauna and flora monitoring and port monitoring in the ports having the most intensive international ship traffic to obtain the required data on NIS for EU MSFD and IAS regulation reporting, for the exemption procedure under the BWM Convention and to obtain data for the needed indicators including the HELCOM core indicator on NIS (trend of new arrivals).

A manuscript has been written and published in Marine Policy in 2015 (Lehtiniemi et al., 2015) in wide international collaboration to present a global review of monitoring approaches, encompassing a range of coastal environments which are especially vulnerable to introductions to provide a conceptual framework for practical monitoring for NIS to fulfil the requirements of present legislative requirements which include the MSFD and the BWM Convention. The importance of early detection of NIS in bridgehead sites and dispersal hubs is highlighted; and different approaches how monitoring for NIS within marine ecosystems may be undertaken is demonstrated.

Additionally, and based on the experience achieved through the surveys conducted, the following points and issues would be important for further discussions and potential decision making regarding the port sampling protocol:

- timing of the spring survey is essentially important, in order to capture the phytoplankton peak;

- in the northern Baltic, it would be sufficient to sample zooplantkon with 100 µm net only, as samples taken with a net having bigger mesh size (400 µm) did not result in additional important information;
- samples taken from fouling communities (by sraping tool and settlement plates) added important information to Ekman grab samples, and therefore, it is essentially important to perform sampling on fouling communities very carefully and thoroughly from as many different substrata as possible;
- it might be worth to consider recording of settling plate community for the upper and lower side separately;
- as spring and autumn surveys resulted in relatively high fish catches, mobile epifauna should be sampled in all three seasons;
- as crab traps always contained more fish than gee minnow traps, use of bait in fish traps should be recommended.

8 References

BSAP, 2007. HELCOM Baltic Sea Action Plan. HELCOM, 101 pp.

BWM Convention, 2004. International Convention for the Control and Management of Ships' Ballast Water and Sediments. International Maritime Organization, BWM/CONF/36, 38 pp.

Conway D. V. P., 2006. Identification of the copepodite developmental stages of twenty-six North Atlantic copepods. MBA, United Kingdom, Plymouth, 27 pp.

Dziubińska A., Janas U., 2007. Submerged objects - a nice place to live and develop. Succession of fouling communities in the Gulf of Gdańsk, Southern Baltic. Oceanological and Hydrobiological Studies 36 (4), 65-78.

EU, 2014. Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species.

HELCOM 2013a. Joint HELCOM/OSPAR Guidelines for the Contracting Parties of OSPAR and HELCOM on the granting of exemptions under International Convention for the Control and Management of Ships' Ballast Water and Sediments, Regulation A-4. HELCOM, 46 pp.

HELCOM, 2013b. Manual for Marine Monitoring in the COMBINE Programme of HELCOM, 406 pp.

HELCOM, 2013c. HELCOM ALIENS 2- Non-native species port survey protocols, target species selection and risk assessment tools for the Baltic Sea. HELCOM, 34 pp.

IMO, 2007. Guidelines for risk assessment under regulation A-4 of the BWM Convention (G7). Resolution MEPC. 162(56), 16pp.

Kasprzak K., 1981. Skąposzczety wodne, I. Rodziny: Aeolosomatidae, Potamodrilidae, Nididae, Tubificidae, Dorydrilidae, Lumbiculidae, Haplotaxidae, Glossoscolecidae, Branchiobdellidae, Państwowe Wydawnictwo Naukowe, 1-226.

Lehtiniemi M., Ojaveer H., David M., Galil B., Gollasch S., McKenzie C., Minchin D., Occhipinti-Ambrogi A., Olenin S., Pederson J. 2015. Dose of truth—Monitoring marine non-indigenous species to serve legislative requirements. *Marine Policy,* 54, 26–35.

Mańkowski W., 1955. Atlas zooplanktonu Bałtyku. MIR, Gdynia, 51 pp.

MSFD, 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive).

Timm T., 2009. A guide to the freshwater Oligochaeta and Polychaeta of the Northern and Central Europe, Lauterbornia, 66, 1-235.

Żmudziński L., 1974. Świat zwierzęcy Bałtyku. Wydawnictwo Szkolno-Pedagogiczne, Warszawa, 196 pp.

Baltic Marine Environment Protection Commission