Evaluation of the environmental state of the sea area in the Lithuanian territorial waters and economic zone adjacent to the Russian oil platform D-6

January 2007

FIMR

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REPORT

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Executive summary

Environmental conditions in the study in 1995-2005 in the area reflect the conditions and trends currently observed in the Baltic Sea in general. However, the transitional zone forms an exception due to the influence of water outflow from the Curonian Lagoon resulting in short-term variations in salinity and temperature as well as higher levels of nutrients. In biota, alterations in zooplankton and macrozoobenthos communities are the most prominent changes during the 10-year observation period. The establishment of invader species has also affected the structure of pelagic and benthic foodwebs.

The highest levels of total oil hydrocarbons (THC) in the surface water were measured in the open-sea area in the mid-1990s, and again in November 2005. In the coastal zone the THC levels were systematically lower. The values are higher than measured in other parts of the Baltic Sea but the comparison is hampered by the use of a different method (IR-technique) used in monitoring studies. 5-17% of the THC values exceed the Maximum Permissible Level (MPL) established by the Lithuanian legislation. Intensive shipping activity (e.g. Klaipeda harbour), illegal discharges and oil spills from ships are potential causes for the peak-type appearance of total oil hydrocarbons (THC) in the surface water between 1995-2005. Presence of oil-oxidizing bacteria indicate the occurrence of oil hydrocarbons in water. According to the 10 year data no increasing trends can be observed at any of the sampled stations although some high peaks in oil oxidizing bacterial numbers have been found.

In studies carried out in 2005 low levels of polycyclic aromatic hydrocarbons (PAH) were observed in sediments and bivalves. However, PAH levels observed at the only true soft-bottom station N-1 signify some degree of hydrocarbon pollution. Grain size of sediment particles at N-1 is small offering large adsorption surface for various chemical compounds. Molecular ratios of indicator PAH compounds imply that hydrocarbon pollution in the study area is mostly of pyrolytic, not petrogenic, origin and apparently from diesel motors.

Heavy metal concentrations in sediments and biota (soft-bottom clam *Macoma balthica*) in the study area were within normal ranges. Concentrations of other hazardous compounds measured in the study area were below detection limit (alkylated phenols) or low (organotins), the latter, however, showing relatively high levels at station N-1.

Biomarker responses in *M. balthica* showed significant differences between the populations. However, some of the enzymatic biomarkers may be affected by temperature differences between the study stations. Cytogenetic damage, measured as frequency of micronuclei, was significantly higher in *M. balthica* from the offshore stations compared to the near-shore. Basing on previous research, exposure to PAH compounds causing the increased levels of DNA damage cannot be ruled out. The clam population at the offshore station N-2 was in the most stressed condition according to the integrated stress response index (IBR) calculated using all 8 biomarkers measured.

In flounder (*Platichthys flesus*) most biomarker responses measured in the two study areas in December 2005 show only small differences with no clear pattern between the two study areas. Compared to the December observations flounder collected in April 2006 showed significantly higher biomarker responses related to potential exposure to organic contaminants (ethoxyresorufin-*O*-deethylase activity, glutathione *S*-transferase and catalase activities, PAH metabolites/1-OH-pyrene in bile). Subsequently, the IBR index was clearly higher in April. The most probable reasons for the elevated biomarker response levels are related to seasonal variability related to reproduction. However, the high concentration of 1-OH-pyrene in bile strongly implies to recent exposure to oil compounds. Prevalence of histopathological lesions in liver as well as external visible fish diseases observed were within the normal range (or lower) than recorded in other parts of the Baltic Sea, implying no marked effects of contaminants affecting the health of flounder in the study area.

Modelling studies showed that, in case of an oil spill at the D-6 platform, the most dangerous winds in regard to oil contamination of the beaches of the Curonian Spit would be from western, southwestern and southern directions. The probability of oil reaching the shore of the Curonian Spit depends on seasonal variability in prevailing wind directions, the risk being the highest in summer and winter due to the prevailing western air mass movement. As an example of modelling scenarios, in stormy conditions (eastward current, speed 50 cm s^{-1}) the oil spill would reach the Curonian Spit (town of Nida) in 14 hours.

Working group and responsibilities

Finland:

Name	Institute	Task
Dr. Eila Lahdes	FIMR	Coordination of the project
Dr. Kari Lehtonen	FIMR	Ecotoxicology
Mr. Hannu Haahti	FIMR	Chemistry and harmful substances
Dr. Kai Myrberg	FIMR	Oil-drift modelling

Lithuania

Ms. Aiste Kubiliute (1.5. – 31.12.2005)	CMR	Coordinator of the Lithuanian party
Ms. Galina Garnaga, (1.1.2006 – 31.1.2007)	CMR	Coordinator of the Lithuanian party Hydrography and hydrochemistry
Ms. Eglė Šupinienė	CMR	Hydrobiology
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FIMR = Finnish Institute of Marine Research
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IGG = Institute of Geology and Geography
CMR = Center of Marine Research
KU = Klaipeda University

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1. INTRODUCTION AND THE AIM OF THE PROJECT

Oil pollution is recognised as one of the greatest hazards for the marine environment, either in the form of large accidents or long-term small-scale spills and leakage. Oil accidents also cause direct economic losses e.g. by affecting fish stocks and spoiling the recreational use of the sea and coastline. From an ecological point of view, damages occur at all levels of the marine food web including birds and mammals. Oil pollution in the aquatic environment originates from sea traffic, harbours, drilling activities, ships, and land-based sources. In regard to oil accidents the effects are - at first – acute, causing visible damage on biota and the environment, but at later stage chronic harmful effects might take place. Leakage during continuous activities such as drilling of oil and gas can cause chronic effects on biota.

The Russian Kravtsovskoye oilfield D-6 began exploitation in July 2004 by the Russian oil company LUKOIL-Kaliningradmorneft. D-6 is situated near the Lithuanian-Russian (Kaliningrad Region) border and Curonian Spit National Park (55°19.4 N; 20°34.3 E.), with minimum distance of 4.3 miles to the sea border and 13.2 miles to the coast of the Curonian Spit. Because of its unique natural and cultural landscape the Curonian Spit is included in the UNESCO World Heritage. It is thus an object of international importance as well as a nature object to be preserved.

Figure 1. Map of the region.

In case of an oil spill in the D-6 oil field, due to the prevalence of western transmission of air masses, the possibility of oil reaching the south-eastern Baltic Sea coast is high. The coastal zone of the Curonian Spit of the Baltic Sea side, characterised by a shallow sandy littoral zone, submarine sand ridges, wide beaches and a high protective dune

ridge, is very sensitive to anthropogenic impacts, especially to oil pollution. If an oil spill occurs in the open sea and reaches the littoral zone it would heavily pollute the coastal zone, beaches and the protective dune ridge, causing multiple damage to the coastal environment. In addition, not only oil but also other by-products originating from the oil production process are harmful if they are dispersed to the environment.

The purpose of this joint Finnish-Lithuanian project was to evaluate the present state of the marine environment of the region in the Lithuanian territorial waters and economic zone, which could be seriously influenced by the operation of the D-6 oil field. The project has been carried out by independent marine experts of the Finnish Institute of Marine Research (Helsinki) and researchers of the Center of Marine Research (Klaipeda), in cooperation with other research institutes in Finland and Lithuania. The Finnish part of the project was financed by the Finnish Ministry of Environment in the framework of the Environmental Cooperation with Neighbouring Countries.

The present report consists of the following parts:

- Long term (1995-2005) monitoring data collected by Lithuanian authorities in the framework of HELCOM COMBINE programme
- Ecotoxicological studies (harmful substances in water, sediments and biota, and biological effect studies) performed by experts and consulting laboratories in Finland, Denmark and Germany.
- Drift modelling of oil
- Recommendations for the future monitoring.

1.1 Curonian Spit

The Curonian Spit is a unique, 98 km long sand dune peninsula, which stretches from the Sambian Peninsula in the south towards Klaipeda where a narrow strait connects the Baltic Sea and the Curonian Lagoon. The northern 52-km stretch of the spit belongs to Lithuania and the rest 46 km to the Russian Federation.

Figure 2. The Curonian Spit and Curonian Lagoon.
(Source: http://en.wikipedia.org/wiki/Curonian_Spit)

The width of the spit varies from a minimum of 0.4 km in the south to a maximum of 3.8 km in the central part. The entire Curonian Spit is protected in the form of National Parks; "Kuršių Nerija" in Lithuania and "Kurshskaya Kosa" in Russia.

The Curonian Spit was inscribed as a cultural landscape (Id.N° 994) on the World Heritage List in 2000 on the basis of Criterion (v): *"The Curonian Spit is an outstanding example of a landscape of sand dunes that is under constant threat from natural forces (wind and tide). After disastrous human interventions that menaced its survival the Spit was reclaimed by massive protection and stabilization works begun in the 19th century and still continuing to the present day"* (http://whc.unesco.org/archive/repcom00.htm#994).

The landscape of the Curonian Spit changes from west to east. On the western coast sandy beach meets the sea. The beach is formed mostly of quartz sand and only between Nida and Preila some gravel grounds occur. An estimated 900 plant species grow in the Kursiu Nerija National Park on Lithuanian side. About 70% of the land in the National Park is covered by forest. The Curonian Spit is an important gateway for migration of birds, both for waterfowls and terrestrial birds. There are also thousands of over-wintering waterfowls in the Spit. An estimated 40 mammalian species inhabit the National Park. On the shoreline grass cover helps to maintain the stability of dune ridges.

In the littoral zone of the Spit, on the Baltic side, bottom sediments consist of fine sand, which greatly determines the species composition of benthic fauna and flora. The diversity is lower than in the littoral zone of other parts of the Lithuanian coast. Long-nosed seals sometimes appear on the shores of the Curonian Spit (Sources: http://en.wikipedia.org/wiki/Curonian_Spit, http://www.nerija.lt/en/, http://whc.unesco.org/sites/994.htm, http://www.coast.lt/?en=1118123334).

A concrete example about the laborious cleaning of exposed sandy beaches and biological impacts of oil pollution and has been followed e.g. after the "Prestige" oil spill on the Spanish coast some years ago (De la Huz et al. 2005).

1.2 International views on the D-6 oilfield

1.2.1 The World Heritage Committee

According to the World Heritage Committee documents (http://whc.unesco.org/en/list/994/), concern has been raised about the possible harmful effects of the D-6 oil platform owned by a Russian company Lukoil, only 22 km from the World Heritage site, Curonian Spit (Decision 26COM 21B.57, 2002, Decision 27COM 7B.70, 2003, Decision 28COM 15B.75, 2004, Decision 29COM 7B.67, 2005, Decision 30BOM 7B.87, 2006).

In these documents, bilateral agreement and collaboration to complete a joint Lithuanian-Russian post-project Environmental Impact Assessment (EIA) was requested. The agreement should include "co-operation in case of pollution accidents, pollution prevention/mitigation, compensation measures and a joint work plan for monitoring". Otherwise, the Curonian Spit would be automatically inscribed on the List of World Heritage in Danger. In 2006, a request was set to provide the World Heritage Centre a detailed and updated report by 1 February 2007 to be examined by the World Heritage Committee at its 31st session in 2007.

1.2.2 The Baltic Marine Environment Protection Commission (HELCOM)

The case of the D-6 oilfield has been under discussions in Commission Meetings and various working groups of HELCOM during the 2000s. Both Lithuania and Russia have presented their concerns and responses against and for oil production in the area. HELCOM Secretariat has also prepared a background paper including the legal aspects and existing regulations concerning the extraction of oil in the Baltic Sea, in general, according to the Helsinki Convention as well as OSPAR regulations (HELCOM HOD 14/2003, Document 7/1).

The Russian Federation submitted a document (HELCOM RESPONSE 4/2004, Document 12/1) containing information on the planned offshore activities at the Baltic shelf *Ecological aspects of Kravtsovskoye (D-6) oilfield exploitation"*. In the document, legal aspects of oil production based on the licence issued by the Ministry of Natural Resources of the Russian Federation (10 August 1999) and principal environmental solutions are introduced. According to the document, the "zero-disposal" principle will be followed meaning "*complete refusal from disposal of all kinds of processing and consumption wastes, grey water inclusive"*. In addition, burning and burial of any substances are prohibited. An underwater pipeline for oil connects the platform and the oil terminal ashore. There will be readiness to control possible oil spills and other accidents and to conduct monitoring survey of environmental parameters in the area and Curonian Spit. In 2004, satellite monitoring of surrounding waters in order to discover oil leaks has commenced (HELCOM 27/2006, Document 9/2).

1.3 Effects of oil production on the environment

Oil contamination is usually caused by an accidental or chronic release of one of three main types of oil: crude oil, heavy fuel and diesel fuel oil. Among the main sources of oil contamination are activities and incidents related to transportation (tanker operations and accidents) and fixed installations (offshore oil production, coastal oil refineries, terminal loading) (Clark 1985). However, the share of other sources to the total input of petroleum hydrocarbons can be surprisingly high with e.g. industrial and municipal wastewaters and river run-off comprising almost half of the load (Clark 1985). Typically oil consists of a highly variable mixture of thousands of compounds with hydrocarbons forming the predominant substance group. Of these, polycyclic aromatic hydrocarbons (PAHs) are regarded as the most toxic to organisms, many of them being potentially carcinogenic (e.g. benzo[a]pyrene). At sub-lethal concentrations, oil constituents cause physiological or behavioural disturbances and, possibly more importantly, may cause developmental abnormalities in organisms almost certainly resulting in their early death (Clark 1986, Midbøe and Persson 2004, Ikävalko 2005 and references herein).

During the past forty years an notable number of major oil accidents have occurred in different sea areas of the world, with the wrecks of *Exxon Valdez* in Alaska in 1989, *Erika* off Bretagne in 1999 and *Prestige* off Galicia in 2003 standing out as the more recent examples. Oil disasters in coastal areas and harbours obviously catch the largest public attention and can cause massive acute damage to local ecosystems, and lead to temporal collapses in local economics related to the exploitation of the sea and the coastline (e.g. fisheries, aquaculture and tourism).

In fact, it appears that long-term chronic contamination by lower levels of oil-derived substances are more harmful to the environment than acute large spills because they

deteriorate the overall conditions in the environment and lead to a constant stress to organisms within the local ecosystem. Chronic contamination can have an effect on the genetic structure of populations of organisms by selecting out the oil-tolerant genotypes, and, likewise, can have an effect on community structure by removing oil-sensitive species and favouring the tolerant ones. Physiological adaptation to chronic sub-lethal levels of oil can cause permanent shifts in cellular energy allocation because of the organisms' need to meet the increased energy costs triggered by contamination (e.g. detoxification of PAHs, metabolic disturbances) that is likely to lead to reductions in growth, general condition and reproductive potential. In addition, the survival capacity of larval stages of marine organisms is often effectively lowered in chronically polluted environments.

In the Baltic Sea, the alarming increase in levels of oil transportation, construction of large oil harbours (e.g. Muuga in Estonia, Primorsk and several others in the Russian territory in the eastern Gulf of Finland) and pipelines during the past two decades in addition to the emerging oil drilling activities all cause concern in all surrounding countries for the increasing threat of deterioration of the state of the marine environment. The probability of the occurrence of a major oil spill has greatly increased during the past few years, while at the same time the marine environment is becoming progressively more contaminated by oil-derived polycyclic aromatic hydrocarbons and other hazardous substances related to oil transportation and production.

1.3.1 Main characteristics of crude oil

Chemically, crude oil (petroleum) is a mixture of hydrocarbons composed of carbon, hydrogen and oxygen, with minor amounts of nitrogen and sulphur as well as heavy metals as impurities. There is variation in chemical composition and physical properties of oil according to the geographical location of oil deposits. Among heavy metals, vanadium and nickel are characteristic constituents of crude oil. Vanadium is usually associated with a high sulphur content and nickel with a low sulphur content of crude oil. Specific gravity of oil generally lies between 0.73 – ca. 1.0. Paraffin-based oils are less heavy than those with naphthenic components. Specific gravity is usually classified according to API (American Petroleum Institute) scale as degrees. An API value > 30° means light and < 22° heavy crude oil (Clark 1986, Clayton & Koncz 1994, http://en.wikipedia.org/wiki/Petroleum).

1.3.2 Specifics of D-6 crude oil

Detailed properties of D-6 crude oil were not available but main characteristics are as follows:

Density 0.830 kg/m^3, viscosity 12.9 mm^2/sec., sulphur content 0.17%, asphaltenes 1.1%, aliphatic hydrocarbons 60.1%, aromatic hydrocarbons 23.0% and polyaromatic hydrocarbons 19.9% and resins 2.29%. This means that API value is >30% and sulphur content is low. These properties are important factors in oil drift models, which will be included in this report (Zdanaviciute 2000, http://www.geo.lt/Litosfera/n4L/n4L_8L1.htm).

1.3.3 Emissions from an oil platform

Despite of all safety measures accidents happen due to human error or natural forces. This in mind we should accumulate information for comprehensive understanding of the possible problems caused by human activities with high-risk operations. A statement by Ståle Johnsen (Statoil, Norway) "*Zero discharge; a myth – zero harm; a real possibility*" gives us a realistic view of oil extraction work in oil fields even in strictly controlled conditions. Harmful effects originate not only from oil spills but several other chemical and physical factors in connection with the operations such as discharges of produced water, mud and cuttings, atmospheric emissions and acoustic pollution (National Research Council of the National Academies 2003, Patin 1999). In the following part a short introduction to some of these factors will be given.

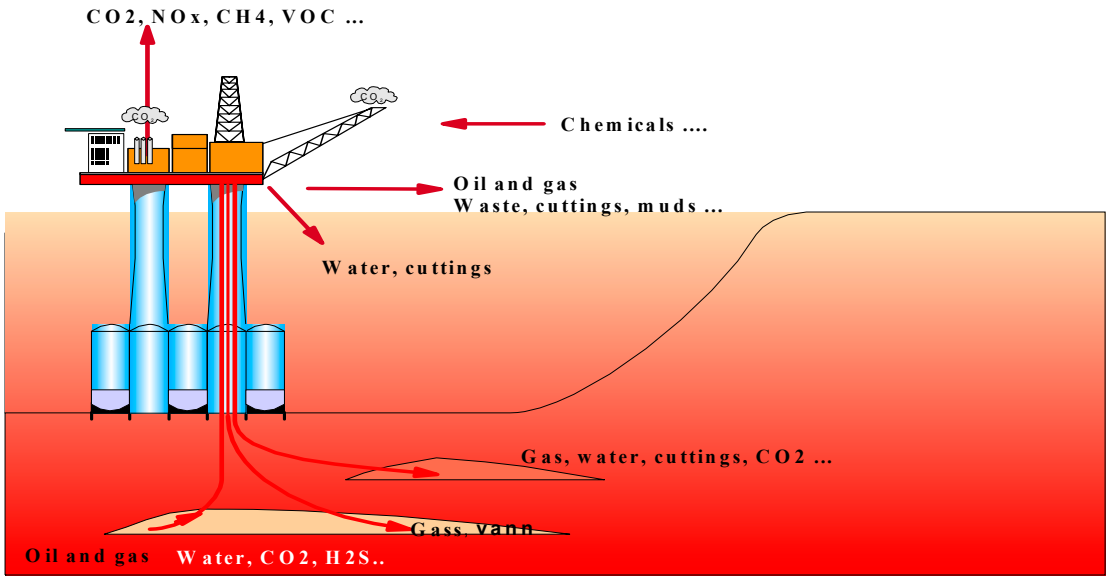

Figure 3. Emissions from an oil platform (Adopted from Johnsen www.naturvern.no/data/f/0/69/90/3_2401_0/Statoil).

Harmful effects of oil production can be estimated by risk assessment of total discharge i.e. calculation of EIF (Environmental Impact Factor). Risk calculations also include the ratio between PEC (Predicted Environmental Concentration) and PNEC (Predicted No Effect Environmental Concentration), which should be < 1. PEC/PNEC > 1 the environmental risk is regarded as unacceptable. In calculations, the volume and chemical composition of discharge as well as physical conditions of the place of discharge should be taken into account. However, numerical risk evaluation calculations were not commissioned for this report.

Effects of an oil spill depend on the characteristics of oil products. Spills may consist of crude oil, fuel oils (http://en.wikipedia.org/wiki/Fuel_oil) or more refined oil products. The spreading, effects and recovery greatly depend on the characteristics of oil. In oil production installations spills concern not only crude oil but also oil-containing products used to run and lubricate installations (cutting oil). After oil tanker accidents several comprehensive studies have been performed to estimate damage to the marine ecosystem (e.g. Bocquené et al. 2004, The Prestige Oil Spill 2006). These studies give a general view of the harmful effects of oil on the marine environment.

1.3.4 Effects of by-products

In connection with oil production several types of by-products are produced that contain harmful compounds to biota. Such products are e.g. produced water, cutting oils and various chemicals. Produced water is the largest emission in oil exploration process. It consists of so-called formation water (the water present naturally in the reservoir) and water injected into the formation to create pressure in the reservoir. Both formation and injected water together with oil (or gas) are produced. In 2005 about 17 million m^3 water was produced daily worldwide and about 40% was discharged offshore. Produced water contains aliphatic and aromatic hydrocarbons, alkylated phenols, PAH compounds, organic acids, inorganic salts, metals, BTEX (Benzene, Toluene, Ethylbenzene, Xylenes), oil droplets and fine sediment particles. After production the produced water is separated from oil. Usually produced water is discharged into the sea or injected back to the reservoir. According to OSPAR regulations (OSPAR 2001, 2006a) discharge of produced water has concentration limits for hydrocarbons; the hydrocarbon content of water should be reduced from 40 mg/l to 30 mg/l by the end of 2006 (Knudsen et al. 2004, OGP 2005).

Effects of produced of water have been studied especially in Norway in the DREAM project (Dose Related Risk and Effects Assessment Model) in early 2000's. An important topic of DREAM was the effects of alkylated phenols on fish reproduction. In laboratory tests no significant risk on reproduction of fish could be observed (Sundt and Baussant 2003, Myhre et al. 2005).

Heavy metals

Concentrations of heavy metals in crude oil vary depending on the oil field and geologic horizons. Toxic effects of heavy metals and bioconcentration processes in marine organisms are well known. In general, acute toxicity originating from heavy metals is minor compared with other hazards of oil after an oil spill. Chronic emissions, accumulation on sediment particles and subsequent transfer into the tissues of benthic organisms can cause disturbances in the long-term. Metals occurring in oil are natural components of the marine environment, and most of them are essential for organisms except for some metals such as mercury and cadmium. Exposure concentrations largely determine their toxicity. Organic forms o metals, like methyl mercury and organotins, are by far the most toxic ones.

As an example, information on the levels of heavy metalscontained in the oil spilled during the accident of the oil tanker "Prestige" in 2002 and the subsequent findings in coastal sediments are given in Table 1 (Prego et al. 2005).

Table 1. Presence of heavy metals in the fuel oil spilled from the Prestige Tanker and their potential influence on the coastal sediment (Prego et al. 2005).

Emulsioned fuel range	Prestige cargo fuel	Coastal sediment	Potential sediment contamination
1000 μg g^{-1}	μg g^{-1}	μg g^{-1}	
Al	264.9	10,700–102,400	null
Si	27.9	70,000–300,000	---
Ca	48.6	29,000	---
Fe	11.3	15,000–33,000	null
Mg	26.5	21,000	---
Ti	?	3,000–3,900	null
100 μg g^{-1}			
Ni	96.5	7–38	should be considered
V	381.8	60–94	should be considered
10 μg g^{-1}			
Mn	?	240–600	null
Mo	3.00	1.5–1.8	should be considered
Zn	4.79	50–136	null
1 μg g^{-1}			
As	1.19	1.5–10.0	low
Co	0.31	4–13	null
Cr	0.54	12–76	null
Cu	2.74	5–35	low
Se	0.34	0.04–0.08	should be considered
< *0.1* μg g^{-1}			
Cd	0.98	0.01–0.20	low
Hg	?	0.02–0.03	null
Pb	0.41	16–78	null
Sn	?	2.5–3.5	null

Drilling fluids and cuttings

During the platform operation, drilling fluid is pumped down the drill pipe. The fluid is usually a mixture of water, clay, a weighting material (usually barite), and various chemicals. Cuttings means solid material consisting of crushed rock and clay, which is brought to the surface by the drilling fluid and may be discharged overboard or re-injected into the well. Among others, OSPAR has made a decision against using organic-phase drilling fluid (OPF), which can harm the marine environment (OSPAR 2000), as well as recommendations for the disposal of cuttings (OSPAR 2006b), which can have negative impact on benthic communities and spawning areas of fish.

Other substances and effects

Perfluoro-octo sulphonate (PFOS), a persistent compound has been extensively used in oil fields as a component of fire-fighting foam ("Light Water AFFF"). PFOS binds with protein molecules, is hepatotoxic and is found ubiquitously in tissues of humans and animals throughout the world.

Acoustic pollution and air pollution

Offshore oil and gas production creates acoustic pollution (drilling, platform machinery, movement of oil in underwater pipelines). Effects of this kind of pollution in the Baltic Sea are not well documented.

2. INVESTIGATIONS IN THE LITHUANIAN SEA AREA ADJACENT TO THE D-6 OIL PRODUCTION PLATFORM

2.1 Description of the study area

The study area is situated in the south-eastern Baltic Sea, representing the southern part of the Lithuanian waters and covering about 40% of the sea area under Lithuanian jurisdiction (Figs. 1 and 2). The area extends to the Curonian Spit in the east and to the borderline of the Russian Federation in the south. The limits of the area are latitude 55°50' in the north and longitude 20°30' in the west.

Continental shelf off Lithuania is shallow (< 75 m) and the sediments are mostly sandy with few silt/mud areas in the deeper parts of the sea (Fig. 2). Salinity in the area is around 6-7 and shows more variation near the coast due to the runoff of less saline water of the Curonian Lagoon (Fig. 2). The sea area can be divided in three categories according to the types of water bodies and bottom stratum.

Table 1. Classification of the water bodies in the study area (Adopted from HELCOM EUTRO "*Assessment of eutrophication status in south-eastern Baltic Sea*" (HELCOM 2005).

Area	Types of water body	Salinity range	Depth range	Wave exposure	Mixing	Main bottom stratum
Coastal waters	Open Baltic sea sandy coast (Curonian Spit)	>5-6	5-30	Exposed	Partly mixed	Sand
Transitional waters	Plume from the Curonian Lagoon into the Baltic Sea	0.5-7	>5	Exposed	Partly mixed	Sand with fields of stones
Open sea	Open sea	>7	30-120	Weak-none	Stratified	Silt

When divided according to depth, five sub-areas can be distinguished in the coastal and open-sea zones, determinining the type of the sampling sites in more detail:

- transitional water area in the sea – the Curonian Lagoon influence area (depth 16 m; Station 4);
- coastal waters area with depth of 13-16 m (Stations 6, N-5, N-7, N-9)
- coastal waters area with depth of 36-37 m (Stations N-4, N-6, N-8)
- open sea area (depths 47 and 66 m for Stations 65 and N-2 respectively)

- deep open-sea region (depth of 70 m; Station N-1).

The coastal area of the Curonian Spit opening to the the Baltic Sea with shallow sandy littoral zone, submarine sand, wide beaches and a high fore dune ridge is very sensitive to anthropogenic impacts, including oil contamination. The coastal area is hydrodynamically a very active environment influenced by wind, waves and water currents. These physical factors naturally shape the biota inhabiting the coastal area.

The transitional area is influenced by the plume from the Curonian Lagoon, creating a continously fluctuating environment in regard to salinity, temperature and nutrient conditions. This variability is reflected in the composition of biota as well.

The open-sea area resembles the south-eastern Baltic Sea in general. In deeper parts the water body is stratified in respect of salinity affecting the conditions of oxygen and nutrients in near-bottom water. Halocline is typically formed at the depth of 60–80 m.

Detailed description of the hydrography, hydrochemistry and biology will be presented in connection with the presentations of the long-term data for the years 1995–2005.

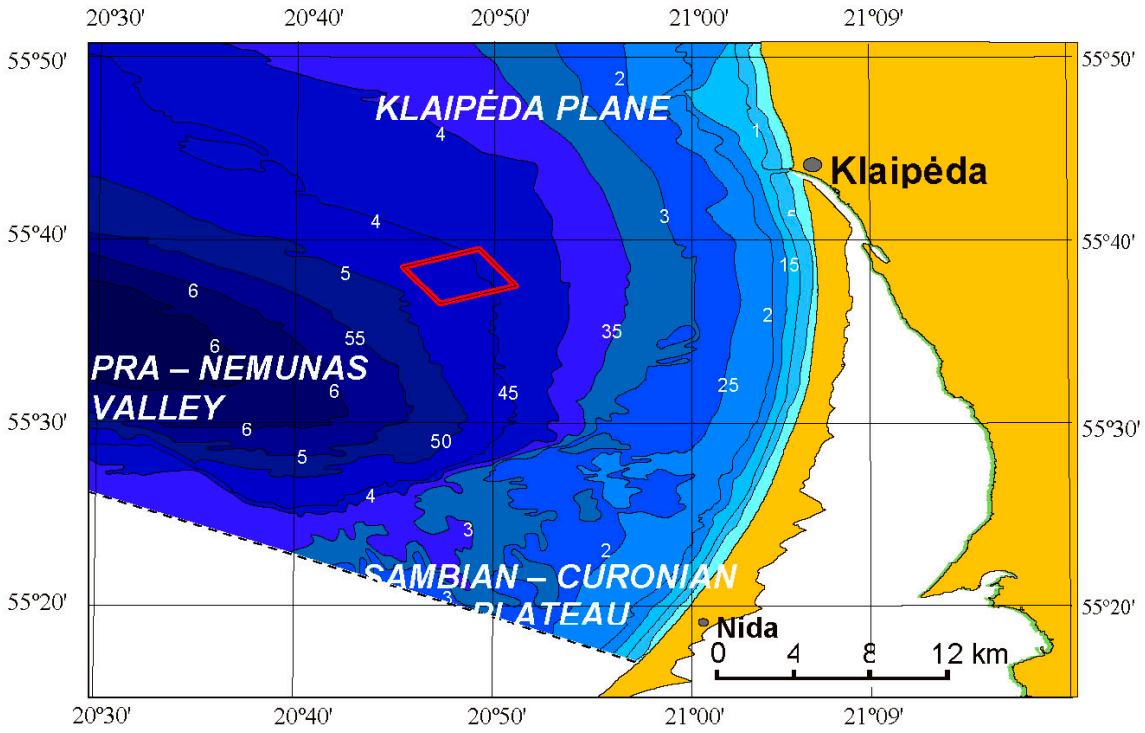

Figure 4. The geographical classification and bathymetric conditions of the research area.

2.1.1 Geographical settings of the area

Responsible scientist: Kęstutis Jokšas (IGG)

Bathymetric conditions, bottom formations and type of bottom sediment are important factors when we are evaluating the state of the marine environment and behaviour and fate of various pollutants entering to the sea.

Factual material

Description of the conditions of the surface layer of bottom sediments and sedimentation are based on the geological mapping of the Baltic Sea bottom carried out by the Baltic Sea team (executive K. Savickas) of oil prospecting expedition for ecological mapping of the Baltic Sea bottom in 1990–1992, for geological mapping of the Baltic Sea bottom in the Klaipėda–Šventoji water area (1:50 000) (executive M. Repečka) in 1993–1996 and in the Nida–Klaipėda water area (1:50 000) (executive Ž. Gelumbauskaitė) in 1998 (Grigelis, Satkūnas, 1997). Results of the research work obtained by other marine expeditions (executive K. Jokšas) in 2005 and published data were also used.

Orography

According to orographic classification of the Baltic Sea bottom, the study area occupies the eastern part of the central Baltic orographic domain (Григялис 1991). It includes four main morphological forms of relief: the nearshore (submarine shore slope), Kuršiai–Sambian Plateau, Pra-Nemunas old valley and Klaipėda submarine slope (or the north-eastern slope of the Gdansk Deep) (Fig. 1).

The submarine slope, extending from north to south, can be described as abrasional–transitional submarine onshore. Its width ranges from 1 to 1.5 km and the slope base can be traced at a depth of 8–12 km. In the sector between Melnragė and northern jetty of the Klaipėda port, the submarine slope reaches 2.5 km in width and the base is situated at a depth of 12–14 m. In this area, the processes of wave transformation and deformation are under a strong effect of Nemunas drift, which moving in north-western and southern directions forms an onshore of weak accumulation. South of the southern jetty of the Klaipėda port till Preila, the width of the submarine shore slope is 2.8–3.7 km and its base is situated at a depth of 23.5–25.0 m. In this sector, the submarine slope is represented by an onshore of intensive accumulation. South of Preila, the submarine slope narrows to 1.2–2.0 km and its base is situated at a depth of 20.0–22.0 m. This sector (between Nida and Juodkrantė) is represented by an abrasional–accumulative onshore (Janukonis 1994–1995, Janukonis 1997, Repečka ir kt. 1997, Žaromskis 1999, Žilinskas, Jarmalavičius 2003, Gelumbauskaitė 2003).

The nearshore gradually merges into the Klaipėda submarine slope (the north-eastern slope of the Gdansk Deep) with a relief of low hills (the relative height of the hills is 2–3 m). At a depth of 40–70 m, the submarine slope merges into the Pra-Nemunas old valley and, beyond the study area, into the Gdansk Deep.

The Pra-Nemunas valley extends from east to west along the edge of Kuršiai–Sambian Plateau. The slopes of this valley are uneven. They are clearly visible at a depth of 40–45–50 m. There is a depression of Pra-Nemunas old valley at a depth of 60–69 m. It is a closed morphological form with a flat silty bottom (Григялис 1991, Trimonis, Gulbinskas 2002).

The Kuršiai–Sambian Plateau is situated at a depth of 20–50 m. It has a varying abrasional–accumulative relief (relative altitude 5–10 m), which is complicated by various deeps, oxbow lakes and terraces and scarps of the shores of old basins in its peripheral part (Gelumbauskaitė ir kt. 1991, Rudenko 2002).

Table 2. Classification of oceanic and marine sediments according to granulometric composition.

Sediment group		Sediment type	Size of dominant grains, mm	Md, mm
Psephites	Blocks		>1000	
	Boulders	coarse-grained	1000–500	
		medium-grained	500–250	
		fine-grained	250–100	
	Gravel	coarse-grained	100–50	
		medium-grained	50–25	
		fine-grained	25–10	
Psammites	Pebbles	coarse-grained	10–5	
		medium-grained	5–2.5	
		fine-grained	2.5–1	
	Sand	coarse-grained	1–0.5	
		medium-grained	0.5–0.25	
		fine-grained	0.25–0.1	
Silts	Silt	coarse-grained	0.1–0.05	
		fine-grained silty mud	0.05–0.01	
Clays		silty-clayey mud	<0.01 (50–70%)	0.01–0.007
		clayey mud	<0.01 (>70%)	<0.007

Bottom sediments

Granulometric type of bottom surface sediments was determined using the decimal classification (Table 1) of recent oceanic and marine sediments based on the median diameter (Md, mm) of grains (Безруков, Лисицин 1960). Trusk‘s sortedness coefficient was used to evaluate the sortedness of sediments (Рухин 1969).

All granulometric types of sediments are widespread in the study area except clayey mud (Figs. 3 and 4). The distribution pattern of sediments is uneven. As a rule, coarse-grained sediments (Md>0.25 mm) occur on the bottom relief elevations whereas fine-grained sediments (Md<0.01 mm) are spread in the deepest part of the study area (>60 m).

Boulders, gravel and pebbles are locally assembled in the shallow zone (beginning with the depth of 8–15 m) north of Klaipėda Strait and on the surface of Kuršiai–Sambian Plateau. These sediments represent remnant surfaces on sandy loam and till. Medium-grained sand (Md=0.5–0.25 mm) is widespread on the Kuršiai–Sambian Plateau.

Fine-grained sand (Md=0.25–0.1 mm) and coarse-grained silty (Md=0.1–0.05 mm) sediments are most widespread in the study area. Fine-grained sand is lying in the shallow zone, southern part of Klaipėda submarine slope and Pra-Nemunas old valley (till the depth of 60 m). Fine-grained sand is rarer on the Kuršiai–Sambian Plateau where it is accumulated in relative declensions of relief (oxbow lakes and hollows). Coarse-grained silt sediments are widespread in the submarine Klaipėda slope north of $55^0$40’latitude. They are bedded within a depth interval from 20–25 m to 60 m. Coarse-grained silty sediments form an intermediate zone (at a depth of 50–65 m) between fine-grained sand and mud sediments. Mud sediments (Md<0.05 mm) were recorded in the

deepest part of the study area. Fine-grained silty (Md=0.05–0.01 mm) and silty–clayey (Md=0.01–0.007 mm) mud is spread on the bottom surface, at a depth of 65 m and more.

Sediments of highly variable granulometric composition (gravel, pebbles and sandy and silty sediments) standing out in the field of fine-grained sand were recorded in the central part (soil dump) of the study area.

Though Trusk's sortedness coefficient is rather unevenly distributed over the whole region (Table 3 and Fig. 5), the study area is predominated by very well, and well sorted sediments (So=1–1.5). The best sorted sediments are concentrated in the shallow zone up to the depth of 20 m where sortedness coefficient is <1.2. Zones of well sorted sediments are found over the whole study area. Worst sorted sediments (So>1.5) are found in the Kuršiai–Sambian Plateau north of the Klaipėda Strait. Sediments in the dump also are badly sorted (So>15). The uneven distribution of sediment types and sortedness is predetermined by recent and past sedimentation processes.

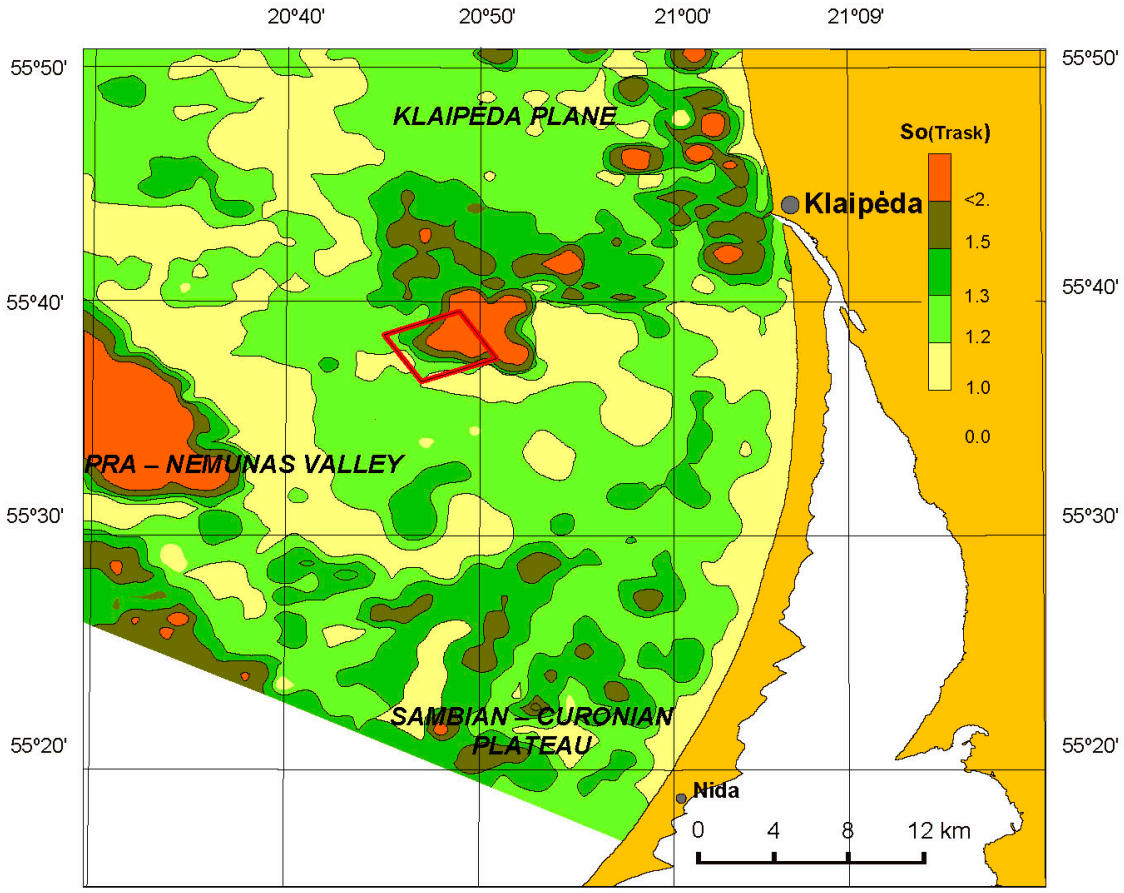

Figure 5. Sediment sortedness coefficient according to Trusk according to Repečka M. 1997 and Radzevičius R. 2001. (Note: In scale <2 should be >2.)

Surface sediments are classified into relict and recent ones (Блажчишин, Усонис 1970, Блажчишин 1976[a], Gulbinskas 1994, Repečka ir kt. 1997, Repečka 1999). Relict sediments formed in the past under different sedimentation conditions. Recent sediments are forming in the present marine sedimentary environment. This classification of sediments is based on the dynamics of mobilization, migration and accumulation cycles of terrigenous material (Блажчишин 1976[a]). Three sedimentation

zones of terrigenous material (clastic material) are distinguished: shallow, transitory-neutral and deep.

The Curonian Lagoon transports silty-clayey particles to the Baltic Sea through the Klaipėda Strait. Coarser fractions get into the sea as a result of coast and bottom abrasion. The impact of intensive wave action on the bottom (in the shallow zone) facilitates accumulation of particles coarser than 0.05 mm. Finer particles are transported to hydrodynamically calmer sedimentation zones (Блажчишин, Шуйский 1973). As was mentioned, sediments in the shallow zone or the onshore accumulate till the depth of 20 m. Fine-grained sand is most widespread.

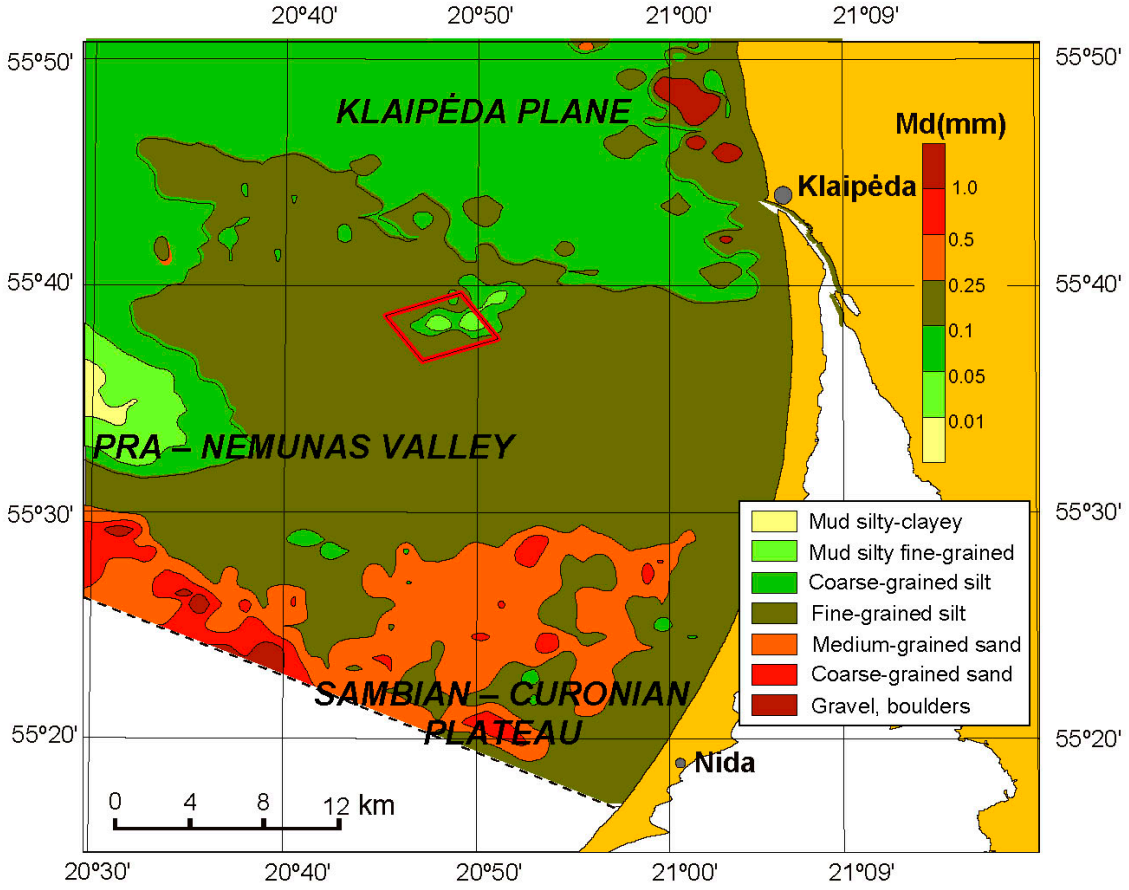

Figure 6. Sediment types and median diameter (Md, mm) of grains (Repečka M. 1997, Radzevičius R. 2001).

Sediment distribution and accumulation in this zone depend on the hydrodynamic regime of water mass, morphological and geological properties of the bottom and source and composition of drift. A sustained sediment flow from south to north exists along the Curonian Spit. Based on the data of detailed morphodynamic investigations and studies of hydrodynamic regime of water mass the shallow zone is subdivided into two parts: Curonian Spit and mainland part north of Klaipėda (Janukonis 1994–1995, Janukonis 1997, Žaromskis 1999, Janukonis 2000). Transverse transport of water mass and sediments takes place in the sector between the northern jetty of Klaipėda port and northern boundary of the study area. Alongshore migration flows with northern resultant are dominant in this sector. In the shallow zone near the Curonian Spit, accumulation and distribution of sediments are in direct dependence on wave direction, duration and intensity. The dominant SSW direction of winds is responsible for dominant sediment transport from south to north. Sediment transit sector between Nida and Juodkrantė and

accumulation zone between Juodkrantė and Kopgalis dune of Curonian Spit are distinguished in the shallow zone near the Curonian Spit.

Particles <0.05 mm accumulate in the deep sedimentation zone. It is assumed that theses particles are transported by near-bottom currents forming individual flows (Блажчишин 1998). Silty sediments containing >50 % of clayey particles form in the deep sedimentation zone. The shallow and deep zones of recent sedimentation are separated by a wide transitory or zero terrigenous sedimentation zone. The zone is marked by abrasion of surface bottom sediments or absence of terrigenous material accumulation.

Polymictic (boulders–gravel–pebbles) and oligomictic (sands and silts) quartziferous–feldspathic sediments are widespread in the shallow and transitory sedimentation zones. In the southern part of the study area, these sediments contain glauconite and phosphorite (polimictic ones) (Блажчишин 1976[a], Блажчишин 1976[b], Блажчишин & Усонис 1970, Лукашев 1986). The fine-grained sediments of the shallow zone are often rich in heavy allothigenous minerals. Their content in the 0.5–0.01 mm fraction near the Curonian Spit ranges from 1.0 % to 4.46 % and account for 2.12 % on the average. The heavy allothigenous minerals of the fine-grained sand and coarse-grained silt sediments of the shallow sedimentation zone are predominated by black metalliferous ones, amphiboles, garnets and zircon whereas sediments of transitory sedimentation zone contain garnets, amphiboles, black metalliferous minerals, and epidote. Silty oligomictic (fine-grained silty mud) feldspathic–quartziferous–micaceous, hydromicaceous and kaolinized–feldpathic (silty clayey mud) sediments are widespread in the deep sedimentation zone. Mica and glauconite are dominant among the heavy allothigenous minerals in the sediments of deep sedimentation zone. Yet the total content of heavy allothigenous minerals does not exceed 1% in the fine-grained sand–coarse-grained silt fractions.

Boulders–gravel–pebbles–coarse-grained and medium-grained sands usually are relict sediments. They are locally found north of the Klaipėda Strait. Usually these sediments form a thin cover on Pleistocene deposits (tills and clays). These sediments are composed of rock debris of different petrographic composition and of various minerals. Fractions <1.0 mm contain quartz, potassium feldspars, amphiboles (hornblende), pyroxenes and other minerals (Table 4).

Coarse-grained and medium-grained sand is most widespread in the southern part of the study area (Kuršiai–Sambian Plateau). It is mostly relict one, i.e. formed in the past sedimentary environments. These sediments locally accumulate in the shallow sedimentation zone near the abraded coast. Coarse-grained and medium-grained sand sediments are predominated by quartz and feldspar. They also contain glauconite, ilmenite, garnets, amphiboles and other minerals (Table 4).

Fine-grained sand and coarse-grained silt are most widespread sediments in the study area. Recent sedimentation of fine-grained sand takes place in the shallow zone. The sand bedding deeper than 20 m is relict. The fine-grained sand is predominated by quartz and feldspar. In the southern part, it contains glauconite. The contained heavy minerals include ilmenite, amphiboles, mica, zircon, phosphates, garnets, limonite, and glauconite (Table 4). Coarse-grained silt accumulates in the shallow zone till the depth of 25 m. In front of the Curonian Lagoon strait, it accumulates till the depth of 30–35 m. Coarse-grained silt in the deeper parts of the sea is relict, i.e. formed in the past sedimentary environments. Its dominant minerals are quartz and potassium feldspars. In the southern part of the zone it contains glauconite and carbonaceous detritus. The contained heavy minerals include ilmenite, amphiboles, garnets, and zircon (Table 4).

Fine-grained sediments accumulate in the deep sedimentation zone. The upper accumulation boundary of these sediments almost coincides with the 60 m isobath. These sediments include two granulometric types: fine-grained silt and silty–clayey mud. These sediments are of similar physical properties, contain large amount of organic material and accumulate in close proximity to each other. Yet their mineralogical composition is different. The fine-grained silty mud is predominated by potassium feldspars and contains considerable amounts of clay minerals (mica). It contains the same heavy minerals as the coarse-grained silt. The silty–clayey mud is predominated by clay minerals. It also contains potassium feldspars, mica, glauconite and metalliferous minerals (Table 4).

Table 3. The most widespread minerals in sediments (compiled according to Блажчишин 1976[b], Блажчишин & Усонис 1970, Лукашев 1986).

Sediment fractions	Light minerals	Heavy-accessory minerals
1.0–0.25 mm	Quartz, potassium feldspars, clastic material	Amphiboles (hornblende), pyroxenes, phosphates, garnets, mica (biotite), (limonite).
0.25–0.1 mm	Quartz, potassium feldspars, plagioclase, carbonaceous clastic material (detritus), glauconite	Ilmenite, garnets, amphiboles, limonite, zircon, glauconite.
0.1–0.05 mm	Quartz, potassium feldspars, carbonaceous clastic material (detritus), glauconite, mica.	Ilmenite, limonite, hematite, magnetite, amphiboles, pyroxenes, garnets, zircon, rutile, tourmaline.
0.05–0.01 mm	Potassium feldspars, quartz, mica.	Mica, metalliferous minerals, amphiboles, zircon
0.25–0.1 mm (in muds)	Mica, quartz, potassium feldspars.	Mica, amphiboles, metalliferous minerals, pyroxene.

Note: “Light glauconite” – polytypical crystalline modification of 1Md hydromica, “heavy glauconite” – polytypical crystalline modification of 1M hydromica.

The mineral composition of sediment fraction 0.1–0.05 mm is best investigated. Based on these investigations it was determined that the sediments of the study area belong to the Sambian–Vistula geological province (Блажчишин & Усонис 1970). The terrigenous material abraded from the coast and submarine slope of Sambian Peninsula played an important role in the formation of the mineral complex of sediments. This is why the studied sediments contain greater amounts of ilmenite, amphiboles, glauconite, and phosphates (especially in the southern part of the area).

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2.1.2 Meteorology

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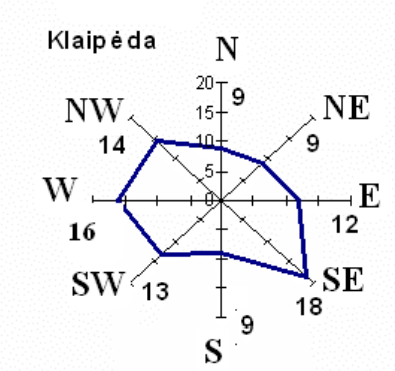
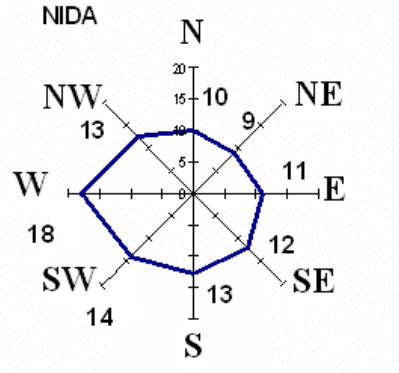
In the southernmost part of the Baltic, the climatic conditions are closer to those over the North Sea, whereas towards the north and east the climatic conditions have a more continental character. The surface layer temperature over the Baltic Sea has thus a considerable amplitude. The horizontal variability in the air temperature above the sea in the summer months lies within 2°C over the entire Baltic Sea, while during the winter months the mean temperature deviates by more than 10°C. The range of variability in the southern Baltic is about 17 °C and in the north up to 27°C. The average monthly air temperatures range from -9°C in winter to 22°C in summer. Monthly rainfall varies between 41–78 mm.

South-eastern wind and the wind from western directions is dominant in the Lithuanian coastal zone in which conditions the water mass is moving in the south-eastern part of the Baltic Sea along the shore from the south to the north. The speed of wind gusts in the Lithuanian (near Nida) seaside reaches and exceeds: Ø ≥15 m/s in average during 67 days per year and, Ø ≥20 m/s in15 days. Wind directions in the Lithuanian coast comprise of strong winds of southwestern, western and southern directions, 38% near Klaipeda and 45% near Nida, located on the Curonian Spit.

More details are discussed in Chapter 4, Modelling of an oil drift.



Wind direction (%) near **Klaipeda** 1994-2004.



Wind direction (%) near **Nida** 1994-2004.

2.2 Long term data on biological, chemical and physical determinants in 1995–2005

A marked of the project consists of the compilation and analysis of 10-years data (1995–2004) data collected by CMR for the HELCOM monitoring purposes (see Appendix 1). In this section (2.2), results of this analysis are presented. Also, according to the project plan (Appendix 1) in connection with the autumn monitoring cruise in 2005 (8–9 November 2005) samples for ecotoxicological studies were collected. For this reason results originating from the November cruise are processed separately in the section 2.4 for hydrography, hydrochemistry and biology and in Chapter 3 for harmful substances and biological effects studies. Methods used in the analyses are presented in Appendix 3.

As described earlier, Lithuanian waters are divided into three categories according to bottom stratum and type of the water body. Data is based on measurements performed on four annual HELCOM COMBINE monitoring cruises. Standard monitoring stations for hydrographical, hydrochemical and biological studies were Stations 4, 6, N-9 (=7), 65 and N-2 (=6B). For this project, number of stations was increased to 12, the added stations being N-1, N-3, N-4, N-5, N-6, N-7 and N-8. The stations are located in the different zones as follows:

- transitional zone – influence area of the Curonian Lagoon (depth 16 m, Station 4)
- coastal waters area with depths 13-16 m (Stations 6, N-5, N-7, N-9)
- coastal waters area with depths 36-37 m (Stations N-4, N-6, N-8)
- open sea area (depths 47, 66 & 42 m, Stations 65, N-2, N-3)
- deep open sea region (depth 70 m, Station N-1).

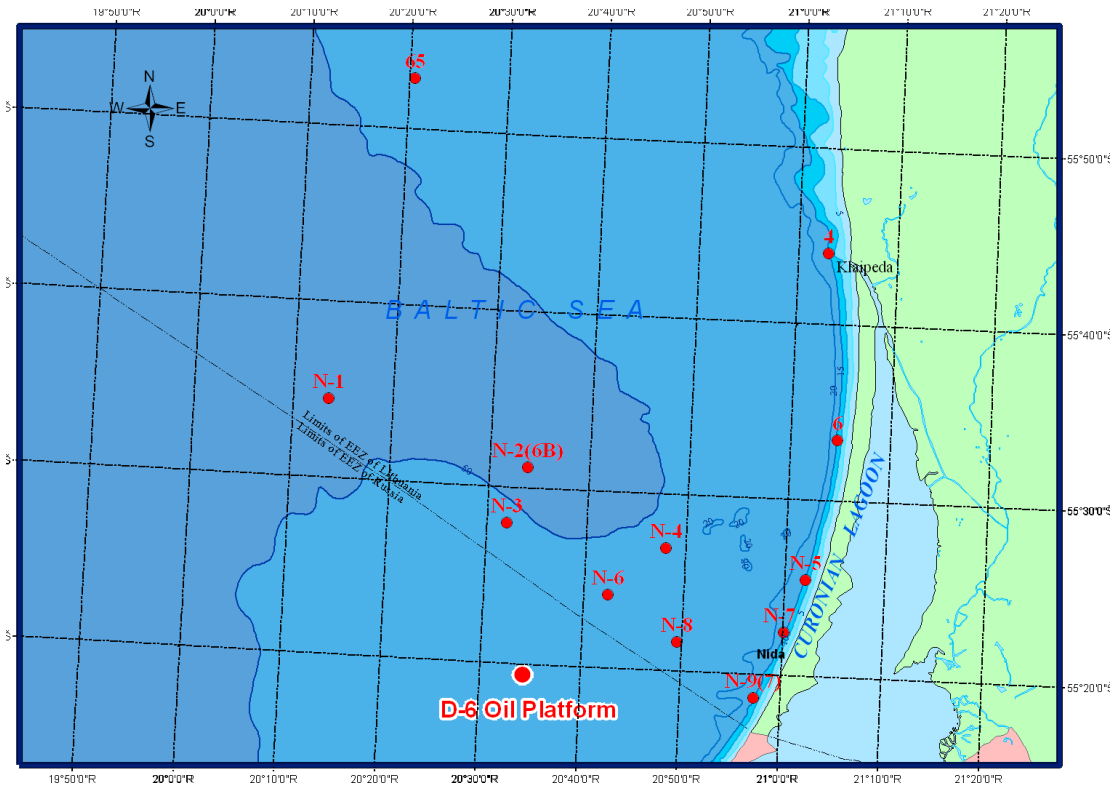


Figure 7. Map and sampling stations in the study region.

Table 4. Sampling stations in 2005. 10-years data (1995–2004) were collected from the stations marked in bold. Regular sampling at the other stations has started later in the 2000s.

Station code	Latitude	Longitude	Depth (m)	Sediment type
N-1	55°34.5	20°13.5	70	Silty mud, fine grained
N-2 (6B)	55°32.2	20°33.8	65	Fine grained sand
N-3	55°28.0	20°32.0	42	Coarse grained sand
N-4	55°27.0	20°48.0	33	Coarse grained sand
N-5	55°25.5	21°02.1	13	Fine grained sand
N-6	55°24.3	20°42.4	36	Fine grained sand
N-7	55°22.5	21°00.1	15	Fine grained sand
N-8	55°21.7	20°49.5	37	Medium grained sand
N-9 (7)	55°18.7	20°57.4	14	Fine grained sand
4	55°44.1	21°03.0	16	Fine grained sand
6	55°33.5	21°04.7	13	Fine grained sand
65	55°52.9’	20°20.5’	47	Coarse grained silt

In the following sections the Figures and Tables have their own numbering as well as the list of references.

2.2.1 Hydrography

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Salinity

The most significant variations in water salinity are observed in the zone of transitional waters (Station 4). In general, surface laer salinities of 4-6 were found in this area. When the wind was from western and north-western directions the water salinity the 1995-2005 seasonal expeditions reached almost 7, and in the autumn of 2000 even exceeding it (7.11). When a strong water outflow from the Curonian Lagoon prevails for a longer period the salinity reduces to 2–3 and at the time of the spring expedition of 1997 it reached only 1.58 (Fig. 1).

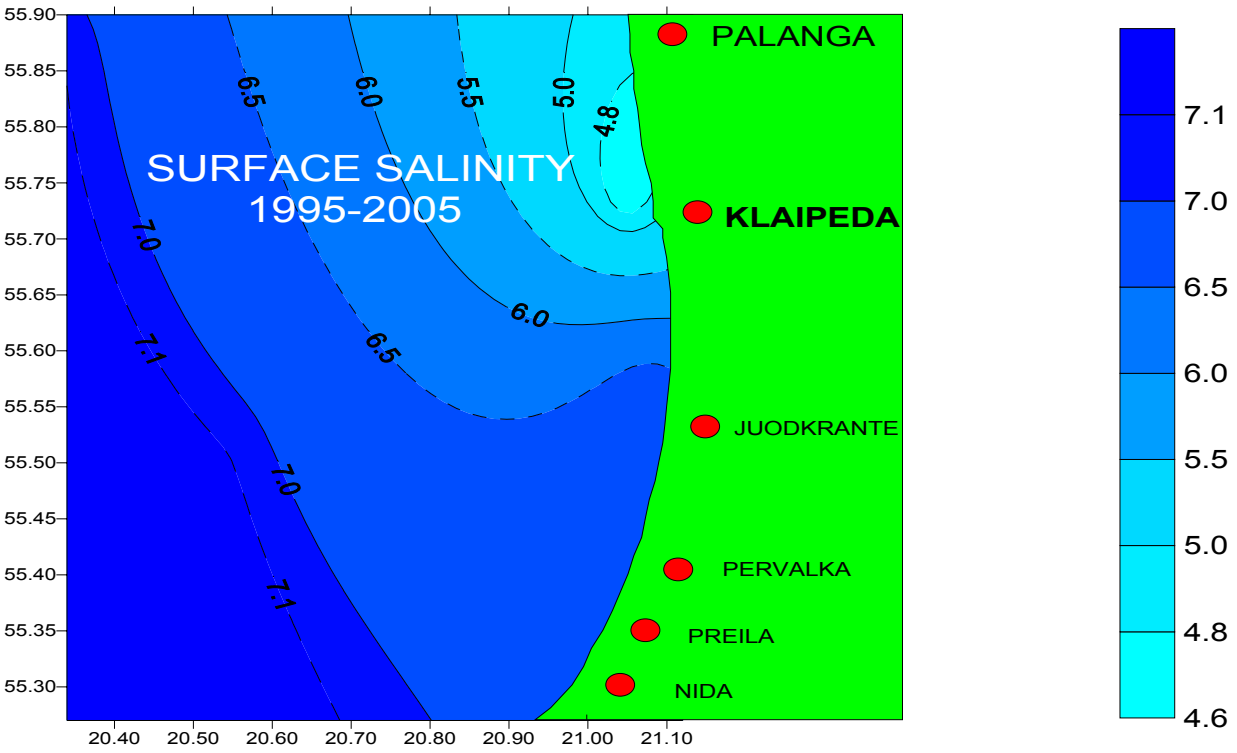


Figure 1. Surface salinity distribution in 1995–2005.

When the depth of the water column in the area increases the bottom water salinity increases as well. The difference in water salinity of the sea surface and near-bottom layers varied from of 0.1 to 5, depending on site. The lowest salinity in the near bottom water during the seasonal expeditions of 1995–2005 was observed in the winter of 1995 (4.06) and highest in winter 2005 (7.27) (Fig. 2).

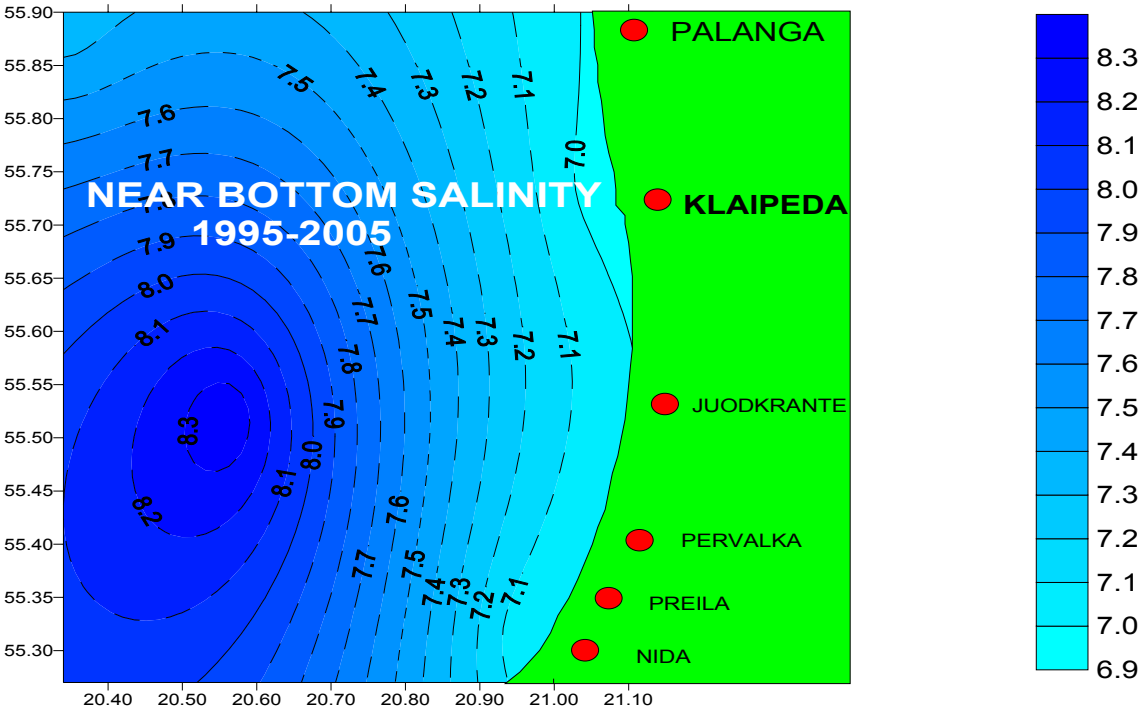


Figure 2. Bottom water salinity distribution in 1995–2005.

The variation in water salinity in the coastal zone water (Stations 6 and 7) is smaller than in transitional waters. The most significant changes are caused by the further spreading of the Curonian Lagoon water transformed in the transitional water zone depending on currents, wind direction, speed and duration. In general, water salinity in the surface layer in this region during the seasonal expeditions of 1995–2005 was between 6 and 7. The lowest salinity (5.86) in the surface layer was measured in spring 1995 and the highest (7.24) in winter 2003.

Salinity stratification at the oceanographic stations is weak with the difference between surface and bottom water salinities of only 0.1. During the seasonal expeditions of 1995–2005 the lowest water salinity in the near-bottom water (6.22) was observed in summer 2001 and the highest, as in transitional water, in winter (7.29).

The impact of the fresh water inflow on the open-sea area (Stations N-2 and 65) was weak. Therefore, the variation in water salinity is insignificant. Water salinity in the surface layer of this area during the period of 1995-2005 fluctuated between 6.62 (spring 1999 and 2002) and 7.37 (winter 2003). In the southern part of the open-sea surface water salinity has a slightly decreasing trend (Station N-2), and in the northern part slightly increasing (Station 65) (Fig. 3).

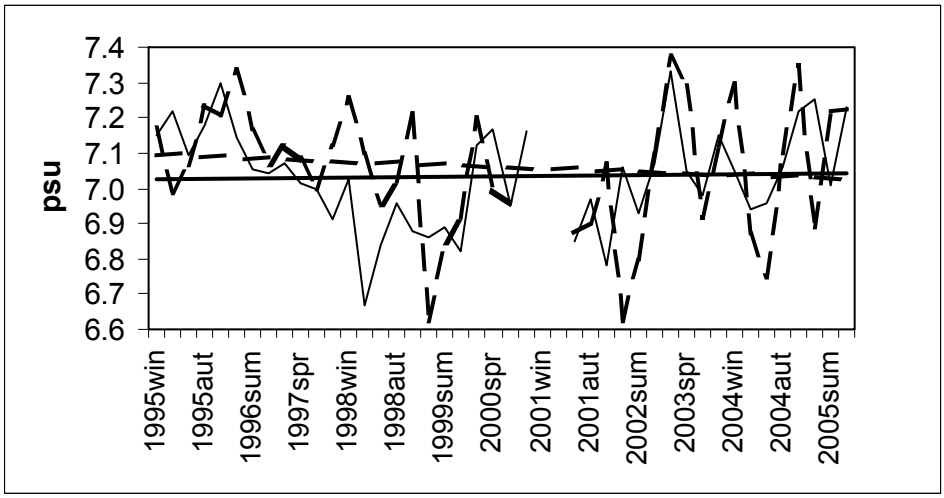


Figure 3. Variation and trends in surface water salinity during 1995-2005 at two open sea stations, Station 65 (broken line) and Station N-2 (solid line).

Salinity slightly increases down to the halocline. The depth of the halocline, which is around 60 meters at this part of the Baltic Sea, the water salinity abruptly increases and exceeds 9 in the near-bottom layer. Because water salinity at the near-bottom water depends on movements of water masses and is characterized by significant changes (from 6.89 to 9.87), there is no point to determine a trend for developments in salinity.

2.2.2 Hydrochemistry 1995–2005

Responsible author: Galina Garnaga (CMR)

A dataset of oxygen and nutrient concentrations in the surface (0–10 m) and bottom water layers for the period of 1995–2005 was taken for this study. The sampling was performed by the Center of Marine Research in the framework of HELCOM COMBINE environmental monitoring programs. These data have been collected from the same stations as the salinity data (*see above*).

Oxygen

Oxygen concentration in the surface water is controlled by fluxes between the sea and atmosphere, by assimilative production and by respiration. Oxygen deficiency causes stress for fish in the open water and animals living on the sea floor at oxygen levels below 3 ml/l. In concentrations < 2 ml/l the situation becomes critical. Oxygen concentration in bottom waters is controlled by vertical mixing, water exchange and oxygen consumption by aquatic organisms. The lowest oxygen concentrations are typically measured at the end of the summer and autumn when decomposition of sinking organic material may use up the oxygen reserve.

Long-term oxygen concentrations in all three areas (open-sea, coastal waters and trasitional waters) are presented in Figures 1 and 2. No noteworthy changes in the surface water oxygen concentration can be observed. Oxygen concentrations (above 12 ml/l) in surface waters of the coastal zone and the open-sea areas were found in spring 2000 when in April an intensive photosynthesis developed due to high air temperature and low water temperature. This may be interpreted as a result of increasing phytoplankton production but could also be due to meteorological or hydrographic changes. In 2001 lower oxygen concentrations were observed in the surface and near-bottom layers in the open sea because the studies were performed only in summer and autumn. In winter and spring the oxygen conditions were good in all three areas.

Oxygen concentrations in the near bottom layer of the open-sea area decreased during 2003. Reduced values (below 3 ml/l) were observed only in the near-bottom layer of the open-sea (>60 m depth) during autumns of 2003–2005.

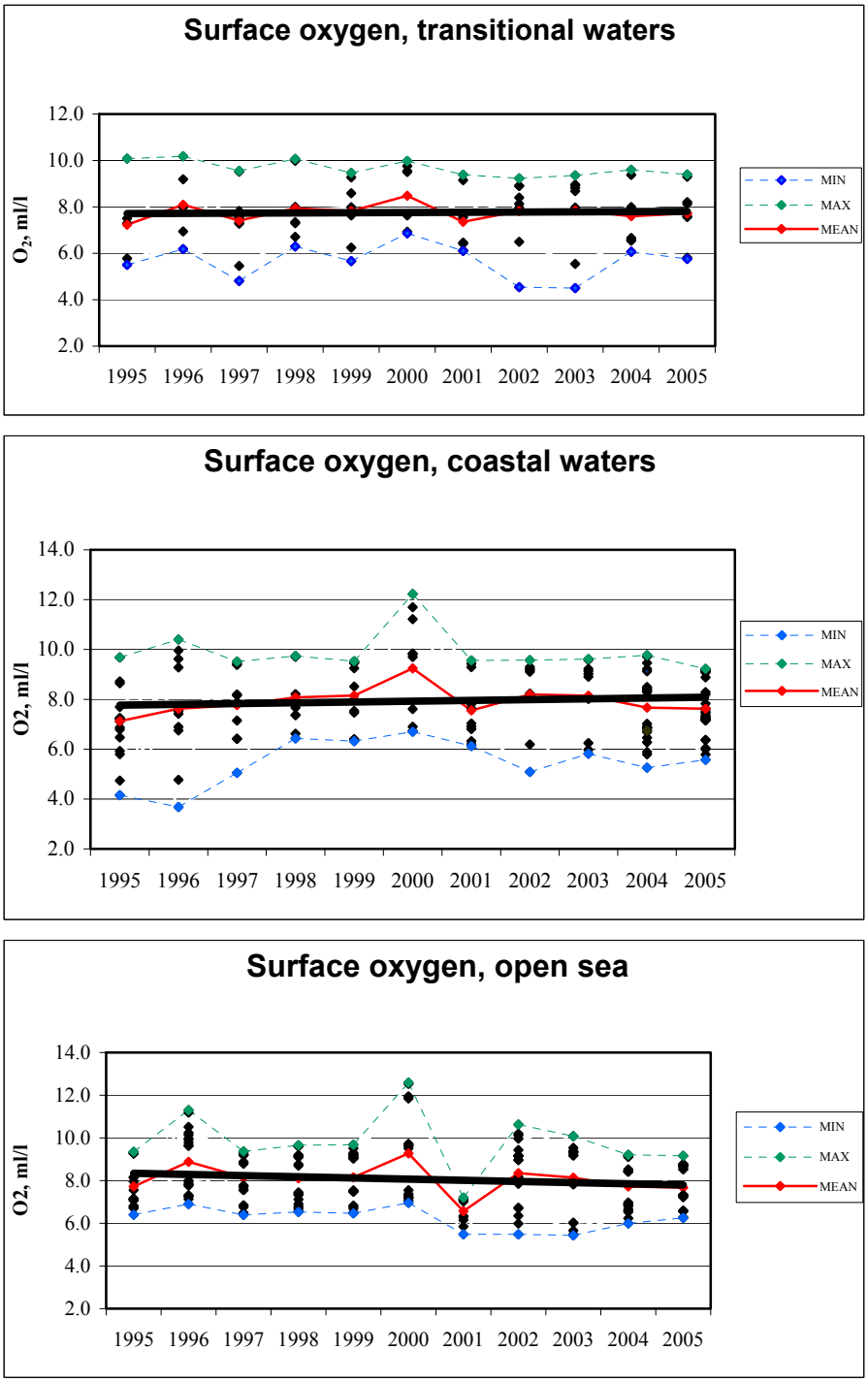


Figure 1. Oxygen concentrations in the surface water (0–10 m depth) in the study area during 1995–2005. The red line represents the annual mean. The green line represents the maximum values and the blue line the minimum values. The black solid line shows the linear trend, which can be considered as indicative development of the oxygen levels in the area.

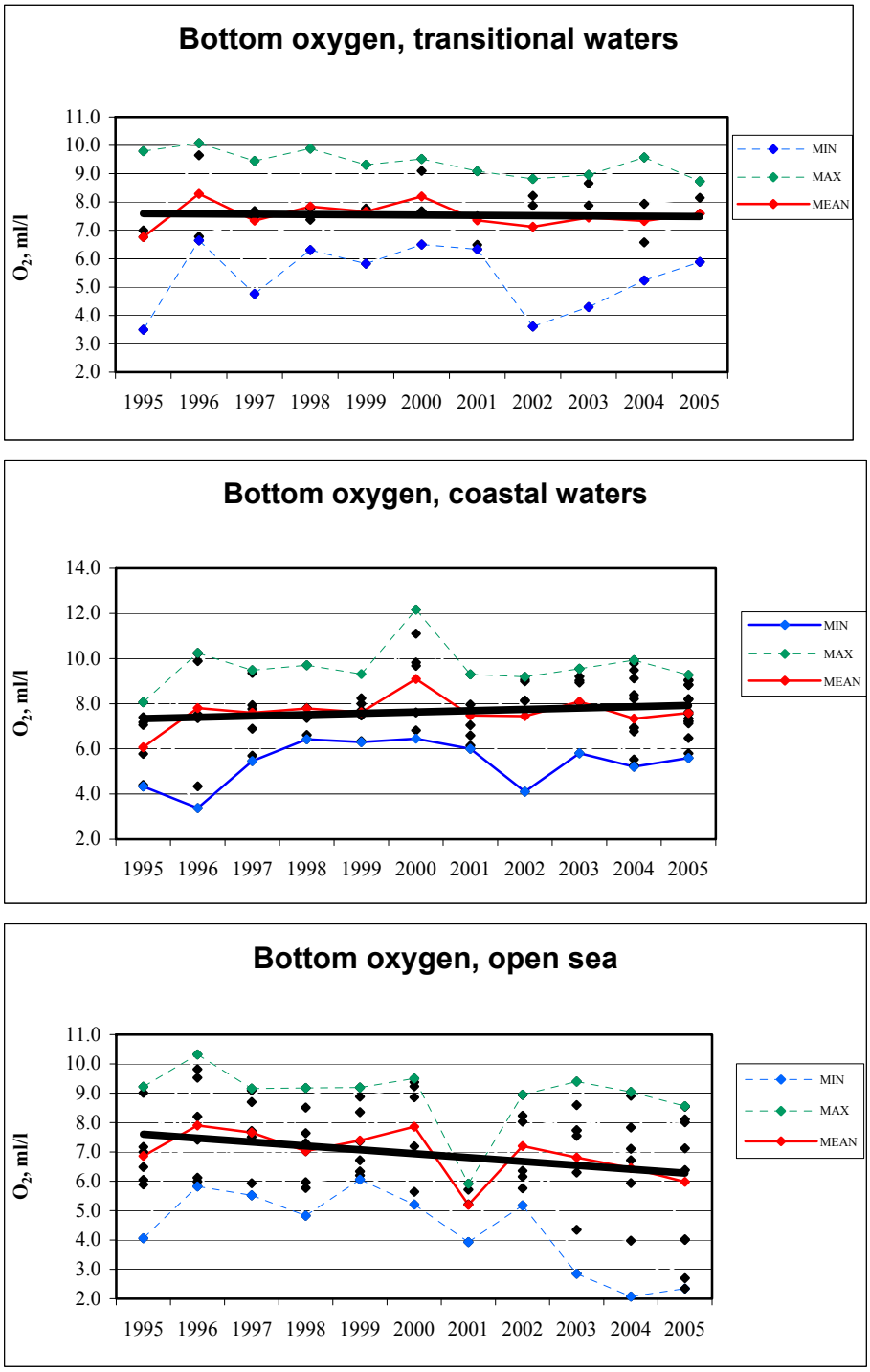


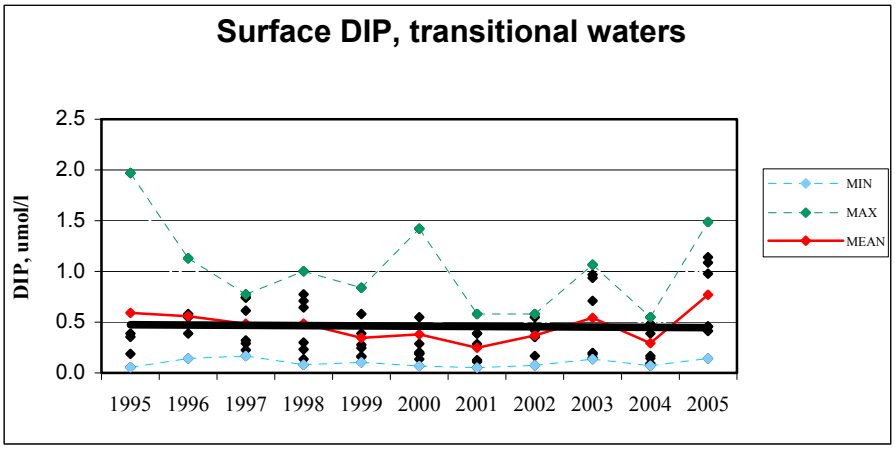
Figure 2. Oxygen concentrations in the near-bottom waters layers in the study areas in 1995–2005. The red line represents the annual mean. The green line represent the maximum values and the blue line the minimum values. The black solid line shows the linear trend, which can be considered as indicative of the long-term development of the oxygen levels in the area.

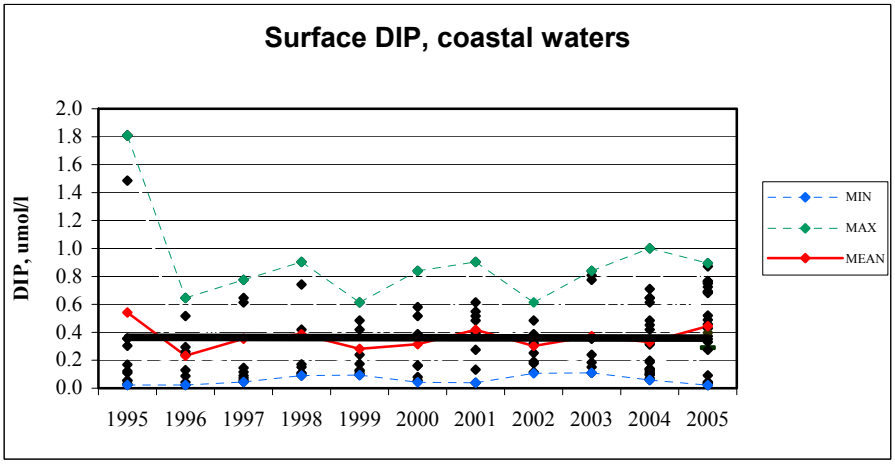
Nutrients

A dataset of nutrient concentrations in the surface and near-bottom water layers for the period of 1995–2005 was taken for this study.

The maximum concentrations were found in winter. Nutrient concentrations decreased from the transitional water towards the open sea. In the transitional zone the values were at elevated level due to the inputs from the Curonian Lagoon.

Long-term dissolved inorganic phosphorus (DIP) values in all three areas are presented in Figures 3 and 4. The increasing trend of DIP has been observed in the surface waters of the open sea since 2002 and near-bottom waters since 2003. The mean concentration of DIP in the surface waters of the open sea for the period 1995–2005 is 0.28 μmol/l, in coastal waters 0.36 μmol/l and in transitional waters 0.46 μmol/l. The mean concentrations of DIP in the near-bottom layer waters were 0.67 μmol/l (open-sea area) and 0.43 μmol/l c(oastal and transitional waters).





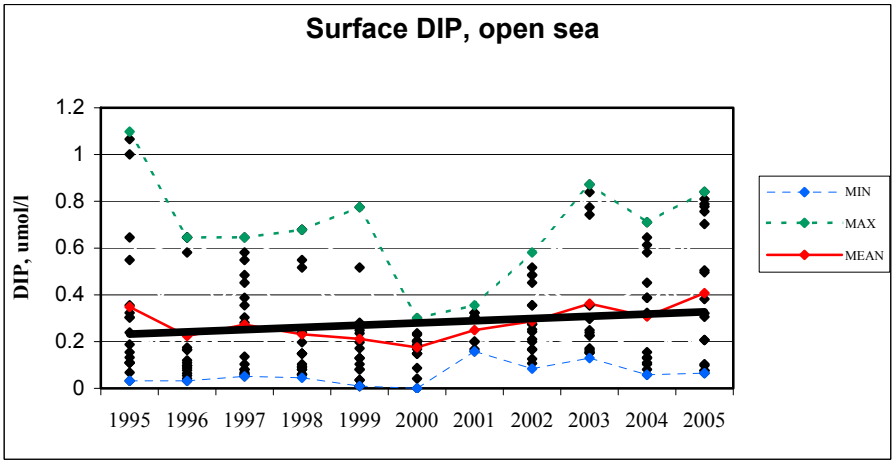


Figure 3. Dissolved inorganic phosphorus (DIP) concentrations in the surface water (0–10 m) in the study areas, 1995–2005. The red line represents the annual mean. The green line represent the maximum values and the blue line the minimum values. The black solid line shows the linear trend, which can be considered as indicative of the development of DIP concentrations in the area.

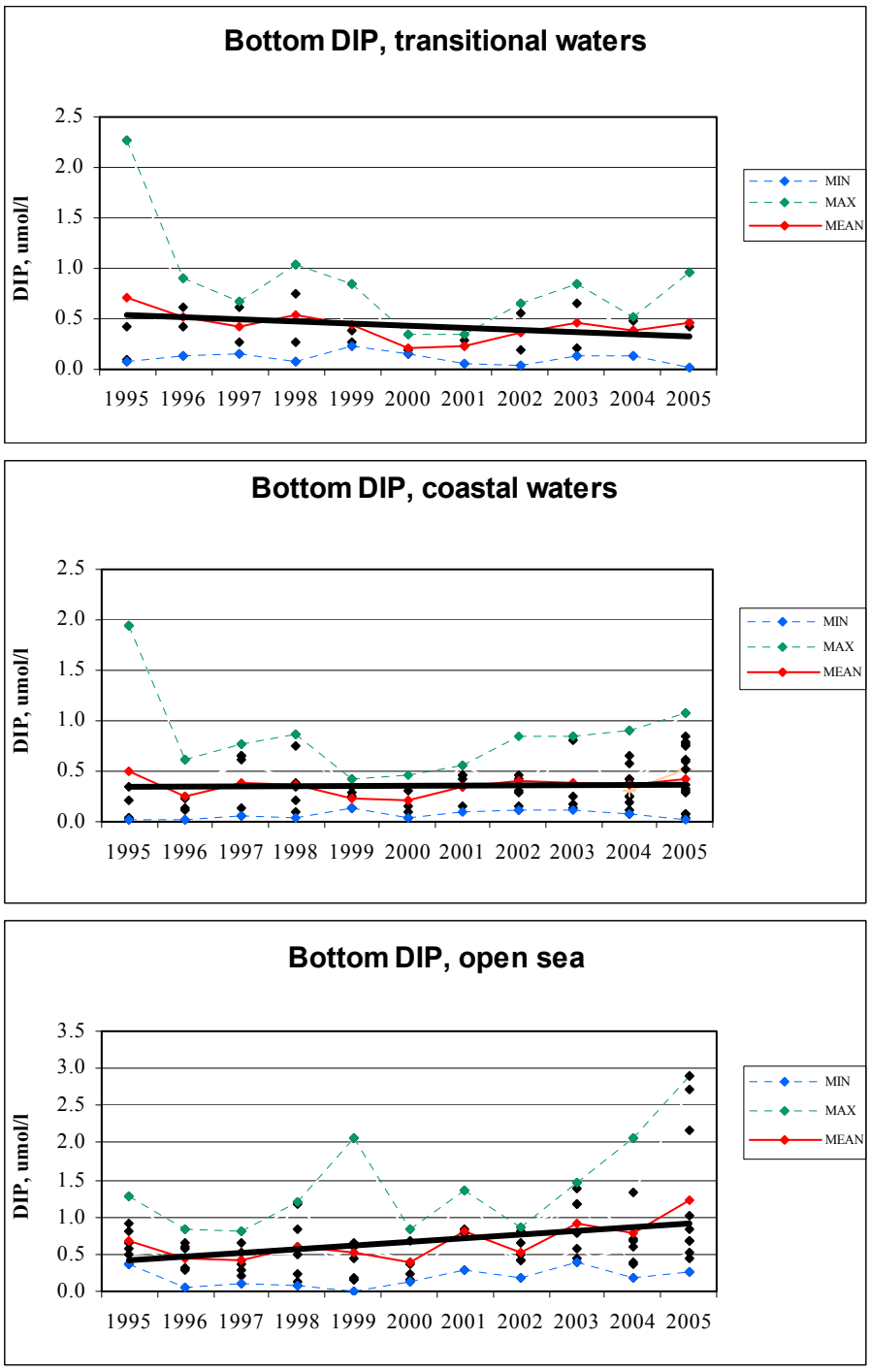


Figure 4. Dissolved inorganic phosphorus (DIP) concentrations in the near-bottom waters layers in the study areas in 1995–2005. The red line represents the annual mean. The green line represent the maximum values and the blue line one the minimum values. The black solid line shows the linear trend, which can be considered as indicative of the development of DIP concentrations in the area.

Long-term total phosphorus (TP) values in all three areas are presented in Figures 5 and 6. An increasing level of TP is determined in the near-bottom layer of the open-sea area. The mean concentration of TP in the surface waters of the open-sea area for the period 1995–2005 is 0.81 µmol/l, 0.87 µmol/l for coastal waters and 1.19 µmol/l for transitional waters. The mean concentration of TP in the near-bottom layer waters for that period was 1.08 µmol/l for the open sea, 0.86 µmol/l for coastal waters and

1.07 µmol/l for transitional waters. The mean concentration of TP varies between 0.9 and 1.2 µmol/l. The reasons for the near-bottom layer water P distribution are obvious: the main process transferring nutrients from the euphotic surface layer to the aphotic near-bottom layer is the sedimentation of plankton detritus. Further analysis of the data on oxygen and phosphorus concentrations in deep waters shows that the high phosphorus value has nearly always been connected to low oxygen values in the bottom layer.

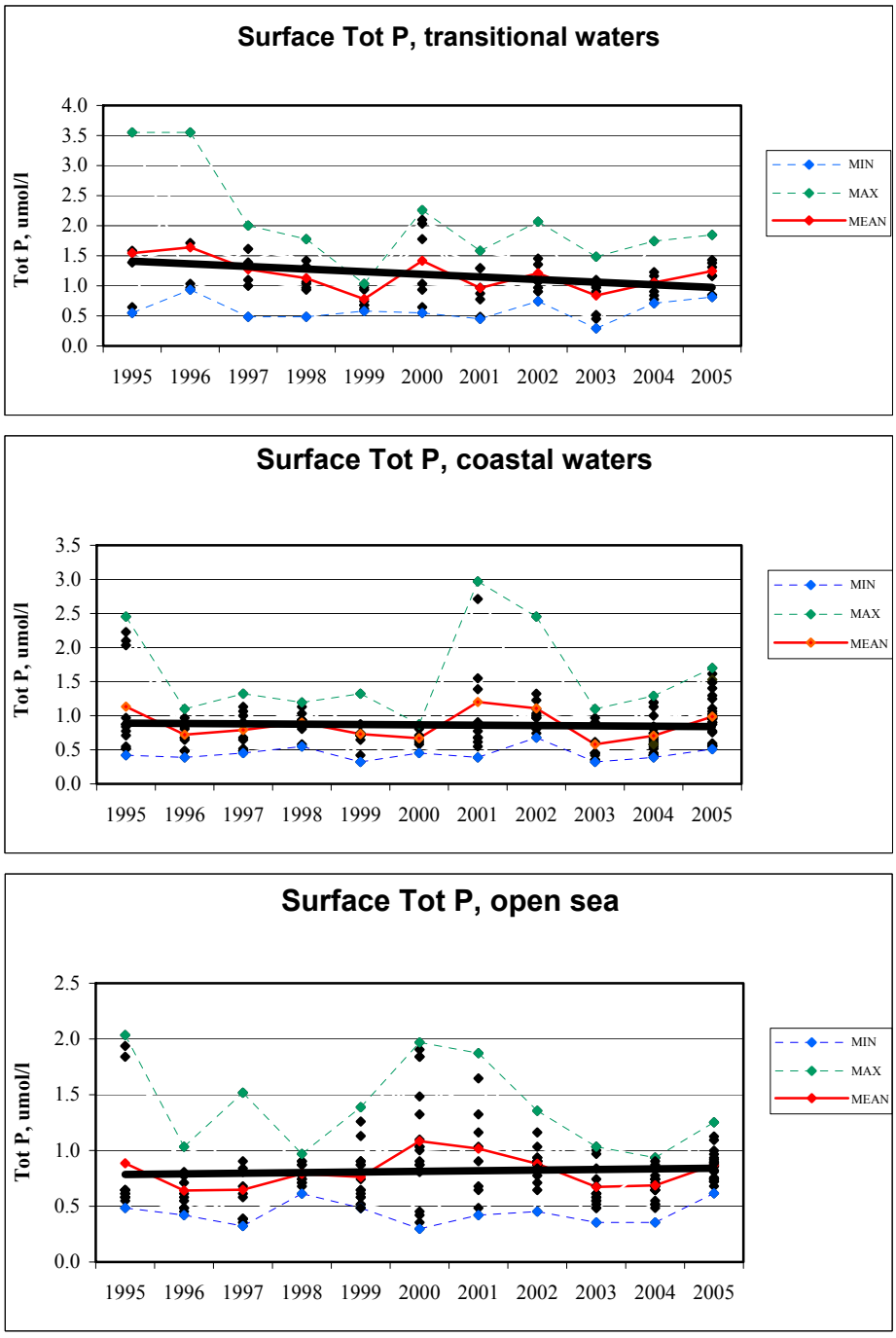
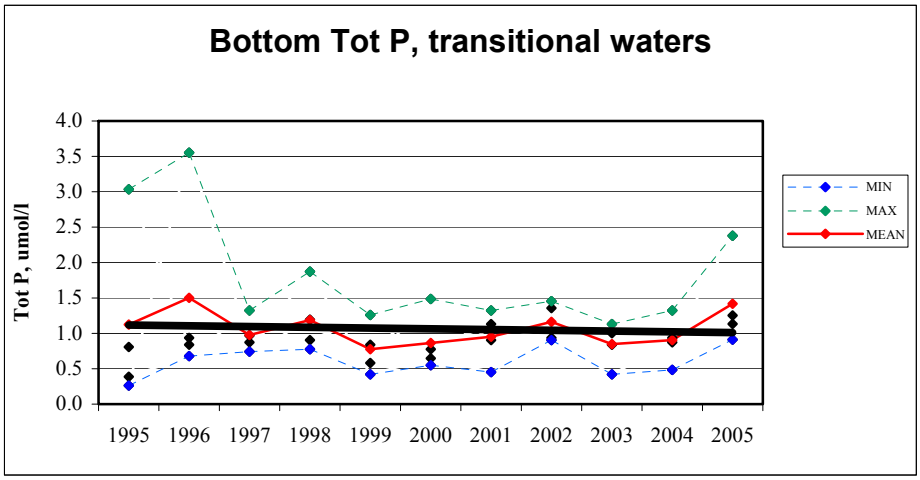
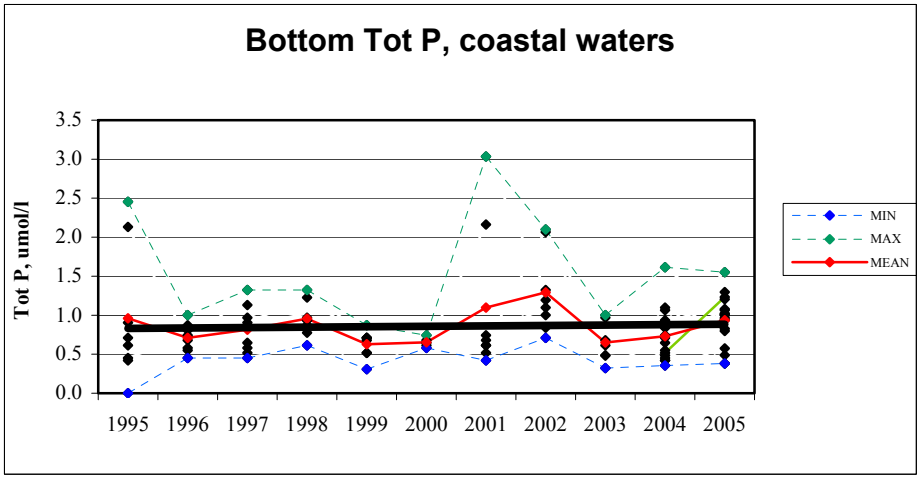


Figure 5. Total phosphorus (TP) concentrations in the surface (0–10 m depth) water in the study areas in 1995–2005. The red line is the annual mean. The green line represent the maximum values and the blue the minimum values. The black solid line shows the linear trend, which can be considered as indicative of the development of TP concentrations in the area.





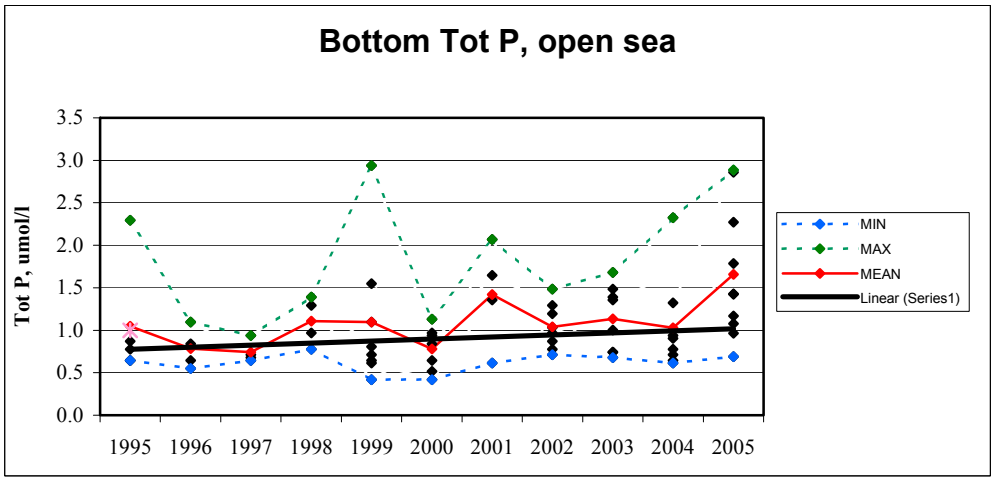


Figure 6. Total phosphorus (TP) concentrations in the near bottom water in the study areas in 1995–2005. The red line represent the annual mean. The green line represent the maximum values and the blue line the minimum values. The black solid line shows the linear trend, which can be considered as indicative of the development of TP concentrations in the area.

Long-term dissolved inorganic nitrogen (DIN) values in all three areas are presented in Figures 7 and 8. The concentration of DIN was calculated as the sum of ammonia, nitrite and nitrate. The concentrations of DIN fluctuate strongly at a high level but without significant trends. The concentrations of DIN have increased in the surface transitional waters and bottom water of the open sea. In surface water layers they are highest during the winter when no primary production can incorporate them into organic

matter The mean concentration of DIN in the surface waters of the open-sea for the period 1995–2005 is 4.9 µmol/l, in coastal waters 7.0 µmol/l and in transitional waters 13.5 µmol/l. Mean concentrations of DIN in the near-bottom layer waters for the period was 6.7 µmol/l for the open-sea area, 7.6 µmol/l for the coastal waters and 10.0 µmol/l for the transitional waters.

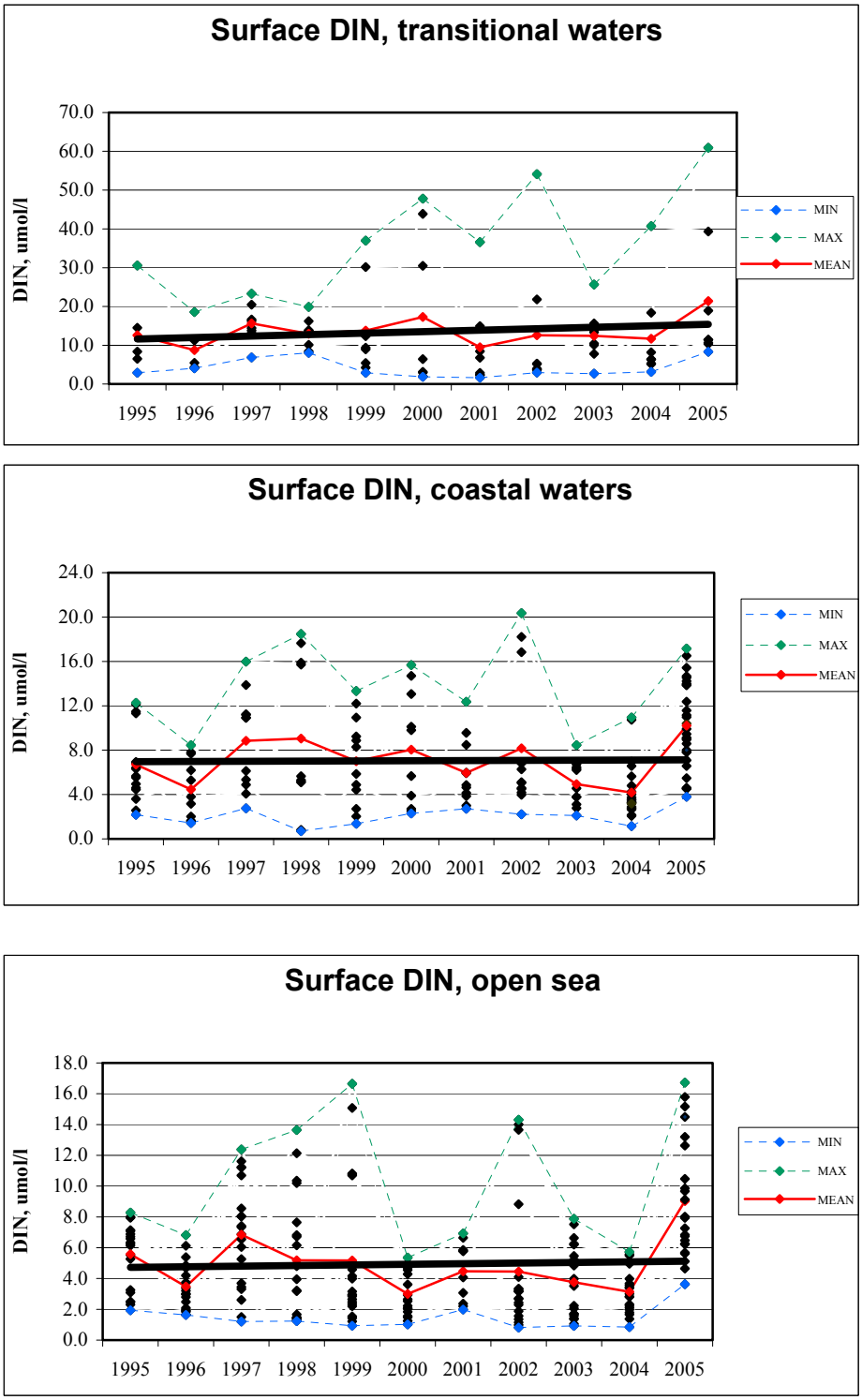


Figure 7. Dissolved inorganic nitrogen (DIN) in the surface water layer (0–10 m depth) in the study areas, 1995–2005. The red line represents the annual mean. The green line represent the maximum values and the blue line the minimum values. The black solid line shows the linear trend, which can be considered as indicative of the development of DIN concentrations in the area.

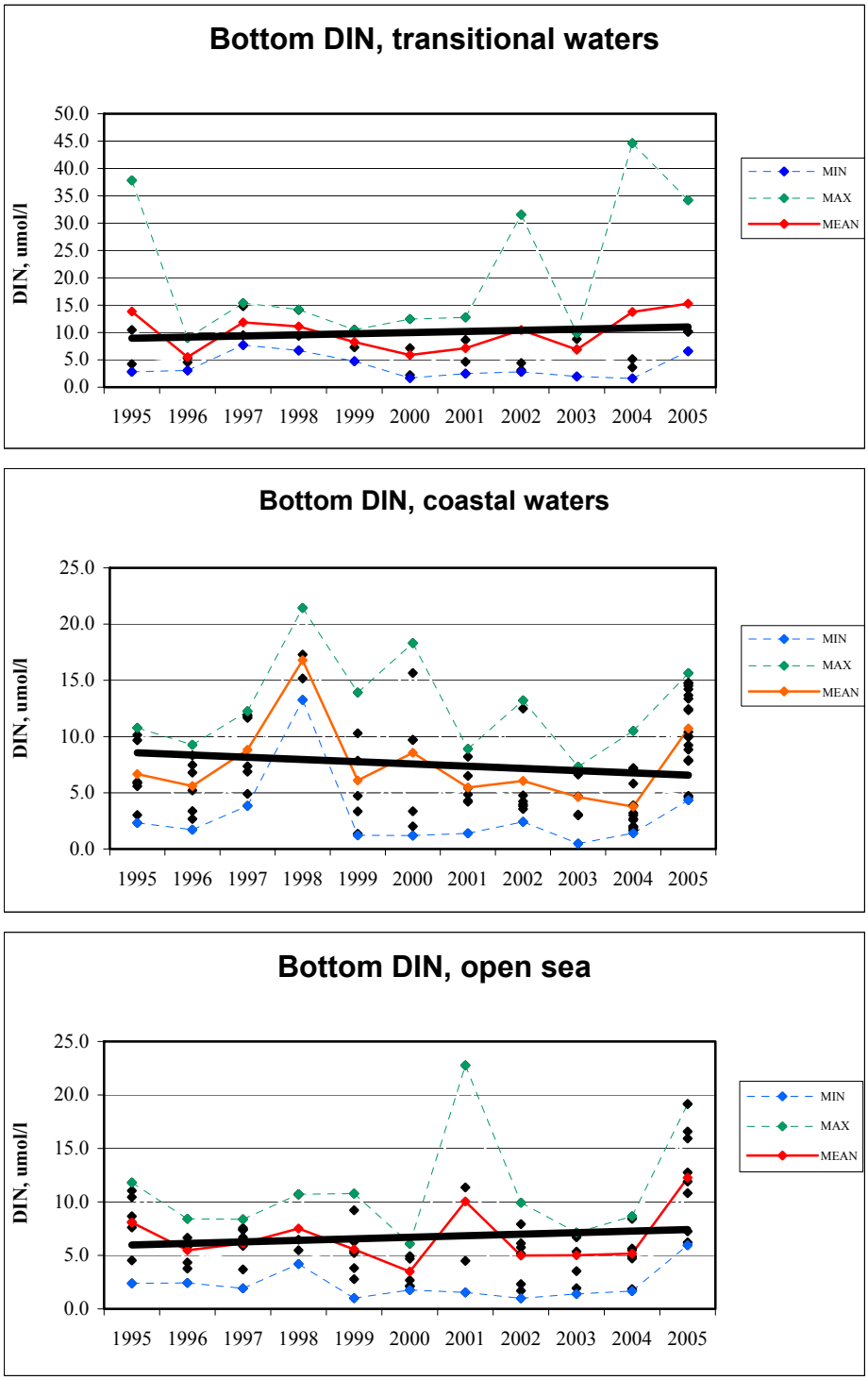
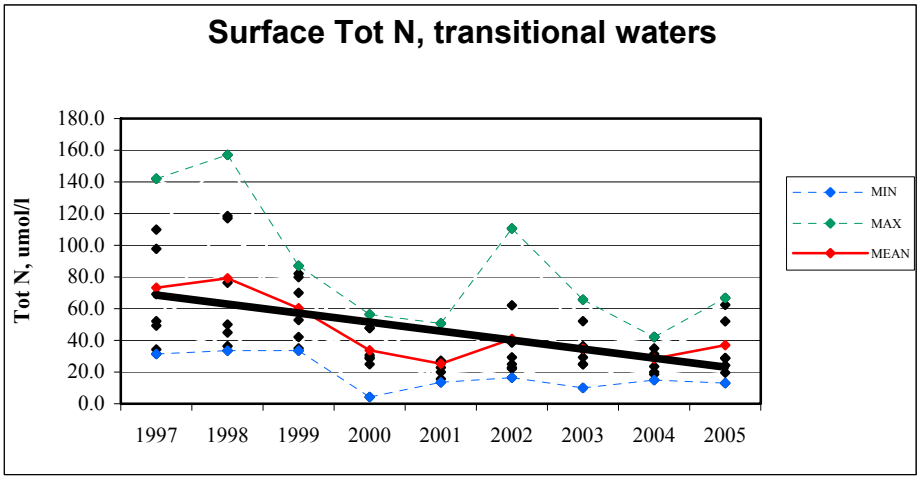
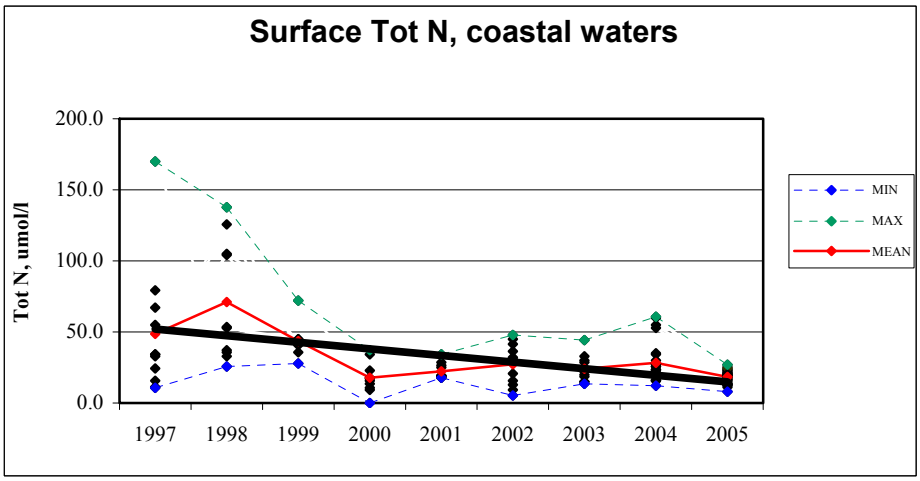


Figure 8. Dissolved inorganic nitrogen (DIN) in the near-bottom waters layers in the study area in 1995–2005. The red line represents the annual mean. The green line represent the maximum values and the blue line the minimum values. The black solid line shows the linear trend, which can be considered as indicative of the development of DIN concentrations in the area.

Long-term total nitrogen (TN) values in all three areas are presented in Figures 9 and 10. TN showed a long-term decrease in all areas. The mean concentration of TN in the surface waters of the open sea for the period 1995–2005 was 29 µmol/l, in coastal waters 33 µmol/l and in transitional waters 45 µmol/l. The mean concentration of TN in the near-bottom waters for that period was 26 µmol/l for the open-sea area, 28 µmol/l

for coastal waters and 34 µmol/l for transitional waters. During the period of 1995–2005 the mean concentration of TN varied between 26 and 45 µmol/l.





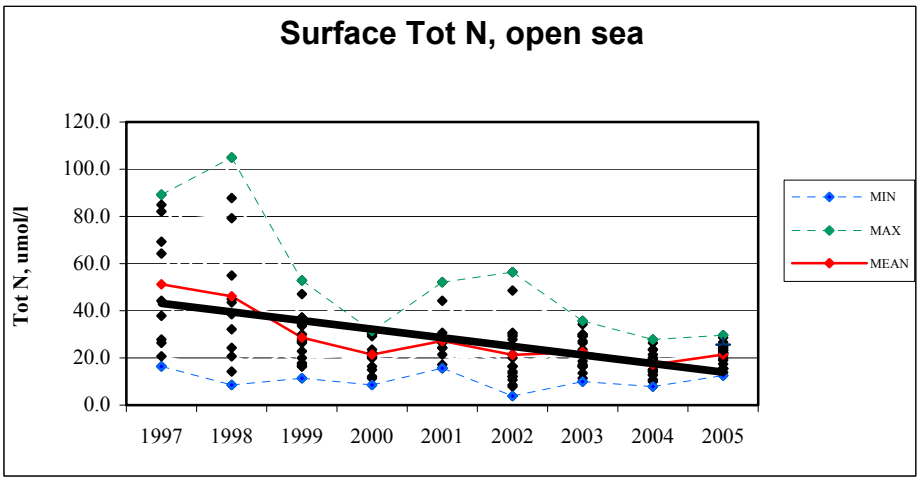


Figure 9. Total nitrogen (TN) contentration in the surface water (0–10 m depth) in the study areas in 1995–2005. The red line represents the annual mean. The green line represent the maximum values and the blue line the minimum values. The black solid line shows the linear trend, which can be considered as indicative of the development of TN concentrations in the area.

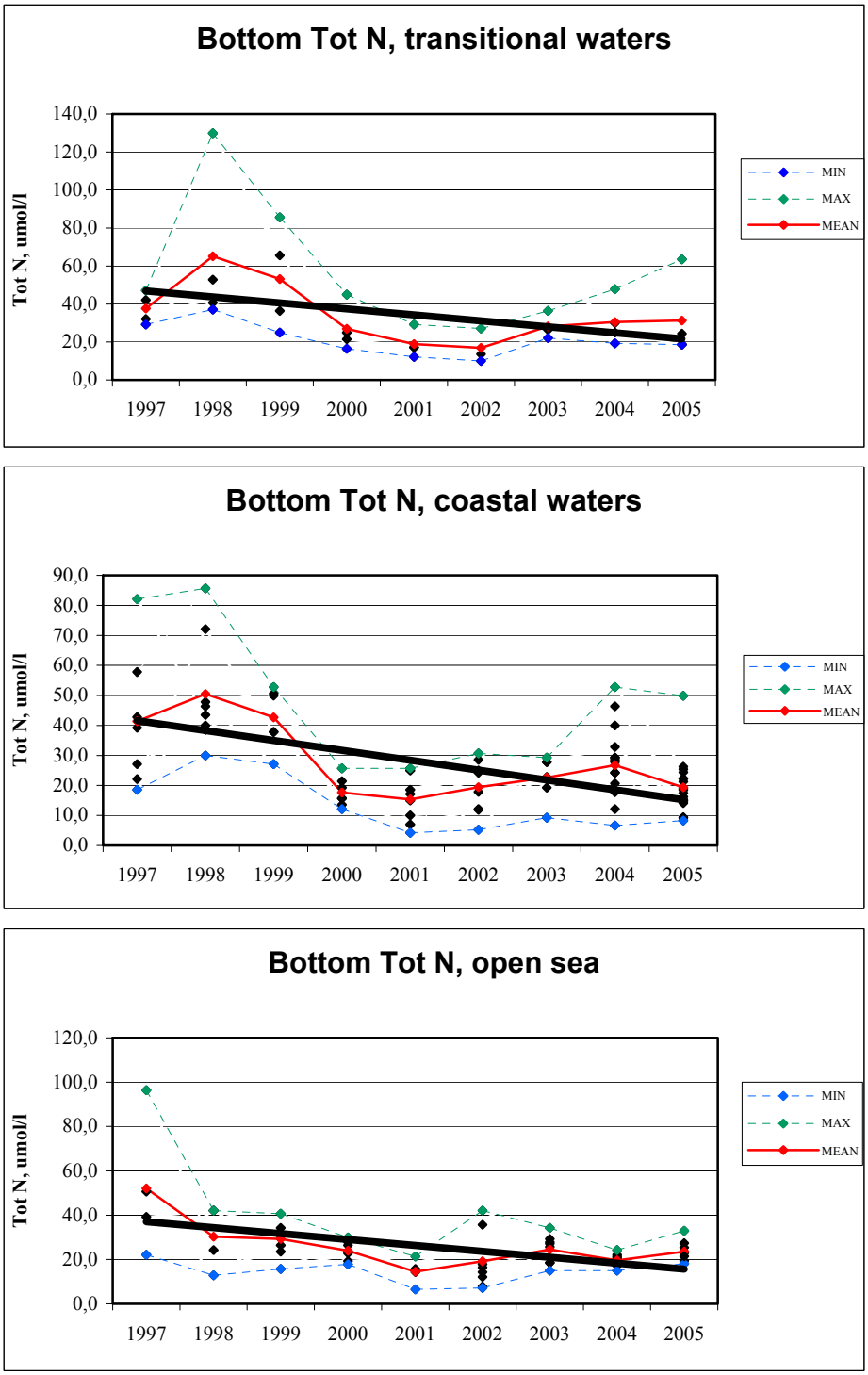


Figure 10. Total nitrogen (TN) contentrations in the near-bottom waters layers in the study areas in 1995–2005. The red line represents the annual mean. The green line represent the maximum values and the blue line the minimum values. The black solid line shows the linear trend, which can be considered as indicative of the development of TN concentrations in the area.

Municipal and industrial waste waters and agriculture are most likely the primary sources for the nutrient increase. In the transitional waters, the values are somewhat higher.

2.2.3 Bacterioplankton 1995–2005

Responsible scientist: Zoja Stukova (CMR)

Anthropogenic activities are often harmful to ecosystems when the natural self-regulation of ecosystem weakens and, consequently, effects of pollution and eutrophication on biological processes intensify.

Microorganisms react fast to changes in environmental conditions. Therefore, they are sensitive indicators of the state of ecosystems. The input of pollutants to the Baltic Sea induces significantly changes the composition of microbiocenosis. Microbiological parameters are regulated by environmental factors, such as water temperature, concentration of organic and inorganic matter, the development of phytoplankton blooms etc. Oil-oxidizing bacteria use dissolved oil hydrocarbons in their metabolic processes. Many of them have constructive enzymatic systems that appear in the association of microorganisms with petroleum hydrocarbons (National Research Council of the National Academies 2003). The capability of oil-oxidizing bacteria to utilize oil hydrocarbons as substrates could be used as a bioindicator of water ecosystem self-purification from oil pollution (Gusev et al. 1985, Platpira 1982).

Monitoring data on bacterial numbers cover the years 1995–2005 for stations 4, N-9 and 65, years 1995–2003 for station 6 and years 2004–2005 for stations N-2 and N-6. Winter values are available since 2000.

Total bacterial numbers (TBN)

The amount of planktonic bacteria in the study area change both in time and space. During the study period, the density of bacterioplankton in this area fluctuated widely from 36,000 cells/ml (winter) to 7.09 x 10^6 cells/ml (summer). The highest concentrations of bacteria are mostly found at station 4 in an area impacted by waters from the Curonian Lagoon (Fig. 1). At the “new” offshore stations N-2 and N-6 the TBN are lower than those recorded in the coastal areas (data not shown).

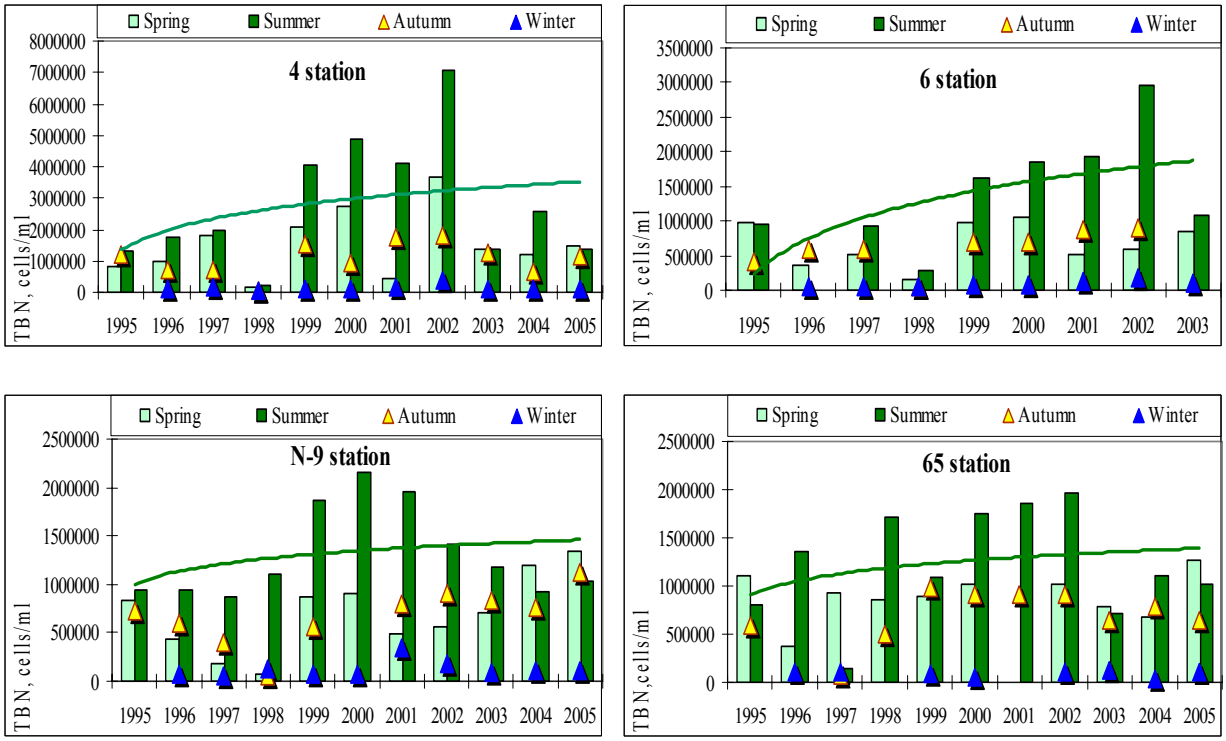


Figure 1. Seasonal distribution of total bacterial numbers (TBN, cells/ml) at the different stations. Station 4, N-9 and 65 (1995–2005), station 6 (1995–2003). Trend lines for summer season are only indicative of the development of the bacterial community.

Saprophytic bacteria (SB)

Saprophytic bacteria (SB) describe the water quality (amount of organic matter saturated in water). The SB in the study area varied from 25 cells/ml to 60,000 cells/ml during the study periods. The highest SB were usually found in the site impacted by waters from the Curonian Lagoon (Station 4) (Fig. 2). At stations N-2 and N-6 the observation period covers only two years. SB at station N-6 was <1000/ml at maximum, the lowest number observed at the study stations. SB at station N-2 is in the same order of magnitude as recorded at the other stations (data not shown). In 2004 spring the SB values at stations N-9 and 65 were higher than recorded previously.

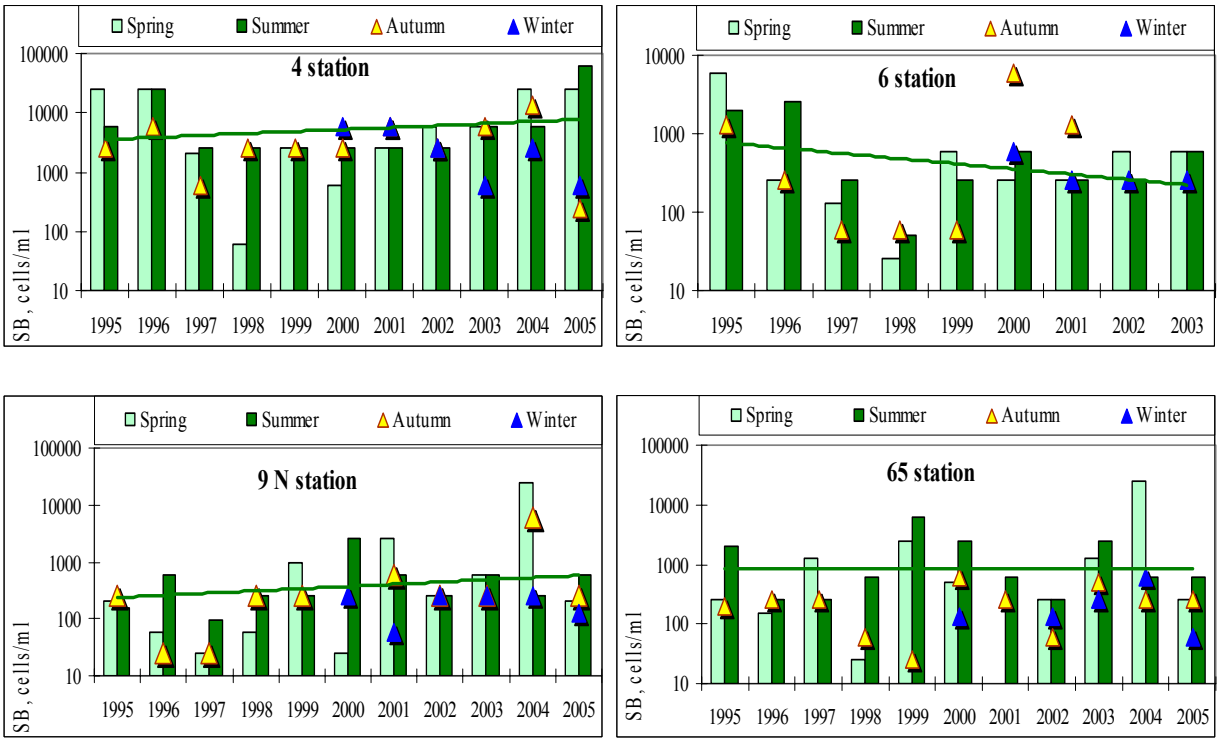


Figure 2. Seasonal distribution of number of saprophytic bacteria (SB) at the different stations. Station 4, N-9 and 65 (1995–2005), station 6 (1995–2003). Winter values were available only from 2000 onwards. Trend lines for summer season are only indicative for the development of the bacterial community.

Oil-oxidizing bacteria (OB)

Oil-oxidizing bacteria (OB) grow in an environment enriched by oil hydrocarbons. OB in the study area varied from 6 cells/ml to 25,000 cells/ml. Results do not show increasing trend in OB in the study region between 1995–2005 in regard to summer values, but in spring 2004 and 2005 higher numbers of OB acan be observed at the coastal stations (Fig. 3).

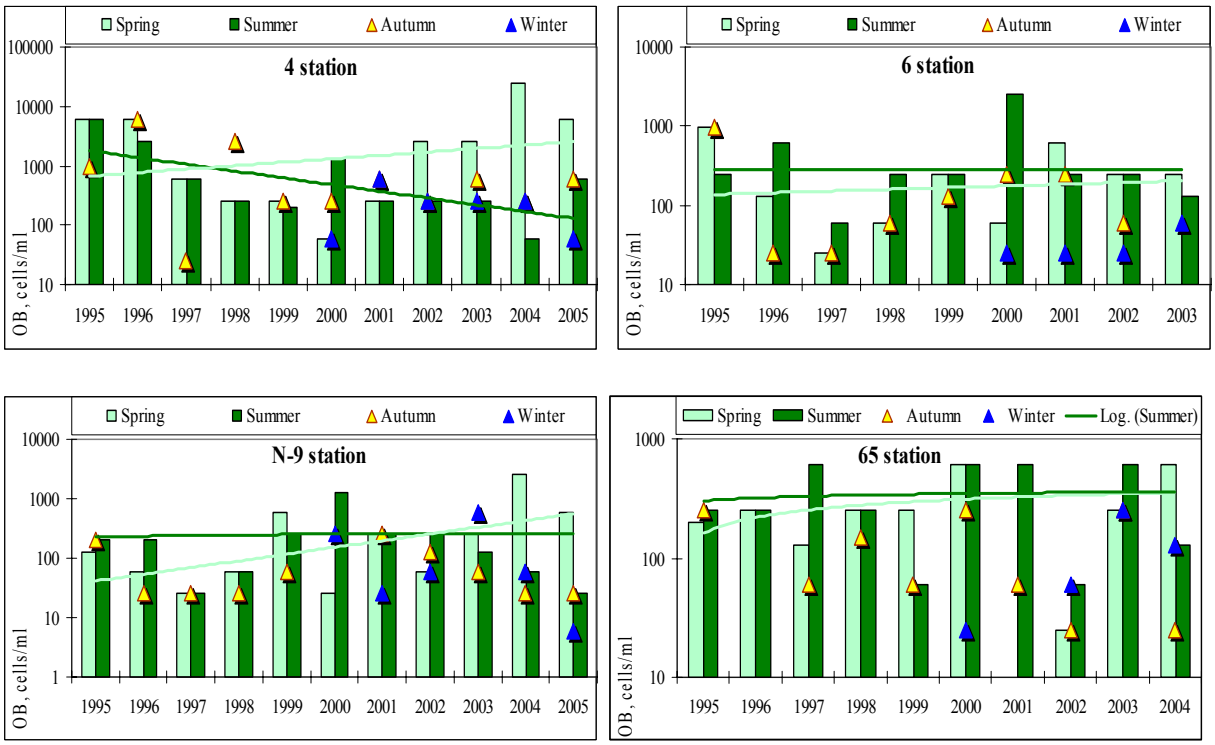


Figure 3. Seasonal distribution of the numbers of oil-oxidizing bacteria (OB, cells/ml) at the different stations: Stations 4 and N-9 (1995–2005), station 65 (1995–2004) and station 6 (1995–2003). Winter values were available only 2000 onwards. Trend lines give only indication for the development of the bacterial community.

Seasonal variation in bacterioplankton

Winter

In winter periods 1995–2005 TBN in study area varied between 0.036–0.409 x 10^6 cells/ml (Fig. 4). The maximum values of TBN occurred in the zone influenced by the Curonian Lagoon (Station 4). The highest TBN were observed in 2002 (Station 4). In winter, SB and OB in the study area varied from tens to hundreds of cells/ml. Temperature, together with the amount of dissolved organic carbon in water, is an important factor regulating TBN and the general intensity of microbial processes. The average water temperature during the study period of 1995-2005 ranged from 1 °C to 5 °C.

Spring

The production period starts in spring following the increase in water temperature by 3–6 °C. TBN increase as well. In May, the TBN increase by 5–6 times compared to winter values. TBN ranged between 0.070 and 3.690 x 10^6 cells/ml during the study period of 1995–2005. The maximum of TBN occurred in 2002 (Station 4). The spring values of SBN and OBN in the study area varied from tens to tens of thousands of cells/ml. The highest SBN occurred in 2005 (Station 4).

Summer

As the season progresses the spring bacterioplankton is replaced by summer forms. As necessary for bacterial cell growth the amount of organic matter is increased. The peak of bacterioplankton development occurred in summer, the TBN being about twice

higher spring periods (Stations 4 and 6) (Fig. 1). The range of TBN was 0.136–7.090 x 10^6 cells/ml. The highest TBN occurred in 2002 in the sea area under the influence of the Curonian Lagoon (Station 4). The abundance of SB and OB in summer was at the same level as during the spring periods from hundreds to thousands of cells/ml (Figs. 5 and 6).

Autumn

From the end of October to the beginning of November an insignificant recession in TBN was found. The level of development of microorganisms is usually higher than in spring and TBN varied between 0.042–1.830 x 10^6 cells/ml.

In autumn SBN and OBN varied from tens to tens of thousands of cells/ml in the study area. The highest SB occurred in 2004 (Station 4). In autumn 2005 the amount of oil-oxidizing bacteria was less than 10,000 cells/ml. The highest concentration of this bacterial group was observed in the zone influenced by waters from the Curonian Lagoon (Station 4).

Conclusions

Results of the microbiological monitoring between 1995–2005 do not show marked changes in the structure of the microbial community. Long-term analysis of the seasonal distribution of bacterioplankton in the different regions discloses some general points: (1) the highest abundance of bacteria is usually observed in the zone under the influence of the plume of the Curonian Lagoon and the lowest at the most remote station from the coast (Station 65), (2) bacterial growth generally reaches maximum during the summer period. However, in the spring periods of 2004 and 2005 saprophytic and oil-oxidizing bacteria at the coastal stations (4 and N-9) reached higher numbers than measured earlier in the same area.

Some increasing trends in TBN can be observed at all the monitored stations that could be interpreted as evidence about the increasing eutrophication going on in the Baltic Sea (Fig. 4).

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2.2.4 Phytoplankton and chlorophyll *a* in 1995–2005

Responsible scientists: Irina Olena and Rima Kavolyte(CMR)

Phytoplankton

The data on phytoplankton are based on only one annual seasonal cruise (spring, summer, autumn, winter). Because of this in some years the spring or summer blooms were missed due to the inadequate sampling frequency and timing of the research cruises. The peak of the bloom was rarely met.

The basic data compilation is summarized in Table 1. As the different stations in the Lithuanian waters are more or less influenced by river water, three groups of stations – open (Stations 65, N-2 and N-6), coastal (Stations 6 and N-9) and plume (Station 4) have been analysed separately.

From the results in different years, a general picture of the seasonal development of phytoplankton in the Lithuanian waters can be drawn.

The lowest phytoplankton abundances and biomasses were found in winter and highest in spring. Low summer biomass values in comparison with summer abundance values might be due to the dominance of small cyanobacterial species from *Aphanocapsa*, *Aphanothece* and *Cyanodictyon* genera. In opposite, the high autumn phytoplankton biomasses versus to autumnal abundances, could be explained by the big size of cells of vegetated species, for example the diatom *Coscinodiscus granii* (Fig. 1).

According to the differences in nutrient concentrations also the amounts of phytoplankton were different in the investigated water areas. The general tendency of decreasing phytoplankton abundance from the plume area (area influenced by freshwaters of the Curonian Lagoon) to the open-sea region is obvious (Table 1). Generally, phytoplankton concentration in the investigated region also depended greatly on water temperature and salinity (Fig. 2).

The higher abundance of phytoplankton in wintertime was connected to the abnormally high water temperature that prevailed for example in 2002. The values of vernal and summer phytoplankton abundances depended on the sampling time. Mostly, if the sampling was performed during blooms, the phytoplankton abundances markedly exceeded the long-term average, and accordingly, if the sampling was performed before or after the blooms, the phytoplankton abundances did not reach the value of the long-term average.

Analysis of the quantitative development of planktonic microalgae in 1995–2005 showed some increasing tendency. In the open-sea area of the Lithuanian waters the summer phytoplankton abundance from 1999 onwards and autumn abundance already from 1995 onwards, greatly exceeded the long-term (1985–2005) seasonal averages. In the coastal area an increasing tendency of phytoplankton abundance in the period of investigation was visible not only in summer and autumn periods, but also in spring. In the plume area the clear increases in phytoplankton abundances was observed only the since year 2000. (Table 1, Fig. 5)

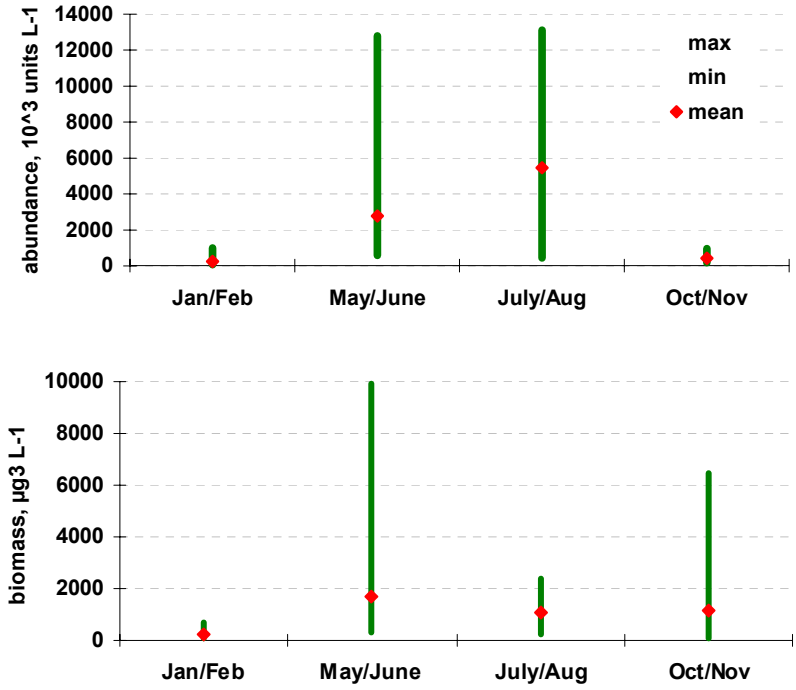

Figure 1. Seasonal dynamics of phytoplankton abundance and biomass in the open-sea area of the Lithuanian waters in 1995–2005.

Table 1. Phytoplankton abundances (10^3 units L^{-1}, mean values for the area) in the different areas of the Lithuanian waters of the Baltic Sea in 1995–2005.

Years	Open area	Coastal area	Plume area
	Winter		
1995	169.9		
1996	73.8		
1997	70.4		
1998	230.3		
1999	92.4		
2000	202.8		
2001	383.7		
2002	991.3		
2003	96.0		
2004	237.5		
2005	129.5		
1984-1994 average	76.8±62.1		
	Spring		
1995	2247.7	2871.9	2973.9
1996	1698.2	914.2	3151.3
1997	2242.3	384.4	1918.0
1998	2193.9	198.4	4387.6
1999	642.5	2089.9	2374.3
2000	2777.9	2598.4	2948.5
2001	1715.4	3398.5	1499.3
2002	4459.1	2937.8	5874.9
2003	1106.6	1241.7	3810.1
2004	2336.9	5146.4	2641.7
2005	250.8	3786.2	5796.3
1984-1994 average	1941.0±2263.1	923.3±1642.81	1852.5±1463.2
	Summer		
1995	2772.5	1660.6	1320.2
1996	1441.3	438.8	1882.4
1997	643.3	1282.7	2841.6
1998	593.4	2507.1	2872.1
1999	3100.4	3368.9	2295.7
2000	7442.9	3372.1	4630.7
2001	2371.8	1101.8	2615.1
2002	8104.3	11397.5	10942.0
2003	1103.9	4299.1	7607.8
2004	11671.9	3832.6	11101.5
2005	3132.2	1769.3	2920.1
1984-1994 average	775.4±602.3	823.6±786.7	2476.8±2601.2
	Autumn		
1995	1002.5	1573.6	1889.1
1996	499.8	580.1	5571.1
1997	162.0	372.4	2118.5
1998	233.9	333.9	406.2
1999	868.5	676.4	1405.2
2000	586.3	928.7	1081.0
2001	1304.8	1402.0	4073.9
2002	1208.9	765.1	4679.2
2003	379.6	893.6	4560.4
2004	406.6	746.1	1482.4
2005	342.7	1404.1	1832.6
1984-1994 average	57.5±43.4	99.4±205.5	525.4±559.8

Open sea area

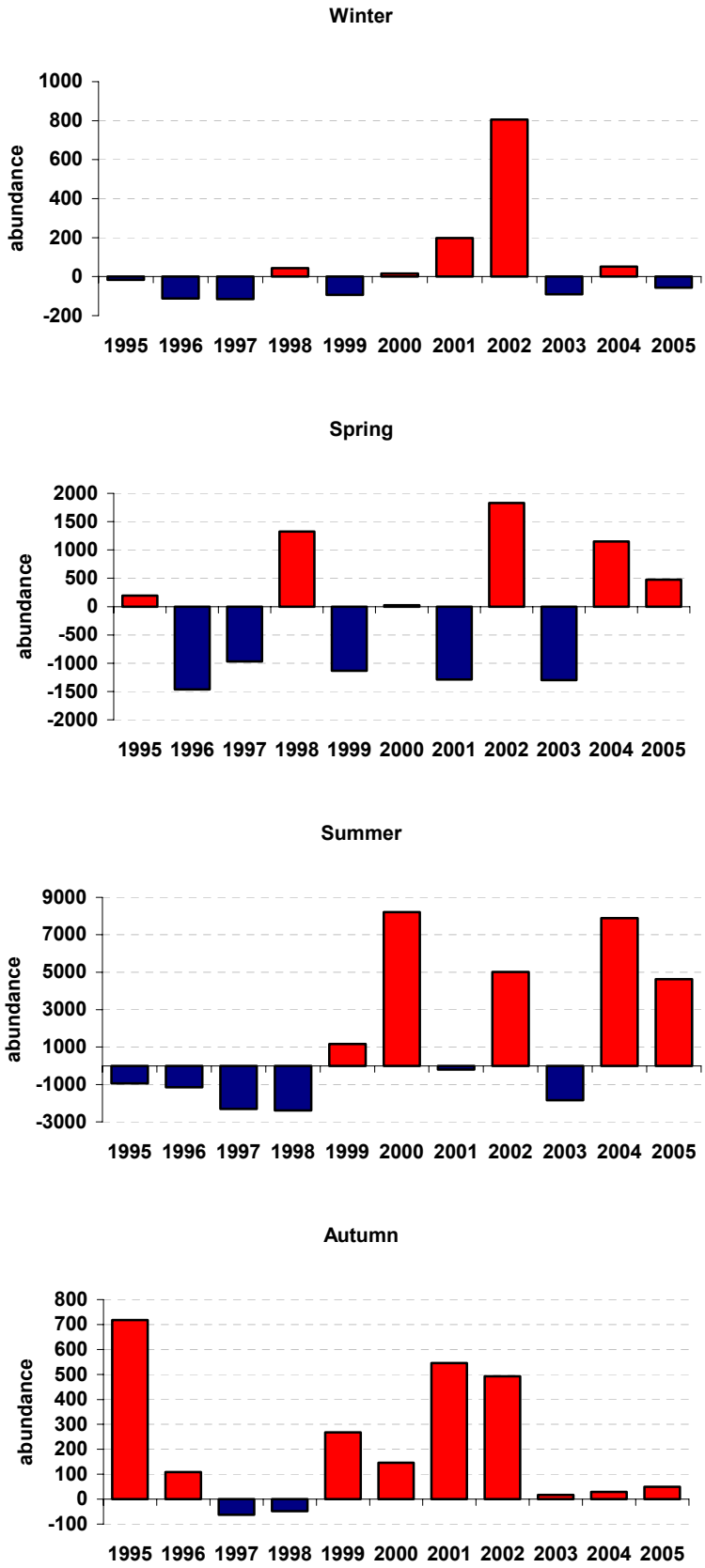

Figure 2. Seasonal 1995–2005 phytoplankton abundances (10^3 units L^{-1}) relative to long-term seasonal average (1984–1994) for the open-sea area of Lithuanian waters of the Baltic Sea. The figures show an increase (red column) or a decrease (blue column) in phytoplankton abundance relative to the long-term average.

Coastal zone

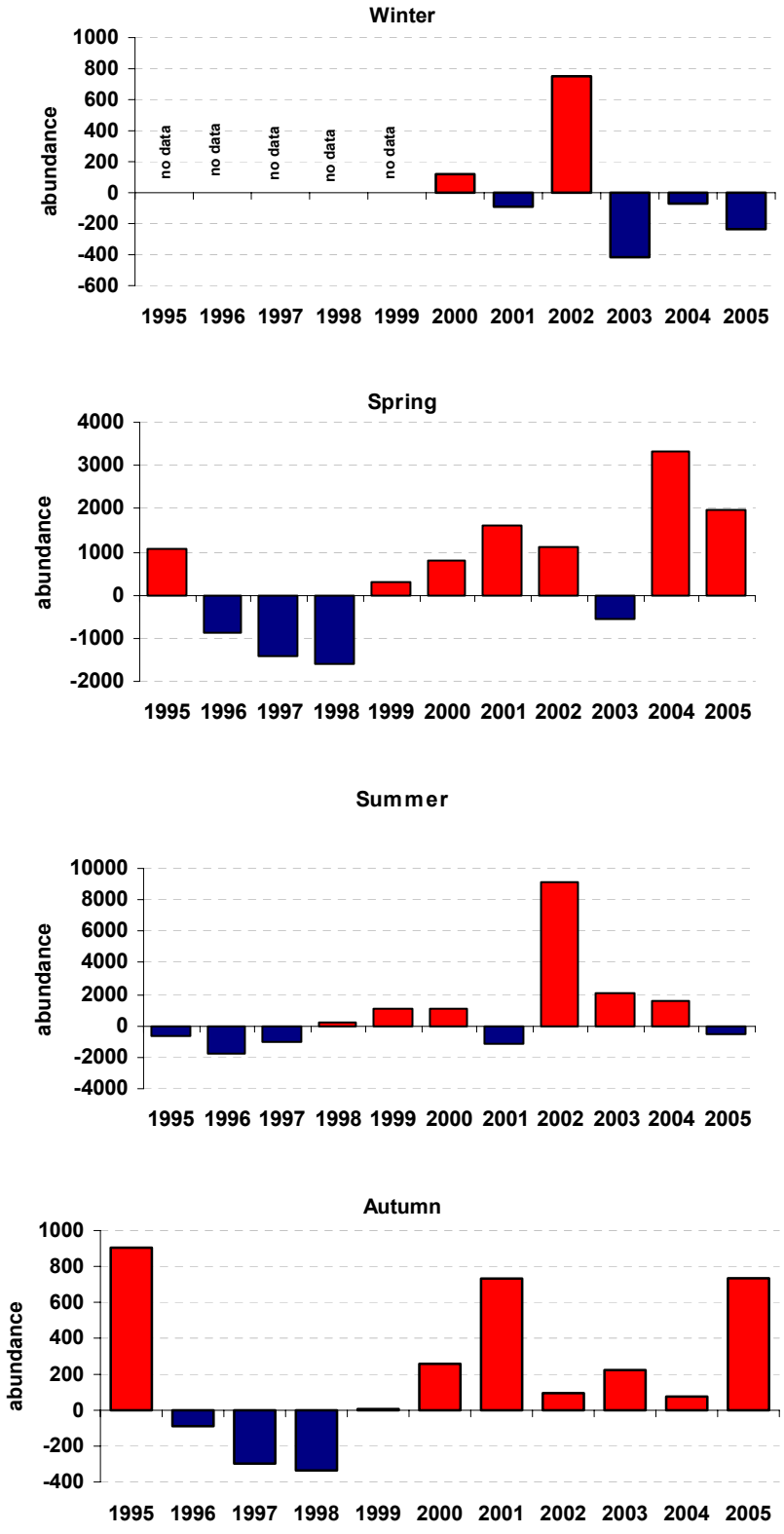

Figure 3. Seasonal 1995-2005 phytoplankton abundances (10^3 units L^{-1}) relative to long-term seasonal average (1984-1994) for the coastal area of Lithuanian waters of the Baltic Sea. The figures show an increase (red column) or a decrease (blue column) in phytoplankton abundance relative to the long-term average.

Plume area

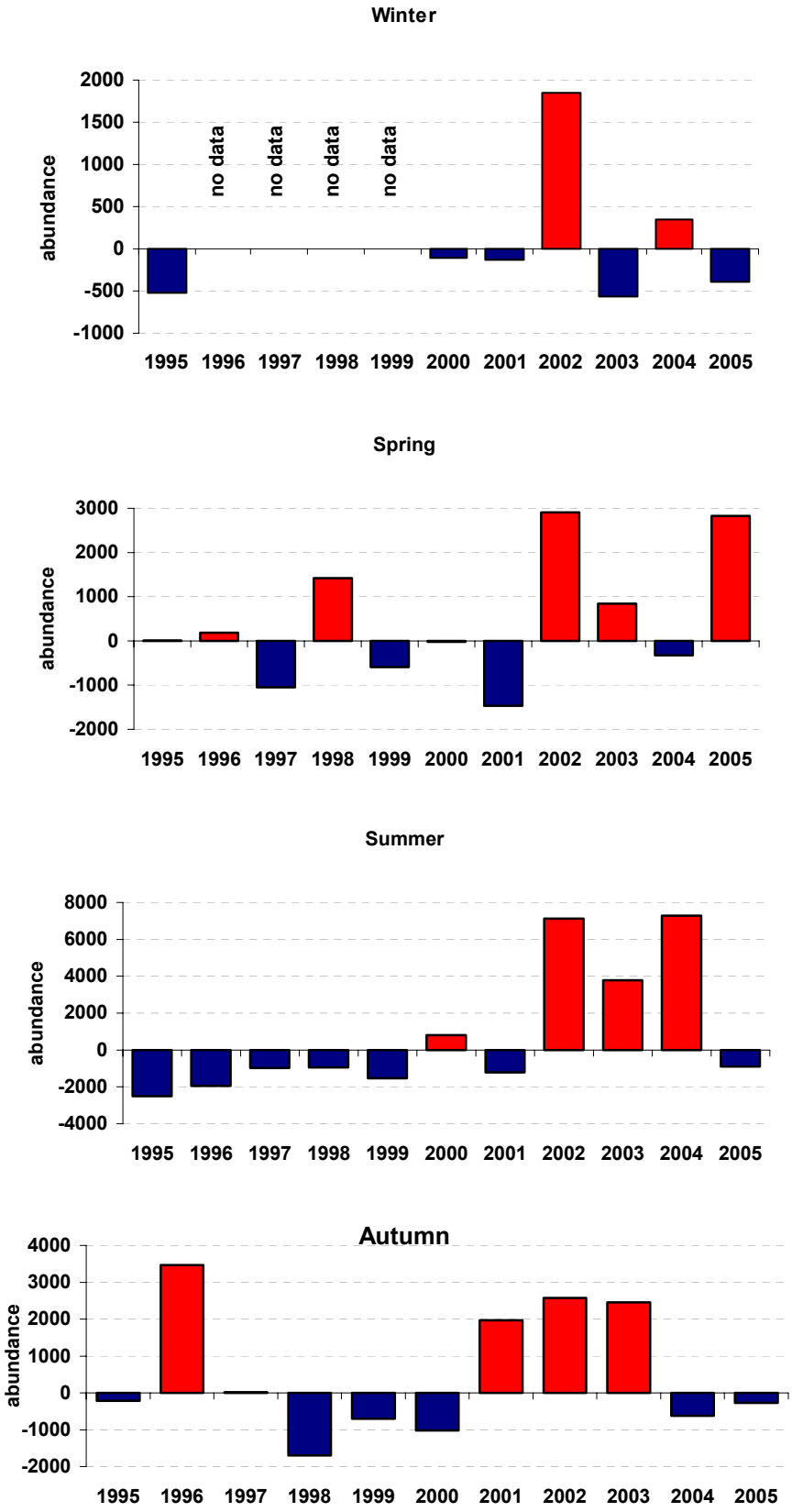

Figure 4. Seasonal 1995-2005 phytoplankton abundances (10^3 units L^{-1}) relative to long-term seasonal average (1984-1994) for the plume area (transitional area) of Lithuanian waters of the Baltic Sea. The tigures show an increase (red column) or a decrease (blue column) in phytoplankton abundance relative to the long-term average.

As an attempt to reveal if there is a tendency in the structural characteristics of phytoplankton during the recent decade, station 65 (open-sea) was analysed as an example (Figs. 6, 7 and 8). Cluster and MDS analysis (Fig. 6) show that there is no clear tendency in fluctuations of the phytoplankton structure. It is obvious, however, that in winter and summer the phytoplankton structure is much more stable (combining all years at level ~40 in both cases) than in spring and autumn (combining at level ~ 20).

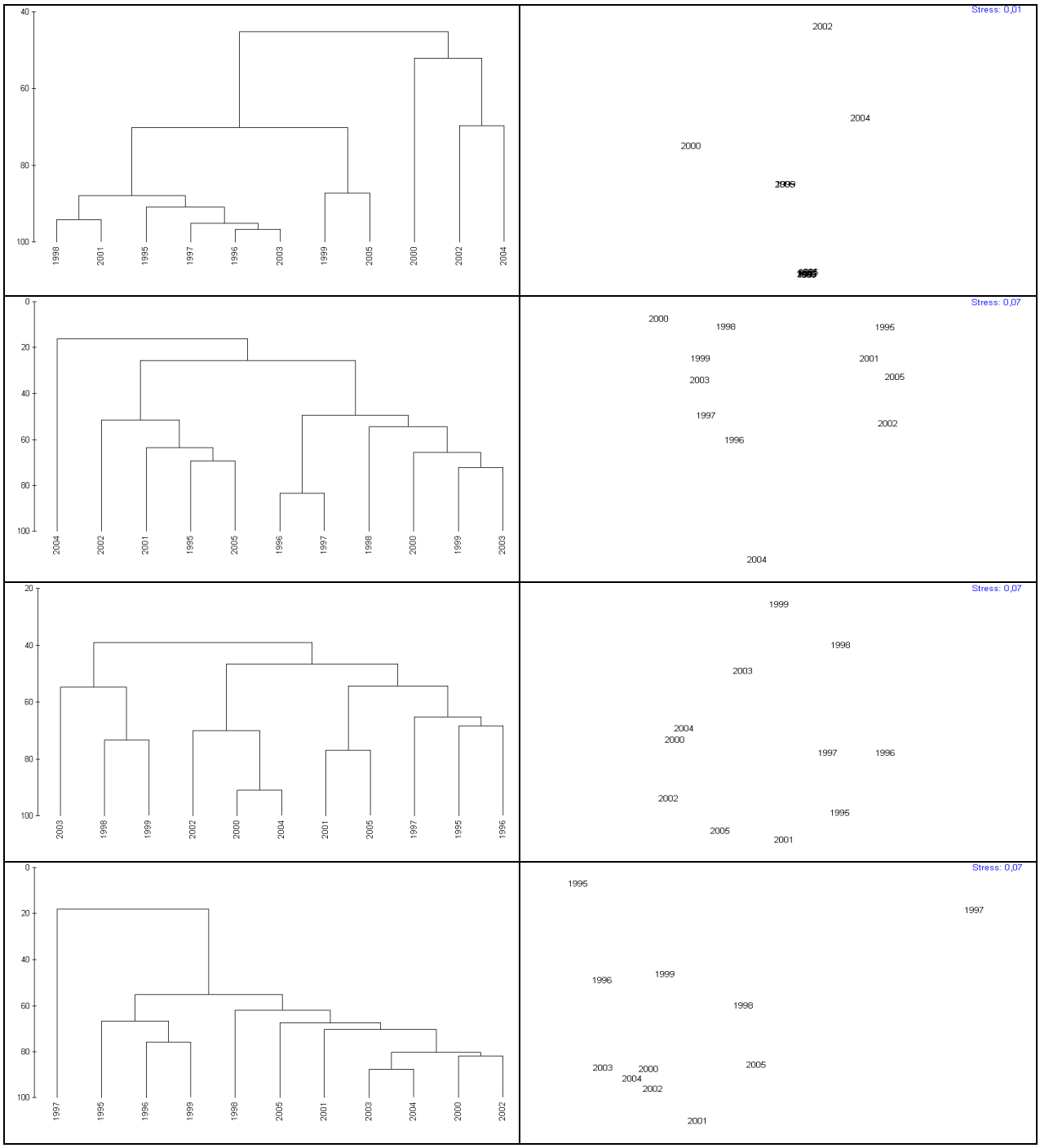

Figure 5. Cluster analysis (left column, Brey-Curtis similarity) and MDS plots (right column) based on phytoplankton structure (relative abundance of main groups) at the station 65 (open-sea area) in winter, spring, summer and autumn (1, 2, 3 and 4 rows, accordingly).

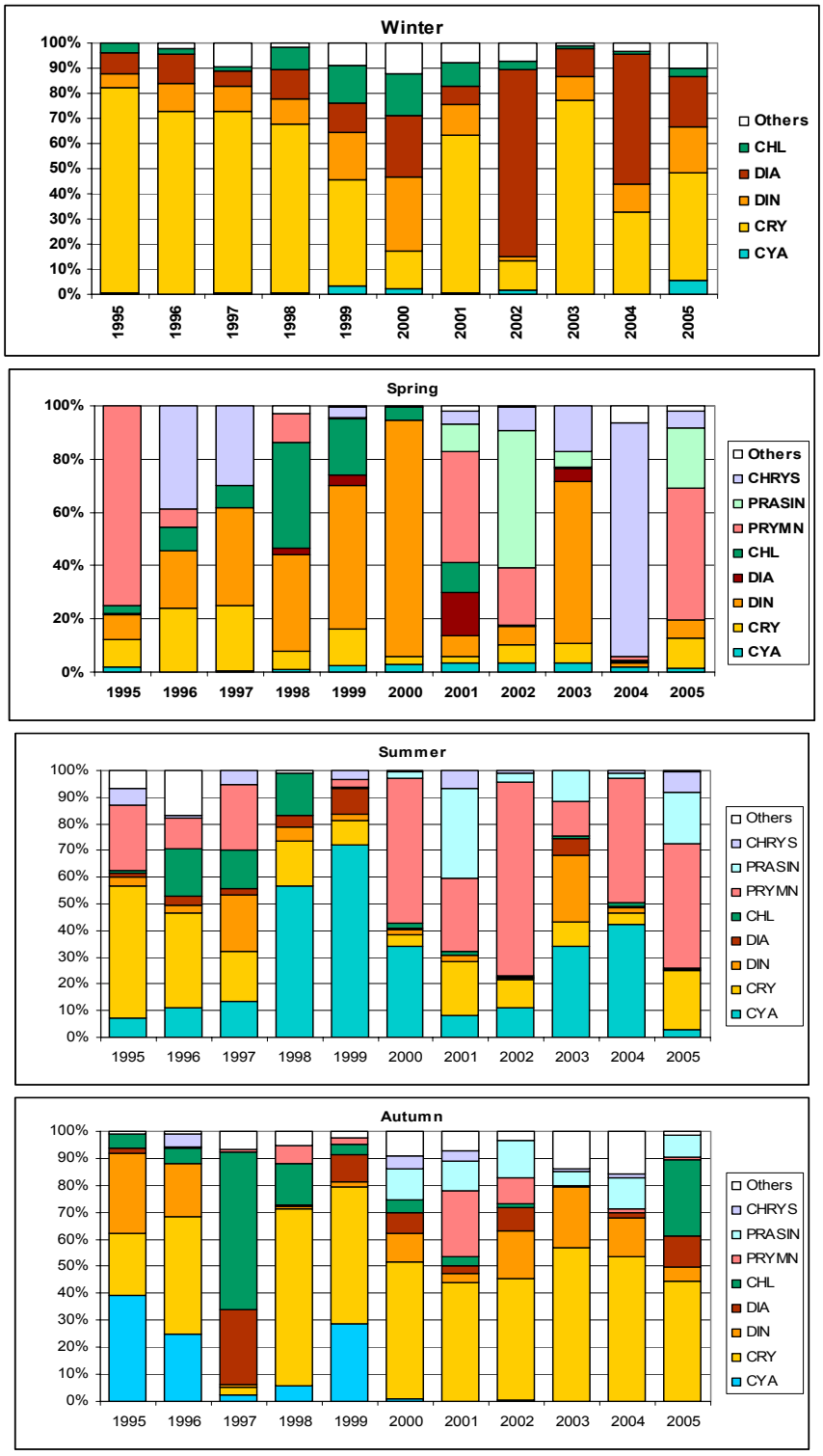

Figure 6. Seasonal variation in phytoplankton structure based on relative abundances of main phytoplankton groups at station 65 (open-sea area).

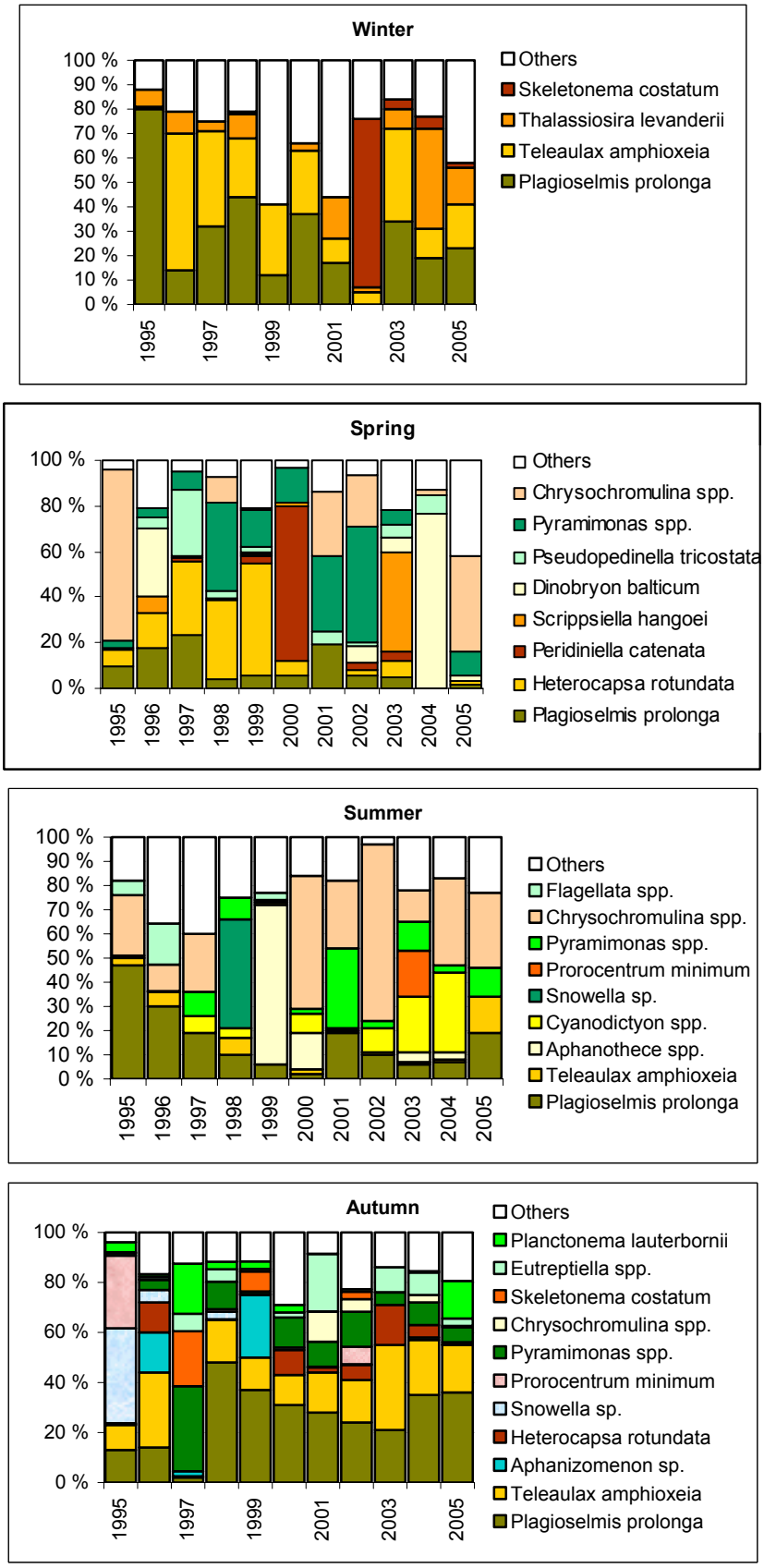

Figure 7. Seasonal variation in phytoplankton structure based on relative abundances of dominant species at station 65 (open-sea area).

According to literature data (Hällfors 1979, 2004, Глезер & Макарова 1974, 1988, Голлербах 1977), some phytoplankton species mostly occur in eutrophied waters and could be used as indicators of eutrophication. Their abundance gradually rises as the level of eutrophication increases. In the Lithuanian coastal waters, there are about 16 phytoplankton species belonging to four algae classes (Cyanophyceae, Dinophyceae, Diatomophyceae and Chlorophyceae) that could reflect the eutrophication status of waters:

SPECIES	DISTRIBUTION	OCCURRENCE
CYANOPHYCEAE		
Aphanizomenon flos-aquae	Whole Sea	Summer/autumn
Limnothrix redekei	Coastal areas	Spring/summer
Planktothrix agardhii	Coastal areas	Summer/autumn
DINOPHYCEAE		
Heterocapsa rotundata	Whole Sea	Spring (not typical)
Peridiniella catenata	Whole Sea	Spring
DIATOMOPHYCEAE		
Asterionella formosa	Coastal areas	Spring
Diatoma tenuis	Coastal areas	Spring
Nitzschia paleacea	Whole Sea	Summer
Skeletonema subsalsum	Coastal areas	Summer
Stephanodiscus binderanus	Areas influenced by Lagoon's waters	Summer
Stephanodiscus hantzschii	Areas influenced by Lagoon's waters	Spring
Stephanodiscus minutulus	Areas influenced by Lagoon's waters	Spring
Stephanodiscus rotula	Areas influenced by Lagoon's waters	Spring
Synedra acus	Coastal areas	All seasons
CHLOROPHYCEAE		
Planktonema lauterbornii	Whole Sea	All seasons
Chlamydomonas sp.	Areas influenced by Lagoon's waters	Summer

The largest part of the selected species show a seasonal occurrence in phytoplankton. Five of them are characteristic for fresh waters and could be abundant only in areas influenced by water from the Curonian Lagoon. Analysis of the species data for the period of 1995–2005 did not show any significant year-to-year increasing tendency in their quantitative development in the study areas (e.g. Fig. 8).

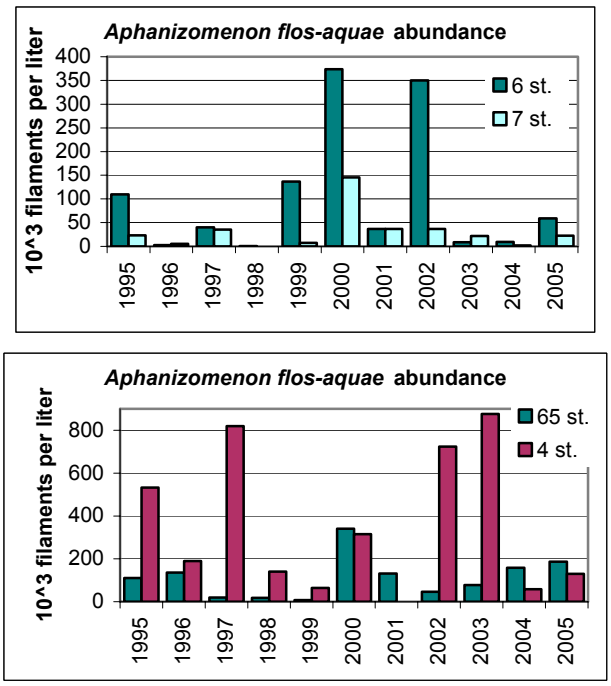

Figure 8. Variation in the abundance of *Aphonizomenon flos-aquae* at different study stations during 1995-2005.

In autumn 1992, the potentially toxic, alien dinoflagellate *Prorocentrum minimum* was observed for the first time near the Lithuanian coast, and now it is a regular component of the late summer and autumn flora. In Lithuanian waters *P. minimum* usually reaches the peak of density in September – October. Its abundance shows very high annual variability: in some years the species have created blooms with abundances up to 10.2×10^3 cells/litre, while during other periods it was absent (Fig. 9). In 1995, 1999, 2002, and 2003 *P. minimum* developed very dense populations in the entire coastal zone and constituted up to 80% of the total phytoplankton biomass.

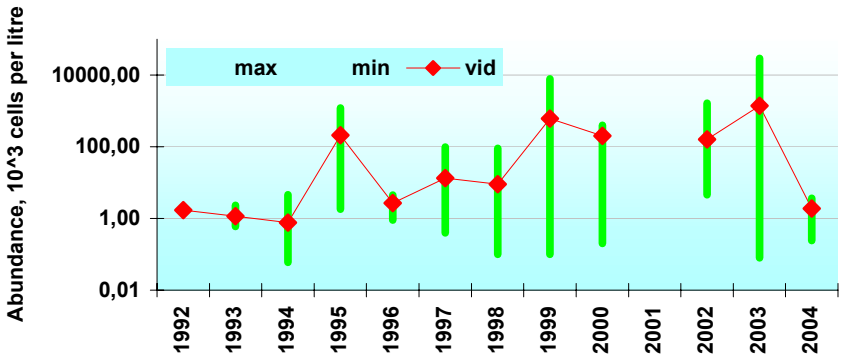

Figure 8. Long-term dynamics of the abundance of the potentially toxic alien dinoflagellate *Prorocentrum minimum* in the Lithuanian waters. Note logarithmic Y-axis scale.

Chlorophyll a concentrations in 1995-2005

The southern part of the study area is outside of the plume area of the Curonian Lagoon. Because of this the area is characterized by low chlorophyll *a* (chl *a*) concentrations. During the period 1995–2005 the highest autumn chl *a* concentrations were observed in the plume zone (Station 4) near Klaipeda. Lower concentrations were typically observed

in the open-sea area (Stations 65 and N-2). Chl *a* concentrations in this area fit to the oligo-mesotrophic classification according to the Vinberg-scale (Vinberg 1954).

Table 2. Integrated (1–10 m depth) mean chlorophyll *a* concentrations (μg/l) in different zones of the Lithuanian waters during the period 1995–2005.

Chlorophyll *a* concentrations	**Transitional waters**	**Coastal sea area**	**Open sea**
Winter mean	2.36	2.21	1.82
Spring mean	8.16	4.26	3.97
Summer mean	12.11	4.64	3.41
Autumn mean	10.90	2.73	3.07
Long-term average	**8.97**	**3.46**	**3.08**

The main difference between the study regions is that the maximum concentrations of chl *a* in the coastal and open-sea zones are observed during the spring and summer seasons, but in transitional waters influenced by waters from the Curonian Lagoon, the highest concentrations are measured usually in summer and autumn.

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2.2.5 Zooplankton in 1995–2005

Responsible scientist: Natalja Demereckiene (CMR)

Zooplankton sampling and analysis were done according to the requirements of marine monitoring methods of the HELCOM COMBINE programme (HELCOM, 2006).

The analysis of zooplankton samples showed a general main tendency of changes in all three zones studied: the abundance of *Copepoda* is decreasing (Figs. 1, 2 and 3). Another group of zooplankton, *Rotifera,* becomes dominant in the coastal zone and the Curonian Lagoon plume, and in spring even in the open-sea area. These cases provide evidence about the increasing trophic level in the Baltic Sea.

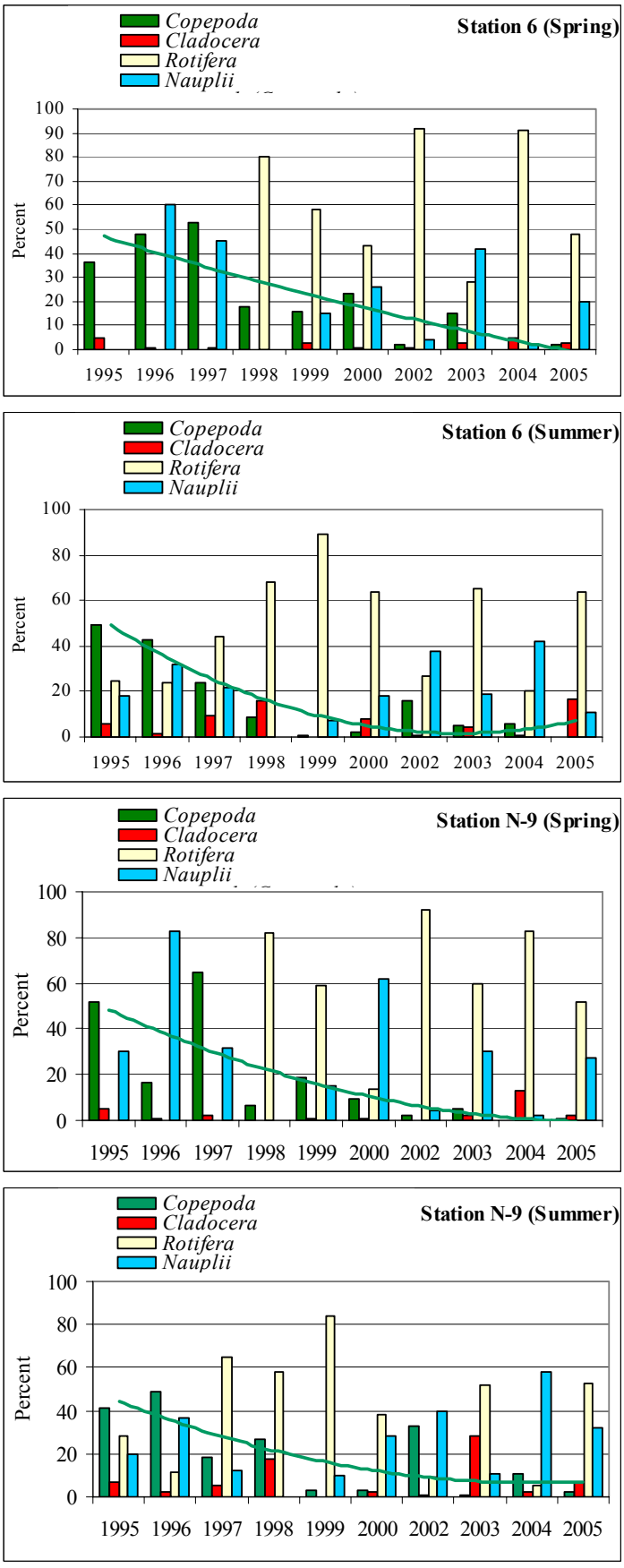

Figure 1. Changes in zooplankton taxonomic groups in different season in coastal waters during period 1995–2005. Nauplii = Copepoda nauplius stages. The green line is a trend line, which gives an estimate for the long-term development of copepods

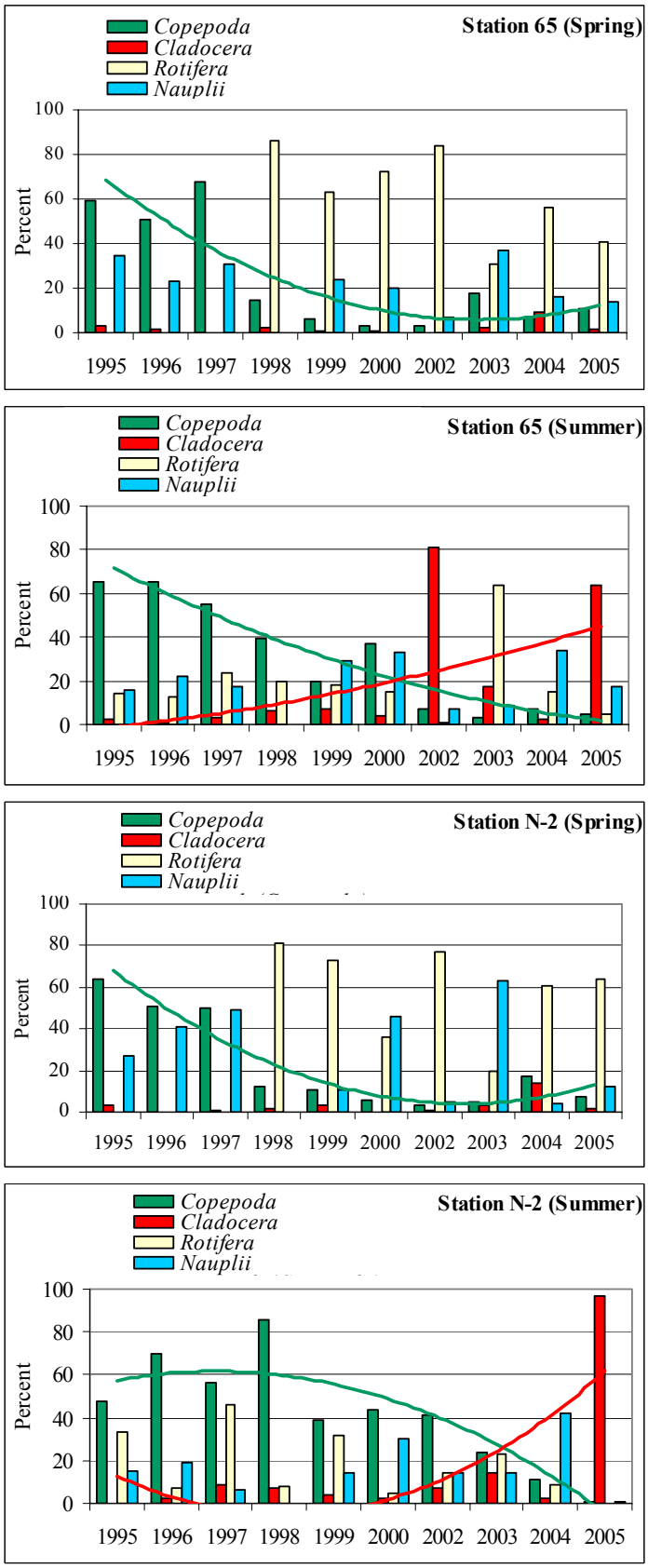

Figure 2. Changes in zooplankton taxonomic group different season in the open-sea area in period 1995–2005. Nauplii = Copepoda nauplius stages. The green line is a trend line, which gives an estimate for the long-term development of copepods.

In summers, from 1999 onwards no taxonomic group exceed 50% of total zooplankton abundance. However, in summer 2002 (station 65) and 2005 (stations N-2 and 65) The domination of *Cladocera* was observed, forming 60–80% of total zooplankton abundance. At station N-2 almost the whole zooplankton community consisted of one *Cladocera* species (Fig. 2).

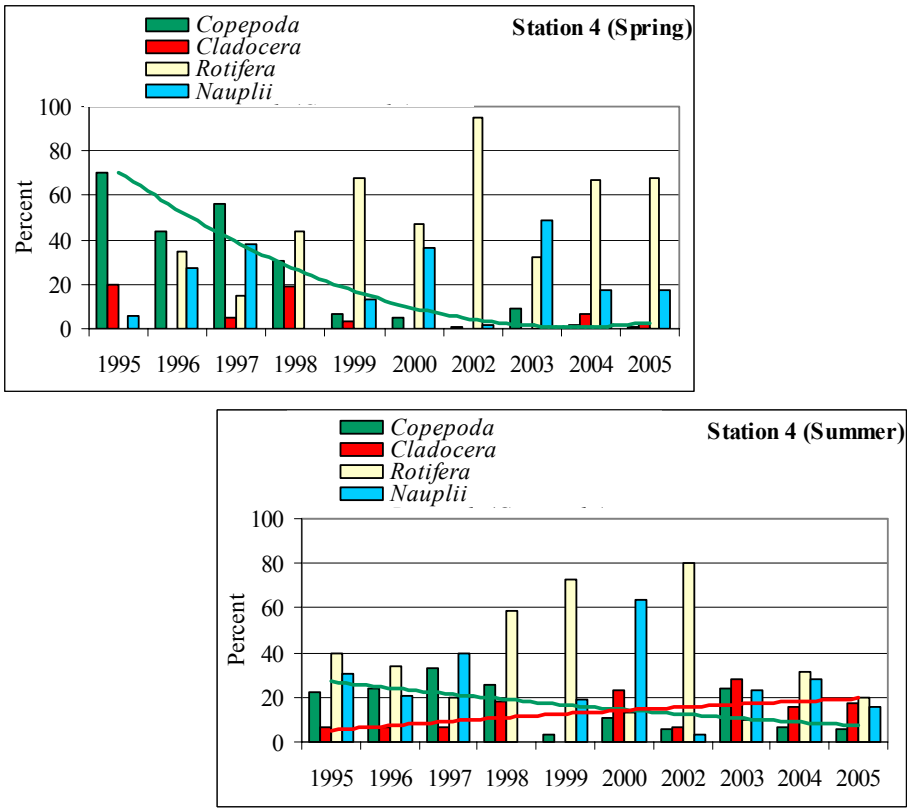

Figure 3. Changes in zooplankton taxonomic groups in different seasons in transitional waters during the period 1995–2005. Nauplii = Copepoda nauplius stages. The green line is a trend line, which gives an estimate for the long-term development of copepods.

The reduction in *Copepoda* abundance is not related to changes in salinity. The correlation coefficient ***r*** describing the dependence of zooplankton on salinity differs in each case and fluctuates between -0.12 and 0.44 (Figs. 4, 5 and 6).

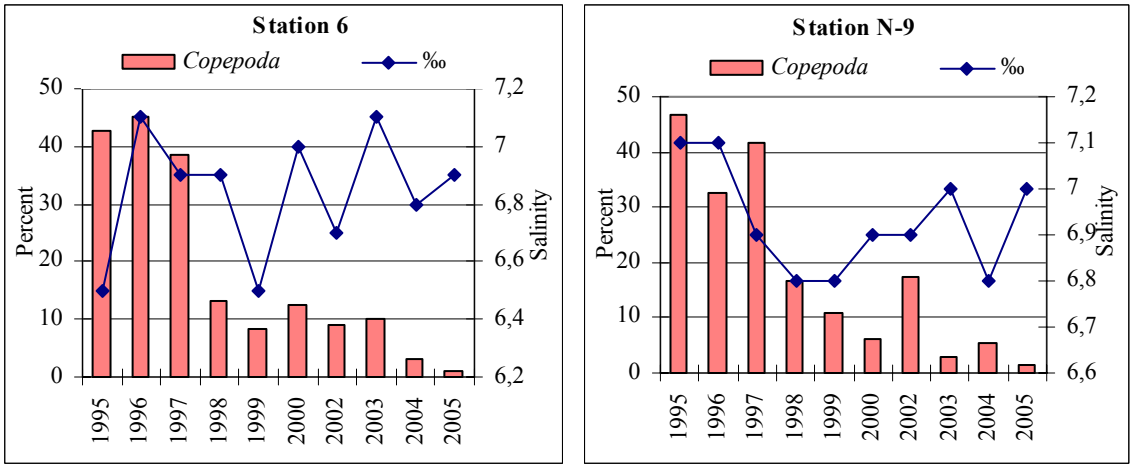

Figure 4. Distribution of the *Copepoda* zooplankton group (%) and salinity of whole water column in coastal waters during the period 1995–2005.

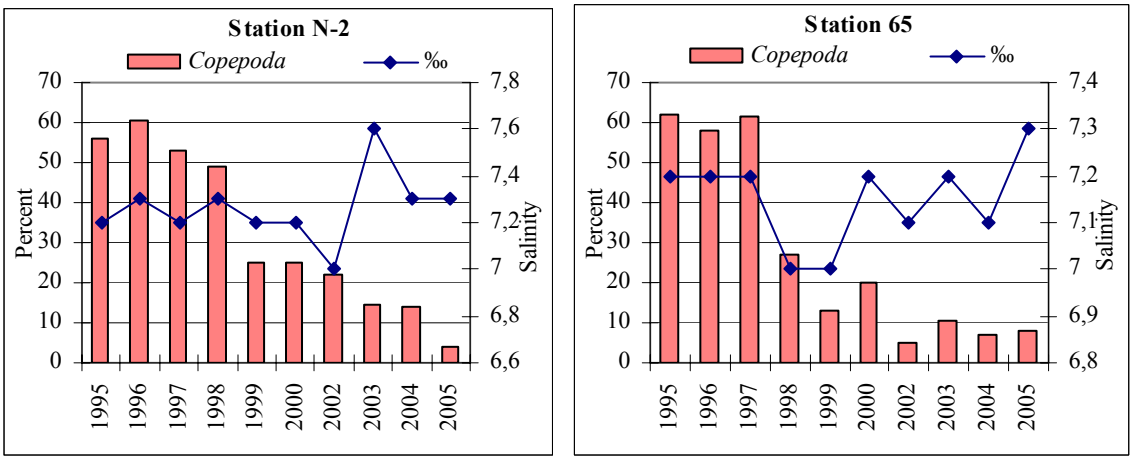

Figure 5. Distribution of the *Copepoda* zooplankton group (%) and salinity of whole water column in open sea during the period 1995–2005.

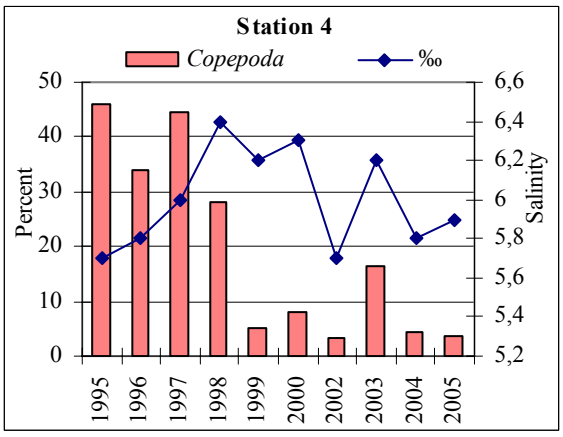

Figure 6. Distribution of the *Copepoda* zooplankton group (%) and salinity of whole water column in transitional waters during the period 1995–2005.

2.2.6 Macrozoobenthos 1995–2005

Responsible scientist: Sabina Solovjova (CMR)

In this report macrozoobenthos is specified as the size group of >0.5 mm. Abundance is expressed as individuals/m^2 (ind./ m^2) and biomass as g/m^2 (wet weight).

Long-term data concerning community structure of macrozoobenthos basing on abundance and biomass are presented. Due to their relative longevity and low mobility macrozoobenthos is universally considered as an appropriate indicator of environmental changes reflecting changes both in the sea bottom and the water phase.

The data on macrozoobenthos monitoring study from three different regions was used for this purpose: open sea region (Stations 65 and N-2), transitional area (Station 4), and coastal area (Stations 6 and N-9).

The results of analyses of monitoring samples collected between 1981–2005 were used for the analysis of long-term dynamics of soft-bottom macrofauna in the open-sea zone (Station 65).

Twenty-six macrozoobenthic taxons were found during these study period (Table 1).

Total abundance of macrozoobenthos was 1053-15840 ind./m^2 (average 7721 ind./m^2) and biomass 7.37–422.0 g/m^2 (average 209.7 g/m^2) until the alien polychaete worm *Marenzelleria viridis* was found in the area for the first time in 1992. Although changes in the benthic community were observed both in abundance and biomass, reduction of number and biomass e.g. of the amphipod *Monoporeia affinis* was not interconnected with the advent of *M. viridis* and concurrent interspecific interactions, because the decline was observed already from 1990 onwards (Fig. 2). Total abundance of macrozoobenthos during the period 1992–2005 was 40–7170 ind./m^2 (average 1862 ind./m^2) and biomass 5.181–231.97 g/m^2 (average 100.729 g/m^2). The same benthos groups dominated during these two periods, but their characteristics differed (Table 1).

Table 1. Abundance ind./m^2 and biomass g/m^2 (wet weight) of dominant macrozoobenghic species during two time periods at the open-sea station 65.

Dominant species	Parameter	Period 1981–1991	Period 1992–2005
Monoporeia affinis	Abundance average	3079 ind./m^2,	496 ind./m^2,
	Biomass average	11.162 g/m^2	0.38 g/m^2
Macoma balthica	Abundance average	1855 ind./m^2	493 ind./m^2
	Biomass average	183.129 g/m^2	86.725 g/m^2
Ostracoda	Abundance average	1676 ind./m^2	40 ind./m^2
Marenzelleria viridis	Abundance average		252 ind./m^2
	Biomass average		1.148 g/m^2

From 1992 onwards the average abundance and biomass of macrozoobenthos were gradually reduced in comparison with 1981–1992 (Fig. 1).

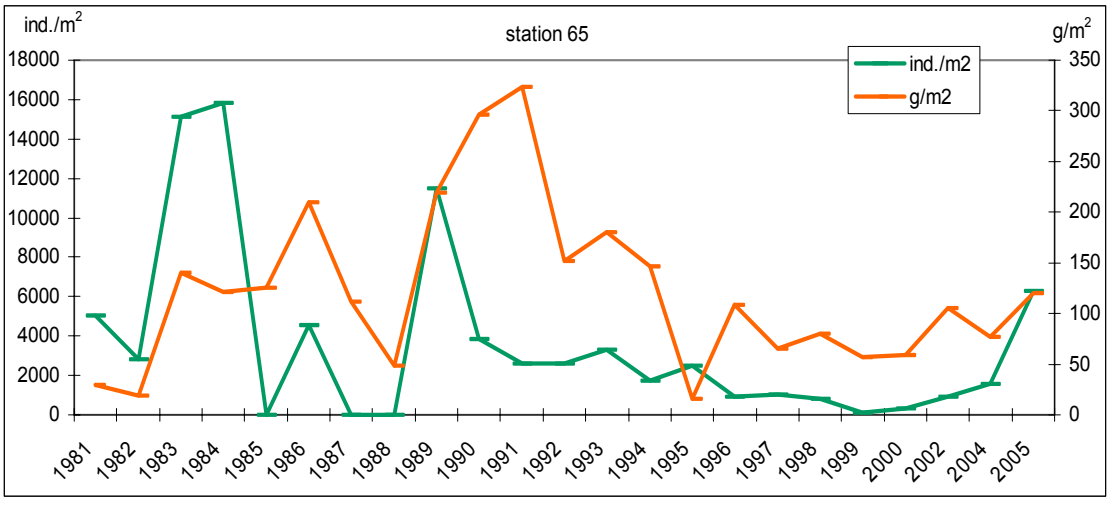

Figure 1. Long-term dynamic and trends in average total abundance (ind./m^2) and biomass (g/m^2 ww) of macrozoobenthos at the open-sea station 65 during 1981–2005.

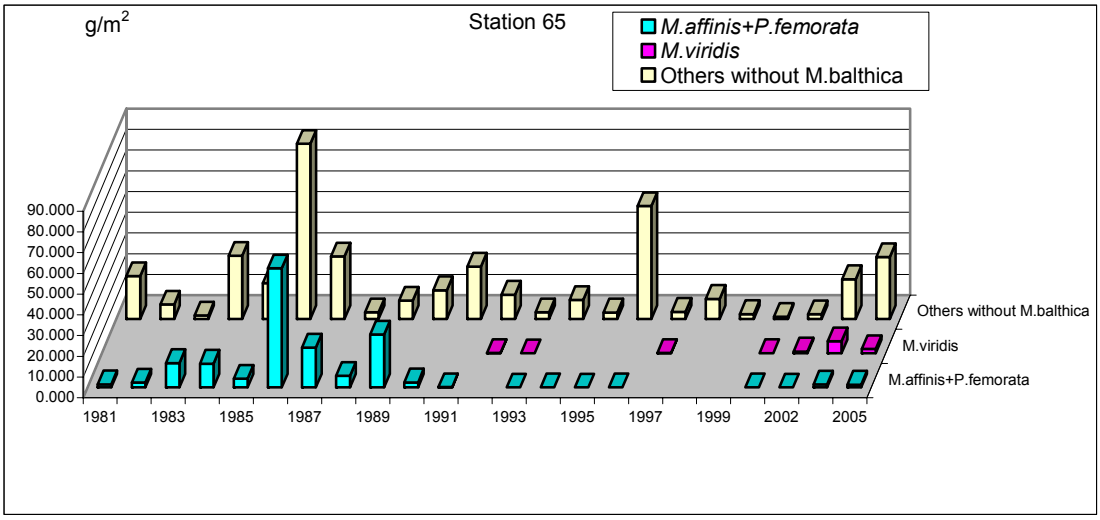

Figure 2. Long-term dynamics in the averages biomass (g/m^2 ww) of *M. affinis, M. viridis* and other species (except the clam *M. baltica*) at the open-sea station 65 during 1981–2005.

The results from the open-sea Station 65 showed a reduction in the abundance and biomass of macrozoobenthos species, but also a change in the community structure (Figs. 1 and 2). The exact causes underlying the changes remain unresolved; with present data it can only be assumed that they are caused either by eutrophication-related factors in the ecosystem, chemical pollution or concurrent interspecific interactions, or combinations of all three.

Station N-2 is characterised by a depth of 65 m and sediments consisting of sand, black mud and detritus. Fifteen macrozoobenthic taxons were found at the stations (2–11 taxons per sample) during the monitoring period of 1995–2005. Macrozoobenthos species such as *Harmothoë sarsi, Monoporeia affinis, Pontoporeia femorata* and *Saduria entomon*, which characterise deep-water ecological systems in the Baltic Sea, were found at this station. *Macoma balthica, Pygospio elegans* and *Marenzelleria viridis* were also found, but they were not as abundant as in the coastal areas. Total abundance was 50–2960 ind./m^2, (average 673 ind./m^2) biomass 0.936–104.08 g/m^2 (average 31.134 g/m^2) (Fig. 3).

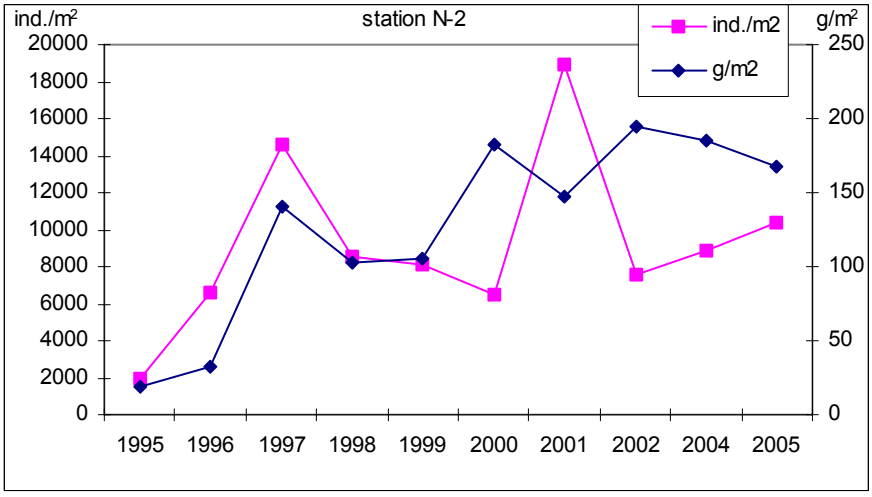

Figure 3. Long-term dynamics and trends in average total abundance (ind./m^2) and biomass (g/m^2 ww) of macrozoobenthos at open-sea station N-2 during 1995–2005.

The abundance of the indicator amphipod species *M. affinis* and *P. femorata* was 10–120 ind./m^2 during 1995–2005 (Fig. 4). Populations of these species decline rapidly under deteriorating environmental conditions, e.g. reductions in near-bottom oxygen concentration.

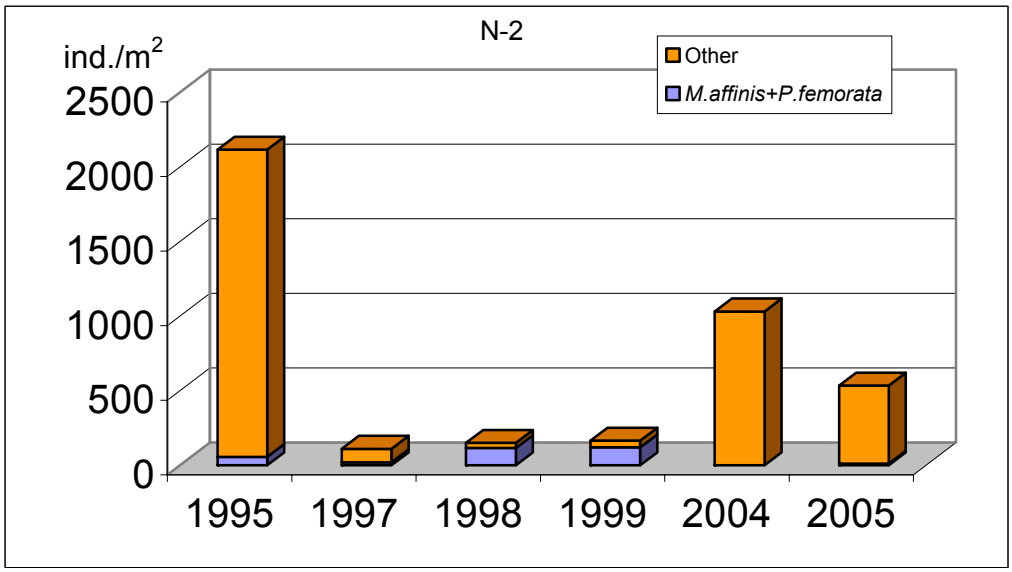

Figure 4. Long-term dynamics in the abundance (ind./m^2) of the indicator zoobentic species *M. affinis* and *P. femorata* and other species at the deep open-sea station N-2 during 1995–2005.

Biomass at station N-2 was dominated by the clam *M. balthica* 0.113-34.585 g/m^2 (Fig. 5).

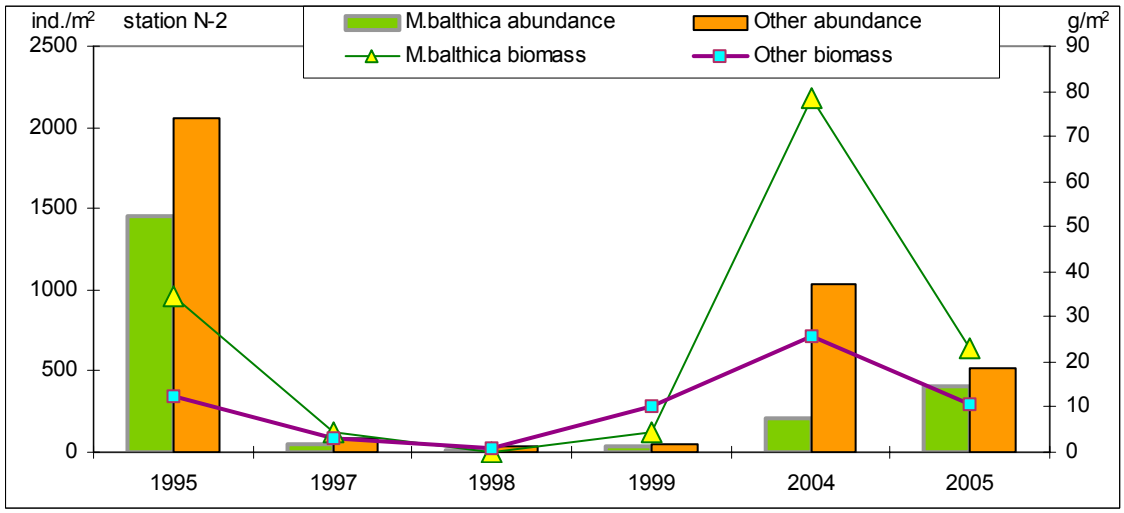

Figure 5. Long-term dynamics in abundance (ind./m^2) and biomass (g/m^2 ww) of the dominant macrozoobenthic species *M. balthica* and other species at deep open-sea station N-2 during 1995-2005.

The 1995–2005 data do not indicate stability in abundance or biomass of the ecological system in the region of station N-2 (Figs. 4 and 5). Only the species composition is relatively stable at this study site.

The transitional water zone represented by Station 4, which is influenced by the Curonian Lagoon waters. Water depth in this station is 15 m, sediments are sand with a layer of black mud, sometimes with smell of H_2S. Twenty-one macrozoobenthic taxons (4–13 taxons per sample) were recorded here during 1995–2005.

Total abundance of the zoobenthos community at Station 4 was ca. 600–30970 ind./m^2 (average 4608 ind./m^2) and biomass 2.26-178.446 g/m^2 ww (average 42 g/m^2 ww) (Fig. 6).

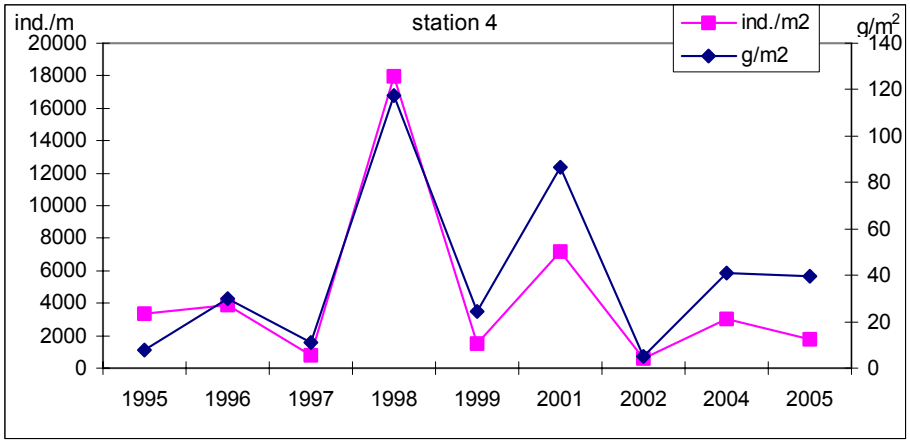

Figure 6. Long-term dynamics in the average of total abundance (ind./m^2) and biomass (g/m^2 ww) during 1995–2005 in transitional waters (Station 4).

No significant trends in the changes in biomass or abundance could be observed in the transitional zone (Fig. 6).

Usually there are six abundant species, which have enough significance to total biomass of the community (Figs. 7 and 8).

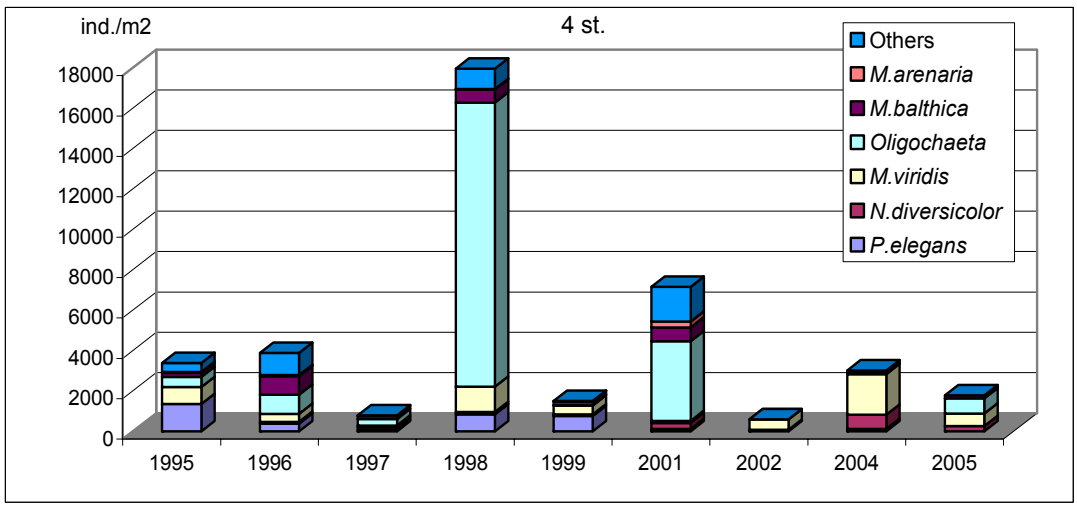

Figure 7. Long-term dynamics in average abundance (ind./m^2) of most important species of the macrozoobenthos community at station 4 during 1995–2005.

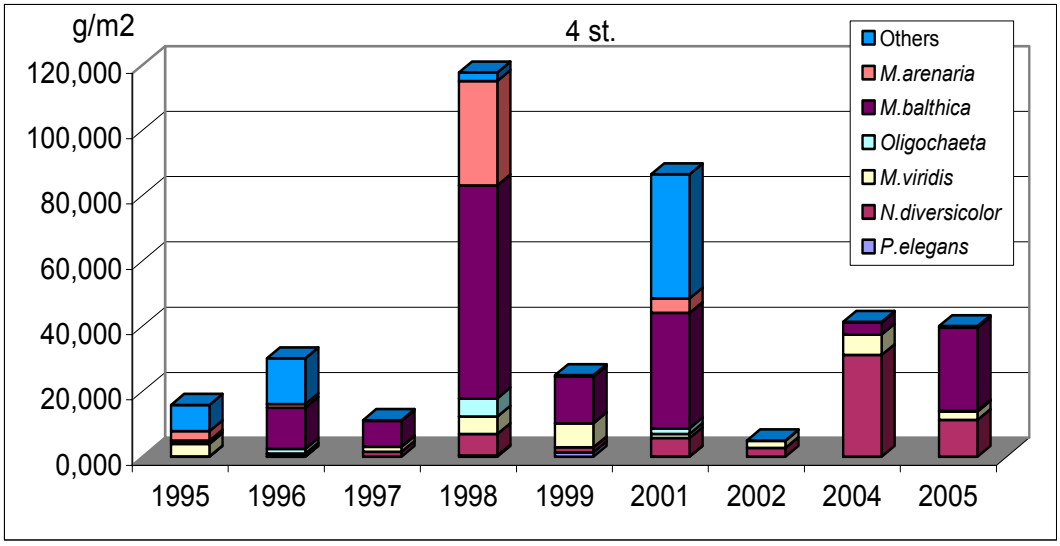

Figure 8. Long-term dynamics of the biomass (g/m^2 ww) of most important species of the macrozoopenthos community at station 4 during 1995–2005.

In the coastal area the monitoring studies were done at Stations 6 and N-9 with fine sand sediment and depth range of 13–15 m. The results showed similarity between the two stations. Fifteen taxons were found (7–8 taxons per sample) at station 6, and 18 taxons (7-11 taxons per sample) at station N-9 during the period of 1995–2005.

Total abundance varied between 820–21570 ind./m^2 (average 9214 ind./m^2) and biomass 1.14 -219.92 g/m^2 (average 105 g/m^2) (Fig. 10). Species, like *Macoma balthica*, *Mya arenaria*, *Pygospio elegans*, *Nereis diversicolor*, *Oligochaeta* sp. and, *Marenzelleria viridis* were found almost in all samples.

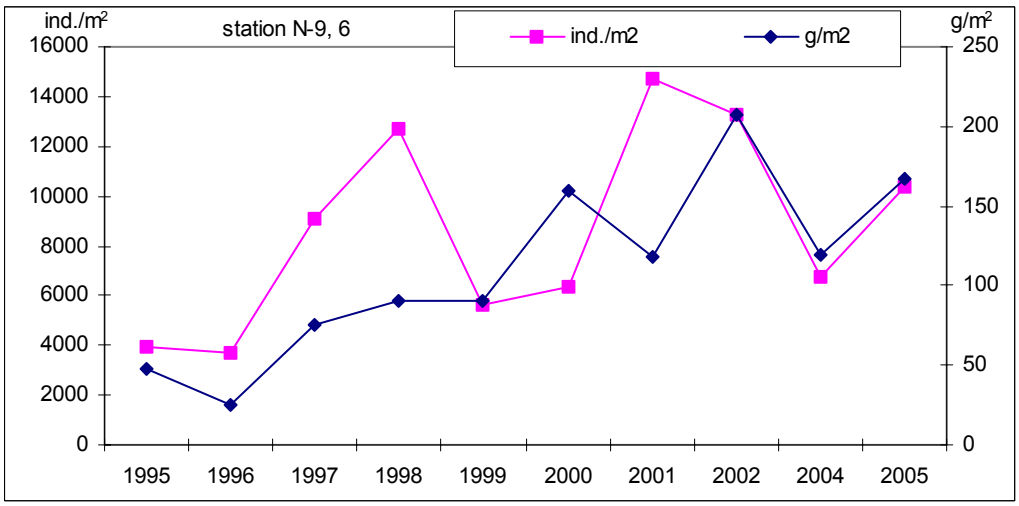

Figure 9. Long-term dynamics in average total abundance (ind./m^2) and biomass (g/m^2 ww) of macrozoobenthos in the coastal zone (station 6) during the period of 1995–2005.

The results of long-term dynamics of macrozoobenthos communities in the coastal zone (stations 6 and N-9) showed increasing trends in abundance and biomass. This could be connected with increasing eutrophication of coastal ecosystems.

An indicator group for eutrophication in macrozoobenthos communities is the family *Oligochaeta*. Some trends exist in their relative abundance (% of total) in the macrozoobenthos samples in the different study regions (Fig. 11) At the coastal stations N-9 and 6 an increased tendency of the percentage of *Oligochaeta* in the samples can be observed whereas at station 65 a decreasing tendency can be seen. No changes can be observed at station 4, influenced by the Curonian Lagoon.

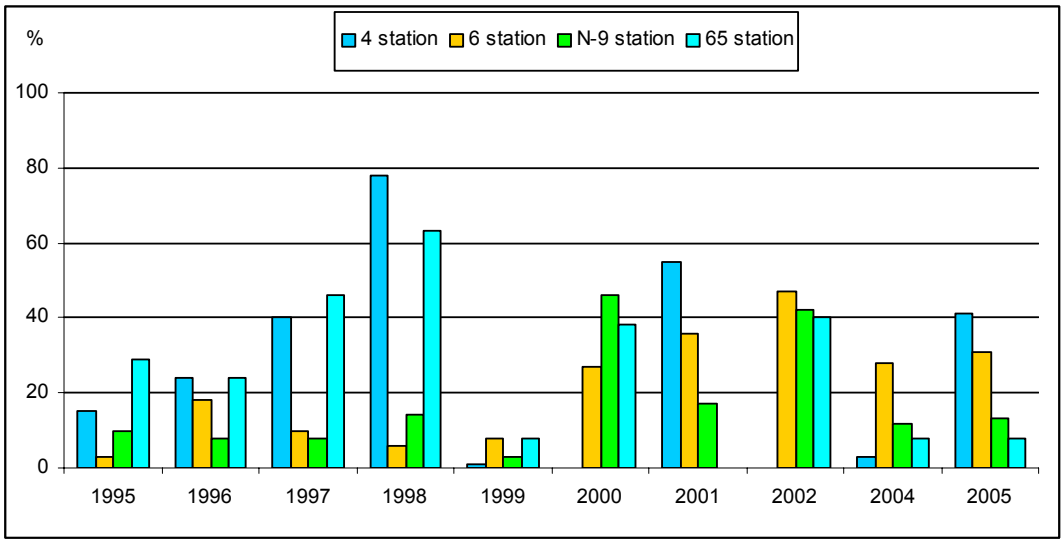

Figure 10. Changes in the relative abundance (%) of *Oligochaeta* in macrozoobenthos samples in different regions in 1995–2005.

2.2.7 Harmful substances 1995–2005

Responsible scientist: Galina Garnaga

A dataset of total oil hydrocarbon concentrations (THC) in surface (1 m) and near-bottom water layers as well as in sediments for the period of 1995–2005 was used for this study. Sampling was performed by the Center of Marine Research in the framework of environmental monitoring programs.

As with other studies (see above), the study region was divided into three areas. Station 4 represents transitional waters under the influence of water from the Curonian Lagoon, stations 6, N-5, N-9 and N-6 represent the coastal zone, and stations 65 and N-2 represent the open-sea area.

Total oil hydrocarbons in water

Total oil hydrocarbon data in water at stations 4, N-9 and 65 is available from 1995 onwards, from station 6 from 2000, while stations N-5, N-6 and N-2 have data starting only from 2004.

A total of 86 samples from the transitional zone (station 4) were analysed for total oil hydrocarbons (Fig. 1). Analyses values are compared with the Maximum Permissible Level (MPL) for total oil hydrocarbons in water, which has been established in the Order of Minister of Environment of Lithuania "Wastewater treatment regulation" (Žin. 2006, Nr. 59-2103). The MPL for total oil hydrocarbons in surface waters is 0.05 mg/l. In transitional waters 13% of the values were above the MPL during the study period 1995–2005. Mean concentration of oil hydrocarbons in water for that period was 0.04 mg/l.

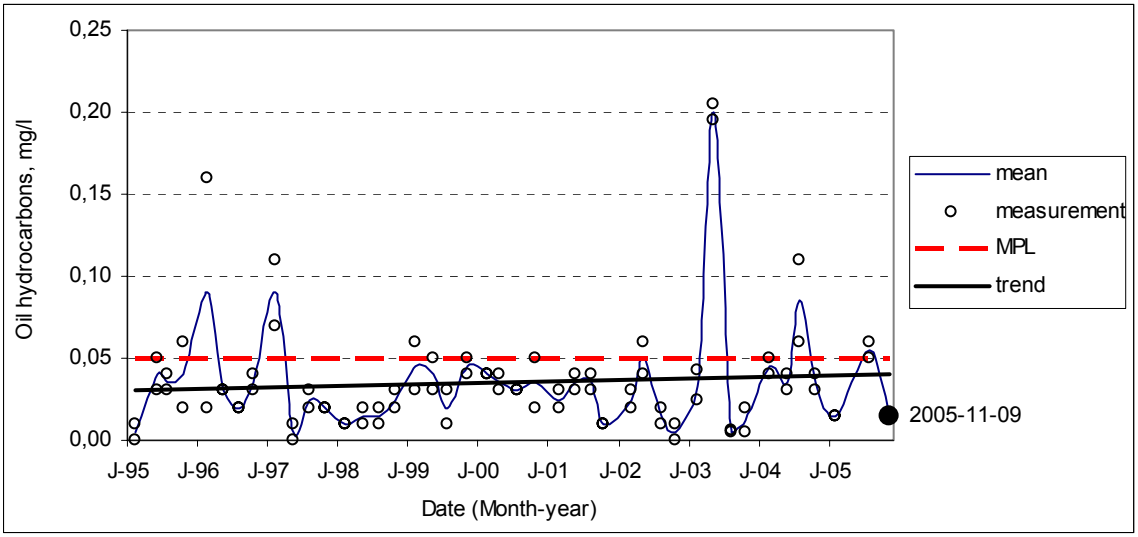

Figure 1. Oil hydrocarbon concentrations in the water (surface and near bottom layers) in transitional zone: station 4 (1995–2005, 86 measurements) (• - measurements from the cruise in autumn, 2005; MPL – Maximum Permissible Level).

Long-term total hydrocarbon values in the coastal zone are presented in Fig. 2. Hydrocarbon measurements at Station N-9 start from 1995, for Station 6 from 2000, for stations N-5 and N-6 from 2004. In total there are 154 measurements for that period in the coastal zone. A slightly increasing trend of total oil hydrocarbons in water in this

area can be noticed. During the first 5 years of the study period (1995–1999) only 5% of the values were above the MPL. Starting from year 2000, the percentage of values above MPL has increased to 17%. Station N-9 alone has a similar trend. The mean oil concentration during the period 1995–2005 in the coastal zone was 0.03 mg/l.

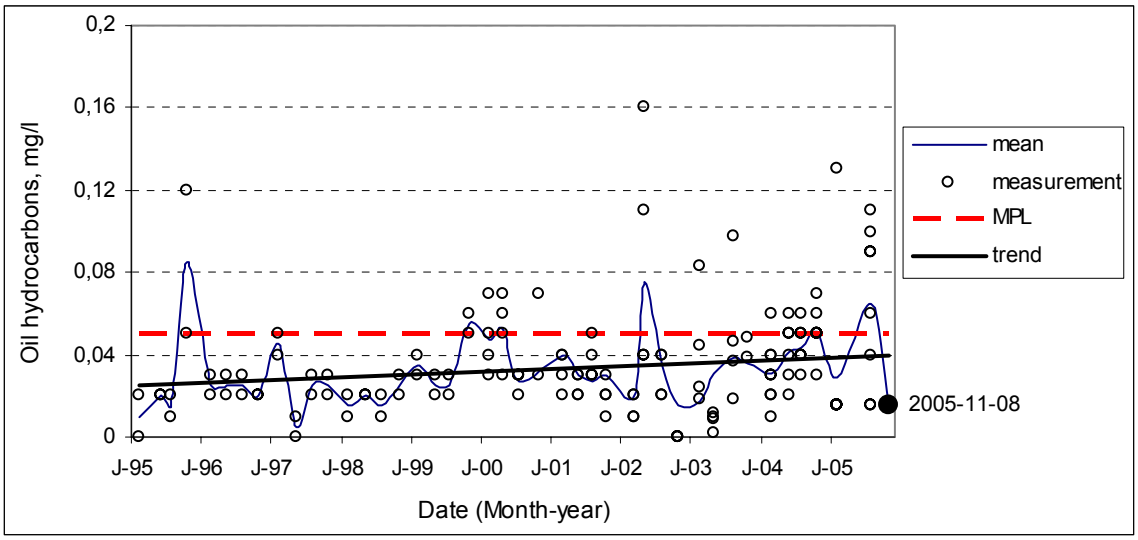

Figure 2. Total oil hydrocarbon concentrations in water (surface and near bottom layers) in the coastal zone: station 6 (2000–2005, 44 measurements), station N-5 (2004–2005, 12 measurements), station N-9 (1995–2005, 84 measurements), station N-6 (2004–2005, 14 measurements). (• - measurements from the cruise in autumn, 2005; MPL – Maximum Permissible Level).

In the open-sea area, measurements at station 65 cover the whole period of 1995–2005 (Fig. 3). Fourteen measurements from station N-2 have been added to the graph from 2004. About 15% of the values were above the MPL during the study period. Some high values in 1995–1997 could be observed but during the period 1998–2002 there were no values above the MPL, and only in 2003 higher values than 0.05 mg/l started to appear again. No long-term temporal trend could be detected in the open sea zone. Mean concentration for the period of 1995–2005 in the open-sea was 0.04 mg/l.

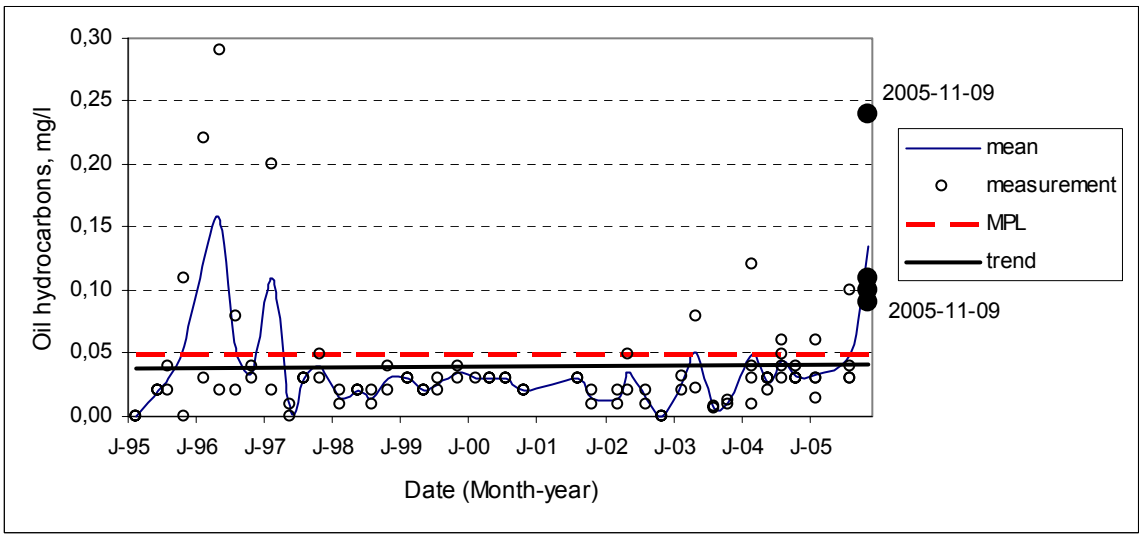

Figure 3. Total oil hydrocarbon concentrations in water (surface and near bottom horizons) in open sea area: station 65 (1995–2005, 81 measurement) and station N-2 (2004–2005, 14 measurements); (• - measurements from the cruise in autumn, 2005; MPL – Maximum Permissible Level).

Compared to the coastal zone, the higher mean concentrations of total oil hydrocarbons (0.04 mg/l) in the transitional waters can be attributed to the intensive shipping activity in the area and the influence of the Klaipėda harbour, as well as to illegal discharges of oil products in the open sea area. Illegal discharges of oil from ships may even create a chronic impact in certain areas (HELCOM, 1996; Pikkarainen and Lemponen, 2005). However, the mean concentrations of total hydrocarbons in the water were not high and did not reach the established MPL level.

It is to be noted that the method used in the analysis of total oil hydrocarbon concentration, Infrared Spectrometry (IR), and therefore the results are not fully comparable. However, the concentrations above the detection level of the IR method seem high compared to those observed in other parts of the Baltic Sea (HELCOM 2002).

Total oil hydrocarbons in sediments

Since no significant regional differences could be detected, the concentration trend curve for the period 1995–2005 is based on data from all the stations in the southern area of the Lithuanian part of the Baltic Sea (Fig. 4). Total oil hydrocarbon measurements in sediments during the whole 1995–2000 period is available for station 65 only. Starting from 2000, data from stations 4, 6 and N-9 have been included, stations N-2, N-5 and N-6 were added from 2004.

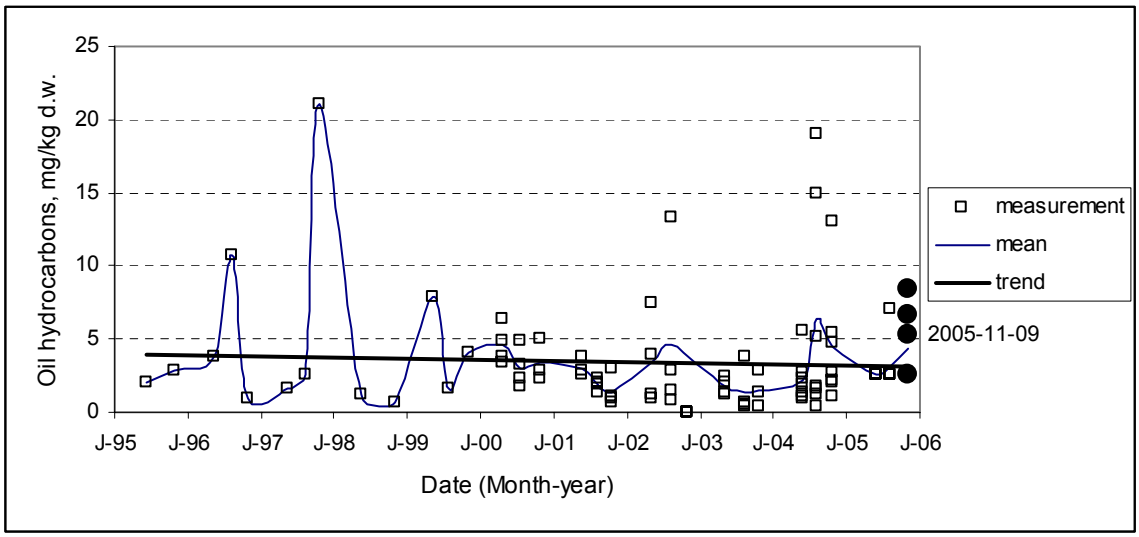

Figure 4. Oil hydrocarbon concentrations in sediments (mg/kg dry weight) in the study area: station 4 (2000–2005, 17 measurements), station 6 (2000–2005, 16 measurements), station N-5 (2004–2005, 6 measurements), station N-9 (2000–2005, 16 measurements), station N-6 (2004–2005, 6 measurements), station 65 (1995–2005, 29 measurement) and station N-2 (2004–2005, 6 measurements) (• - measurements from the cruise in autumn, 2005).

Due to large variability no long-term temporal trend could be detected. Most of the data (about 87%) lay under the method detection limit of 5.1 mg/kg dw.

Heavy metals in sediments

Determination of heavy metals in sediments (Cd, Cr, Cu, Ni, Pb and Zn) have been added to the environmental monitoring program in 2003. Surface layer (~ 1 cm) of sediments was sampled for heavy metal analysis.

Fig. 5 presents metal concentrations in sediments in three areas: transitional waters (Station 4), coastal zone (Stations 6 and N-9) and open sea (Station 65).

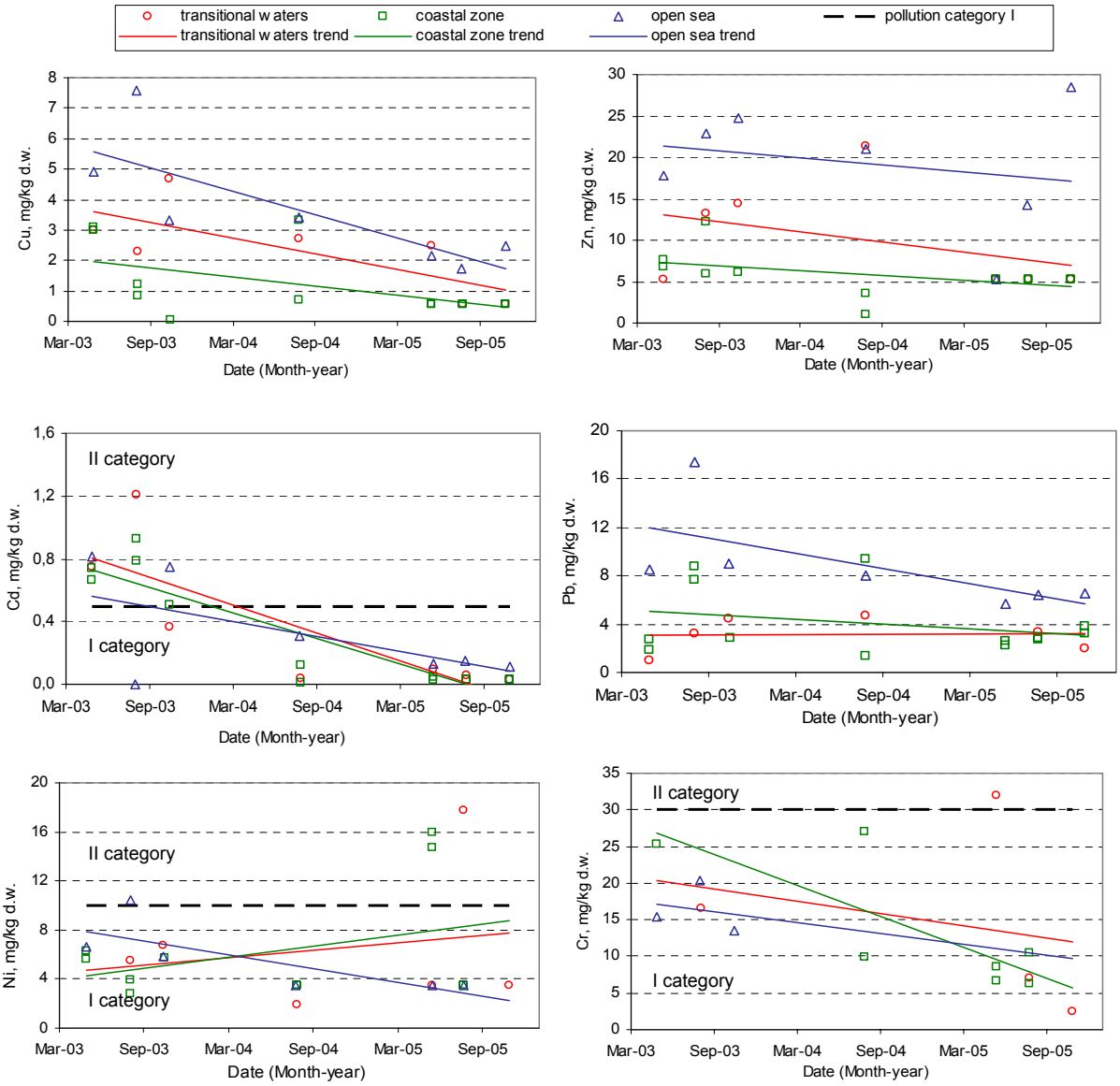

Figure 5. Concentrations of heavy metals (mg/kg dry weight) in sediments in 2003–2005 in transitional waters (the plume zone) (station 4, ~ 7 measurements for each metal), coastal zone (stations 6 and N-9, ~ 13 measurements for each metal) and open sea (station 65, ~ 7 measurements for each metal).

According to the Lithuanian legislation document (Order of Minister of Environment of Lithuania) on "Sediment dredging in sea and sea-port areas and dredged sediment treatment rules" [Žin., 2002, Nr. 27-976] there are 4 categories of sediments according to pollution by heavy metals and oil hydrocarbons. Concentrations of Cu, Zn and Pb in the study region fall within the cleanest category I (< 10 mg/kg dw for Cu; < 60 mg/kg dw for Zn; < 20 mg/kg dw for Pb). Cd concentrations in 2003 have exceeded the values of the category I (< 0.5 mg/kg dw) up to 1.2 mg/kg dw. However, in 2004-2005 all Cd values were inside category I. There are only some Ni and Cr values above the limit of the pollution category I (< 10 mg/kg dw for Ni and < 30 mg/kg dw for Cr) (Fig. 5).

There are decreasing concentration trends for almost all metals (except Ni) in all three areas. However, the shortage of data permits the making of more covering conclusions.

For some metals (Cu, Pb and Zn,) the concentrations are higher at the open-sea station (65) compared to the other stations. This can be due to sediment type differences. According to sediment type data (see Geology part of the report) the sediment type at station 65 is coarse grained silt (medium grain size is 0.085), other stations (4, 6 and N-9) has fine grained sand (medium grain sizes are 0.133, 0.140 and 0.,132 respectively).

Concentration ranges (mg/kg dw) for each metal for the period of 2003–2005 are presented in Table 1.

Table 1. Heavy metal concentration ranges in sediments of the stations 4, 6, N-9 and 65 for the period of 2003–2005.

Cu	**Zn**	**Pb**	**Cd**	**Ni**	**Cr**
		mg/kg dry weight			
<1.1–7.6	<10–28	1.0–17	<0.05–1.2	<6.9–18	2.4-32

References

HELCOM, 1996. Third periodic assessment of the state of marine environment of the Baltic Sea, 1989–93; Background document. Baltic Sea Environment Proceedings No. 64 B.

Pikkarainen, A.-L., Lemponen, P., 2005. Petroleum hydrocarbon concentrations in Baltic Sea subsurface water. Boreal Environment Research 10, 125–134.

Žin. 2002, Nr. 27-976. Order of Minister of Environment of Republic of Lithuania on LAND 46-2002 "Sediment dredging in sea and sea-port areas and dredged sediment treatment rules" (26.02.2002, Nr. 77).

Žin., 2006, Nr. 59-2103. Order of Minister of Environment of Republic of Lithuania "Wastewater treatment regulation" (17.05.2006, Nr. D1–236) (in Lithuanian).

2.3 Investigations in November 2005

According to the research plan of the current project, specified parameters were agreed to be studied in November – December 2005 by the independent experts coordinated by FIMR. The measured parameters were

(1) bottom geology and geography
(2) total hydrocarbons and PAHs in biota and sediments
(3) heavy metals in biota and sediments
(4) organotins in biota and sediments
(5) alkylated pehenols in sediments
(6) biological effects in biota.

In addition, other including histopathology and external fish diseases and sampling of flounder also in 2006 were included in the study programme.

This sampling occasion was continuation to ongoing monitoring activities in the region. However, it also served as a starting point for the future monitoring of some special parameters in order to follow the environmental conditions in Lithuanian waters adjacent to the D-6 oil field in the Russian continental shelf. The station network is based on the standard monitoring stations in the region, where long-term data exists.

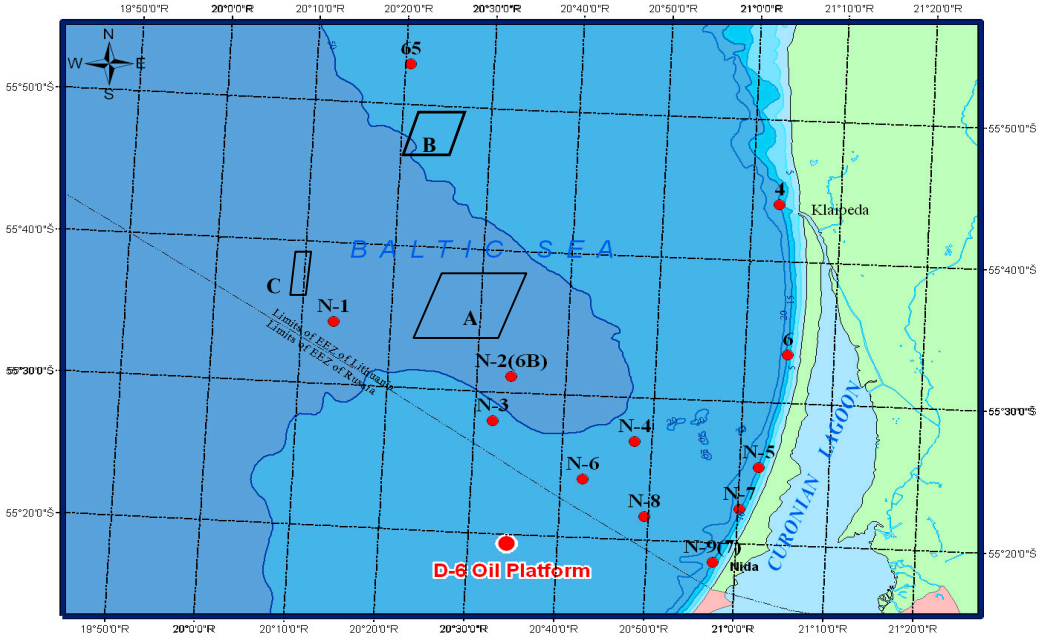

Figure 1. Map of the sampling stations (red dots) and trawling sites, areas A and B in November and December 2005 and area C in April 2006.

Table 1. Sampling stations and trawling sites in 2005–2006. 10-years data (1995–2004) from stations marked in bold. Regular sampling at other stations has started later in 2000's.

Station code	Latitude	Longitude	Depth (m)	Sediment type
N-1	55°34.5	20°13.5	70	Silty mud, fine grained
N-2 (6B)	55°32.2	20°33.8	65	Fine grained sand
N-3	55°28.0	20°32.0	42	Coarse grained sand
N-4	55°27.0	20°48.0	33	Coarse grained sand
N-5	55°25.5	21°02.1	13	Fine grained sand
N-6	55°24.3	20°42.4	36	Fine grained sand
N-7	55°22.5	21°00.1	15	Fine grained sand
N-8	55°21.7	20°49.5	37	Medium grained sand
N-9 (7)	55°18.7	20°57.4	14	Fine grained sand
4	55°44.1	21°03.0	16	Fine grained sand
6	55°33.5	21°04.7	13	Fine grained sand
65	55°52.9'	20°20.5'	47	Coarse grained silt

Trawling sites. Coordinates for the begin and end of a trawl indicated

55.3286N, 20.3024E – 55.3689N, 20.2710E	9 December 2005 (Area A)
55.3974N, 20.2763E - 55.3607N, 20.2321E	9 December 2005 (Area A)
55.3491N, 20.2768E - 55.3926N, 20.2779E	9 December 2005 (Area A)
55.4756N, 20.2770E – 55.4990N, 20.2079E	9 December 2005 (Area B)
55.3820N, 20.1000E - 55.3670N; 20.1000E	4 April 200 (Area C)

2.3.1 Temperature and salinity conditions in November 2005

Light wind (3-4 m/s) from south-western directions blew at the beginning of the expedition in November 2005. Later the wind turned to the southeast and increased in strength to 6-10 m/s. Waves height increased from 0.5 to 2 m during the course of the cruise. Atmospheric pressure fluctuated insignificantly: between 1029 and 1031 hPa. Air temperature at the time of the research varied from 9 to 11°C, and humidity – from 76 to 95%. The sky was entirely covered by low-hanging clouds. Visibility changed from 8 to 20 km. Water transparency was 4-4.5 m**.**

Salinity

Water salinity in the surface layer (Figure 2) of the transitional water zone (Station 4) was 6.95, one of the highest values when compared to the seasonal expedition data of 1995-2005. With increasing depth the water salinity increased and reached 7.2 at the near bottom layer (Fig. 3).

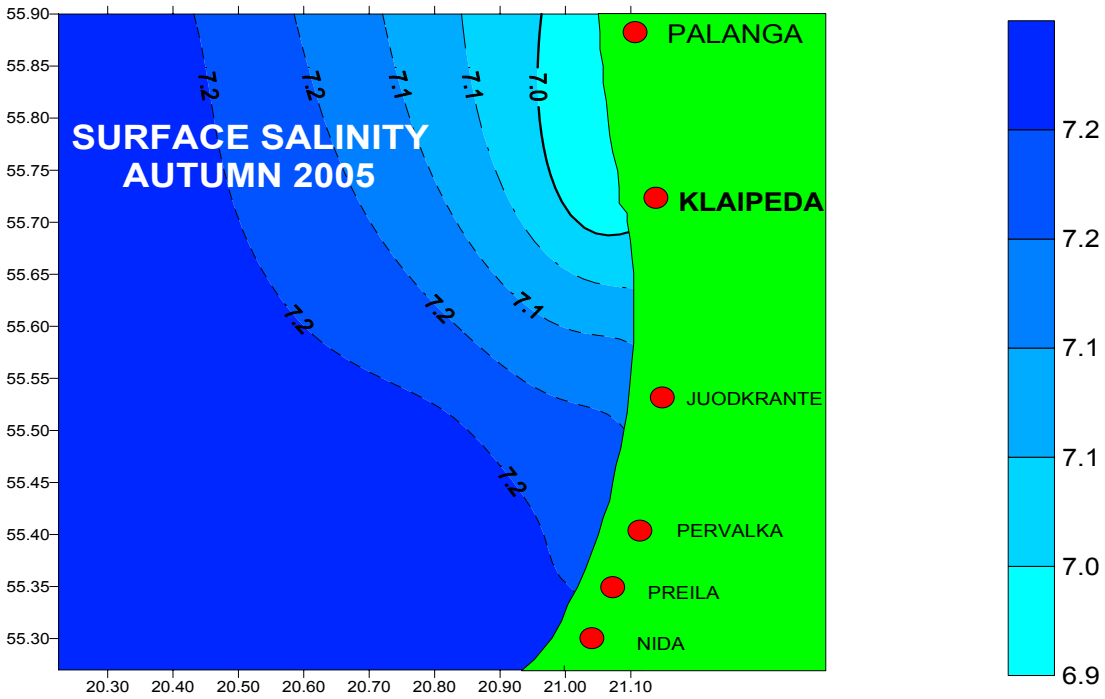

Figure 2. Surface salinity distribution in November 2005.

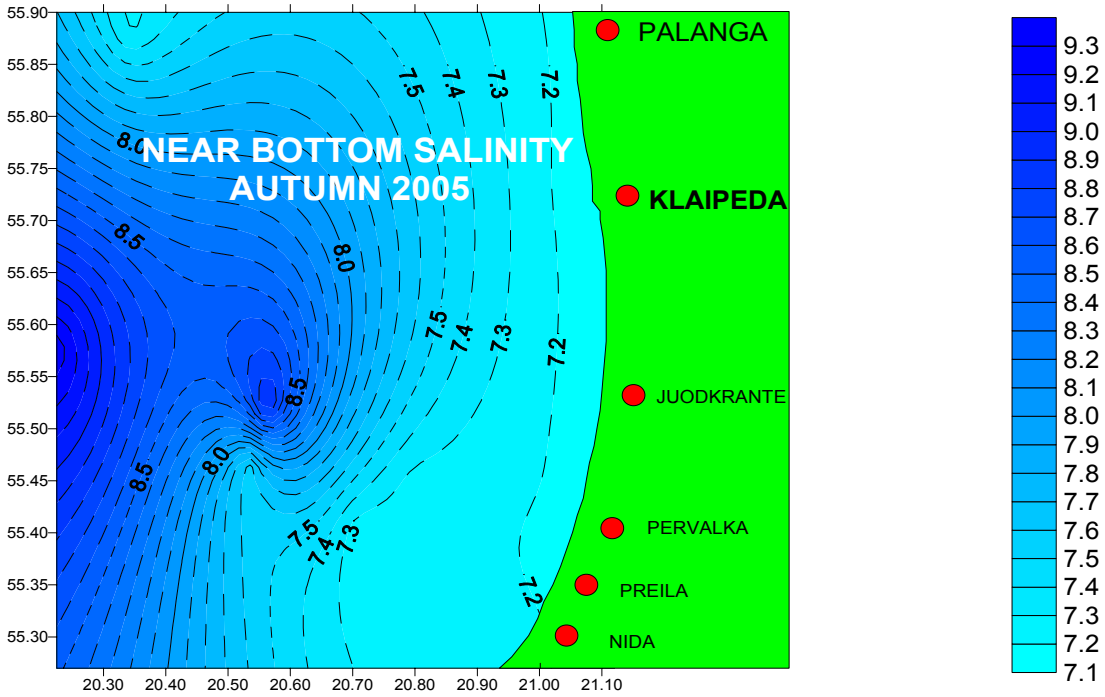

Figure 3. Near bottom salinity distribution in November 2005.

Water salinity in the surface layer of the coastal water zone (Stations N-4, N-5, N-6, N-7, N-8, N-9 and 6) fluctuated between 7.1 and 7.2 being 0.3–0.4 units higher than the mean water salinity measured during the 1995-2005 seasonal expeditions. Such a high water salinity was caused by the absence of significant outflows from the Curonian Lagoon. The lowest salinity (7.13) in the surface layer was in the coastal zone near Juodkrante, and the highest (7.24) level was in the area that was further off the coast. As that area was not deep, water salinity increased insignificantly, by 0.01 ‰, as the depth increased. Water salinity in the near bottom water increased the same way as in the sea surface – from the coast towards the open sea (Fig. 2).

Water salinity in the surface water layer in the open sea area (Stations N-1, N-2, N-3 and 65) was equal everywhere (7.2). In the autumn of 2005 surface water salinity was by 0.2 units higher than the mean water salinity during the seasonal expeditions in 1995-2005. Water salinity was almost equal until the depth of 50 m reaching 9.38 in the deepest area under investigated (near bottom water at N-1 station).

Temperature

Water temperature recorded during the 2005 autumn expedition (Fig. 4) was close to the mean water temperature of the 1995–2005 autumn expeditions. Water temperature of the surface layer in the transitional water zone was 9.4 °C, while in the coastal zone it varied from 9.7°C to 11.1°C, and in the open sea it remained between 10.6°C and 10.8°C. Water temperature in the sea surface increased from the coast towards the open sea. The lowest temperature of the surface water layer (9.66 °C) was recorded in the transitional water zone, near Klaipeda (Station 4) and maximum (11.07°C) was in the coastal zone (Station N-4).

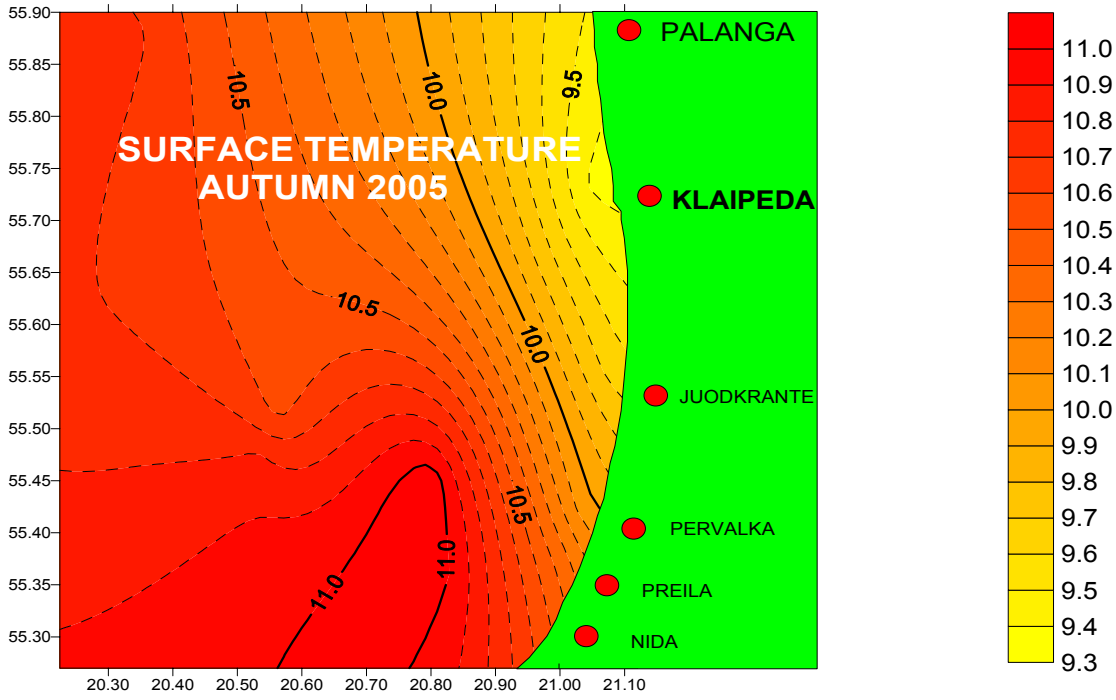

Figure 4. Surface temperature distribution in November 2005.

No thermocline was observed in the areas with the water depth < 40 m. Below that depth, the temperature was lower and at the depth of 60 metres it was <4°C. Water temperature of the transitional water only increased as the colder water from the Curonian Lagoon was in the surface layers. The lowest water temperature (3.32°C) was measured at the depth of 53 m at station N-1. Deeper water temperature increased and exceeded 4°C in the near bottom. Water temperature in the near bottom layer decreased from the coast towards the deeper sea areas (Fig. 5).

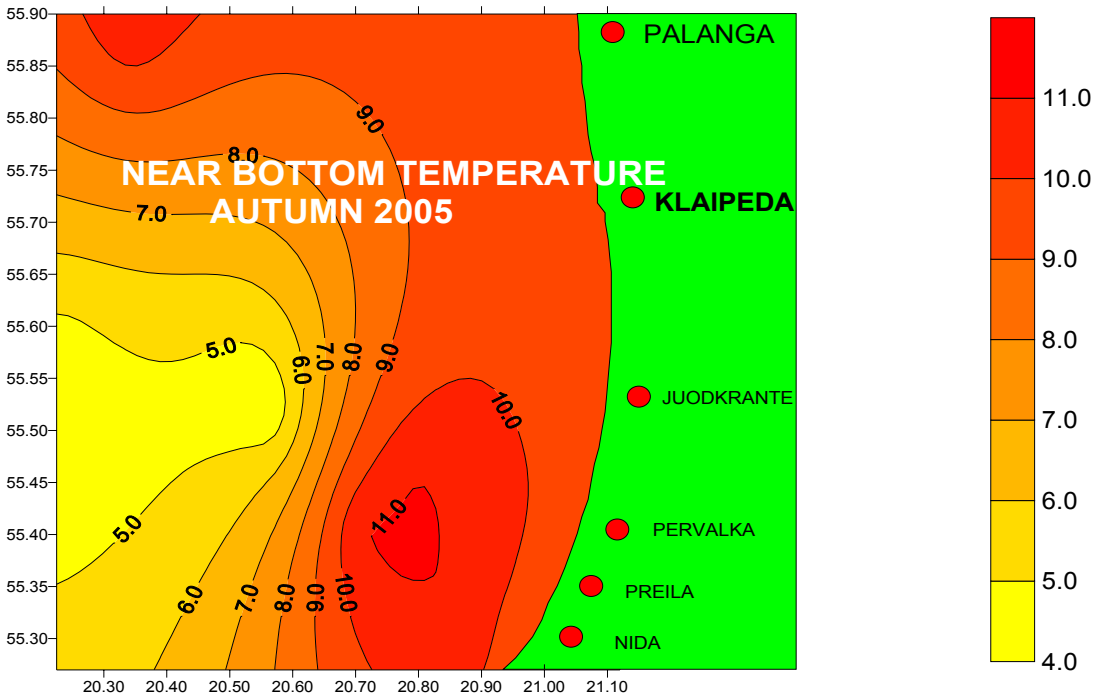

Figure 5. Near bottom temperature distribution in November 2005.

2.3.2 Hydrochemistry

The division of the study region to different areas was the same as before. In November 2005 additional sampling sites were included in the programme: transitional waters (Station 4), coastal zone (Stations 6, N-4, N-5, N-6, N-7, N-8, and N-9) and open-sea area (Stations 65, N-1, N-2 and N-3). (*See also Appendix 2, Table 1*).

Oxygen

The mean concentration of oxygen in the surface waters in all study areas was 7.3 ml/l (Fig. 6). This is close to the mean long-term (1995-2005) autumn concentration.

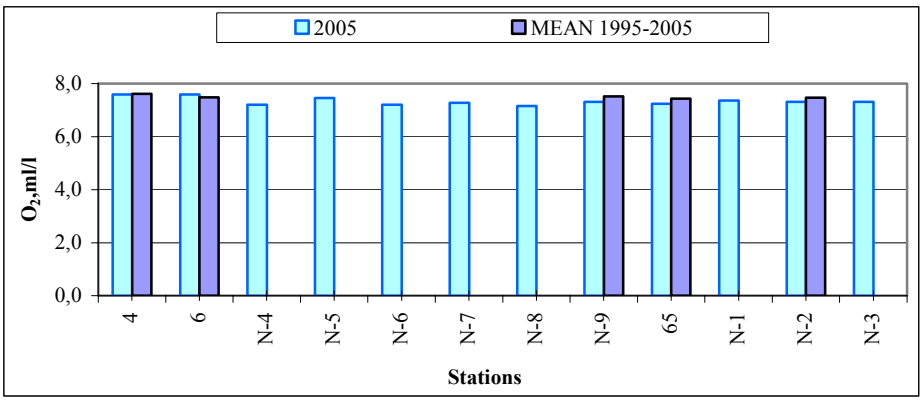

Figure 6. Oxygen concentration in surface waters in autumn 2005 and mean values between 1995–2005.

Decreased values (below 3 ml/l) were observed only in the near bottom layer of the open sea (below 60 m) at Stations N-1 and N-2. Compared to the mean long-term (1995–2005), oxygen concentration, it showed a decreased level in the near bottom layer of the open-sea area (Station N-2) (Fig. 7).

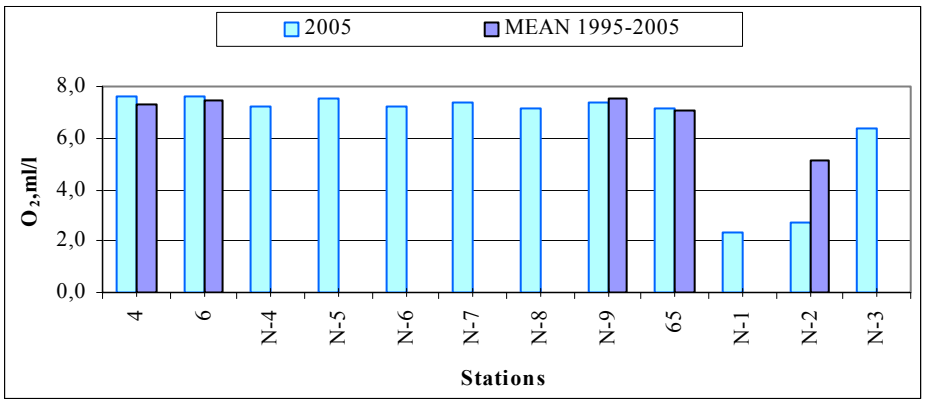

Figure 7. Oxygen concentration in the near-bottom water layers in autumn 2005 and mean values between 1995–2005.

The pH value of the surface layer fluctuated between 8.19 and 8.32. The mean pH value in the surface layer was 8.24, close to the mean long-term average (Fig. 8).

Slightly lower of pH values (7.61–7.79) were found only in the near-bottom layer of the open sea (below 60 m) at Stations N-1 and N-2 (Fig. 9).

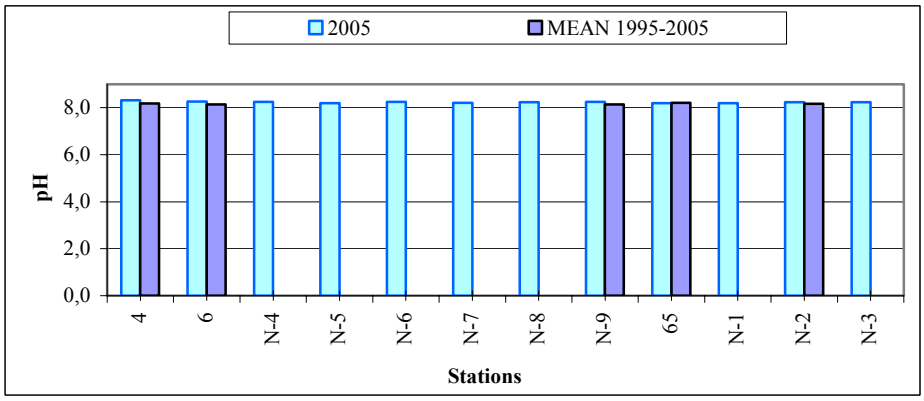

Figure 8. pH values in surface waters in autumn 2005 and mean values between 1995–2005.

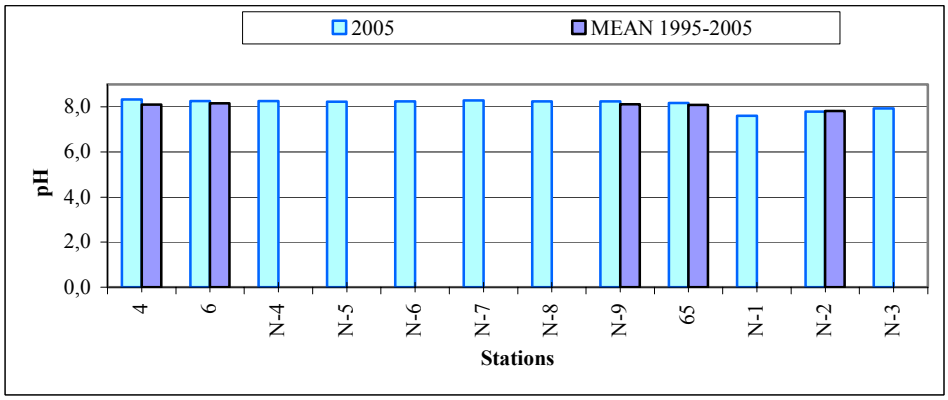

Figure 9. pH in near-bottom water layers in autumn 2005 and mean values between 1995–2005.

Nutrients

Dissolved inorganic phosphorus (DIP)

At the time of the cruise, the highest DIP concentration (0.98 µmol/l) was found in the surface of the transitional water at Station 4 (Figure 10). In comparison to the mean long-term (1995-2005) concentrations, the autumn DIP concentrations were higher in most parts of the area under investigation.

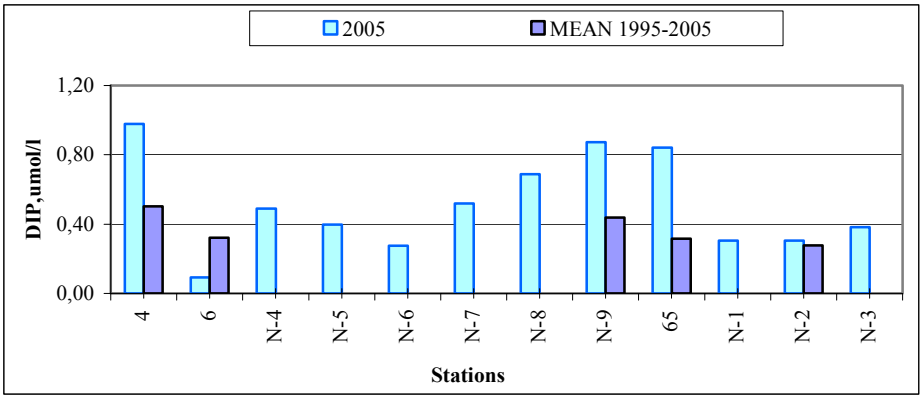

Figure 10. Dissolved inorganic phosphorus (DIP) concentrations in surface waters in autumn 2005 and mean values between 1995–2005.

DIP concentrations in the near-bottom layer concentrations (2.7 µmol/l; 2.9 µmol/l) were observed at Stations N-1, N-2 of the open sea-area (deeper than 60 m, Fig. 11).

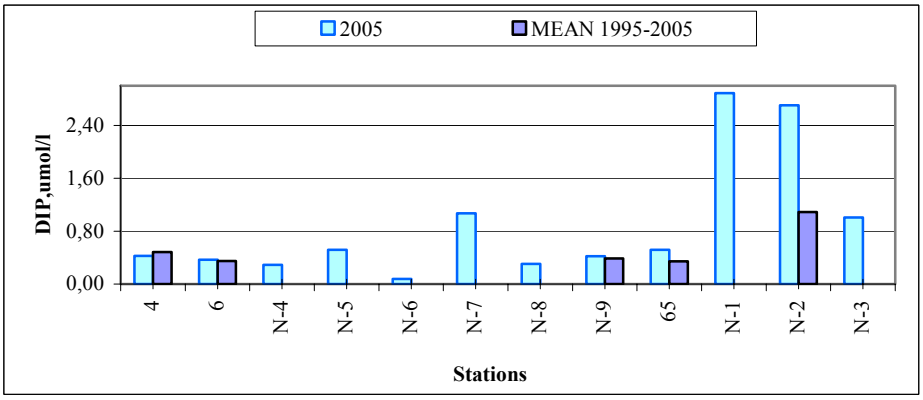

Figure 11. Dissolved inorganic phosphorus (DIP) concentrations in near-bottom waters in autumn 2005 and mean values between 1995–2005.

Total phosphorus (TP)

The concentration of TP (1.2 µmol/l) in the surface was determined in transitional water (Station 4) and coastal water (Station N-7). In comparison with the mean long-term (1995–2005) concentrations, the concentration of TP in the autumn of 2005 was higher. (Fig. 12).

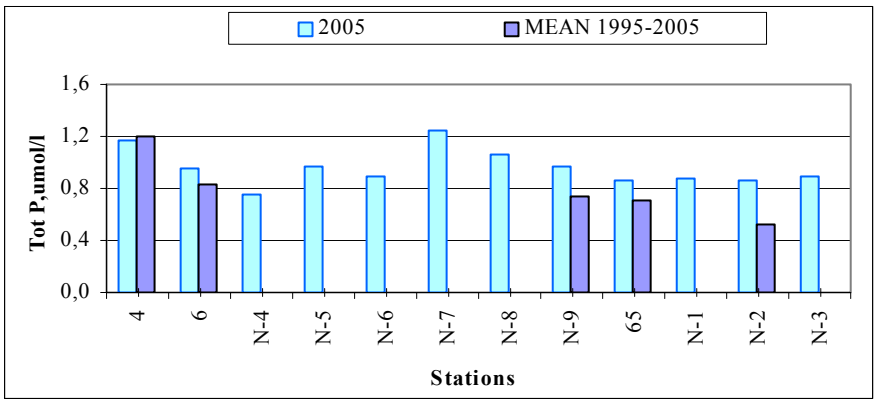

Figure 12. Total phosphorus (TP) concentrations in surface waters in autumn 2005 and mean values between 1995–2005.

The highest concentration of TP (2.9 µmol/l) in the near-bottom layer was significantly higher than the mean long-term one, and was determined in the open sea (stations N-1 and N-2), deeper than 60 m (Fig. 13).

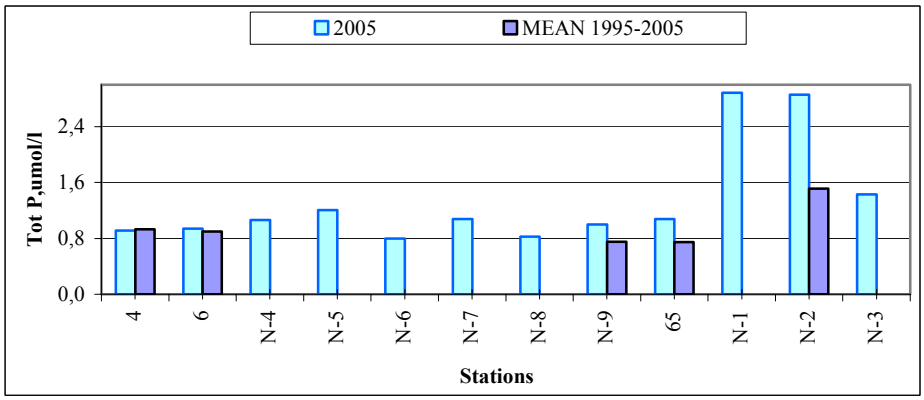

Figure 13. Total phosphorus (TP) concentrations in near-bottom waters in autumn 2005 and mean values between 1995–2005.

Dissolved inorganic nitrogen (DIN)

The concentration of dissolved inorganic nitrogen (DIN) was calculated as a sum of ammonia, nitrite and nitrate.

The concentrations of DIN in the surface layer of the area under investigation were higher than the mean of the long-term autumn concentrations. The highest concentration (ca. 16 µmol/l) was observed in coastal water (Station N-7) and the open-sea area (Station N-3) (Fig. 14).

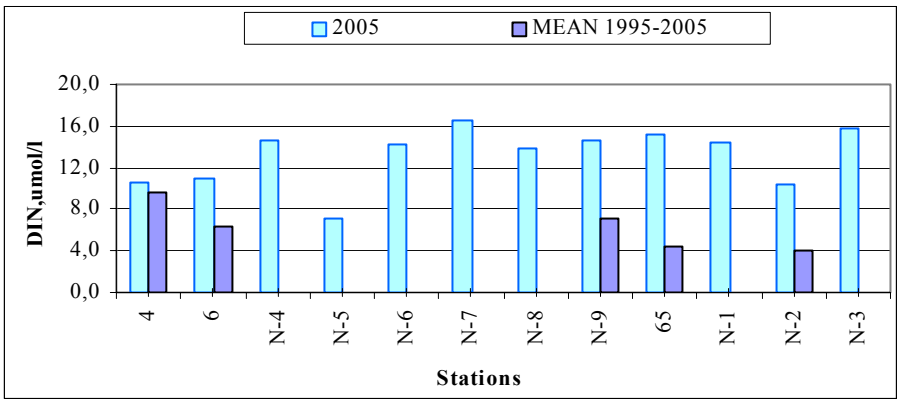

Figure 14. Dissolved inorganic nitrogen (DIN) concentrations in surface waters in autumn 2005 and mean values between 1995–2005.

DIN (19 µmol/l) in the near-bottom water was observed in the deepest (67 m) place of the open sea under investigation. (Station N-1, Fig. 15).

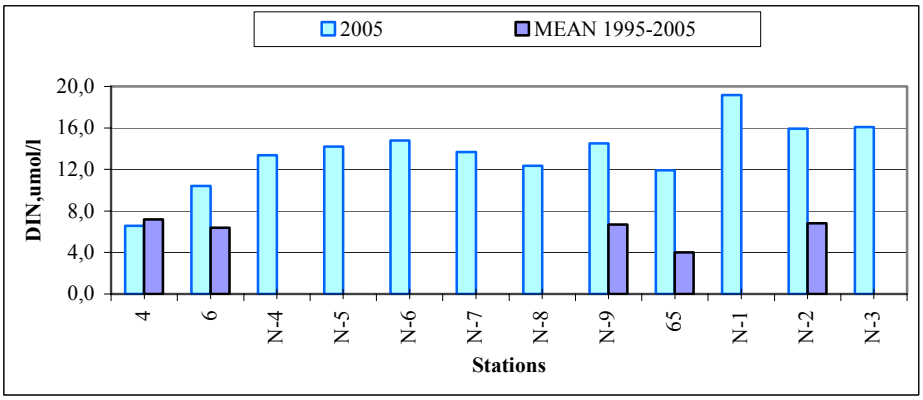

Figure 15. Dissolved inorganic nitrogen (DIN) concentrations in near-bottom waters in autumn 2005 and mean values between 1995–2005.

Total nitrogen (TN)

During the autumn cruise of 2005the TN concentrations were lower than the mean long-term autumn concentrations. The highest concentration (above 25 µmol/l) in the surface water was observed in the coastal water (Station N-8) and the open-sea area (Stations N-1 and N-2) (Fig. 16).

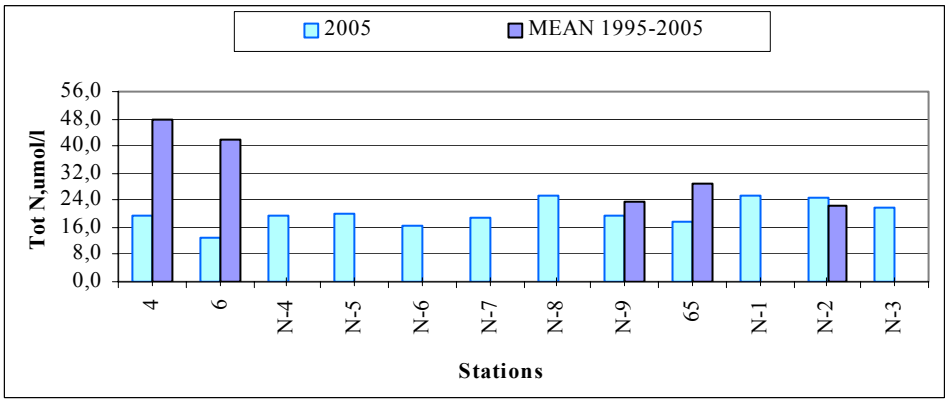

Figure 16. TN concentrations in surface waters in autumn 2005 and mean values between 1995–2005.

The highest concentration of TN (50 µmol/l) in the near-bottom waters was observed in the coastal area (Station N-6) (Fig. 17).

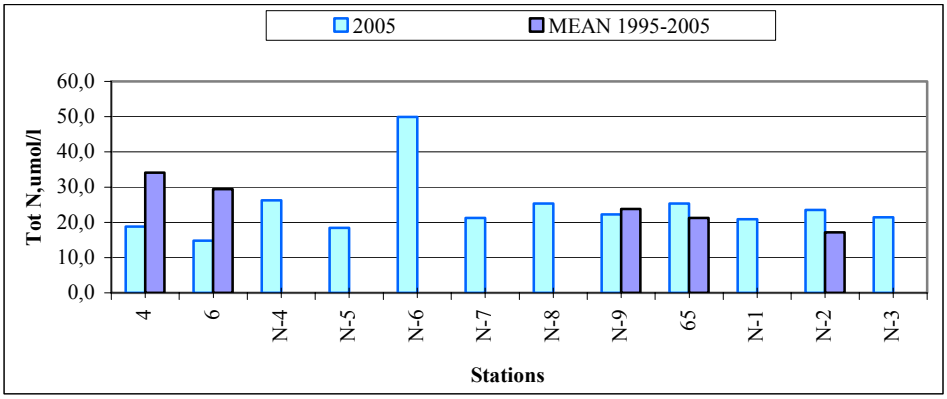

Figure 17. TN concentrations in near-bottom waters in autumn 2005 and mean values between 1995–2005.

Silicate

The concentration of silicate in the surface layer of the most part of the area under investigation were lower than the mean long-term concentration. Only in the open-sea area (Station 65) the concentration was higher than the long-term average reaching 12 µmol/l. Similar concentrations of silicate were found in coastal waters (Stations N-7 and N-8) while in the open-sea area were lower (Fig. 18).

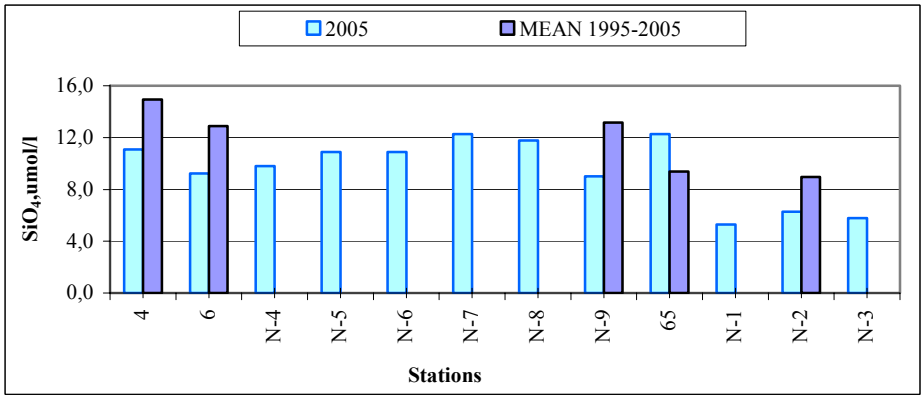

Figure 18. Silicate concentrations in surface waters in autumn 2005 and mean values between 1995–2005.

The highest concentrations of silicate in the near-bottom water were determined in the open-sea area (deeper than 60 m) at Station N-2 (37 µmol/l), and station N-1 (35 µmol/l) (Fig. 19).

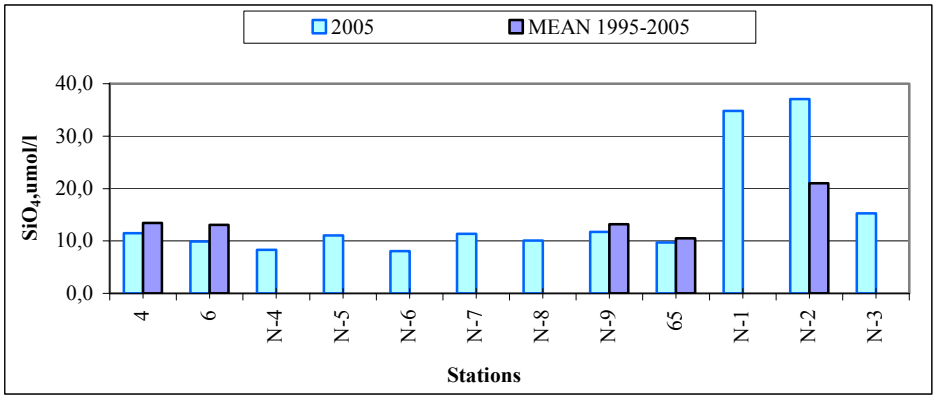

Figure 19. Silicate concentrations in near-bottom waters in autumn 2005 and mean values between 1995–2005.

2.3.3 Bacterioplankton in November 2005

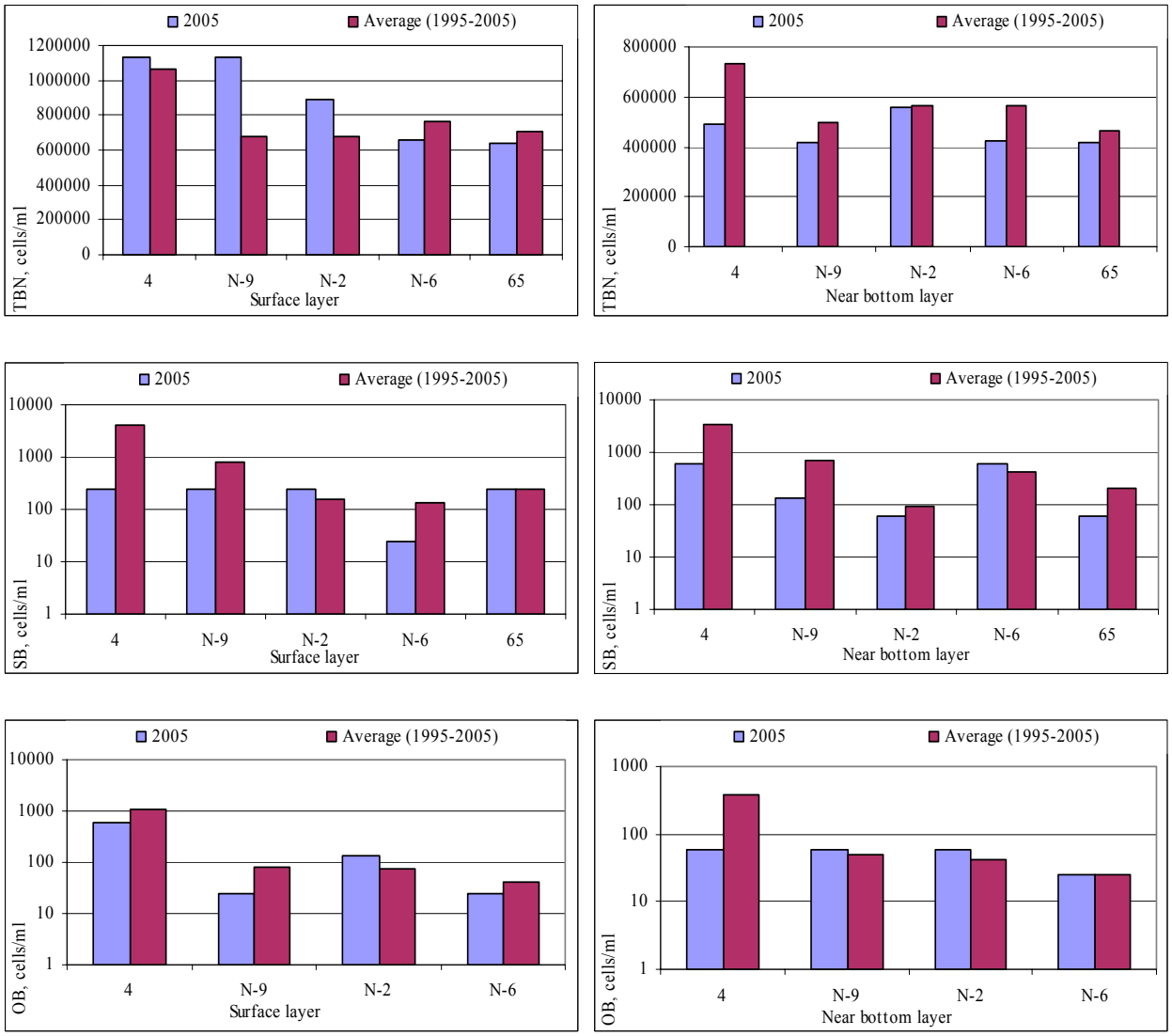

Figure 20. Comparison of long-term autumn averages with the corresponding values measured in November 2005. TBN – total bacterial number, SB – saprophytic bacteria, OB – oil-oxidizing bacteria. Data for the comparison: Stations 4 and N-9, 1995-2005, Stations N-2 and N-6 2004–2005.

In autumn 2005 the level of total bacterial numbers (TBN) was mostly higher than the average (1995-2005) in the surface layer, but lower in near-bottom layer (Fig.20) Level of saprophytic bacterial numbers (SB) was lower than the long-term average. The highest concentration of oil-oxidizing bacteria (OB) was found in the waters influenced by the Curonian Lagoon. However, the results on bacterioplankton from autumn 2005 are statistically in same line as the results of the long-term averages during 1995-2005.

2.3.4 Phytoplankton and chlorophyll a in November 2005

Phytoplankton

In November 2005 the abundance of phytoplankton was not high, 1491.7 x 10^3 cells/l and biomass 4.3 mg/l in the transitional waters (the plume area). The most abundant groups in this area were Cyanophycea, Cryptophycea and Diatomophycea.

In the coastal zone the average phytoplankton abundance was lower than in transitional waters being 1123.8 x 10^3 cells/l and biomass 7.6 mg/l. In the open-sea area the abundance of phytoplankton was even lower (250.8 x 10^3 cells/l, biomass 2.0 mg/l). In the coastal zone phytoplankton taxonomic classes were Prasinophyceae, Chlorophyceae and Cryptophyceae, while in the open-sea area Cryptophyceae and Chlorophyceae dominated.

In the beginning of November 2005, a mass development of marine species *Cerataulina pelagica, Chaetoceros brevis, Dactyliosolen fragilissimus* (*=Rhizosolenia fragilissima*) was observed in the Lithuanian waters with a total density of up to 0.5 x 10^3 cells/l and biomass of 3.8 mg/l. These species are known as marine species with brackish-water affinity, occurring in the Kattegat and Öresund, but almost absent in the Baltic Sea (Snoeijs and Vilbaste 1994, Hällfors, 2004). They have never before been observed in the southeastern part of the Baltic Sea. According to the monitoring data these species were present in all (20) samples collected in the Lithuanian coastal waters between 3–9 November 2005. The water salinity in the area ranged from 6.27 to 7.26 and water temperature from 8.98 to 10.8ºC. All these species were more abundant at the near shore sampling sites, with the highest cell density found 4 km off the shore at the salinity of 6.55 (Fig. 2). The aggregate abundance of these three species reached up to 33 % (16% ± 10%) of the total phytoplankton abundance and up to 82% (59% ± 24%) of the total biomass. *D. fragilissimus* had the highest abundance (Fig. 21, Table 2).

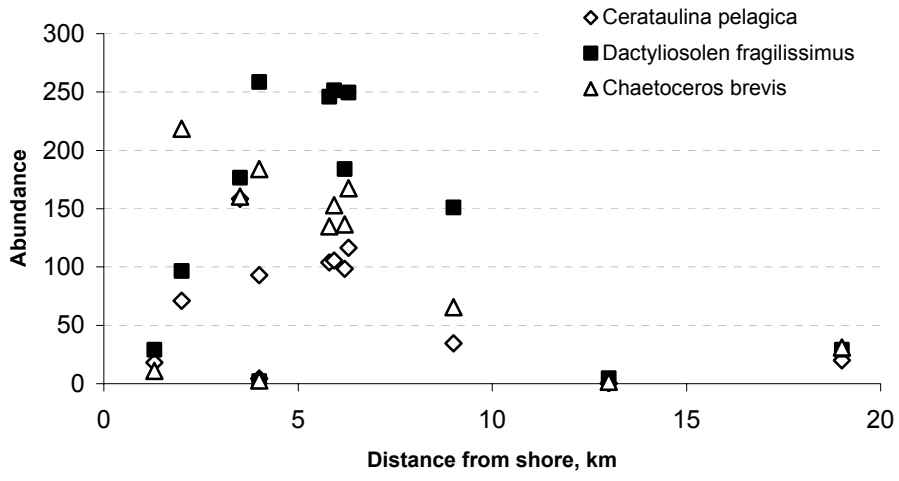

Figure 21. Abundance (cells 10^3 l^{-1}) of *Cerataulina pelagica, Dactyliosolen fragilissimus* and *Chaetoceros brevis* in the Lithuanian coastal waters in November 2005.

The penetration mechanism of these marine phytoplankton species from the Kattegat–Öresund area to the Baltic Proper is not clear, but could be due to a transportation of saline water from the Kattegat or transfer in ballast waters of ships.

Table 2. Abundance and biomass of marine species *Cerataulina pelagica*, *Dactyliosolen fragilissimus* (=*Rhizosolenia fragilissima*) and Chaetoceros brevis in November 2005.

	Abundance, 10^3 cells l^{-1}			Biomass, $mg \cdot l^{-1}$		
	MIN	max	mean	min	max	mean
Cerataulina pelagica	0.3	158.5	68.7 ± 51.6	0.004	1.36	0.57 ± 0.45
Dactyliosolen fragilissimus	2.2	258.7	139.9±103.1	0.03	2.97	1.51±1.12
Chaetoceros brevis	1.7	218.6	105.6±78.3	0.006	0.90	0.39±0.32

Chlorophyll a

Chlorophyll *a* (chl *a*) concentrations in November were lower than in summer being lower in the coastal zone than in the open-sea area. As common, the highest chll *a* concentrations were observed in transitional waters or in the plume area influenced by the Curonian Lagoon (Station 4) (Fig. 22).

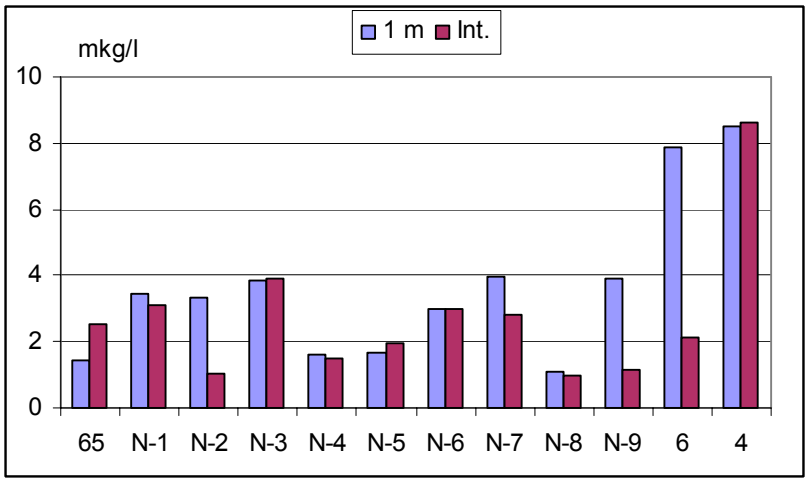

Figure. 22. Chlorophyll *a* concentrations (µg/l) in the depth of 1 m and in the integrated layer 1-10 m in November 2005.

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2.3.5 Zooplankton in November 2005

In autumn 2005, copepoda-nauplii made 20–60 % of total zooplankton abundance in study region. The importance of *Copepoda* declines but *Rotifera* elevates towards the coastal zone (Fig. 23, Appendix 2, Table 8).

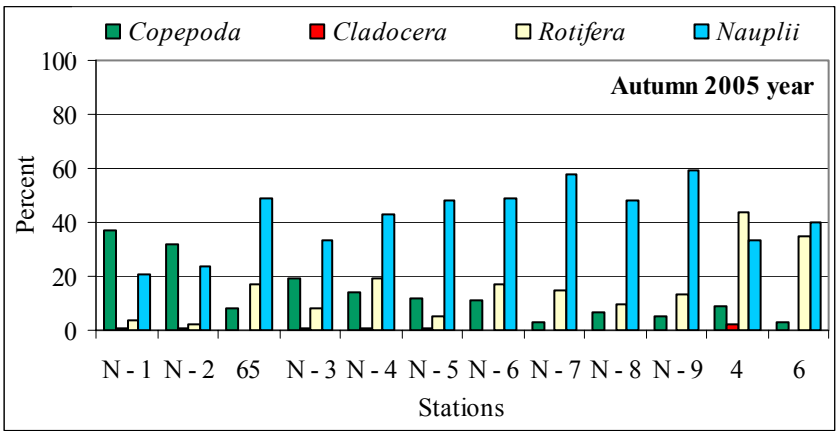

Figure. 23. Distribution of zooplankton taxonomic groups in autumn 2005.
Nauplii = Copepoda nauplius stage.

2.3.6 Macrozoobenthos in November 2005

The samples for macrozoobenthos studies were taken from 11 stations located in five regions, in view of the depth, distance from the coast, and distance from the mouth of the Curonian Lagoon.

Eighteen taxons were found in the research regions: *Halicryptus spinulosus, Pygospio elegans, Marenzelleria viridis, Nereis diversicolor, Harmothoe sarsi,* Oligochaeta, *Monoporeia affinis, Pontoporeia femorata, Gammarus* sp., *Saduria entomon,* Ostracoda, *Crangon crangon, Hydrobia, Mya arenaria* sp., *Macoma balthica, Cardium glaucum, Electra crustulenta* and *Chironomidae* (Appendix 2, Table 6).

The transitional water zone (Station 4) is influenced by the Curonian Lagoon waters. Depth of this station is 15 m, sediments consists of sand and layer of black mud. At this station six taxons were found.

Average of total abundance of the macrozoobenthos community was 1460 ind./m^2, and biomass 6.429 g/m^2 (Fig. 24). *M. viridis* was dominated both by abundance (77% of total) and biomass (66% of total). *M. balthica* was a subdominant species by biomass consisting 17% of total zoobenthic biomass.

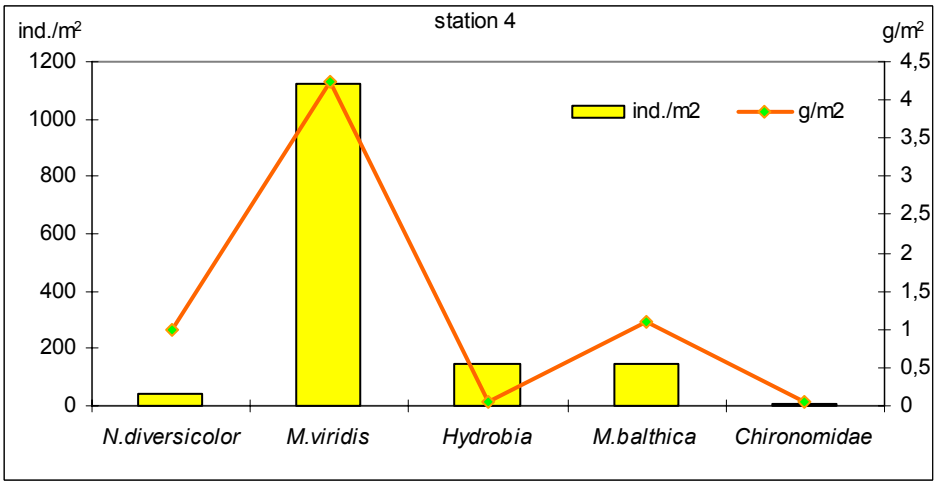

Figure 24. Average of abundance (ind./m^2) and biomass (g/m^2 ww) of the macrozoobenthos species in transitional water area in autumn 2005.

The coastal area with depths between 13–16 m was represented by Stations 6, N-5, N-7 and N-9. The sediments consist of fine-sand at all stations and fine sand with fine stones at Station N-9. Eight macrozoobenthic taxons were found in this area.

Average of total abundance of macrozoobenthos community in the coastal zone was 6880 ind./m^2 and biomass 208.223 g/m^2. *M. viridis* was the dominant species by abundance. *M. balthica* dominated by biomass, being also subdominant by abundance in this area (Figs. 25 and 26).

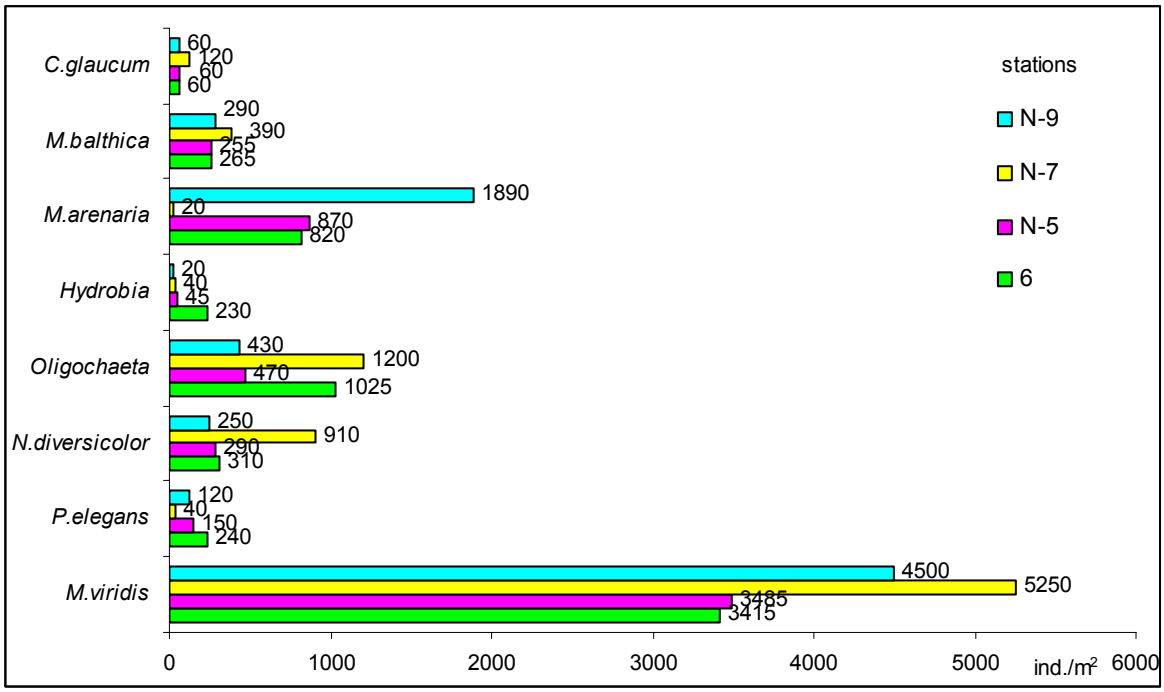

Figure 25. Averages of abundance (ind./m^2) of the macrozoobenthos species in the coastal waters in November 2005.

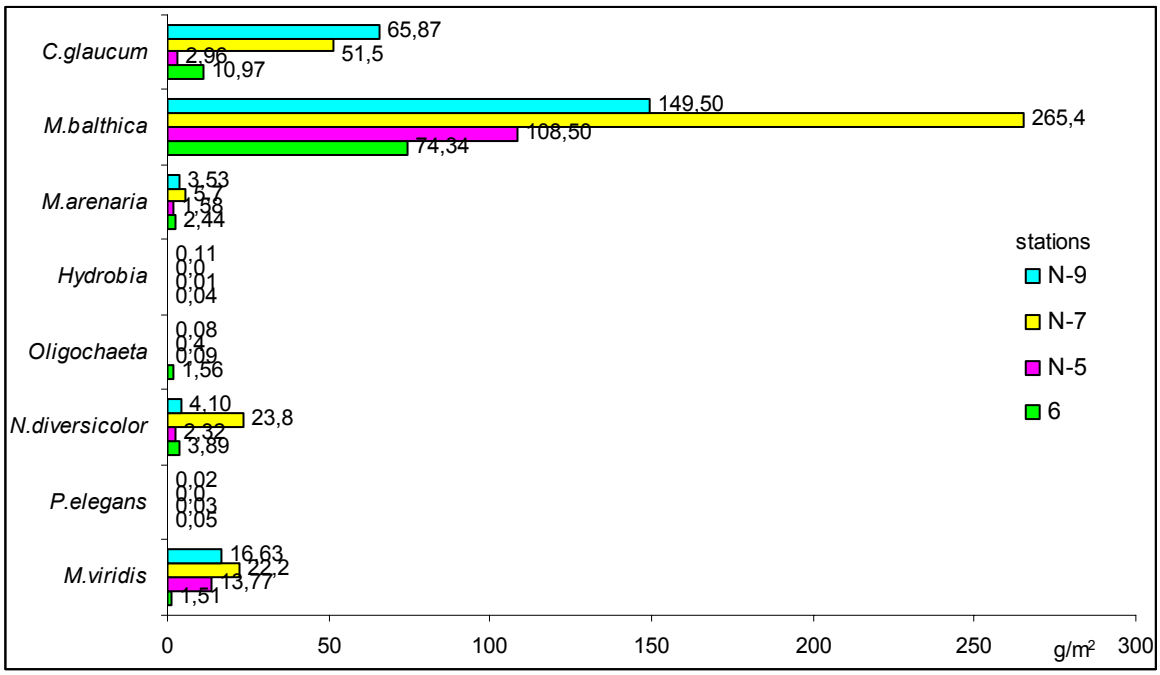

Figure 26. Averages of biomass (g/m^2 ww) of the macrozoobenthos species in the coastal area in November 2005.

Another area in the coastal zone characterized by the depth of 36–37 m is represented by Stations N-4, N-6, N-8. Eight macrozoobenthic taxons were found in coarse sand bottoms here.

Average total abundance of macrozoobenthos community in the deeper coastal zone was 666 ind./m^2 and biomass 30.90 g/m^2. *M. viridis* was dominant species by abundance, while *M. balthica* dominated by biomass and was subdominant by abundance (Figs. 27 and 28).

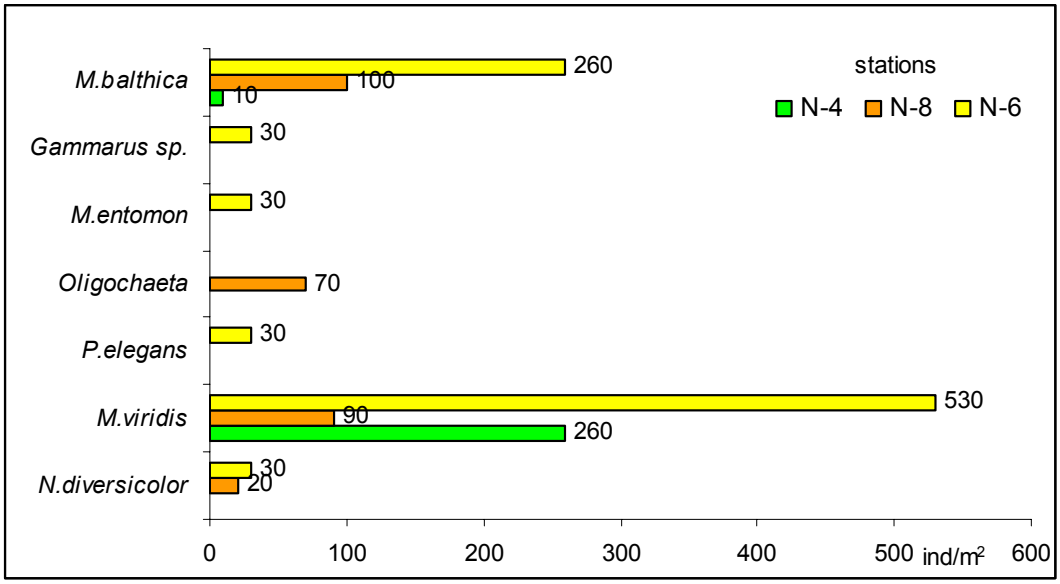

Figure 27. Average abundance (ind./m^2) of the macrozoobenthos species in the deeper coastal area in November 2005.

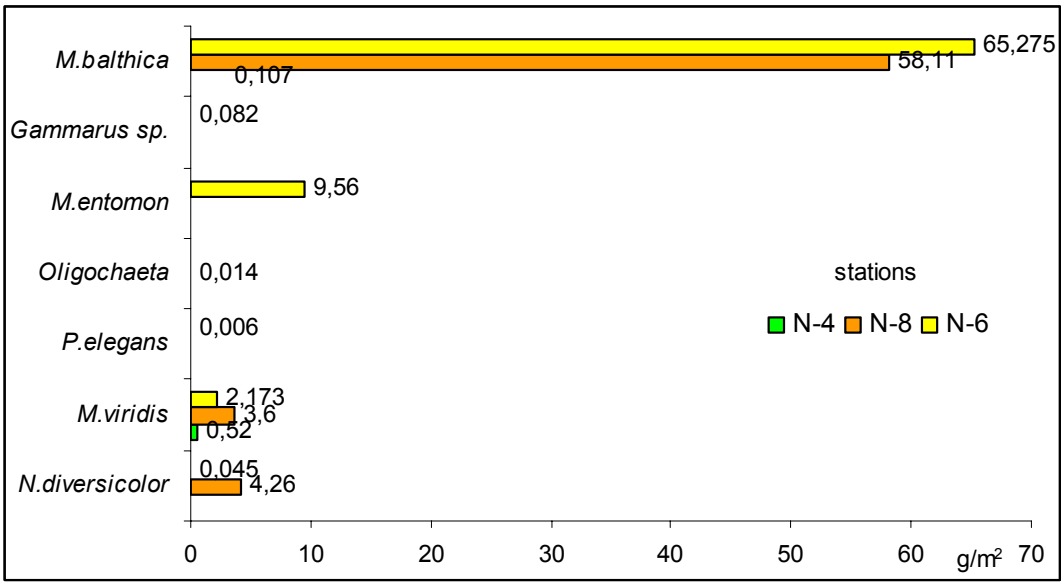

Figure 28. Averages biomass (g/m^2) of the macrozoobenthos species in the deeper coastal waters area in autumn 2005.

The open-sea area is represented by Stations 65 and N-2. Nine macrozoobenthos taxons were found at Station 65 (sand with detritus)(depth 47 m) and 10 taxons were found at Station N-2 (depth 66 m) (sand with more detritus than at Station 65).

Average total abundance of macrozoobenthos community was 1445 ind./m^2 at the station 65 and 940 ind./m^2 at Station N-2. Average of the total biomass was 129.84 g/m^2 at Station 65 and 67.51 g/m^2 at Station N-2. *M. baltica* dominated by abundance and biomass at both stations. *M. viridis, M. affinis* and Ostracoda were subdominant species/groups by abundance, and *S. entomon* by biomass (Figs. 29 and 30).

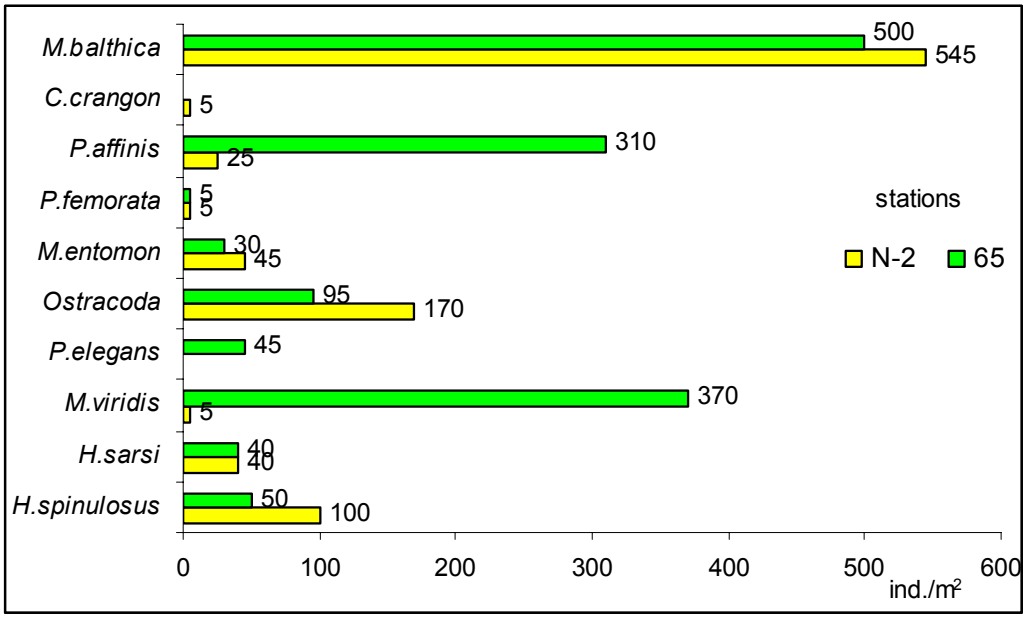

Figure 29. Average abundance (ind./m^2) of the macrozoobenthos species in the open-sea region in November 2005.

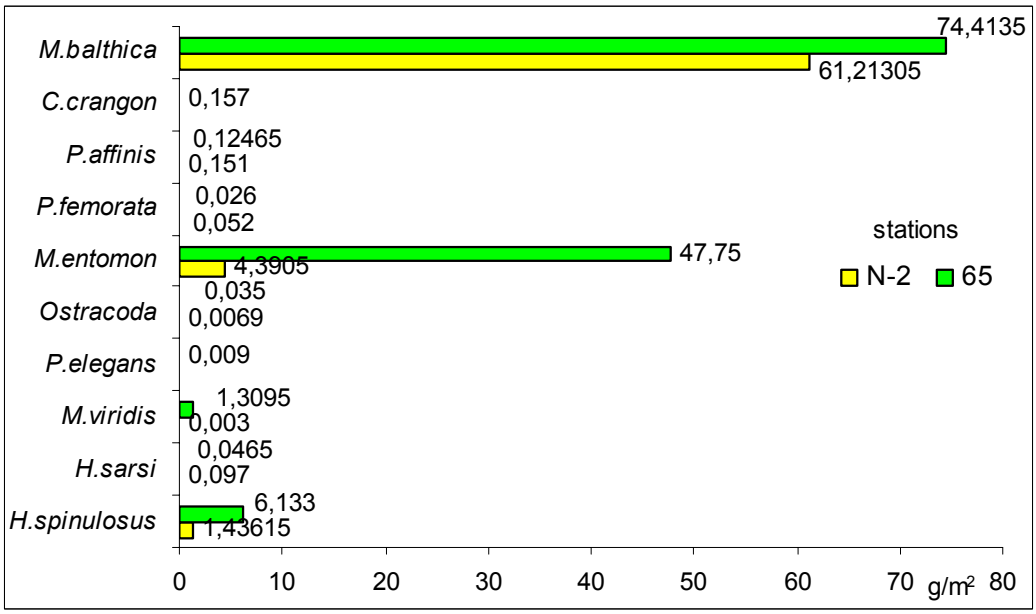

Figure 30. Average biomass (g/m^2 ww) of the macrozoobenthos species in the open-sea region in November 2005.

Only one species, *M. baltica*, was found at the deep open-sea Station N-1 in black aleurite bottom with an abundance of 10 ind./m^2 and biomass 2.971 g/m^2.

2.3.7 Harmful substances

Results on total oil hydrocarbons in water and sediments will be discussed in Chapter 3 (Ecotoxicology).

2.3.8 Geology

Fig. 31 shows that bottom sediments are mostly formed of coarse grain size particles. Only in the southern part, in the open sea zone (Stations N-1, R-7) areas of finer sediments are found with higher content of organic matter (see Chapter 1, Description of the area). Fig. 34 illustrates the type of bottom sediments in the research area and location of the research stations in regard of the bottom type.

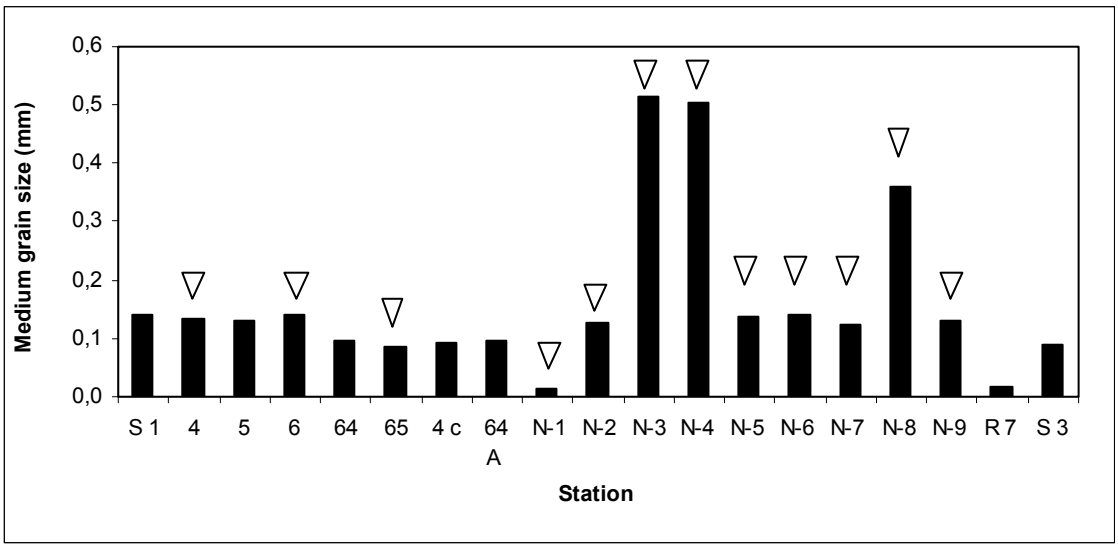

Figure 31. Medium grain size of the bottom sediments (0–3 cm). Stations including the standard monitoring programme are marked with an arrow.

Differences in the type of bottom sediments are substantial, which is illustrated in Fig. 32. Station N-1 is located in an area with muddy and silty sediments with a grain size < 0.16 mm. On the other hand, at Station N-3 the grain size is more evenly distributed and only ~ 1% belongs to the < 0.16 mm fraction (Figure N-1 and N-3).

Geological formations of the bottom and type and quality of sediments are important factors when results of chemical sediment analyses are interpreted. This information is also necessary in comparisons between values obtained from different areas. The importance of the sediment type and grain size becomes evident in metal and hydrocarbon contents of the bottom sediments in this study.

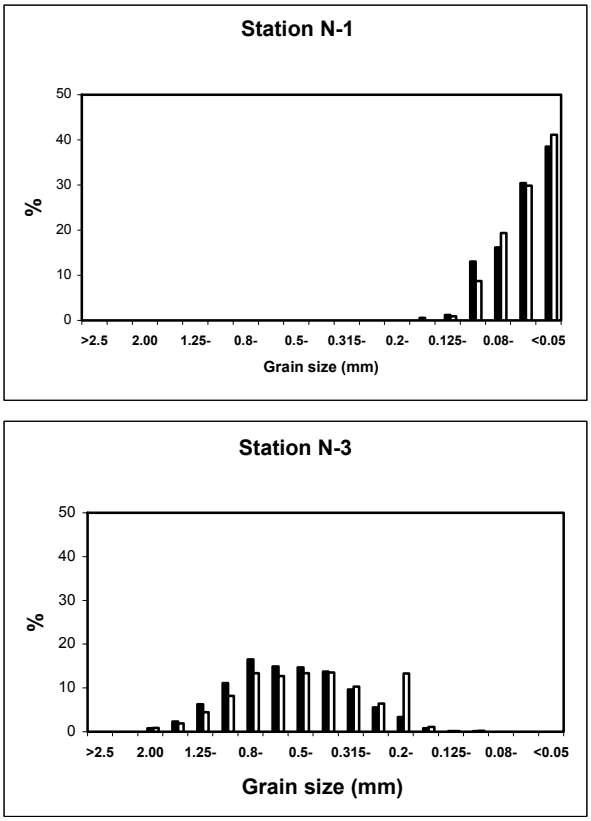

Figure 32. Grain size distribution at the stations N-1 and N-3 in the 0–3 cm sediment layer.

Grain size also gives estimation about the content of organic matter, which is higher in muddy than in sandy sediments. In sandy and rocky bottoms the sedimented organic matter is easily flushed away without permanent sedimentation (Fig. 33). Only stations N-1 and R7 represent areas with "organic" sediment.

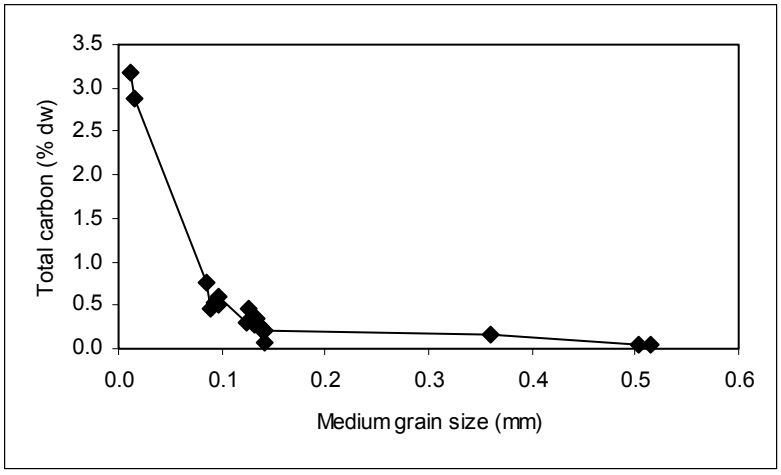

Figure 33. Total carbon content vs. medium grain size of the sediment in the sampled stations.

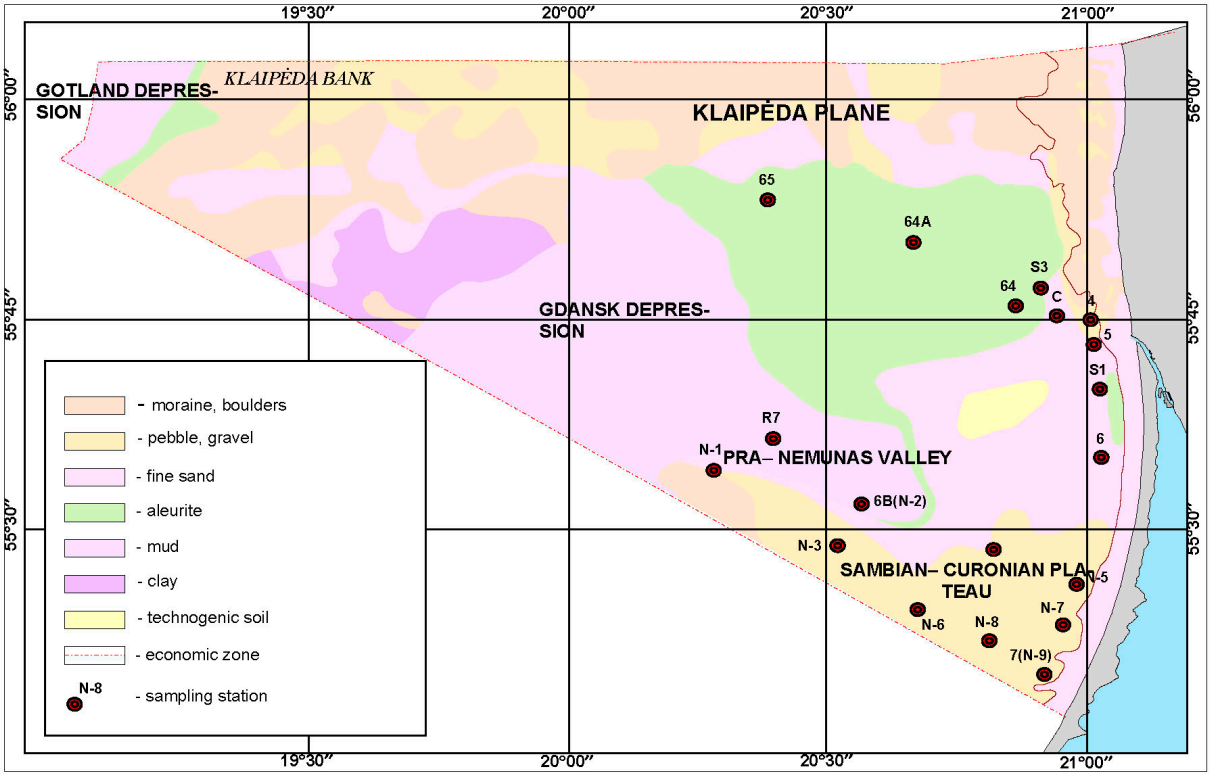

Figure 34. Location of sampling stations in relation to sediment types (compiled by S. Gulbinskas, R. Žaromskis, R. Repečka, 2005) in November 2005.

2.4 Summary on the environmental state and changes in hydrography, hydrochemistry and biology during 1995–2005 based on the monitoring data collected by the Center of Marine Research (CMR), Klaipeda

- Environmental conditions in the study area follow the general development of hydrochemistry and hydrography in the Baltic Sea except in the plume area where considerable variations in salinity and nutrient concentrations might occur caused by the outflow from the Curonian Lagoon. Since only few deeper sampling sites were included low oxygen values (close to 2 ml/l) are only occasionally measured in the near-bottom water. A slightly decreasing trend in salinity was observed at Station 65, which is influenced by processes in the Baltic Proper. Near to the coast no constant changes during the 10 years' period could be seen despite large interannual variations.

- Dissolved inorganic nutrient (DIN and DIP) concentrations in the surface water are in general highest in the transitional zone, which is influenced by outflow from the Curonian Lagoon. In the bottom water the highest levels of DIP are found in the open-sea area and for DIN in the transitional area. In all zones interannual variation was wide but both DIN and DIP levels have increased in the open sea since 2001 both in the surface as well as in bottom water, following changes observed in other sea areas in the Baltic Proper (Andersson and Andersson 2006). In coastal and transitional zones where strong fluctuations in nutrient concentrations occur and increasing concentrations during recent years have been measured only a slight increasing trend is seen. On the contrary, concentrations of total nitrogen (TN), have a decreasing trend in all areas. In the bottom water, nutrient concentrations are related to near-bottom oxygen levels. Measurements in November 2005 showed

increased values for some stations for DIP and DIN compared with seasonal mean values from 1995–2005.

- A vast array of microbial species (bacteria, fungi, algae and cyanobacteria) are able to use both low- and high-molecular-weight PAHs such as naphthalene, acenaphthene, anthracene, fluoranthene, pyrene and chrysene as their sole carbon and energy sources (Leahy and Colwell 1990, and references herein, Van Hamme et al. 2003). The long-term data collected by CMR compose a good base for the future monitoring. According to the 10 year data no increasing trends can be observed at any of the sampled stations but elevated numbers of oil-oxidizing bacteria in spring have been found.
- Species composition of plankton organisms is largely dependent on general physico-chemical factors such as salinity and temperature. In Lithuanian waters the correlation between abundance of copepods and salinity is weak. However, interspecific changes connected to salinity changes are known to occur in Copepoda. Species able to live in lowered salinity conditions have a lower nutritional value for fish affecting their physiological condition (Flinkman et al. 1998). A fast drop in Copepoda abundance in 1998–1999 is evident and they have been replaced by rotifers and cladocerans.
- The invader zooplankter *Cercopagis pengoi* was established in the Lithuanian waters only in 1999. Its effects on the pelagic ecosystem are possibly not yet seen in the long-term data.
- Strong interannual variation can be seen between relative abundances of phytoplankton groups and species. According to the 10 year data, the changes observed reflect the general eutrophication process in the Baltic Sea rather than changes in water quality.
- An invader species, the potentially toxic dinoflagellate *Prorocentrum minimum*, which first appeared in Lithuanian waters in 1992, is now an established species of the phytoplankton community, in some years making up around 80% of the phytoplankton biomass in the coastal zone.
- In regard to open-sea macrozoobenthos, a general strong reduction in total abundance and biomass of as well as changes in the species composition can be observed. In coastal areas abundance and biomass are increasing. Typical Baltic Sea species like *Monoporeia affinis* and *Macoma balthica* have suffered a sharp decline in 1990's. In *M. affinis*, same trend can be found around the Baltic Sea (http://www.helcom.fi/environment2/ifs/ifs2005/benthos_folder/en_GB/benthos/). General eutrophication and changes in plankton community are reflected in the bottom community due to the changes in the quality of the composition of the organic matter settling to the bottom. In November 2005 in the open-sea stations *M. balthica* dominated both in abundance and biomass. In the coastal zone the abundance of *M. viridis* was very high compared with long-term average. In the transitional zone, *M. viridis* dominated in abundance and biomass.
- Macrozoobenthic invader species *Marenzelleria viridis* has most probably had an effect on population structure, although the drop in abundance and biomass of *M. affinis* started already before its establishment.
- The highest peaks in total oil hydrocarbons (THC) were measured in the open-sea area in mid-1990's and again in November 2005. In the coastal zone THC levels are lower with few peaks. THC concentrations are considerably higher than those measured in other areas of the Baltic Sea (HELCOM 2002, Pikkarainen and

Lemponen 2005). Due to the differences in the method used in the analysis of total oil hydrocarbon concentration, the results are not fully comparable. 5–17% of the values exceed the Maximum Permissible Level (MPL) (0.05 mg/l) established by the Lithuanian legislation (Žin. 2006). Intensive shipping activity and illegal discharges occur in this sea area and oil spills from ships is a potential cause for the peak-type appearance of THCs in the surface water. Same type of fluctuation can be observed in sediment TCH concentrations although most of the data remain under the detection limit (< 5.1 mg/kg dw).

- Data of heavy metals in surface sediments cover the period of 2003–2005. According to maximum values for each metal given by Lithuanian legislation to classify sediments into "cleanness" categories (Žin. 2002), Cu, Zn and Pb fall to the cleanest category, while for Ni and Cr only few values exceed the limit of Category I. In Cd, only concentrations measured in 2003 exceed the Category I limit. Thus, the studied areas can be regarded relatively unpolluted by heavy metals .

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3. ECOTOXICOLOGICAL STUDIES

Ecotoxicological studies concerning the potential impact of the D6 platform were carried out by examining the levels of selected harmful substances in different environmental matrices (water, sediment and biota) and their biological effects using local indicator species. Samples were taken in November (concentration measurements and biological effect studies on the soft-bottom bivalve *Macoma balthica*) and December 2005 (biological effect studies on flounder), and an additional sampling was performed in April 2006 (biological effect studies on flounder). Sampling and analysis methods are described in detail in Appendix 3. The results of the studies are presented in the following sections.

3.1 Contaminant concentrations in water, sediments and biota

Responsible scientists: Galina Garnaga (CMR): total oil hydrocarbons in water and sediments; Mirja Leivuori (FIMR): metals in sediments and biota; Eila Lahdes, Kari Lehtonen (FIMR): coordination of sample analysis (PAHs, alkylated phenols, organotins) carried out in consultant laboratories (Nablabs, Finland; NERI, Denmark).

The following contaminant groups were regarded as the most relevant ones in investigating the potential impact of the D6 oil platform in the adjacent sea area. Their levels in sediments and tissues of the soft-bottom clam *Macoma balthica* were studied from material collected during a sampling campaign in November 2005. In addition, total oil hydrocarbons in seawater were determined by CMR as part of the seasonal monitoring programme.

- **Polycyclic aromatic hydrocarbons (PAHs)** are the most toxic components of crude oil and oil products. Uptake of PAH compounds by marine organisms occurs either directly from water by diffusion through cell membranes or via ingested food particles, and are readily absorbed due to their lipophilic nature. For deposit feeders such as *M. balthica* contaminated sediments also serve as a source of PAHs.
- **Heavy metals** are essential for the cellular functions of most organisms except for few toxic ones such as cadmium (Cd), mercury (Hg) and lead (Pb). In higher concentrations also essential metals become toxic. Presence of oil in the marine environment is generally accompanied by increased concentrations of heavy metals contained in oil, especially vanadium (V) and nickel (Ni).
- **Alkylated phenols** are found in produced water originating from oil drilling. These compounds are potentially causing disturbances in reproduction of fish (Sundt and Baussant 2003, Myhre et al. 2005). Alkylated phenols are readily degraded in water and metabolised in fish and no substantial accumulation has been observed under experimental conditions (Myhre et al. 2005).
- **Organotins** (e.g. TBT) are used e.g. in pesticides, wood preservatives and antifouling agents in boat and ship paints. Especially in the latter use they have been found to be extremely harmful to marine life, especially near harbour areas. Although a total worldwide ban concerning the use of TBT comes into effect in 2008, marked environmental hazards related to organotin substances prevail.

3.1.1 Metals

Metals in sediments

The type of bottom sediment greatly determines their metal concentrations. Grain size distribution of sediments shows that in most of the sampling areas large-size sandy sediments prevail. Only at seven geological stations the medium grain size was <0.1 mm. Among them were stations N-1 and 65, which were included in the ecotoxicological sampling programme. Heavy metal levels correlated negatively with the grain size of the sediment (Fig. 1) due to the larger efficient adsorption surface of sediment grains. A similar trend has been observed in earlier studies in the Lithuanian sea area (Jokšas 1994, Radzevičius 2000).

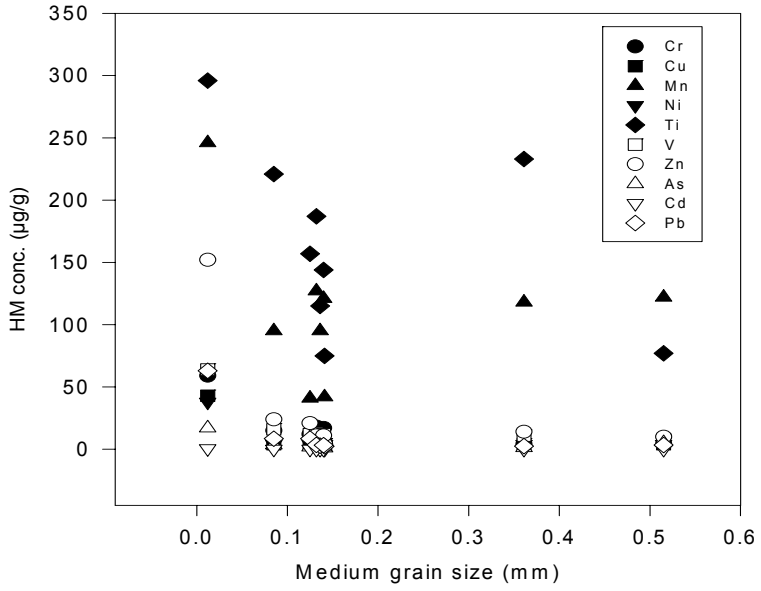

Figure 1. Concentrations of metals vs. medium grain size of the bottom sediment, based on the results obtained from samples collected in November 2005, including all sampling sites. Aluminium, calcium and iron have been excluded from the picture due to different concentration levels.

Concentrations of heavy metals are presented in Table 1. When evaluating the results it should be noted that the use of HNO_3 digestion results in lower levels for aluminium (Al), chromium (Cr), titanium (Ti) and vanadium (V) compared to the "total digestion" method using *aqua regia* and hydrofluoric acid (Appendix 4, intercalibration).

Table 1. Metal concentrations in sediments collected from the study stations. Samples were digested in nitric acid (HNO_3). In the analyses, two instrument techniques (ICP-OES and GF-AAS) were used depending on the metal. Note: concentrations of Al, Ca and Fe are given as % of dry weight.

ICP-OES

Station	**dw-%**	**Al** %	**Ca** %	**Cr** µg/g	**Cu** µg/g	**Fe** %	**Mn** µg/g	**Ni** µg/g	**P** µg/g	**Ti** µg/g	**V** µg/g	**Zn** µg/g
N-1	25.3	3.11	0.38	59	43	3.70	246	38	892	296	64	152
N-2	58.0	0.48	0.14	12	<5	0.61	41	<5	441	157	12	21
N-3	89.1	0.18	0.27	<5	<5	0.67	122	<5	413	77	7.5	10
N-5	76.4	0.40	0.66	13	<5	0.71	95	<5	850	115	11	9.0
N-6	78.0	0.15	0.16	<5	<5	0.22	42	<5	289	75	3.9	4.2
N-8	85.6	0.22	0.68	<5	<5	0.64	118	<5	360	233	11	14
N-9	76.9	0.28	0.77	18	<5	0.77	127	<5	796	187	13	11
6	77.1	0.26	0.54	17	<5	0.76	121	<5	675	144	12	11
65	58.1	0.56	0.48	15	<5	0.89	95	<5	615	221	16	24

GF-AAS

Station	**As** µg/g	**Cd** µg/g	**Pb** µg/g
N-1	17.0	0.634	63.0
N-2	1.8	0.141	8.6
N-3	5.2	0.019	3.4
N-5	1.7	0.019	2.9
N-6	0.9	0.008	2.4
N-8	1.3	0.009	2.5
N-9	1.9	0.027	3.5
6	1.9	0.023	3.3
65	3.4	0.147	8.5

It should be noted that in the present study Station N-1 is the only sampling site characterised by fine-grained sediments and therefore differs markedly from the other stations in grain size distribution.

At Station N-1 trace metal concentrations are in the same order of magnitude as in the northern Baltic Sea (Gulf of Finland, Gulf of Bothnia and Gulf of Riga) in the 1990s (Leivuori 1998, Leivuori et al. 2000), with the exception of manganese (Mn) and V that show 18 and 1.5 times higher values. V is of special interest because it is considered as an indicator of oil pollution. The level recorded here at Station N-1 is close to the highest values measured in the northern Baltic Sea (Leivuori 1998, Leivuori et al. 2000) and also compared to the levels measured in the southeastern Baltic Sea in the 1990s (Radzevičius 2000).

Concentrations of metals considered as harmful substances (arsenic [As], Cd, Pb, Zn) are comparable with those recorded in other Baltic Sea areas (Leivuori 1998, Leivuori et al. 2000). However, results from other sampling stations than N-1 are not directly comparable due to the highly coarser sediment type. Likewise, comparisons between all the stations within this study are difficult to make because metal concentrations are strongly linked to the grain size distribution and not necessarily to anthropogenic input.

Lithuanian legislation (Žin. 2002, Nr. 27-976) determines four categories for the cleanness of sediments regarding heavy metal pollution. According to this classification the levels of Cu, Zn, Pb, Cd, chromium (Cr) and nickel (Ni) at station N-1 are all above the concentration limits set for the cleanest Category I while all other stations belong to Category I. Station N-1 does not belong to the network of standard monitoring stations and no previous CMR monitoring data from the site exists.

To trace the source of metal pollution, aluminium (Al) as a conservative element has been used as a reference to normalise metal concentrations. In environments clearly contaminated by anthropogenic sources normalisation can give an estimate about the origin of metals accumulated in the area (Schropp et al. 1990). However, in bottoms where sediments are transported from various areas normalization is not a valid procedure. In order to study differences between sampling areas metal-Al ratios can be calculated. This kind approach has been used as a tool for localise contaminated areas in the Gulf of Gdansk (Ebbing et al. 2002).

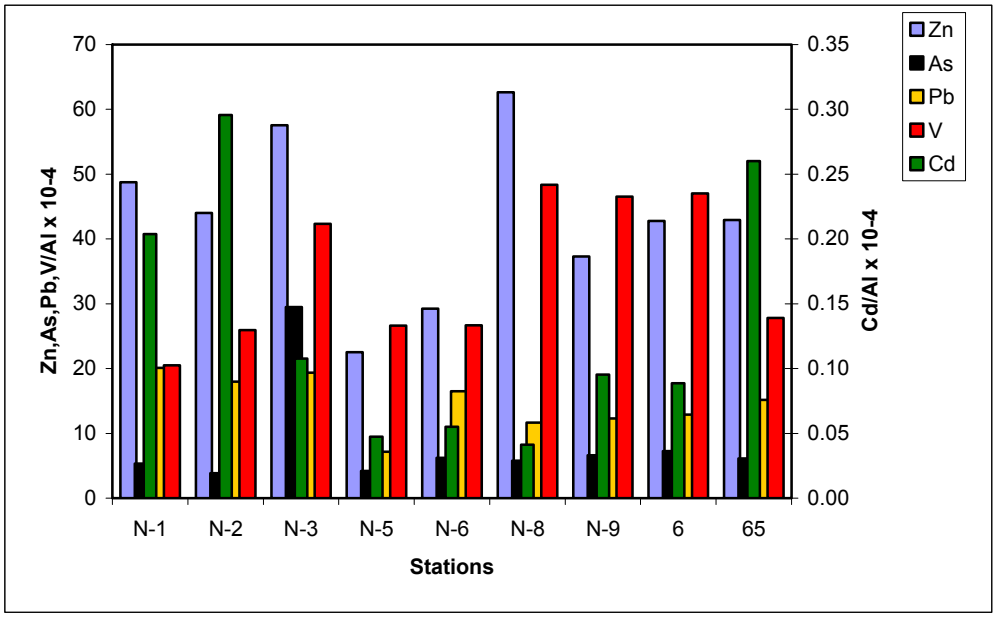

Figure 2. Arsenic, lead, vanadium and zinc vs. aluminium ratios at different sampling stations.

Anomalies in metal-Al ratios were found at stations N-3, N-8, N-9 and 6 for V, N-2 and 65 for Cd, N-3 and N-8 for Zn, and N-3 for As (Fig. 2). Highest ratios were found in coastal areas. However, if the criteria of JMG (1992) that only sediments with more than 20% of material under 0.063 mm grain size should be considered for monitoring purposes is followed only station N-1 would be relevant for the normalisation purposes.

Metals in M. balthica

Heavy metal concentrations in the soft-bottom clam *M. balthica*, measured at FIMR, are presented in Table 2.

Table 2. Metal concentrations in the soft tissues of the clam *M. balthica* collected from the study stations. In analyses, two instrument techniques (ICP-OES and GF-AAS) were used depending on the metal.

Station	ww (g)	dw (g)	dw %	Cd		Cu		Pb		Zn	
				µg/g dw	µg/g ww	µg/g dw	µg/g ww	µg/g dw	µg/g ww	µg/g dw	µg/g ww
65	11.98	9.81	15.9	0.636	0.101	172.7	27.5	3.12	0.50	480.7	76.5
N-2	13.58	10.18	14.7	0.330	0.048	134.3	19.7	2.69	0.40	268.2	39.4
N-3	11.70	9.93	15.7	0.676	0.106	39.6	6.21	1.60	0.25	419.6	65.9
N-8	13.95	10.59	15.5	0.704	0.109	27.8	4.31	0.75	0.12	320.5	49.7
N-9	12.82	10.03	17.0	0.618	0.105	24.7	4.19	0.75	0.13	319.8	54.3

Concentrations of copper (Cu) and Pb were higher in *M. balthica* collected at Stations N-2 and 65. For the other metals measured the concentrations among study stations were more similar. Levels of Cu were in the range measured in *M. balthica* in the Baltic Sea previously, being higher than in the Gulf of Gdansk (Sokolowski et al. 2002, 2004) but lower than in the Gulf of Finland (Lehtonen et al. 2006, Jankovski and Simm 1996). Accumulation of metals is known to de partly salinity-dependent, explaining some of the regional differences observed in the Baltic Sea.

3.1.2 Hydrocarbons

Total oil hydrocarbons in water

Total oil hydrocarbon concentrations in surface and near-bottom water layers in autumn 2005 are presented in Fig. 3, measured by CMR. Original data are presented in Appendix 2, Table 9.

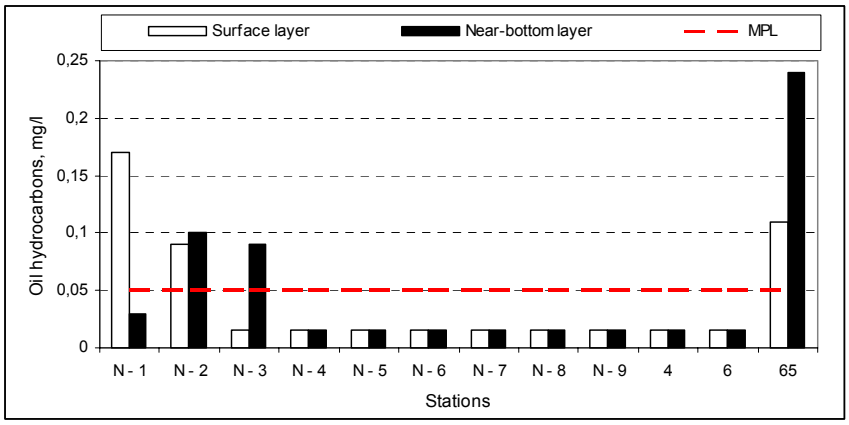

Figure 3. Total oil hydrocarbon concentrations (mg l^{-1}) in surface and near-bottom water layers in autumn 2005. MPL = Maximum Permissible Level (0.050 mg l^{-1}).

At all open-sea stations (N-1, N-2, N-3 and 65) oil hydrocarbon concentrations exceeded the Lithuanian criteria of Maximum Permissible Level (MPL) of 0.05 mg l^{-1} (Žin. 2006, Nr. 59-2103). At Station 65 the oil concentration in the near-bottom water layer was 5 times higher than MPL and in the surface layer 2 times higher than MPL. At station N-2 the oil levels were 2 times higher than MPL in both depth zones. At Station N-1 3 times MPL were recorded in the surface layer while at Station N-3 the near-bottom water oil concentrations were 2 times higher than MPL. Concentrations of total oil hydrocarbons at other study stations were at level with (N-1, surface) or below the detection limit of the method used (0.03 mg l^{-1}). As noted in Chapter 2, the method used in analysis is based on infrared spectrometry (IR) and the results are not therefore fully comparable with results observed in other parts of the Baltic Sea.

The results indicate the presence of oil in the water phase in the open-sea region of the study area. However, the data cannot define its source.

Total oil hydrocarbons sediments

Total oil hydrocarbon concentrations in sediments in November 2005, measured by CMR, are given in Fig. 4. Original data are presented in Annex 2. (The values are also included in of Section 2.2.7 Figure 4 showing the long-term trends.)

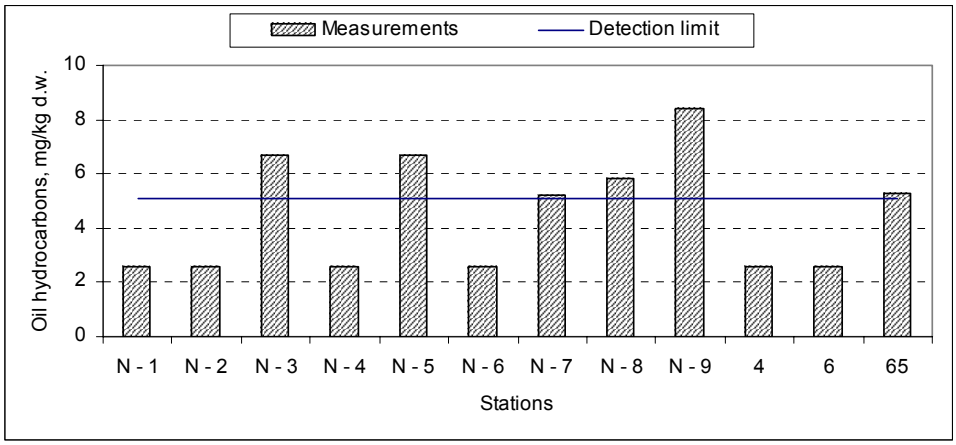

Figure 4. Total oil hydrocarbons in sediments at the study stations in November 2005.

Total oil hydrocarbon concentrations in sediments were mostly near or below the detection limit of the method used (5.1 mg kg dw^{-1}).

Total oil hydrocarbon concentrations in sediments, collected from the same sampling sites, and measured by the Finnish consultant laboratory (Nablabs) were all below the detection limit of the method used (Appendix 2, Table 15).

Total hydrocarbons in Macoma balthica

Total hydrocarbon concentrations in *Macoma balthica* measured by the Finnish consultant laboratory (Nablabs) were all below the detection limit of the method used (Appendix 2, Table 14).

PAHs in sediment

Concentrations of 16 PAH species in the surface sediments measured by Nablabs were analysed and the results are given in Table 3 (original report in Appendix 2, Table 11).

Table 3. PAH compounds in sediment (µg kg dw^{-1}). Note: the sum consists only of compounds above the detection limit (>10 µg kg dw^{-1}).

PAH compound	**N-1**	**N-2**	**N-3**	**N-6**	**N-8**	**N-9**	**6**	**65**
Naphthalene	<10	<10	<10	<10	<10	<10	<10	<10
Acenaphthylene	<10	<10	<10	<10	<10	<10	<10	<10
Acenaphthene	<10	<10	<10	<10	<10	<10	<10	<10
Fluorene	<10	<10	<10	<10	<10	<10	<10	<10
Phenanthrene	11	<10	<10	<10	<10	<10	<10	<10
Anthracene	<10	<10	<10	<10	<10	<10	<10	<10
Fluoranthene	110	37	<10	<10	<10	<10	<10	21
Pyrene	40	13	<10	<10	<10	<10	<10	13
Benzo(*a*)anthracene	25	<10	<10	<10	<10	<10	<10	<10
Chrysene	33	12	<10	<10	<10	<10	<10	12
Benzo(*b*)fluoranthene	110	23	<10	<10	<10	<10	<10	16
Benzo(*k*)fluoranthene	100	23	<10	<10	<10	<10	<10	17
Benzo(*a*)pyrene	42	14	<10	<10	<10	<10	<10	11
Indeno(*1,2,3-cd*)pyrene	150	31	<10	<10	<10	<10	<10	17
Dibenz(*a,h*)anthracene	25	<10	<10	<10	<10	<10	<10	<10
Benzol(*g,h,i*)perylene	130	24	<10	<10	<10	<10	<10	15
Sum PAH	**780**	**180**						**120**

Levels of PAHs over the detection limit were found only at Stations N-1, N-2 and 65. At these stations eight compounds were found; benzo(*g,h,i*)perylene, indeno(*1,2,3-cd*)pyrene, benzo(*a*)pyrene, benzo(*k*)fluoranthene, benzo(*b*)fluoranthene, chrysene, pyrene and fluoranthene. At Station N-1 also small amounts of dibenzo(*a,h*)anthracene, benzo(*i*)anthracene and phenanthrene were measured. As a summary, concentrations were not particularly high and the distribution of PAHs was similar to other parts of the Baltic Sea (Pikkarainen 2004a). All the measured values were also below the OSPAR Ecotoxicological Assessment Criteria (EAC) (OSPAR 2000a and b).

Also in regard to PAHs the type of bottom sediment affects the concentration levels. The levels and number of different PAHs found at station N-1 were higher than at the other sampling sites. On that basis it is difficult to estimate whether that is due to a certain source of contamination or are the findings dependent on the absorption characteristics of the sediment particles.

Ratios of specific PAHs (”molecular indices”) can be used to distinguish between their pyrolytic and petrogenic origins (Kavouras et al. 2001, Swartz et al. 2003). Ratios of phenantrene to anthracene <10 and fluoranthene to pyrene > 1 indicate a pyrolytic origin and values >15 and <1 a petrogenic one, respectively. Diesel engine source is evaluated by calculating ratios of fluoranthene to fluorathene+pyrene and indeno(*1,2,3-*

cd)pyrene to indeno(*1,2,3-cd*)pyrene+benzol(*g,h,i*)perylene. Molecular indices from study Stations N-1, N-2 and 65 where detectable concentrations of these compounds were found are shown in Table 4.

Table 4. Molecular indices indicating the source of PAH compounds at three study stations.

PAH ratios	**Stations**			**Molecular index interpretation**
	N-1	**N-2**	**65**	
Phenantrene / Anthracene	2.20	-	-	petrogenic > 15, pyrolytic < 10
Fluoranthene / Pyrene	2.75	2.85	1.62	petrogenic < 1, pyrolytic > 1
Fluoranthene / Fluoranthene + Pyrene	0.73	0.74	0.48	diesel engines 0.60 - 0.70
Indeno(*1,2,3-cd*)pyrene / Indeno(*1,2,3-cd*) pyrene + Benzol(*g,h,i*)perylene	0.60	0.56	0.53	diesel engines 0.35- 0.70

The molecular index values indicate that the source of PAHs occurring in the sediment is pyrolytic at all three stations, possibly of diesel engine origin. Although these reference values are taken from studies on urban air emissions they have been applied to bottom sediment studies as well (e.g. Pikkarainen 2004a).

PAHs in M. balthica

Concentrations of 16 PAH species in soft tissues of *M. balthica* were analysed by Nablabs and the results are given in Table 5.

Table 5. PAH compounds in the soft tissues of the clam *M. balthica* (µg kg ww^{-1}). Note: the sum consists only of compounds above the detection limit (>5 µg kg ww^{-1}).

PAH compound	**65**	**N-2**	**N-3**	**N-8**	**N-9**
Naphthalene	19	21	35	36	32
Acenaphthylene	<5	<5	<5	<5	<5
Acenaphthene	8	7	<5	9	11
Fluorene	<5	<5	<5	<5	<5
Phenanthrene	<5	<5	<5	<5	<5
Anthracene	<5	<5	<5	<5	<5
Fluoranthene	<5	<5	<5	<5	<5
Pyrene	10	<5	<5	<5	<5
Benzo(*a*)anthracene	<5	<5	<5	<5	<5
Chrysene	<5	<5	<5	<5	<5
Benzo(*b*)fluoranthene	8	7	5	<5	<5
Benzo(*k*)fluoranthene	5	<5	<5	<5	<5
Benzo(*a*)pyrene	16	17	23	18	15
Indeno(*1,2,3-cd*)pyrene	9	<5	6	<5	7
Dibenz(*a,h*)anthracene	<5	<5	<5	<5	<5
Benzo(*g,h,i*)perylene	<5	<5	<5	<5	<5
Sum PAH	81	52	69	63	65

In tissues of *M. balthica* only seven PAH compounds (in concentrations above detection levels) were found, with naphthalene and benzo(a)pyrene showing the highest concentrations. The total concentrations (Sum PAH) are in the range of those measured earlier in the Baltic Sea (Pikkarainen 2004b)

The source of PAHs in the tissues of *M. balthica* assessed the same way as for the sediment (see above). However, concentrations of PAH compounds used for calculations were under the detection limit.

3. 1.3 Alkylated phenols in sediment

Concentrations of alkylated phenols in sediment were all below the detection limit of the method used (Appendix 2, Table 13).

3.1.4 Organotins

Organotins in sediments

Concentrations of organotins (TBT, DBT and MBT) in the surface sediments were analysed and the results are given in Table 6.

Table 6. Organotin compounds in surface sediments. Conversion factors from µg Sn kg^{-1} to TBT, DBT and MBT were 2.44, 1.9 and 1.48, respectively. Dry weight (DW) % was used to convert fresh weight concentrations to dry weight concentrations.

Station	TBT µg Sn kg^{-1} ww	TBT µg kg^{-1} ww	TBT µg kg^{-1} dw	DBT µg Sn kg^{-1} ww	DBT µg kg^{-1} ww	DBT µg kg^{-1} dw	MTB µg Sn kg^{-1} ww	MTB µg kg^{-1} ww	MTB µg kg^{-1} dw	DW %
N-1	90.0	219.6	807.4	2.4	4.7	17.3	<2	<2.96	<10.9	27.2
N-2	1.1	2.7	4.0	<1	-	-	<1	-	-	66.6
N-3	<1	-	-	<1	-	-	<1	-	-	92.7
N-5	<1	-	-	<1	-	-	<1	-	-	76.3
N-6	<1	-	-	<1	-	-	<1	-	-	77.7
N-8	<1	-	-	<1	-	-	<1	-	-	86.3
N-9	<1	-	-	<1	-	-	<1	-	-	76.6
6	<1	-	-	<1	-	-	<1	-	-	76.2
65	1.6	3.9	6.0	<1			<1	-	-	65.2

Sediment concentrations of organotins were above the detection limit at Stations N-1, N-2 and 65. The values were high only at Station N-1 characterised by fine sediment. The levels of TBT measured at station are relatively high for a non-harbour environment. In the Gulf of Finland TBT concentrations in reference areas was ca. 50 µg kg^{-1} dw, in small boat marinas 48-330 µg kg^{-1} dw, and shipyards and harbours up to >6000 µg kg^{-1} dw) (Munter et al. in press). Szpunar et al. (1997) recorded high TBT levels ranging from 1150 to 8500 µg kg^{-1} dw in harbour areas of Gdynia and Gdansk (Poland) but low in other areas of the Gulf of Gdansk (< 1 to 100 µg kg^{-1} dw).

Organotins in M. balthica

Concentrations of organotins (TBT, DBT and MBT) in soft tissues of *M. balthica* were analysed and the results are given in Table 7.

Table 7. Organotin compounds in the clam *M. balthica*. Conversion factors from µg Sn kg^{-1} to TBT, DBT and MBT were 2.44, 1.9 and 1.48, respectively. Dry weight (DW) % was used to convert fresh weight concentrations to dry weight concentrations.

Macoma balthica	TBT µg Sn kg^{-1} ww	TBT µg kg^{-1} ww	TBT µg kg^{-1} dw	DBT µg Sn kg^{-1} ww	DBT µg kg^{-1} ww	DBT µg kg^{-1} dw	MTB µg Sn kg^{-1} ww	MTB µg kg^{-1} ww	MTB µg kg^{-1} dw	DW %
65	4.3	10.5	66.0	1.3	2.5	16.0	1.3	1.9	12.1	15.9
N-2	5.1	12.4	84.7	0.6	1.2	8.0	<0.6	-	-	14.7
N-3	3.9	9.5	60.6	1.2	2.4	15.0	<0.5	-	-	15.7
N-8	2.2	5.4	34.6	0.5	1.0	6.3	<0.4	-	-	15.5
N-9	1.9	4.6	27.3	0.5	1.0	5.8	<0.3	-	-	17.0

The levels of organotins in the tissues of *M. balthica* (highest value: 12.4 µg TBT kg ww^{-1}, station N-2) are 16-43 times lower than recorded in the Gulf of Finland off Helsinki (204-544 µg kg ww^{-1}) (Munter et al. in press) and probably represent more-or-less typical levels currently observed in open-sea areas affected by ship traffic in the Baltic Sea.

Summary

- The levels of heavy metals in sediments and *M. balthica* in the study area are within normal ranges and reveal no particular environmental impacts.
- The levels of total hydrocarbons in seawater in November 2005 showed the highest levels at the open-sea stations, especially in the near-bottom water at the northernmost ("reference") station 65 and in the surface water at station N-1. These data indicate that some oil contamination from unknown sources was present in the whole open-sea study area. Since the region is intensively used for maritime traffic (e.g. shipping to the Klaipeda harbour) the contamination is most likely resulting from these activities. In sediments the levels of total hydrocarbons were very low showing no marked oil contamination.
- In general, low levels of PAH compounds were observed in sediments and *M. balthica*. However, PAH levels observed at the only soft-bottom station N-1 signify some degree of hydrocarbon pollution. The few "molecular index" ratios of indicator PAH compounds that could be calculated from the results obtained imply that hydrocarbon pollution in the study area is mostly of pyrolytic (not petrogenic) origin and apparently coming from the use of diesel motors.
- The levels of the other hazardous compounds measured in the study area showed generally low levels, with alkylated phenols in sediments remaining below the detection limit and of organotins in *M. balthica* and sediments showing low levels except for the soft-bottom open-sea station N-1 with a relatively high sediment concentration of TBT.

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3.3 Biological effect studies

Responsible scientists: Kari K. Lehtonen, Mikko Putkonen, Susanna Hyvärinen (FIMR): acetylcholinesterase activity; ethoxyresorufin-O-deethylase activity; metallothionein induction; acyl-CoA oxidase activity; glutathione S-transferase activity; oxidative stress enzyme activities (glutathione reductase, catalase, superoxide dimutase); Janina Baršienė, Aleksandras Rybakovas (Institute of Ecology of Vilnius University, Lithuania): micronuclei frequency; Pekka J. Vuorinen (Finnish Game and Fisheries Research Institute), Susanna Eerola (Finnish Food Safety Authority): PAH metabolites in bile; Thomas Lang (Federal Research Centre for Fisheries, Institute for Fishery Ecology, Cuxhaven, Germany): external visible fish diseases and parasites; histopathology (flounder).

- Measuring the concentrations of pollutants from sediments, water and biota give information of their levels and distribution patterns in the marine environment. What they cannot provide is information on biological effects of contaminants.
- A large suite of methods has been developed to indicate such effects, with some of them being indicators of general stress, some showing exposure to specific groups of pollutants (ICES WGBEC 1999). The application of biological "early-warning" endpoints, the so-called biomarkers, markedly improves the detection capacity of exposure and biological effects of contaminants.
- Biomarkers are widely used in the detection of various types of contamination and evaluation of their effects in monitoring and assessment programmes in many sea areas, including The North Sea and The Mediterranean (ICES WGBEC 1999). In the Baltic Sea they have so far been little applied although very recent development has been achieved mainly during the biomonitoring activities carried out during the EU funded BEEP project (www.beep.u-bordeaux1.fr).
- After the wrecks of *Erika* and *Prestige*, in addition to the collecting of data on the levels of PAH the health status of different living communities was investigated through the use of biomarkers to reveal eventual molecular, cellular or physiological disorders due to the exposure to oil (Bocquené et al. 2004, Orbea et al. 2006). The ongoing development calls urgently for increased research on topics related to the effects of oil on organisms and ecosystem of the Baltic Sea, as well as the development of early warning systems for the efficient detection of oil contamination.

Bioindicator species in the D-6 study

- Baltic clam (*Macoma balthica*) is a widely distributed species in the Northern Hemisphere and also in the Baltic Sea. It lives buried in soft sediments, being mainly a deposit feeder but also a facultative filter feeder. Due to its feeding mode *M. balthica* is affected differently by pollutants compared to suspension feeders and is considered a highly potential species for biomonitoring (Rainbow and Phillips 1993, Lehtonen et al. 2006).
- Flounder (*Platichthys flesus*) is a benthic fish living in close connection with sediments. Numerous biological effects data are available from this species, especially from the North Sea (e.g. Köhler and Pluta 1995) and very recently also from the Baltic Sea (Baršienė et al. 2006, Kopecka et al. 2006, Lang et al. 2006, Vuorinen et al. 2006).

Biomarkers used in the D6 study

Most of the biomarkers used here are recommended or promising techniques for biological effects monitoring (WGEBC 1999). Many of them arise from the detoxification mechanisms expressed by organisms exposed to xenobiotics or metabolites as a result of detoxification, or oxidative stress caused by excessive oxyradical formation in detoxification reactions. A biomarker of genotoxicity is included since PAHs are well-known genotoxic compounds. The application of several biomarkers representing more or less the same defined level of response (biotransformation, oxidative stress) is made to examine the sensitivity of each biomarker.

- Neurotoxic effects: Acetylcholinesterase activity (AChE). AChE is commonly found in the nervous tissue, brain red blood cells, and muscle tissues. AChE inhibition has been used widely as an indicator of exposure to organophosphate and carbamate pesticides in various marine taxa (Bocquené and Galgani 1998), but shows sensitivity also to other pollutants groups (Payne et al. 1996).
- Exposure to metals: Metallothionein (MT) induction. MTs are low molecular weight (6-8000 D), cysteine-rich (20-30%), metal binding proteins. Their neosynthesis represents a specific response of the organisms to pollution by heavy metals such as Cu, Zn, Cd and Hg (Viarengo 1989). Binding of metal cations by *de novo* synthesised apothioneins produces non-toxic forms, thus reducing the deleterious effects of metals (Roesijadi 1990).
- Exposure to polyaromatic compounds – biotransformation: Ethoxyresorufin-*O*-deethylase (EROD) induction. The induction of cytochrome P450 (CYP) is among the best-characterised biomarkers of polycyclic aromatic hydrocarbon (PAHs) and halogenated aromatic hydrocarbon (HAHs) exposure (Stegeman and Lech 1991). Cytochrome P450s are a superfamily of hemoproteins that catalyse the oxidative metabolism of diverse compounds. In addition, the biotransformation through P450 plays an important role in the formation of carcinogenic compounds.
- Exposure to polyaromatic compounds – peroxisome proliferation: Acyl-Coenzyme-A oxidase (AOX) activity. A peroxisomal response to contaminant exposure, in bivalves used as an alternative to cytochrome P_{450}-based biomarkers (e.g. EROD) that show poor induction (Cajaraville et al. 2003).
- Exposure to polyaromatic hydrocarbons: PAH bile metabolites (PAH). PAHs are converted by P450s to hydroxylated and conjugated species that are secreted into the bile. Hence, the concentration of fluorescent aromatic metabolites in fish bile can be used to assess exposure to their parent hydrocarbons (Ariese et al. 1997).
- Exposure to organic pollutants - conjugation: Glutathione-S-transferase (GST). The family of GSTs plays an important role in conjugating different xenobiotics to facilitate their excretion (Habig et al. 1974).
- Oxidative stress: Many toxicants increase free radical production in cells and tissues, i.e. reactive oxygen species (ROS). Glutathione reductase (GR). Induction of GR protects the cell of oxidative damage and therefore reflects exposure to pollutants causing oxidative stress (Carlberg and Mannevik 1975). Catalase (CAT). CAT activity may increase as a result of exposure to certain kinds of pollutants (DiGiulio et al. 1989). Superoxide dismutases (SOD): a

family of metalloenzymes that catalyse dismutation (a reaction in which two identical molecules have different fates) (McCord and Fridovich 1969).

- Genotoxicity: Micronucleus frequency (MN). Extensive chromosomal rearrangements such as MN are well-recognised consequences of genome instability (Fenech et al. 1999). The MN test is among the most widely used tools in ecogenotoxicology. Variation in the frequency of MN in the cells of organisms provides an index of integrated influence of genotoxic compounds during the life span of the cells.

General health parameters

In addition to biomarkers, flounder collected from the study sites were subjected to the analysis of external visible diseases, parasite infections and liver histopathology.

Sampling and sample treatment

Sampling of *M. balthica* was carried out aboard the Lithuanian r/v "Vejas" between 8 – 10 November 2005. Several van Veen grab samples were taken from each of the sampling stations (Stations N-2, N-3, N-8, N-9 and 65). The sediment samples were sieved through a 1 mm stainless steel mesh and *M. balthica* were collected. The collected specimens were kept in the fridge in water buckets containing water from the sampling site and dissected for target tissues within 8 hours of sampling.

For AChE, foot tissues (*M. balthica*) from 5 individuals were pooled in one Eppendorf tube, with 5 tubes from each station. For MT and AOX, SOD and GR the digestive glands of 6 specimens were pooled in one tube, with 5 tubes per station. The digestive glands of 12 individuals were stored separately for the analysis of GST and CAT. Each sample was deposited in liquid nitrogen immediately after dissection and stored at –80°C until analysis.

For pollutant analyses the bivalves were dissected, frozen and maintained at –20°C until analysis. For heavy metal (HM) analyses 15 individuals were taken for each sample while for the measurement of organic contaminants (PAH, alkylated phenols and TBT) the amount of specimens was 30 each. During the dissection of soft bodies from the shells all precautions were taken to avoid sample contamination. Acid-washed glass vials were used for sample storage.

Flounder were trawled during ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM) cruise aboard German r/v "Walther Herwig III" in December 2005 at 4 stations north of the D6 oil production site. Stations 14-16 (Area A) were closer to D6 than Station 17 (Area B), which, therefore, was regarded as the "reference" site. Methodologies applied for obtaining samples (fish sampling, dissection, tissue sampling (biomarkers), and fixation (histology) were according to Lang et al. (2006). Bile was collected by piercing the exposed gall bladder wall with a needle fitted into a 1-ml disposable syringe, sucking bile into syringe and emptying it into a 1-ml dark glass vial. Samples were stored in -20 ºC until sent to the laboratory packed in dry ice. In the laboratory the samples were further stored in -80 ºC until being analysed. Only females were analysed. In April 12, an additional sampling was made further west from the Area A, called Area C, using fishermen's vessel used in local monthly fish monitoring. The flounder were kept and brought ashore in water buckets and treated in the local laboratory. Eight females and 7 males were examined and dissected for biomarker analyses.

Biomarker measurements

The selected biomarkers were measured in *M. balthica* and flounder according to Table 8. Detailed information about the methods used is found in Appendix 3.

Table 8. Biomarkers/biological endpoints measured in *M. balthica* and flounder (*P. flesus*).

Biomarker/endpoint	Short name	Indicator	Baltic clam (*Macoma balthica*)	Flounder (*Platichthys fesus*)
Ethoxyresorufin-*O*-deethylase activity	EROD	Biotransformation Phase I	---	X
Acyl-CoA oxidase activity	AOX	Exposure to organic contaminants	X	---
Glutathione S-transferase activity	GST	Biotransformation Phase II/ oxidative stress	X	X
Catalase activity	CAT	Oxidative stress	X	X
Superoxide dismutase activity	SOD	Oxidative stress	X	---
Glutathione reductase	GR	Oxidative stress	X	---
PAH metabolites in bile (fluorescent aromatic compounds/1-OH-pyrene)	FAC/ 1-OH-PYR	Exposure to PAH	---	X
Acetylcholinesterase activity	AChE	Neurotoxicity/general stress	X	X
Metallothionein induction	MT	Exposure to heavy metals/ general stress	X	X
Micronuclei frequency	MN	Genotoxicity	X	X
External visible diseases	---	Health	---	X
Liver histopathology	---	Health	---	X

Integrated Biomarker Index (IBR)

For both species an Integrated Biomarker Index (IBR, Beliaeff and Burgeot 2002) was calculated using biomarkers of neurotoxicity (AChE), metal exposure (MT), genotoxicity (MN), biotransformation and detoxification (mussels: AOX; flounder: EROD, and GST) and oxidative stress (*M. balthica*: combined parameter determined from CAT, SOD and GR; flounder: CAT). The IBR is calculated by summing up triangular Star Plot areas calculated for each two neighbouring biomarkers in a given data set. The procedure described below was used (from Broeg and Lehtonen 2006):

For each biomarker: (1) Calculation of mean and SD for each station. (2) Standardisation of data for each station: $x_i' = (x_i - \text{mean } x) / s$, where x_i' = standardised value of the biomarker, x_i = mean value of a biomarker from each station, mean x = mean of the biomarker calculated for all the stations, and s = standard deviation calculated for the station-specific values of each biomarker. Result: variance = 1, mean = 0. (3) Using standardised data, addition of the value obtained for each station to the absolute (=non-negative) value of the minimum value in the data set: $B = x_i' + |x_{min}|$.

Result: adjusts the lowest value in the set to zero. For all the biomarkers treated this way: (4) calculation of Star Plot areas by multiplication of the obtained value of each biomarker (B_i) with the value of the next biomarker, arranged as a set, dividing each calculation by 2 and (5) summing-up of all values: $\{[(B_1 \times B_2) / 2] + [(B_2 \times B_3) / 2] + \ldots [(B_{n-1} \times B_n) / 2]\}$. Result: IBR (average of different arrangements of biomarkers in the set). Since the value of IBR is obtained by summing up the parameters derived from the actual biomarker values, i.e. after the calculation steps 1 to 4, it is directly dependent on the number of biomarkers in the set. Thus, the values of IBR are given divided by the number of biomarkers used and termed as IBR/n.

3.3.1 Biomarker responses in *Macoma balthica*

Neurotoxicity: AChE activity. Differences in AChE activity between the study stations was almost significant (KW statistic 9.452, p = 0.051) (Fig. 5). The activity values (mean ± SE) ranged from 16.4 ± 2.1 (Station N-3) to 26.4 ± 2.8 nmol ACTC min^{-1} mg^{-1} protein (Station N-9).

Exposure to organic contaminants – peroxisome proliferation: AOX activity. In AOX activity no statistically significant differences between the study stations could be observed (KW statistic = 6.993, p = 0.136) (Fig. 5). AOX activity (mean ± SE) in digestive gland tissue ranged from 0.50 ± 0.07 (Station N-8) to 0.88 ± 0.10 mUnits mg^{-1} protein (Station 65).

Phase II – conjugation: GST activity. GST activity showed significant variability between the study stations (KW statistic = 16.923, p = 0.002)(Fig. 5). GST levels (mean ± SE) in digestive gland tissue ranged from 900 ± 60 (Station 65) to 1290 ± 153 µmol min^{-1} mg^{-1} protein (Station N-8).

Oxidative stress: CAT, SOD and GR activity. In activity levels of the three oxidative stress enzymes varied followingly:

CAT: In the levels of CAT activity no statistically significant differences between the study stations could be observed (Fig. 5). CAT levels (mean ± SE) in digestive gland tissue ranged from 143 ± 18 (Station N-2) to 216 ± 27 µmol min^{-1} mg^{-1} protein (Station N-9).

SOD: In the levels of SOD activity statistically significant differences between the study stations could be observed (KW statistic = 15.699, p = 0.003)(Fig. 5). SOD levels (mean ± SE) in digestive gland tissue ranged from 20.5 ± 1.0 (Station N-8) to 33.7 ± 1.8 Units mg^{-1} protein (Station 65).

GR: In the levels of GR activity statistically significant differences between the study stations could be observed (KW statistic = 10.870, p = 0.028) (Fig. 5). GR levels (mean ± SE) in digestive gland tissue ranged from 10.9 ± 0.9 (Station N-8) to 17.2 ± 2.0 nmol min^{-1} mg^{-1}protein (Station 65).

Exposure to metals: MT content. In the levels of MT no statistically significant differences between the study stations could be observed (KW statistic = 2.301, p = 0.681)(Fig. 5). MT levels (mean ± SE) in digestive gland tissue ranged from 174 ± 36 (Station N-8) to 266 ± 50 µg g^{-1} wet wt (Station N-9).

Genotoxicity: micronuclei frequency. MN frequency showed significant variability between the study stations (KW statistic = 15.597, p = 0.004)(Fig. 5). MN levels (mean ± SE) in gill cells ranged from 0.73 ± 0.26 (Station N-9) to 2.52 ± 0.39 MN 1000 cells^{-1} (Station N-2). MN frequencies were clearly higher at the offshore stations N-2 and N-3 compared to nearshore Stations N-8 and N-9, while the population at the offshore station 65 showed intermediate values.

Integrated Biomarker Index (IBR): Clearly highest IBR (IBR/n, see above) value, indicating highest integrated response, was calculated for the population inhabiting Station N-2 (Fig. 6). Second-6highest index values were observed at Stations N-3 and 65. Clams at the near-shore Stations N-8 and N-9 showed the lowest index values with Station N-8 standing out clearly as the station with the lowest integrated biomarker response.

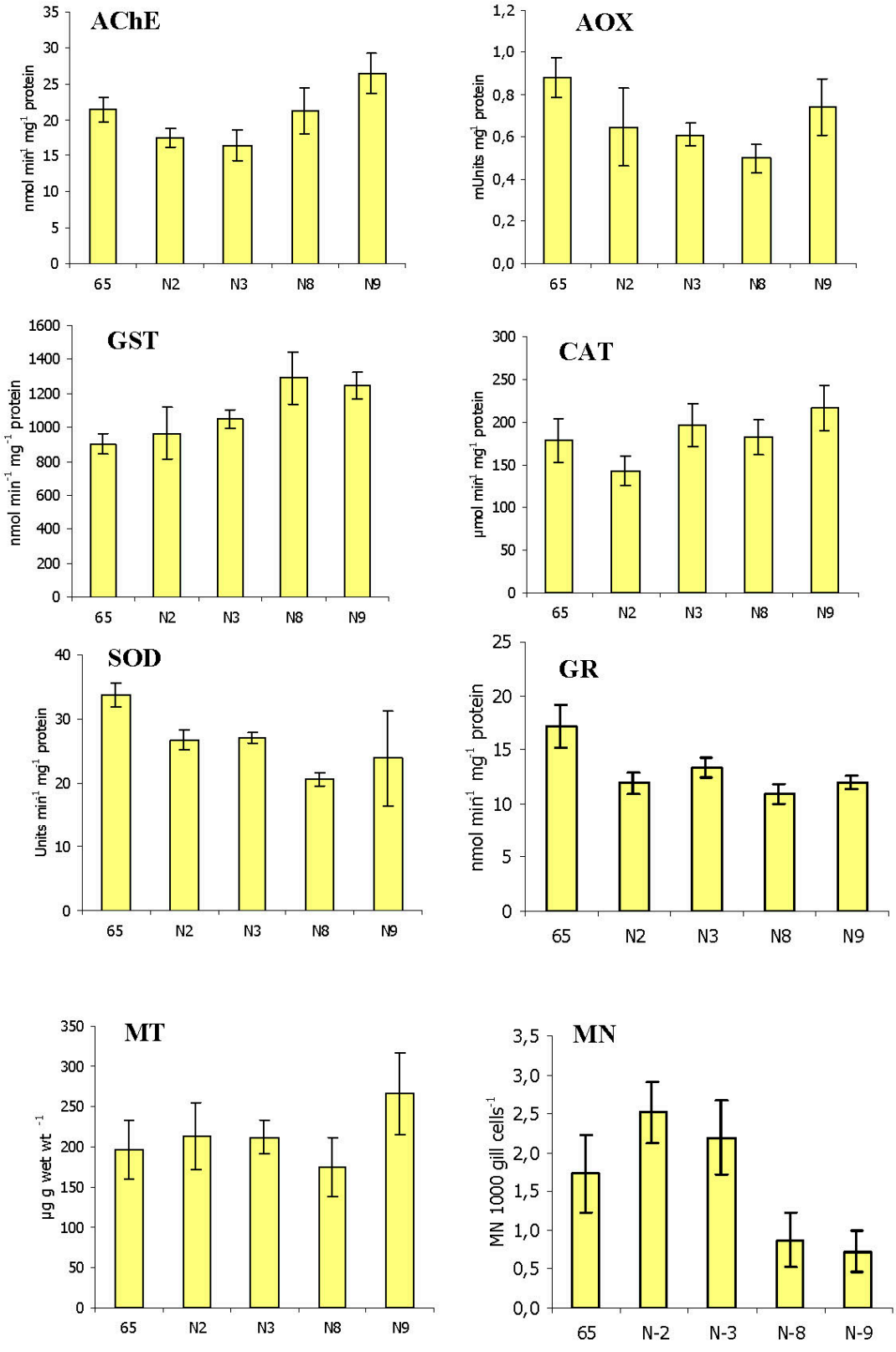

Figure 5. M. balthica. Biomarker responses (mean ± SE) measured in individuals collected from the study stations in November 2005. Abbreviations: AChE – acetylcholinesterase activity, AOX – acyl-CoA oxidase activity, GST – glutathione S-transferase activity, CAT – catalase activity, SOD – superoxide dismutase activity, GR- glutathione reductase activity, MT – metallothionein content, MN – micronuclei frequency.

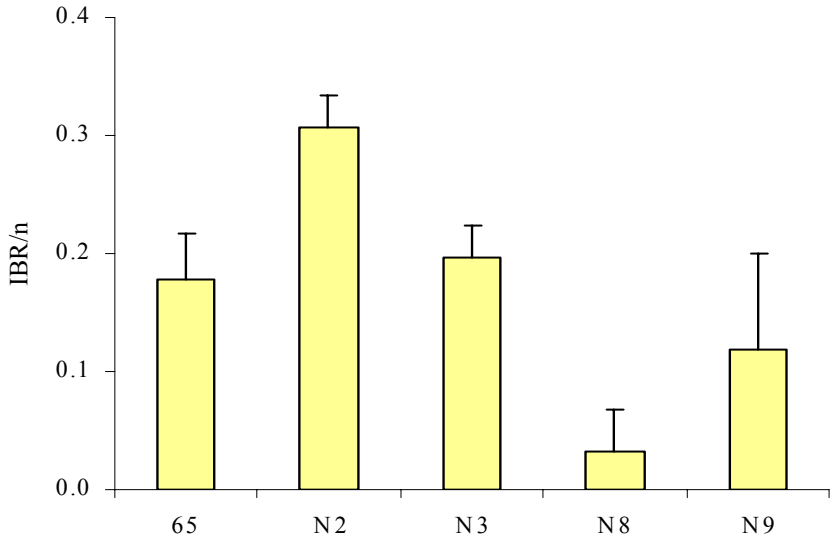

Figure 6. M. balthica. Integrated Biomarker Index (IBR), calculated using biomarkers of neurotoxicity (AChE), metal exposure (MT), genotoxicity (MN), biotransformation and detoxification (AOX and GST) and oxidative stress (combined parameter determined from CAT, SOD and GR). IBR is given here as IBR/n, n being the number of biomarker parameters used in the calculation of the index.

In *M. balthica*, biomarker responses in the study area in November 2005 showed greatest variability between the near-shore Stations N-8 and N-9 and open-sea Stations N-2, N-3 and 65. These main areas differ in important physical properties such as water depth, near-bottom temperature and oxygen conditions and somewhat also on sediment type. Since many of the biomarkers measured here are known to respond to changes in temperature the responses observed have to be carefully studied to avoid misinterpretations. Table 9 shows a compilation of these factors to be considered.

Table 9. Hydrographic features of the *M. balthica* sampling stations.

Station	Sediment type	Depth (m)	Temperature (ºC)	Salinity	Oxygen (ml l^{-1})
65	Coarse-grained silt	47	10.7	7.3	7.1
N-2	Fine-grained sand	65	4.0	8.9	2.7
N-3	Coarse-grained sand	42	5.4	7.5	6.4
N-8	Medium-grained sand	37	11.0	7.2	7.1
N-9	Fine-grained sand	14	9.7	7.2	7.3

The most striking differences were recorded in the genotoxicity biomarker MN that exhibited significantly increased incidences in populations inhabiting the open-sea Stations N-2 and N-3 compared to the near-shore Stations N-8 and N-9. The frequency of MN (mean ± SE) in clams was from 0.73 ± 0.26 (Station N-8) and 0.88 ± 0.36 (N-9) to 2.19 ± 0.48 (N3) and 2.52 ± 0.39 MN 1000 $cells^{-1}$ (N2). *M. balthica* from the

offshore Station 65 showed also a markedly higher MN frequency (1.73 ± 0.39 MN 1000 cells^{-1}) compared to the two near-shore stations.

Low temperature has been suggested to reduce MN formation because of very low mitotic activity in low temperatures (Baršienė et al. 2006a) such as those recorded at the open-sea Stations N-2 and N-3 in November (4.0 and 5.4ºC, respectively). However, despite this hypothesis the MN frequency in *M. balthica* collected from these stations was significantly higher compared to the near-shore Stations N-8 and N-9 and the northernmost open-sea Station 65 characterised by temperatures between 9.7–11.0ºC. This indicates a markedly higher genotoxicity of the sediments at these open-sea stations compared to the near-shore area.

In the Baltic Sea, higher frequencies of MN have been detected in blue mussels (*Mytilus edulis*) close to oil terminals and marine port areas (Baršienė and Baršytė Lovejoy 2000, Baršienė 2002). In laboratory exposure studies elevated frequencies of MN have been noted after treatment with 0.25-0.5 ppm of Lithuanian crude oil (Baršienė et al. 2006e). Significant elevations of MN level in mussels 30 days post-oil spill and persistence of the cytogenetic damage have been described to extend for up to 100 days (Parry et al. 1997), 8 months (Baršienė et al. 2004, 2006a, b) and even 10 years (Bolognesi et al. 2006). Cells with MN have been found to increase in the gills or hemolymph of marine molluscs treated with benzo[*a*]pyrene (Burgeot et al. 1995, Venier et al. 1997, Siu et al. 2004) and dimethylbenzo[*a*]anthracene (Bolognesi et al. 1996). Conclusively, the observed high levels of MN in the open-sea area can result from exposure to PAHs although other chemical compounds and environmental factors can also be behind the observed elevations.

The original "reference" Station 65 stands out in many ways as a potentially contaminated environment with the highest mean values observed in AOX, SOD and GR activities, as well as the relatively high incidence of MN already mentioned. Oil concentrations in surface and especially near-bottom waters were elevated in this area, while total PAH compounds in soft tissues of *M. balthica* were the highest recorded in this study, although the differences between stations were not very large. These might reflect the effects of intensive ship traffic in this sea area and/or contaminated water from the Klaipeda Strait. There also exists a dumping site of dredged sediments relatively close to this site.

The "integrated response", calculated as the IBR index basing on all eight biomarkers measured on *M. balthica*, shows the highest stress level at the open-sea Station N-2, followed by the other open-sea Stations N-3 and 65. Since more-or-less similar bottom sediments typify these sites the exposure conditions can in this sense be regarded comparable. However, at the time of sampling the temperature at Station 65 was markedly higher (10.7ºC) compared to Stations N-2 and N-3 (4.0 and 5.4ºC, respectively). Therefore, masked by the temperature difference, some of the enzymatic biomarkers – as well as MN frequency – might have shown an even higher response at the "colder" stations compared to the "warmer" ones, and, subsequently, the IBR an even clearer distinction between stress levels among the stations. Conclusively, the *M. balthica* population inhabiting Station N-2 is clearly the most stressed one of those measured in this study. The apparent difference in the stress level of the two near-shore populations implies a more elevated contaminant level closer to the shoreline (N-9) compared to the more transitional zone (N-8).

3.3.2 Biomarker responses in flounder (Platichthys flesus)

Neurotoxicity: AChE activity. Significant variability in AChE activity between the study areas was observed (KW statistic 6.238, p = 0.044) (Fig. 8). The activity values (mean ± SE) in muscle tissue ranged from 248.6 ± 26.7 (Area C/Spring) to 353.9 ± 25.7 nmol ACTC min^{-1} mg^{-1} protein (Area B).

Biotransformation Phase I: EROD activity. In EROD activity, statistically significant variability between the study areas could be observed (KW statistic 7.198, p = 0.027) (Fig. 8). EROD activity (mean ± SE) in liver tissue ranged from 0.25 ± 0.02 (Area A) to 6.05 ± 7.7 pmol min^{-1} mg^{-1} protein (Area C/Spring).

Biotransformation Phase II (conjugation): GST activity. In the levels of GST activity no statistically significant variability between the study areas could be observed, although the variability was almost significant (KW statistic 5.097, p = 0.078) (Fig. 8). GST levels (mean ± SE) in liver tissue ranged from 320.7 ± 20.3 (Area B) to 487 ± 74.7 nmol min^{-1} mg^{-1} protein (Area C/Spring).

Oxidative stress: CAT activity. In the levels of CAT activity statistically significant variability between the study areas could be observed (KW statistic 22.154, p < 0.001) (Fig. 8). CAT levels (mean ± SE) in liver tissue ranged from 37.8 ± 13.1 (Area A) to 109.4 ± 49.5 µmol min^{-1} mg^{-1} protein (Area C/Spring).

Exposure to metals: MT content. In the levels of MT statistically significant differences between the study areas could be observed (KW statistic 13.785, p = 0.001) (Fig. 8). MT levels (mean ± SE) in liver tissue ranged from 710 (Area C/Spring) to 973.3 ± 39.2 µg g^{-1} wet wt (Area A).

Micronuclei frequency. MN frequency showed no variability between the study areas (KW statistic = 0.236, p = 0.889)(Fig. 8). MN levels (mean ± SE) in liver cells ranged from 0.05 ± 0.03 (Area C/April) to 0.07 ± 0.03 MN 1000 $cells^{-1}$ (Area B). In April 2006 the analysis of MN was performed in three tissues, mature (blood) and immature erythrocytes (kidney and liver). The highest response was observed in cephalic kidney and lowest in liver (results not presented here). (See also Fig. 7. for images of typical micronucleated cells.)

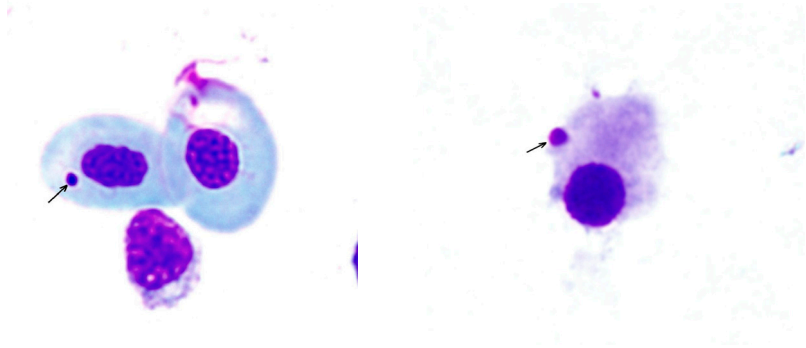

Figure 7. Micronuclei (arrows) in (A) mature erythrocyte of flounder and (B) gill cell of *M. balthica*. 1000 × magnification.

PAH metabolites in bile. PAH metabolites in bile (FAC, fluorescent aromatic compounds/1-OH-pyrene) showed almost significant variability between the areas (KW statistic = 5.866, p = 0.053)(Fig. 8). FAC levels (mean ± SE) ranged from 6.7 ± 1.6 (Area B) to 26.7 ± 9.9 fluorescence units (Area C/Spring).

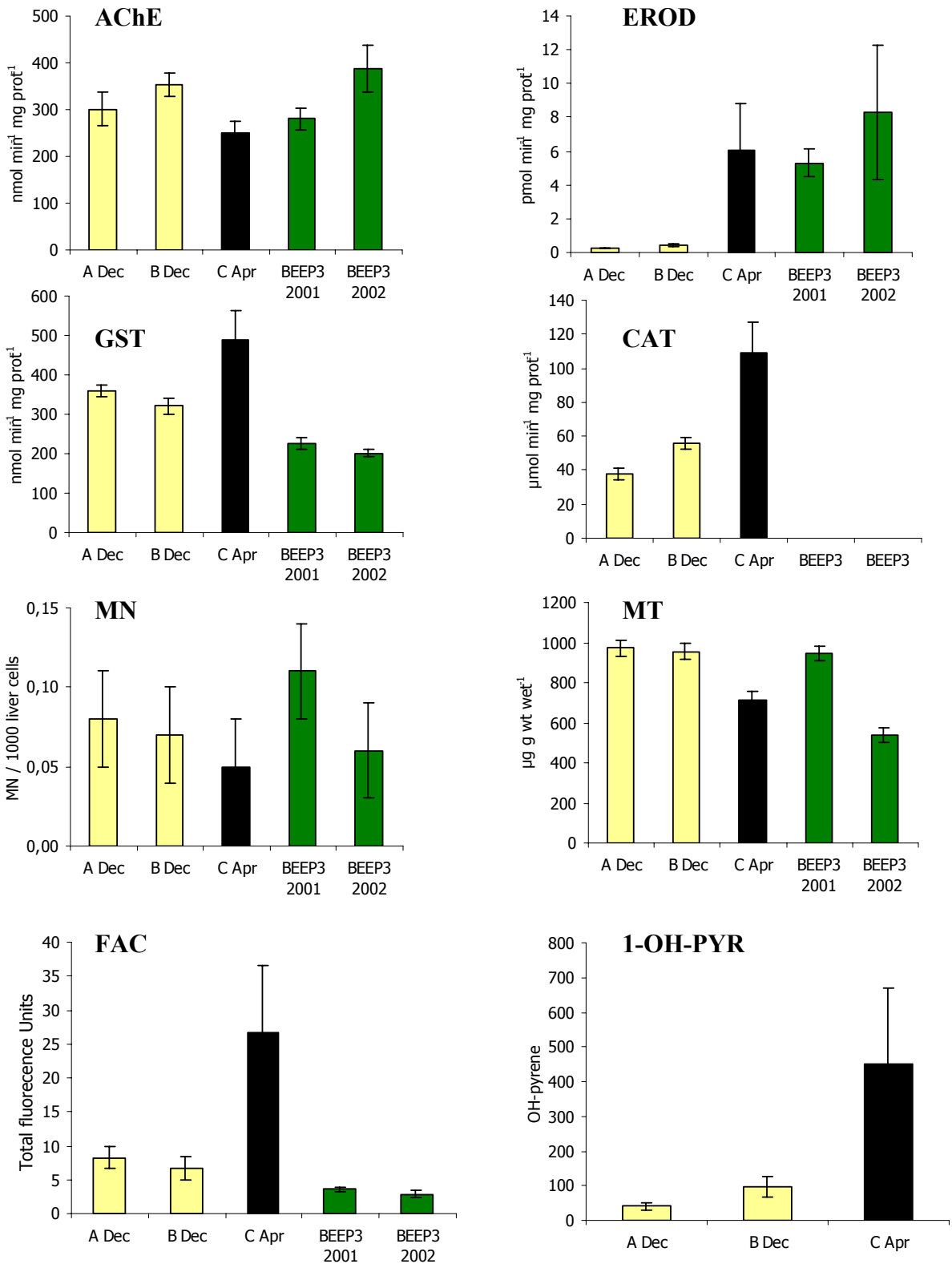

Figure 8. Flounder (Platichthys flesus). Biomarker responses (mean ± SE) measured in individuals collected from Areas A and B in December 2005 (yellow bars) and Area C in April 2006 (black bar). Results from flounder studies carried out during the EU BEEP project in December 2001 and 2002 in the study area (Area BEEP 3) have been included for comparison (green bars). Abbreviations: AChE – acetylcholinesterase activity, EROD – ethoxyresorufin-O-deethylase activity, GST – glutathione S-transferase activity, MN – micronuclei frequency, MT – metallothionein content, FAC – fluorescent aromatic compounds (PAH metabolites), 1-OH-PYR – 1-hydroxypyrene (PAH metabolites).

Integrated Biomarker Index (IBR)

Clearly highest IBR (IBR/n, *see above*) value, indicating highest integrated response, was calculated for Area C sampled in April (Fig. 9). Populations at Areas A and B sampled in November showed no difference between them.

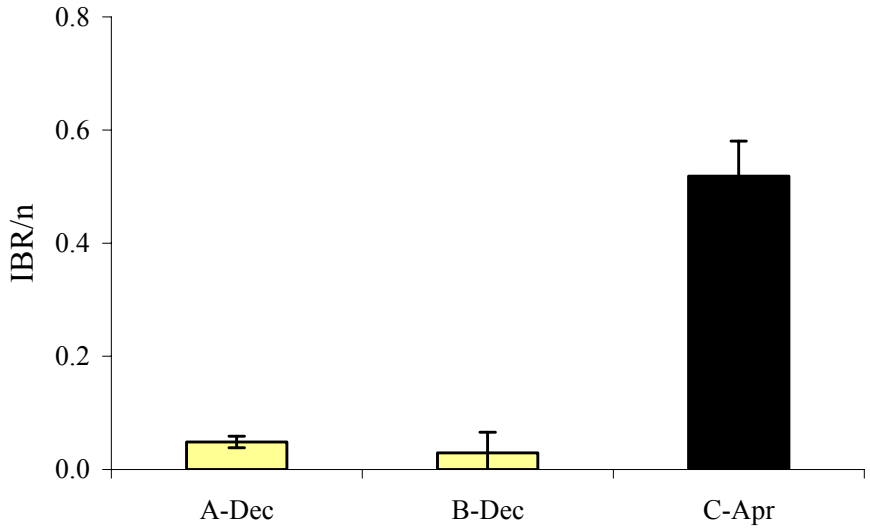

Figure 9. Flounder (*P. flesus*). Integrated Biomarker Index (IBR), calculated using biomarkers of neurotoxicity (AChE), metal exposure (MT), genotoxicity (MN), biotransformation and detoxification (EROD and GST) and oxidative stress (CAT). IBR is given here as "IBR/n", n being the number of biomarker parameters (7) used in the calculation of the index.

Both FAC and 1-OH-pyrene concentrations in bile were significantly ($p < 0.001$) higher in spring (in Area C) than in late autumn (in Areas A and B). In April, opposite to earlier observations, mean FAC values were higher in females than in males (Vuorinen et al. 2006) but the number of individuals was smaller (seven males, eight females) while only females were collected in December. FAC concentrations in bile observed in December were of the same order as reported from autumns 2001 and 2002 for flounders caught further up the Lithuanian coast (Klaipeda-Butinge region in spring and autumn 2001 and 2002 (ca. 3–9 fluorescence units)(Baršienė et al. 2006a), and in December 2001 and 2002 in the same open-sea region (ca. 3–4). In contrast, FAC values recorded in April (26.7 ± 9.9, mean ± SE) were considerably higher than the values observed in spring 2001 and 2002. Methodological differences cannot account for the observed differences since the samples were analysed by the same laboratory using the same determination method.

FAC and 1-OH-pyrene concentrations in flounder bile correlated significantly with high coefficients of determination (78.6–96.8%, $p < 0.01$) as detected earlier in flounder and also in other fish species (Vuontisjärvi et al. 2004, Vuorinen et al. 2006). In the April samples both FAC and 1-OH-pyrene concentrations in females showed a significant positive correlation with the liver somatic (LSI) and gonadosomatic (GSI) indices (Table 10). In December in Area B the correlations were weaker while in Area A there were no correlation between these parameters. Previously, a significant correlation between FAC and GSI was detected in female flounder, eelpout (*Zoarces viviparus*) and perch (*Perca fluviatilis*)(Vuorinen et al. 2006) but the coefficients were considerably weaker than observed in the present study. Regarding the above it is possible that the high bile FAC and 1-OH-pyrene concentrations observed in April can be related to the reproductive cycle of the species. However, the observed elevated concentrations may

also originate from an environmental source of PAHs. FACs in bile can occur as a result of non-PAH sources but the presence of 1-OH-pyrene points to an increased exposure to PAH compounds.

Table 10. Flounder (*Platichthys flesus*). Pearson correlation coefficients between bile FAC and 1-OH-pyrene concentrations and weight, length, liver somatic (LSI) and gonadosomatic index (GSI) of females collected in December 2005 and April 2006. Statistically significant correlations are indicated as superscripts: * = $p < 0.05$ and ** = $p < 0.01$.

Sampling		**Weight**	**Length**	**LSI**	**GSI**
December 2005, Area A	FAC	-0.266	-0.228	0.097	0.149
	1-OH-pyrene	-0.198	-0.245	-0.151	0.003
December 2005, Area B	FAC	0.238	0.269	0.482	0.588*
	1-OH-pyrene	0.641*	0.560	0.595*	0.592*
April 2006, Area C	FAC	0.710	0.303	0.949**	0.877**
	1-OH- pyrene	0.820*	0.440	0.928**	0.805*

In flounder, the elevations in EROD, GST and CAT activities observed in April 2006 compared to December 2005 suggests increased detoxification of organic compounds, including increased biotransformation and conjugation reactions and increased neutralisation of oxyradicals possibly produced during biotransformation of these compounds. It is also notable that mean AChE activity was significantly decreased in Area C in April compared to Area A (by 17%) and Area B (30%), indicating neurotoxic effects and, in the absence of organophosphate and carbamate pesticides, non-specific stress. All the recorded responses may indicate spatial differences in exposure to organic compounds between Areas A and B compared to Area C, or a seasonal change in exposure situation in the study area as a whole.

However, it is emphasised that many biomarkers, including EROD activity and PAH metabolite concentrations in fish bile show seasonal fluctuations related to the reproductive cycle (e.g. Förlin et al. 1984), which may therefore be, at least partly, behind the observed elevations in these parameters observed in April. The seasonal variability in EROD activity in female flounder observed in this study has also been reported in the North Sea (Broeg et al. 1999). The observed differences in EROD activity can be caused by variable stages of gonad maturity or LSI (Whyte et al. 2000), and the higher EROD activity recorded here in spring can therefore be interpreted as resulting from spawning stress (Eggens et al. 1995, 1996, Kirby et al. 1999, Rotchell et al. 1999). Similarly related to seasonal fluctuations, the observed decrease in MT in flounder collected in April compared to December is most likely related to the reproductive cycle of the species as observed in previous studies in the Baltic Sea (Baršienė et al. 2006a, Kopecka et al. 2006). In addition, even the elevated EROD activity levels in April (mean < 6 pmol min^{-1} mg $protein^{-1}$) can be regarded as relatively low (Förlin et al. 1984).

Conclusively, although a number of elevated biomarker responses in flounder collected in April compared to December were recorded these can at least partly be attributed to seasonal variations caused by biotic (e.g. reproduction) and abiotic factors. However, the fact that the levels of 1-OH-pyrene were greatly elevated in Area C in April (also in regard to previous measurements in the Lithuanian coastal region in springs 2001 and 2002) indicates that recent, increased exposure to a PAH source is possible.

The current study revealed very low MN frequency in liver immature and blood mature erythrocytes of flounder (0.04–0.08 MN 1,000 cells^{-1}). These values are assumed to be the baseline level in this fish species in the Baltic Sea offshore zones (Baršienė et al. 2006b). A significantly higher frequency of MN (0.21 MN 1,000 cells^{-1}) was found in flounder cephalic kidney immature erythrocytes compared to mature blood erythrocytes (results not shown here). Previous studies on turbot (*Scophthalmus maximus*) and Atlantic cod (*Gadus morhua*) have showed, after treatment with crude oil, frequencies of MN ca. 2-fold higher in the cephalic kidney than in mature erythrocytes from peripheral blood (Baršienė et al. 2006c). It is known that in teleost fish the main erythropoietic tissue is the cephalic kidney. In fish, mature erythrocytes are not dividing cells (Grisolia and Cordeiro 2000). Since MN can arise after cell division the MN test in peripheral blood show comparatively low response to genotoxic agents. On the other hand, inter-tissue differences in the frequency of MN could be a result of elimination of damaged erythrocytes from the peripheral blood system. There are literature data showing the spleen activity in selective removing of micronucleated erythrocytes from peripheral blood system (Meier et al. 1999, Cristaldi et al. 2004). The observed low level of MN in flounder liver could be explained by the high frequency of fragmented-apoptotic cells; such type of cells is recognized as one of main ways in elimination of micronucleated cells (Micic et al. 2002).

Liver histopathology

The results of the histopathological study in December 2005 are summarised in Fig. 10 and provided in full in Appendix 1. In total, 38 flounder (16 from station 14, one each from station 15 and 16 [all forming Area A]) and 20 from station 17 (Area B) were examined, 30 (78.9 %) of which were afflicted with liver lesions. Twenty-five (65.8 %) specimens showed non-specific lesions, 4 (10.5 %) early non-neoplastic lesions, 7 (18.4 %) foci of cellular alteration (putative pre-neoplastic lesions), 3 (7.9 %) benign liver tumours (all classified as adenoma), and none malignant tumours. The mean score in flounder from stations 14-16 (Area A) was 0.5 and was, thus, slightly lower than the score in flounder from station 17 (Area B) (1.0). This was largely due to the fact that pre-neoplastic lesions and benign tumours were more prevalent at station 17 than at stations 14-16.

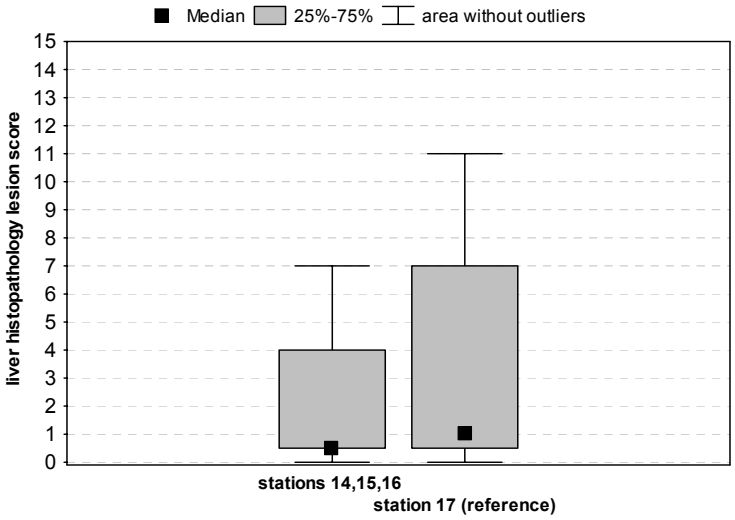

Figure 10. Mean histopathological liver lesion scores in flounder (*P. flesus*) from stations 14-16 (Area A) and station 17 (Area B), respectively.

The types of histopathological liver lesions recorded in flounder in the present study and the resulting mean lesion scores calculated were in the range recorded previously in areas off the Lithuanian coast (Lang et al. 2006, Lang et al. [in preparation]). The differences between the sampling sites (Area A vs. Area B) were minor. The only difference was the slightly higher prevalence of putative pre-neoplastic and neoplastic lesions recorded in Area B. However, when interpreting the results, it has to be taken into account that the distance of all 4 stations to the oil production site was considerable (in the range of 14.4 [station 14] to 30.2 [station 17] nautical miles). It can, therefore, not be excluded that all 4 stations represent areas that are non-affected by the oil production activities.

External fish diseases

Compared to other areas examined in the Baltic Sea during the WKFDM flounder in the study area (marked here as BEEP 3) as a whole showed the lowest prevalence of acute/healing skin ulcerations, while the other externally visible diseases did not differ markedly from other sea areas (Fig. 11).

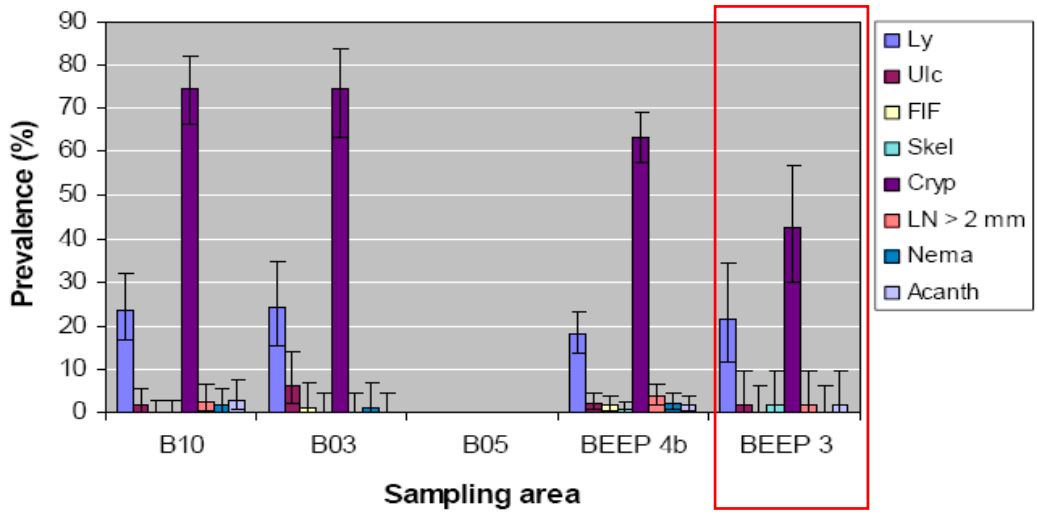

Figure 11. Prevalences (with 95% confidence intervals) of externally visible diseases in Baltic flounder (*Platichthys flesus*) recorded during the Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM). BEEP 3: Lithuanian coast, both study areas (A and B) pooled. (Ly: lymphocystis; Ulc: acute/healing skin ulcerations; FIF: acute/healing fin rot/erosion; Skel: skeletal deformities; Cryp: *Cryptocotyle* spp.; LN > 2 mm: liver nodules > 2 mm in diameter (not yet histologically confirmed; Nema: liver nematodes; Acanth: liver acanthocephalans). Adapted from "Report of the ICES/BSRP Sea-going Workshop on Fish Disease Monitoring in the Baltic Sea (WKFDM), 5–12 December 2005. ICES Baltic Committee ICES CM 2006/BCC:02".

Summary

- Biomarker responses in the clam *M. balthica* show significant differences between populations from the study stations. However, since some of the enzymatic biomarkers may be affected by temperature differences between the stations (higher rates at higher temperatures), interpretations are not straightforward.
- Frequency of MN was significantly higher in *M. balthica* from the open-sea Stations N-2 and N-3 compared to the near-shore Stations N-8 and N-9, indicating the presence of higher genotoxicity in the offshore sea area. The relatively high levels of MN recorded at the "reference" offshore Station 65

indicate an elevated degree of genotoxicity also in this sea area. Basing on previous research, potential exposure to PAH compounds causing the observed increased levels of DNA damage cannot be ruled out.

- Basing on the integrated stress response (IBR index) the *M. balthica* population at the offshore Station N-2 was in the most stressed condition. To some degree this is reflects differences in the sediment structure and subsequent contaminant levels between open-sea and near-shore habitats. However, the clear distinction in IBR values between Station N-2 and the two other offshore stations (N-3 and 65) demonstrates spatial differences unlinked to sediment structure and singles out Station N-2 with the most unfavourable environmental conditions.
- In flounder, most biomarker responses measured in the two study areas in December 2005 (Areas A and B) show only small differences with no clear pattern between the two study areas. The areas were probably two close to each other and/or too far from any significant pollution source for any marked differences to be seen.
- Compared to the December 2005, observations on flounder collected in April 2006 (Area C) showed significantly higher biomarker responses related to potential exposure to organic contaminants (EROD, GST, CAT and PAH metabolites/1-OH-pyrene in bile). Subsequently, the IBR index was clearly higher in April. However, the most probable reasons for the elevated biomarker response levels are related to known seasonal variability related to reproduction, but the high concentration of 1-OH-pyrene in bile implies to recent exposure to oil compounds.
- Prevalences of histopathological lesions in the liver of flounder as well as external visible fish diseases observed are within the normal range (or lower) than recorded in other parts of the Baltic Sea, implying no marked effects of contaminants seriously affecting the health of flounder in the study area.

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4. OIL DFIFT MODELLING

Responsible scientists: Inga Dailidienė (CMR/KU) and Kai Myrberg (FIMR)

4.1 Introduction

One of the main ecological problems of the Baltic Sea is spillage of oil products. After the occurrence of an accident and the spillage of oil products into the sea all the organisms living and feeding in the seawater i.e. the whole ecosystem is under threat. Therefore, all preventive measures allowing anticipatory evaluation of every possible consequence is necessary.

Forecast calculations of the oil drift in different hydro-meteorological conditions give means for the planning of effective measures in the case of an accident. The forecast model presented in this report is based on the forecast program Seatrack Web on oil and chemical contaminants which CMR has worked in cooperation with the Swedish Meteorological and Hydrological Institute, and on the hydro-meteorological data collected in the study area.

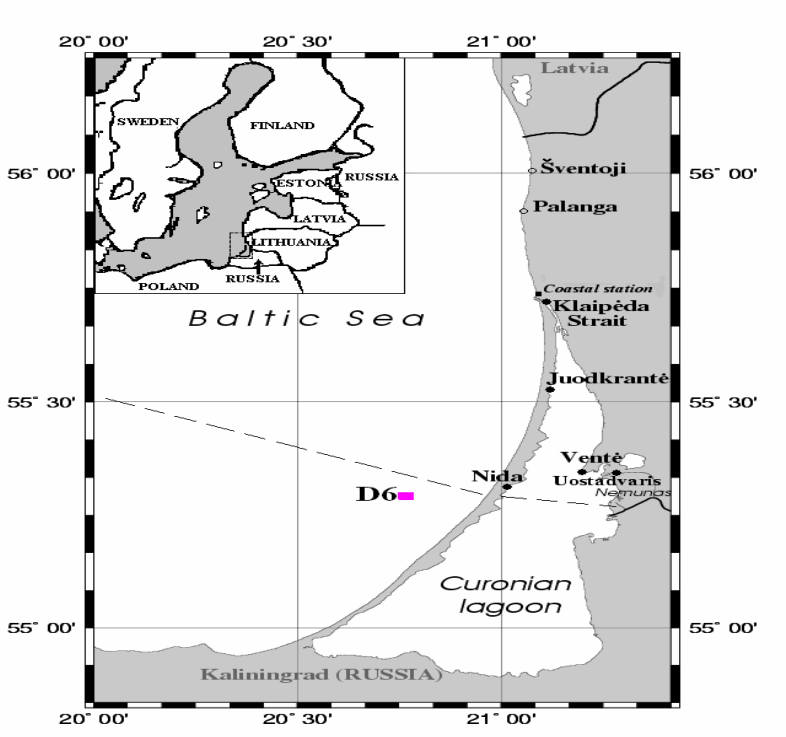

Figure 1. Scheme of the location of D6 oil platform in Baltic Sea.

4.2 General meteorological conditions in the Baltic Sea area

The Baltic Sea is situated in latitudes where the inter-annual variations in solar radiation, pressure, wind and temperature are rather high (Mälkki and Tamsalu 1985). The distribution of the oceans and continents, and also the orographic effects of the Norwegian mountains determine the prevailing condition and modify the routes and life history of weather disturbances. In the free atmosphere westerly winds generally

prevail, and although their direction and magnitude at different heights have a clear seasonal cycle, the pattern is fairly regular. During autumn-winter when the surface pressure has its greatest gradients and winds, blowing from western direction, have their largest speeds. During the spring and early summer the pressure field over the Baltic is rather uniform and the wind speeds are most of the time weak. During this period there often exist long-lasting blocking highs.

In the southernmost part of the Baltic, the climatic conditions are closer to those over the North Sea, whereas towards the north and east the climatic conditions have a more continental character. So, the surface layer temperature over the Baltic sea has a considerable amplitude. The horizontal variability in the air temperature above the sea in the summer months lies within 2°C over the entire Baltic Sea, while during the winter months the mean temperature deviates by more than 10°C. The range of variability in the southern Baltic is about 17 °C and in the north up to 27°C.

4.3 Wind distribution in the area studied

The oil accident risk in this part of the Baltic Sea is high due to the prevailing hydrological and meteorological conditions in this region. In case of oil spillage due to the prevailing western air mass movement in this part of the Baltic Sea (Fig. 2.), the possibility for the oil to reach the south-eastern coast of the Baltic Sea is very high. South-eastern wind and the wind from western directions is dominant in the Lithuanian coastal zone which conditions where the water mass is moving in the south-eastern part of the Baltic Sea along the shore from the south to the north.

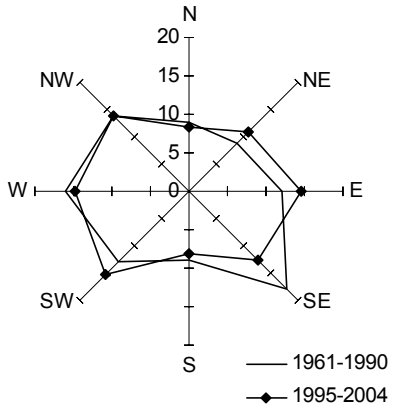

Figure 2. Wind direction (%) rose, in the south-eastern part of Baltic Sea near Klaipeda city, 1961–1990 and 1994–2004.

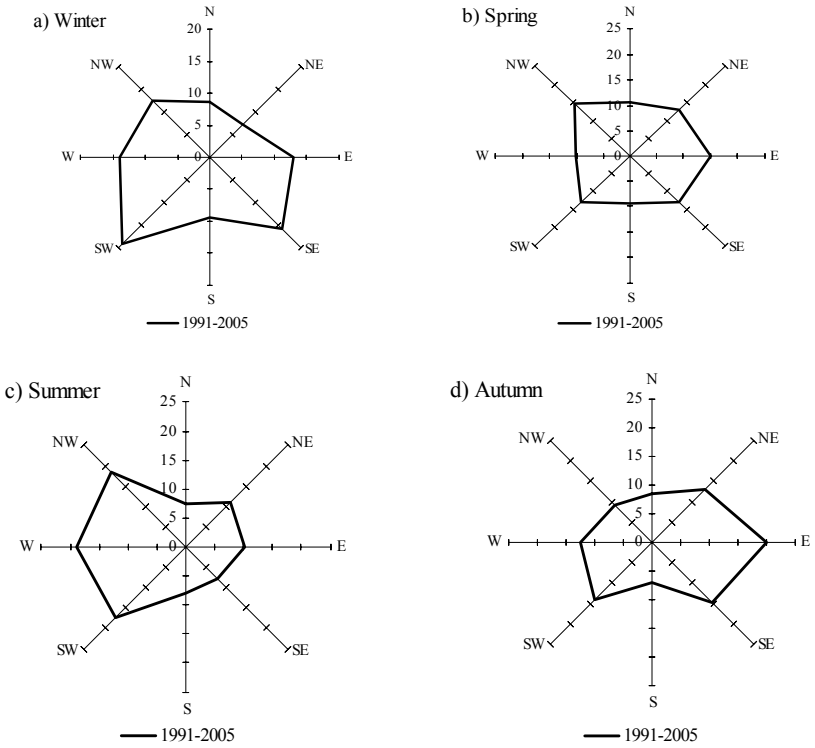

Figure 3. Season wind direction (%) rose, in the south-eastern part of Baltic Sea near Klaipeda city, 1961-2005

The possibility for the oil to reach the south-eastern coast of the Baltic Sea is the prevailing western air mass movement during summer and winter seasons. However, the wind directions from south to north-west have a key role in the wind distribution patterns during all seasons. It means that the possibility of oil to be transported towards the eastern coast of the Baltic Sea is high during all seasons (Fig. 3).

The wind, with a speed of not more than 15 m/s, becomes already dangerous for the coastal area. During a storm extreme hydro-meteorological conditions are formed which limit work at the sea. The wind in gusts at the Lithuanian seashore reaches and exceeds 15 m/s on average 67 days per year, 15 days – 20 m/s. The strongest wind is usually from western directions (Dailidienė 2001; Dailidienė et. al. 2004).

4.4 Current distribution in the area studied

In the open coastal zone of the south-eastern Baltic Sea, which is without islands and is shallow, a complicated system of waves and water mass movement is formed. It is complicated to present and evaluate more detailed information about currents in this part of the Baltic Sea due to the lack of measurements. According to the historic measurement of currents in the coastal zone of the south-eastern part of the Baltic Sea, they are usually directed to the north when the wind is of W, SW, S and to SE directions and when the wind is from the N, NW, E and NE the stream of currents usually is directed to the south (Dailidienė et al. 2004).

While analyzing the data of near-bottom current flow it was noticed that in one of the oceanographic measurement station, which reflects the southern shallow region of the Lithuanian zone, currents to the north prevail (Žaromskis and Pupienis, 2003). The

bottom relief and the exposition of the shoreline have also influence to the existing flow patterns in addition to the dominant wind conditions.

According to the long-term data of current measurements it is possible to conclude that near-bottom and surface water currents in the southern part of the Lithuanian area are directed 7 times more often to the north than to the south (Žaromskis and Pupienis, 2003). That means that during an accident the possibility of oil products to spread into the Lithuanian territory is very high. This can do much harm for fish spawning grounds, birds, recreational part of the coastal zone.

4.5 Scenario simulations of oil drift

According to long-term wind statistics and current data we made general scenarios the oil products drift for the situations when wind blows from the west and from south-west. Scenarios are made to evaluate possible oil spread in the south-eastern part of the Baltic Sea. Calculations of oil drift were made using Seatrack Web model system. The results gained showed in general that oil drift with the abovementioned wind conditions cause potentially damage for the Curonian Spit and the south-eastern part of the Baltic Sea.

Calculations of oil spread according to scenarios were made by using the Sea Track Web system. The Seatrack Web system has tree parts. The first is an operational weather and ocean forecasting system, which provides the necessary wind and current fields. Presently system users wind from the weather model HIRLAM (High Resolution Limited Area Model) and currents from 3-dimensional baroclinic circulation model HIROMB (High Resolution Operational Model for the Baltic Sea), which is run twice a day using forcing fields from HIRLAM and produces 48-hour forecasts of currents, sea level, temperature, salinity and ice conditions for the Baltic Sea – North Sea area. HIROMB calculates current velocities in a regular grid with a horizontal resolution of one nautical mile and a vertical resolution in the range of 4-60 m with increasing resolution towards the surface (Ambjörn 2005). Both HIRLAM and HIROMB are run operationally in the SMHI (Swedish Meteorological and Hydrological Institute). The forecasts are post-processed to a format suitable for Seatrack Web, in which forecast fields 2 days ahead and 10 days back in time are accessible. Detailed description of the Seatrack Web system is given by Ambjörn in Manual of Seatrack Web (the address of the system is http://pro.smhi.se/seatrack) Seatrack Web is the HELCOM system for forecasting of oil drift, and the primary users are oil combating authorities in the countries surrounding the Baltic Sea. It has been in operation since the early 1990´s, and a lot of experience in its use has been gathered among users and developers (Ambjörn 2005).

Seatrack Web is a drift forecasting system developed for emergency purposes. The drift model calculates the three-dimensional movements of substances at sea, including sinking, stranding and turbulent dispersion. For oil evaporation, emulsification and wave-induced vertical dispersion are also calculated. The Seatrack Web system helps us to make the oil spill scenarios in the Baltic Sea near D6 oil platform. These calculations only partially reflect the real situation, but the calculation of the trajectory of the possible oil drift in a specific case will allow foreseeing preventive means and the plan of effective actions for the liquidation of accident outcome.

After making oil spread calculations using the Seatrack Web programme, where the wind blows from various directions, we gain the results to be unfavorable to the

territory of Lithuania and the Curonian Spit. If the wind was from the south-western direction, oil products would appear near Klaipeda (Smiltyne), if the wind blew from the west –near Nida, if the wind was from the south, the oil would spread to the Lithuanian economic waters, if the wind was from the east, south-east and north-east, the oil would drift to the open sea. The oil would reach the shore of the Curonian Spit near Rybacij and Lesnoy settlements if the wind were from the north-west and west.

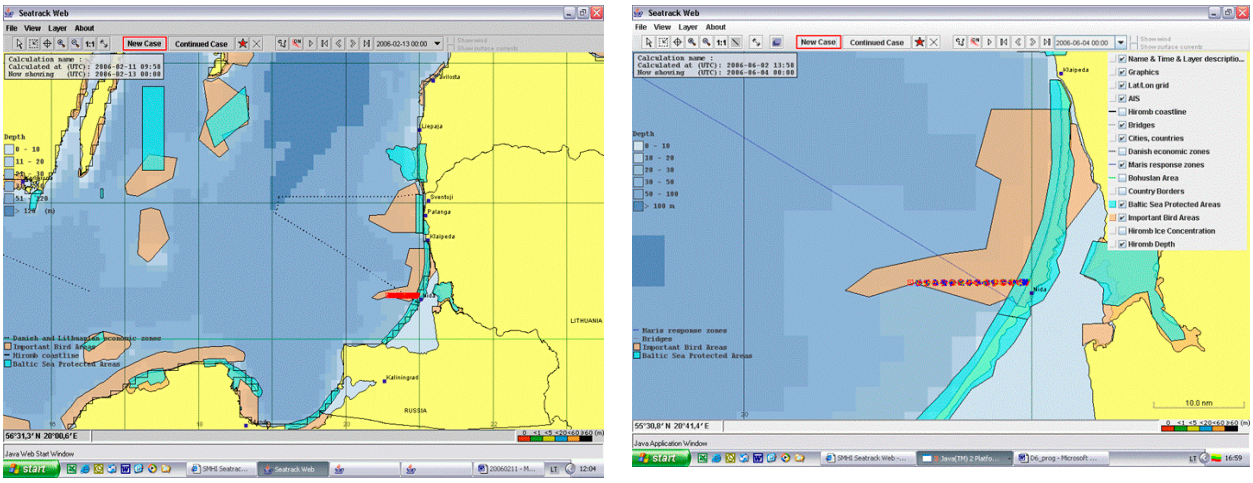

Figure 4. Two days scenario run in a case when currents are directed to the east..

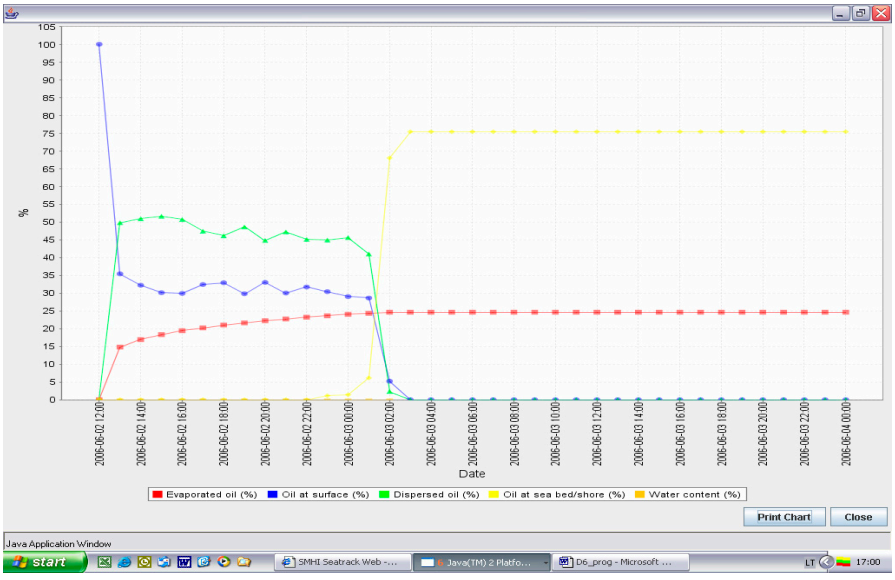

Figure 5. Scenarios where currents are directed eastwards and the current speed reach 50 cm/s. The volume of oil products is 100 m^3. In this case oil products would reach the ground and shores near the Nida city after about 14 hours.

The two days scenario run (Fig. 4) where the currents are directed eastwards show that if current speed would be 50 cm/s (stormy conditions) oil products would reach the ground and shores in 14 hours near the settlement of the Curonian Spit (and Nida city) (Fig. 5). If the speed of currents 20 cm/s oil products will reach the ground in approximately after one and a half days.

A two-days scenario (Fig. 6), where currents are directed north-eastwards, show that if current speed would be 50 cm/s the oil would spread towards Klaipeda and reach the shore after 24 hours, approximately after 2 days, and if current speed would be 40 cm/s – after 3 days if current speed would be 20 cm/s.

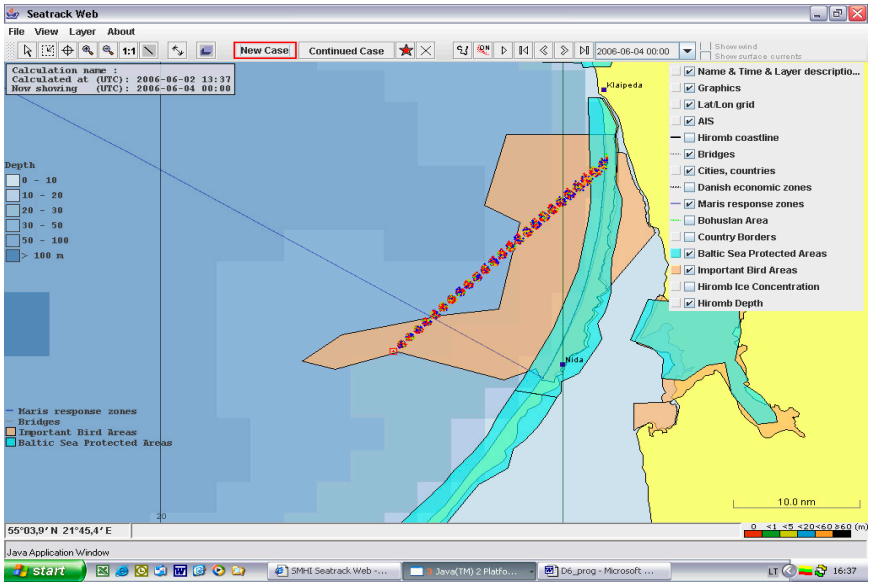

Figure 6. Scenario when currents are directed to north-east.

In a real situation hydrological and meteorological conditions change very quickly, due to constantly changing weather situations and corresponding changes in the water circulation patterns. This would lead the oil products to be spread over a large area of the Baltic Sea than according to the scenario simulations with constants winds. In reality, in an accident the possibility for oil to reach the southeastern Baltic Sea coast is very large due to the prevailing high westerly winds (Fig. 7).

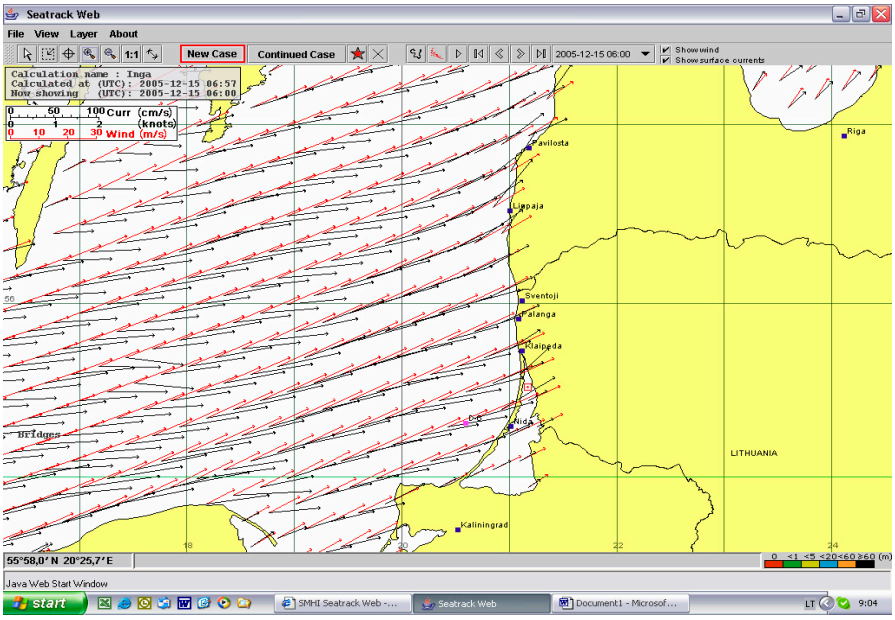

Figure 7. Forecast for December 15, 2005 when a storm took place in the Baltic Sea.

In Fig.8 it is presented an example with real (forecasted) hydro-meteorological conditions. The current field is for the time June 3, 2006 at 20.00 GMT, while the oil drift patterns represent for the period June 2 at 13.24 GMT until June 3, 20.00 GMT. The red arrows represent wind speed in (m/s) and black arrows represent currents speed in (cm/s). The amount of released oil is 100 m^3 of crude oil. This situation shows that even if the oil spill does not reach the coastal area, it may cause damage to the bird emigration area of big importance (Fig. 7).

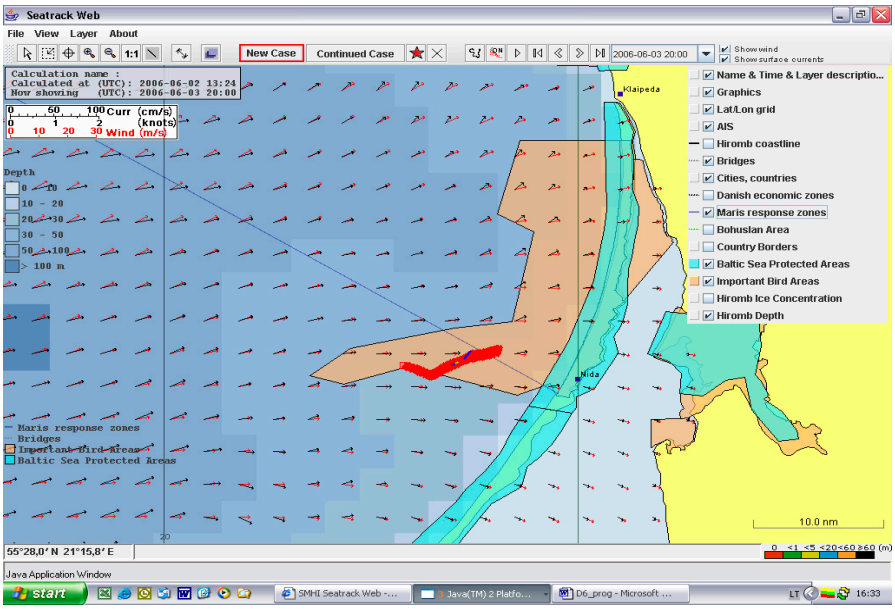

Figure 8. It is presented an example with real (forecasted) hydro-meteorological conditions. The current field is for the time June 3, 2006 at 20.00 GMT, while the oil drift patterns represent for the period June 2 at 13.24 GMT until June 3, 20.00 GMT. The red arrows represent wind speed in (m/s) and black arrows represent currents speed in (cm/s). The amount of released oil is 100 m^3 of crude oil. This situation shows that even if the oil spill does not reach the coastal area, it may cause damage to the birds emigration area of big importance (pink area).

4.6 Summary

After making oil spread calculations using the Seatrack Web system when the wind is blowing from different directions, we can conclude that results are unfavorable to the territory of Lithuania and the Curonian Spit If the wind was from the south-western direction, oil products would appear near Klaipeda, if the wind blew from the west – near Nida, if the wind was from the south, it would spread to the Lithuanian economic waters, if the wind was from the east, southeast and northeast, the oil would drift to the open sea. The oil would reach the shore of the Curonian Spit near Rybacij and Lesnoy (Russia) settlements if the wind were from the north-west and west. The most dangerous wind for Lithuania would be of the western, south-western and southern directions. The possibility for oil to reach the southeastern coast of the Baltic Sea after an oil spillage is very big due to the prevailing westerly winds.

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5. CONCLUSIONS OF THE PROJECT

- During 10-years observation period (1995-2005) environmental conditions in the study area have followed the general development of hydrochemistry and hydrography in the Baltic Sea except in the transitional zone where considerable variations in salinity and nutrient concentrations might occur caused by the outflow from the Curonian Lagoon. Nutrient concentrations are highest just in the transitional zone. In the open sea area levels of phosphorus and nitrogen nutrients are slightly increased. Near to the coast no constant changes during the 10 years' period could be seen despite large interannual variations.
- Most prominent changes in the biota in the studied area in zooplankton and benthic communities. One is the sharp decline of zooplankton group Copepoda since late 1990's and Copepods are important food source for fish and changes might be seen in physiological condition of fish. Another observed phenomenon is the decline of two important benthic species, clam *Macoma balthica* and amphipod *Monoporeia affinis* during the period of 1992-2005 compared with the figures in 1981-1991. Species composition and biomass of plankton organisms are largely dependent on general physico-chemical factors such as salinity, temperature and nutrient conditions. Accordingly, general eutrophication and changes in plankton community are reflected in the bottom community due to the changes in the quality of the composition of the organic matter settling to the bottom.
- Since 1990's new invader species have established in the Lithuanian waters. Such species are potentially toxic dinoflagellate *Prorocentrum minimum* in phytoplankton, predatory cladoceran *Cercopagis pengoi* in zooplankton and effectively spreading benthic species *Marenzelleria viridis*. Occasionally *Prorocentrum minimum* already create blooms in autumn when there is less competition of other species.
- Oil-oxidizing bacteria appear to be a promising parameter for the monitoring of oil spills because their presence indicate the occurrence of oil hydrocarbons in water. According to the 10 year data no trends can be observed at any of the sampled stations although some high peaks in numbers have been found.
- Monitoring data of heavy metals in sediments cover only the period of 2003-2005. According to maximum values for each metal given by Lithuanian legislation Cu, Zn and Pb fall to the cleanest category, while for Ni and Cr only few values exceed the limit of Category I. In Cd, only concentrations measured in 2003 exceed the Category I limit. Thus, the studied areas can be regarded relatively unpolluted by heavy metals.
- The levels of heavy metals in *M. balthica* analyzed in samples collected in November 2005 in the study area are within normal ranges and show no particular environmental impacts.
- The highest peaks in total oil hydrocarbons (THC) were measured in the open-sea area in mid-1990's and again in November 2005. In the coastal zone THC levels are lower with few peaks. The values are higher than measured in other parts of the Baltic Sea but the comparison is hampered by the different methodology used in monitoring studies. 5-17% of the values exceed the Maximum Permissible Level (MPL) established by the Lithuanian legislation. Intensive shipping activity (e.g. shipping to the Klaipeda harbour) and illegal

discharges occur in this sea area and oil spills from ships is a potential cause for the peak-type appearance of THCs in the surface water and the source of the oil cannot be specified.

- In general, low levels of PAH compounds were observed in sediments and bivalves collected in November 2005. However, PAH levels observed at the "true" soft-bottom station N-1 signify some degree of hydrocarbon pollution. The very few "molecular index" ratios of indicator PAH compounds imply that hydrocarbon pollution in the study area is mostly of pyrolytic, not petrogenic, origin and apparently from diesel motors.
- The levels of the other hazardous compounds measured in the study area were below detection limit (alkylated phenols) or low (organotins).
- Biomarker responses in the *M. balthica* show significant differences between populations from the study stations. However, since some of the enzymatic biomarkers may be affected by temperature differences between the stations (higher rates at higher temperatures), interpretations are not straightforward.
- Micronucleus frequency (MN) was significantly higher in *M. balthica* from the offshore stations compared to the near-shore stations, indicating the presence of higher genotoxicity in the offshore sea area. Basing on previous research the effect of exposure to PAH compounds on the increased levels of cytogenetic damage cannot be ruled out.
- Basing on the integrated stress response (IBR index) the *M. balthica* population at the offshore station N-2 was in the most stressed condition. To some degree, this is caused by and reflects differences in sediment structure and subsequent contaminant levels between offshore muddy-sand and near-shore sandy habitats. However, the distinction in IBR between N-2 and other demonstrates spatial differences unlinked to sediment structure and singles out station N-2 with the most unfavourable environmental conditions. Since many of the biomarkers measured here are known to respond to changes in temperature the responses observed have to be carefully studied to avoid misinterpretations.
- In flounder, most biomarker responses measured in the two study areas in December 2005 (Areas A and B) show only small differences with no clear pattern between the two study areas. The areas were probably too close to each other and/or too far from any significant pollution source for any marked differences to be seen.
- Compared to the December observations flounder collected in April 2006 (Area C) showed significantly higher biomarker responses related to potential exposure to organic contaminants (EROD, GST, CAT and PAH metabolites/1-OH-pyrene in bile). Subsequently, the IBR index was clearly higher in April. The most probable reasons for the elevated biomarker response levels are related to seasonal variability related to reproduction. However, the high concentration of 1-OH-pyrene in bile strongly implies to recent exposure to oil compounds.
- Prevalences of histopathological lesions in the liver of flounder as well as external visible fish diseases observed are within the normal range (or lower) than recorded in other parts of the Baltic Sea, implying no marked effects of contaminants affecting the health of flounder in the study area.
- The Seatrack Web system was used to simulate currents using different meteorological forcing scenarios. In case of oil spillage due to the prevailing western air mass movement, the possibility for the oil to reach the south-eastern

coast of the Baltic Sea is very high. At the Lithuanian coast south-easterly winds in addition to winds from western directions are dominant. According to model results during these conditions the water mass is moving in the south-eastern part of the Baltic Sea along the shore from the south to the north. With southwestern winds oil products would appear near Klaipeda, with western winds to the Curonian Spit near Nida. If the wind was from the south, it would spread to the Lithuanian economic waters, if the wind was from the east, southeast and northeast, the oil would drift to the open sea. In stormy conditions (current speed 50 cm/s), where the currents are directed eastwards oil products, would reach the shore in 14 hours near Nida, with the current speed of 20 cm/s in 1.5 days. When currents are directed north-eastwards and with the same current speeds oil would spread reach the shore near Klaipeda in 24 hours and in three days, respectively.

6. RECOMMENDATIONS FOR THE FUTURE MONITORING OF THE LITHUANIAN SEA AREA ADJACENT TO THE D-6 OILFIELD

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Kai Myrberg (FIMR)

These recommendations are based on results obtained from this study, previous observations from the study area, and some generally accepted measures regarding the monitoring of oil pollution in the marine environment. Although these recommendations do not have any binding effects by legislation to any party, they serve as rational suggestions worth given serious consideration if improvements in the monitoring and protection of the marine environment are to be achieved.

The present monitoring data collected by CMR form a good basis for the future monitoring activities and evaluation of the development of the environmental state of the Lithuanian sea area. Emphasis of the monitoring work done so far lies on hydrographical, hydrochemical and biological parameters, which as such are obligatory for the assessment of the state of the marine environment. In regard to harmful substances, total hydrocarbons in water and sediments as well as heavy metals in sediments have been monitored.

The recommendations presented here are based mainly on the monitoring and assessment of the effects of long-term chronic contamination by low levels of oil-derived substances, which can be argued to be more harmful to the environment than acute large oil spills. Large spills are visible to the eye and their effects are acute and more-or-less predictable. Chronic low-level exposure to pollution causes constant stress to organisms and is often difficult to recognise.

Practical suggestions for the improvement oil pollution monitoring in the Lithuanian sea area

Causes related to oil pollution for the need of increased monitoring activities in the area include:

- The possibility of the occurrence of (major) oil spills at the D-6 oil platform.
- Increase in contamination of the marine environment caused by oil drilling operations, including the accidental introduction of produced water and other by-products into the seawater.
- Potential increase in contamination of the marine environment caused by chronic leakage of small amounts of oil hydrocarbons from the D-6 oil platform.
- An ongoing increase in pollution by oil hydrocarbons of the marine environment caused by increased shipping activities in the whole Baltic Sea area, including Lithuanian waters.

Causes related to specific environmental characteristics for the need of improved monitoring activities in the area includes the following:

- Among the most potential recipient areas of an oil spill occurring at the D-6 oil platform as well as the target of chronic oil exposure is the Curonian Spit, an ecologically and culturally valuable World Heritage Site, also an important recreational area.

Specific recommendations for improving environmental monitoring in the area include the following:

- The sampling sites are now concentrated on the coastal area and close to the Lithuanian–Russian border. To monitor the effects an oil accident, depending on the direction of the oil spread a gradient of monitoring stations should be established along the most probable drift direction of the spill. Using this strategy the extent of the area impacted, seriousness of the effects and post-spill recovery can be followed in a more efficient way.
- The sampling station network for sediment chemistry should include different sediment types with strong emphasis on fine-grained sediments. Different sediment types have different adsorption characteristics and, subsequently, accumulate different concentration levels of harmful substances. This would also enable comparisons with other parts of the Baltic Sea.
- The development of an early-warning system in regard to oil pollution based on selected chemical and biological parameters, such as the occurrence of elevated levels of oil hydrocarbons in water, elevated vanadium concentrations in sediments, evaluation of hydrocarbon origin by using PAH molecular ratios, oil "fingerprinting", oil-oxidizing bacterial numbers, and biomarker responses potentially related to recent exposure to oil compounds (e.g. PAH metabolites in fish bile or bivalves, detoxification enzyme activities [e.g. EROD and GST], genotoxicity [e.g. micronuclei frequency]).
- More detailed studies on selected compartments of the marine environment, e.g. population structure of benthic key species.
- Active searching and testing of "new" cost-efficient parameters for future implementation in monitoring and assessment programmes.
- Ensuring that the oil spill forecast system should be maintained and strengthened by Lithuanian authorities in international co-operation.

Furthermore, the following should be acknowledged:

- Sufficient resources should directed for the general improvement of the general infrastructure and technical development in regard to monitoring of hazardous substances and their biological effects in Lithuanian waters,
- Co-operation between national institutes dealing with the marine environment should be strengthened in order to assure the best use of the available infrastructure, equipment and facilities for cost-efficient monitoring,

Finally:

- Since the area likely to be affected by both chronic leakage of oil and accidental oil spills from D-6 oil platform situated on the border of Lithuania and the Russian Federation, both countries are therefore advised to upgrade their co-

operation in the monitoring of oil pollution, which is also a request by the World Heritage Committee in order to ensure the safety of the World Heritage site, Curonian Spit. In addition to being more effective, this kind of co-operation would also be economically beneficial for both neighbouring countries.

APPENDIX 1

Project plan (29 August 2005) to the Finnish Ministry of Environment for the project:

Evaluation of the environmental state of the sea area in Lithuanian territorial waters and economic zone adjacent to the Russian oil platform D-6

Finnish Institute of Marine Research

Project group:

Hannu Haahti
Eila Lahdes
Kari Lehtonen
Kai Myrberg

Evaluation of the environmental state of the sea area in Lithuanian territorial waters and economic zone adjacent to the Russian oil platform D-6

Introduction

Oil emerges as one of the greatest hazards for the marine environment, either in the form of large accidents or long-term small-scale spills and leakage. Oil accidents also cause direct economic losses e.g. by affecting fish stocks and spoiling recreational use of the sea and beaches. From an ecological point of view damages occur at all levels of food web including birds and mammals.

The D-6 oil field belonging to the Russian Federation started exploitation in July 2004 by the Russian oil company LUKOIL. D-6 is situated near the Lithuanian-Russian (Kaliningrad Region) border and Curonian Spit National Park (55°019.4 N; 20°034.3 E.), with minimum distance of 4.3 miles to the sea border and 13.2 miles to the coast belonging to the Republic of Lithuania. Because of its unique landscape the Curonian Spit is included in the UNESCO World Heritage. It is thus an object of international importance as well as a nature object to be preserved.

There are no guarantees that the oil extraction started in the area will not pose any threat to the environment. In case of an oil spill, because of the prevalence of western transmission of air masses, the possibility of oil reaching the south-eastern Baltic Sea coast is high. The coastal zone of the Curonian Spit of the Baltic Sea side, characterised by a shallow sandy littoral zone, submarine sand ridges, wide beaches and a high protective dune ridge, is very sensitive to anthropogenic impacts, especially to oil pollution. If oil spills occur in the open sea and reach the littoral zone it would be heavily pollute the coastal zone, beaches and the protective dune ridge cause multiple damage to the coastal environment.

Aim of the project

The purpose of this joint Finnish-Lithuanian project is to evaluate the present state of the marine environment of the region in the Lithuanian territorial waters and economic zone, which could be seriously influenced by the operation of the D-6 oil field. In addition, recommendations for future monitoring activities will be prepared and an oil drift forecast will be worked out. The project will be carried out by independent marine experts of the Finnish Institute of Marine Research (Helsinki) and the Center of Marine Research (Klaipeda), in cooperation with institutes in Finland and Lithuania.

Implementation of the project

Timetable

Start of project: 1 September 2005.
End of project: 31 December 2006.

Study area

Preliminary station net includes 12 stations (see chart):

Station code	Latitude	Longitude	Depth (m)	Sediment type
N-1	55°34.5	20°13.5	70	Stones, pebble, sand, clay
N-2	55°32.2	20°33.8	65	Fine sand, clay loam
N-3	55°28.0	20°32.0	42	Gravel, coarse sand
N-4	55°27.0	20°48.0	33	Coarse sand, sand
N-5	55°25.5	21°02.1	13	Sand, fine sand
N-6	55°24.3	20°42.4	36	Sand, fine sand
N-7	55°22.5	21°00.1	15	Sand
N-8	55°21.7	20°49.5	37	Coarse sand, clay
N-9	55°18.7	20°57.4	14	Gravel, sand
4	55°44.1	21°03.0	16	Coarse sand
6	55°33.5	21°04.7	13	Sand
20A	55°39.0	20°50.0	43	Gravel, coarse sand, clay

Additional/substitutive stations are possible if biota or sediment cannot be collected. Ecotoxicological studies (see below) will be carried out only on selected stations.

Structure of the project

The project work consists of four Work Packages (WP) comprising several Tasks.

WP 0 Coordination

Center of Marine Research owns a great number of relevant previous data appropriate to the sea area (see Study area) originated from HELCOM monitoring and other long-term studies. Also a background assessment of the possible impact of D-6 oil field on the condition of the environment has been prepared after a research cruise in 2002 (HELCOM MONAS 5/2003, Document 7/2/INF). It is essential for the project to compile data and evaluate the possible previous changes in the area. It is important also for the monitoring activities in evaluation of the development of marine environmental state in the future.

Task	Content of the Task	Laboratory
Task 0.1	Collection of all existing 10 year data (biota, chemistry, hydrography, pollutants), relevant to the project.	CMR
Task 0.2	Analysis of the collected data.	CMR
Task 0.3	Evaluation of data • biota: community structure, species composition and long-term changes • chemistry: concentrations of nutrients (including near bottom water) and pollutants; long-term changes • pH • dissolved oxygen (including near bottom water) • hydrography: long-term changes in salinity, temperature, water transparency (including near bottom water) • currents	CMR

WP 1 Hydrography and hydrochemistry and drift modeling

Hydrography and hydrochemistry are basic elements in marine environment and determine largely the living conditions of marine biota. Therefore these determinands are obligate in the project and long-term changes in these parameters also indicate environmental state of the sea area. Also the behavior of raw oil depends on physico-chemical characteristics of sea water.

Forecast calculations of the oil drift in different hydro-meteorological conditions give means for the planning of effective measures in the case of an accident. CMR has worked in cooperation with the Swedish Meteorological and Hydrological Institute on development and adaptation of the forecast program Seatrack Web on oil and chemical contaminants. With this experience and collection of hydro-meteorological data CMR will prepare a forecast analysis of oil drift for the area.

Task	Content of the Task	Laboratory
Task 1.1	Planning of the cruise on RV Vejas in early October 2005	CMR, FIMR
Task 1.2	Sampling on RV Vejas • nutrients, pH, dissolved oxygen • CTD-data (salinity, temperature) • transparency data • meteorology •	CMR, FIMR
Task 1.3	Analysis of the cruise samples (standard methods)	CMR
Task 1.4	Data analysis of the cruise data	CMR
Task 1.5	Reporting of the cruise data • combining the cruise data with that in Task 1	CMR
Task 1.6	Forecast of oil drift by Seatrack Web	CMR

WP 2 Ecotoxicology

Measuring the concentrations of pollutants from sediments, water and biota may give information regarding their level and distribution patterns but they do not yield information on their biological effects. Therefore, it is rational to study also the effects of environmental pollutants at molecular, cell, tissue as well physiological functions levels where the adaptive or toxic responses occur and are rapidly displayed.

A large suite of methods developed to indicate biological effects of pollutants, some of which are more general stress indicators and some indicating exposure to specific groups of pollutants. Although many of the effects of toxicants are reversible, causing no permanent damage to the organisms or reduce their fitness or reproductive capacity, a great number of them do have potential links to genotoxicity, disease and reproductive disorders which are features that may strongly affect populations and communities.

Although relatively frequently performed in many other coastal sea areas these kinds of "bioeffect" studies have been carried out in the Baltic Sea very rarerly. However, an urgent need for the development of pollution monitoring strategies and assessment tools for the Baltic Sea exists. Thus, the planned study serves as one of the ground-breaking field surveys in this sea area and is valuable as such. The deliverables of this WP include "baseline" ecotoxicological data from various stations of the study area and practical recommendations for continuous pollution monitoring activities in the area.

Task 2.1: Sampling takes place on RV Vejas in early October, integrated with the sampling programme listed in WP 1. *Co-operation with local fishermen must be considered in regard to the catching of flounder.* The involvement of experts in taking samples for biological effects (from IE/VU) is essential.

All chemical and biological parameters listed in Table 1 and Table 2 will be measured from samples collected from all the chosen study stations in autumn 2005 with the following limitations:

- Availability of target species at the station

- Availability of an adequate number of specimens/amount of tissue for the specific analyses
- Successful sampling of sediment at hard sandy bottoms
- Suitability of the new method for the target species (AOX)

Task 2.2: Analysis of samples

In the framework of this project integrated measurements of chemical and biological determinands from environmental samples (sediment and biota) will be performed in Finland and Denmark with the exception of some metal analyses. For tissue samples collected for the analysis of biomarkers, snap-freezing (liquid nitrogen) aboard the vessel and conditions during maintenance [liquid nitrogen or ultrafreezer (-80ºC)] and transportation (dry ice) is essential**.** Concentrations of selected chemical substances that are generally related to oil drilling and production activities and corresponding indicators of exposure/effects (biomarkers) on target organisms are planned to be measured as presented in the tables below.

Table 1. Chemical compounds planned to be measured during the project.

Compound group	Specifics	Laboratory	Matrix	Notes
Polynuclear aromatic hydrocarbons (PAH)	16 common ones	Consulting (Finland)	*Macoma* (*Mytilus*) Sediment	
Phenols	Total phenols Nonylphenols (check) Octylphenols (check)	Consulting (Finland)	Flounder, *Macoma* (*Mytilus*) Sediment	
Organotins	Tributyltin (TBT) and derivatives (DBT, MBT)	Consulting NERI (Denmark)	*Macoma,* (*Mytilus*) Sediment	
Metals	Ni, Cr, Pb, Cu, Cd, As, Zn, V, Al ←	CMR FIMR	Flounder, *Macoma* (*Mytilus*) Sediment	Including intercalibration exercise

For the evaluation of pollutants' distribution in sediments such parameters as sediment type, granulometric composition, sedimentation rates will be included in the programme. Sampling and measurements will be carried out by specialist from the Institute of Geology and Geography (IGG/VU).

Table 2. Biological endpoints (biomarkers) planned to be measured during the project.

Biomarker	Indicator	Laboratory	Target species	Notes
Acetylcholinesterase activity (AChE)	Neurotoxicity/general stress	FIMR	Flounder *Macoma* (*Mytilus*)	
Glutathione-S-transferase activity (GST)	Biotransformation Phase II/oxidative stress	FIMR	Flounder *Macoma* (*Mytilus*)	
Catalase activity (CAT)	Oxidative stress	FIMR	Flounder *Macoma* (*Mytilus*)	
Metallothionein induction (MT)	Exposure to heavy metals/general stress	FIMR	Flounder *Macoma* (*Mytilus*)	
Acyl-CoA oxidase activity (AOX)	Exposure to organic contaminants	FIMR	*Macoma* (*Mytilus*)	At development stage at FIMR
PAH metabolites in bile	Exposure to PAH	FGFRI/ EELA	Flounder	
Micronuclei frequency (MN)	Genotoxicity	IE/VU	Flounder *Macoma* (*Mytilus*)	
Ethoxyresorufin-*O*-deethylase activity (EROD)	Biotransformation Phase I	FIMR	Flounder	At development stage at FIMR

WP 3 Ecology

Bacterioplankton, phytoplankton and zooplankton are pelagic organisms which, in the case of oil accident, among the first ones will be the affected. This is result of acute toxicity, toxication via food and soiling by oil. Raw oil is complex mixture of chemical compounds; e.g. aliphatic, cyclic and aromatic hydrocarbons, sulfur and nitrogen compounds and metals. Damages in some part of the food web cause problems at all trophic levels. Therefore the whole pelagic food web has to be studied for the project and future monitorin purposes. The effects on benthic fauna are direct on the shallow shore line but with delay on those living in deeper areas.

Task	Content of the Task	Laboratory
Task 3.1	Planning of the cruise on RV Vejas in early October 2005	CMR, FIMR
Task 3.2	Sampling on RV Vejas • Phytoplankton (qualitive & quantitative sampling) • Mesozooplankton • Macrozoobenthos • Bacterioplankton	CMR
Task 3.3	Analysis of the cruise samples (standard methods)	CMR
Task 3.4	Data analysis of the cruise data	CMR
Task 3.5	Reporting of the cruise data • combining the cruise data with that in Task 1	CMR

WP 4 Data analysis and reporting

This WP includes the preparation of the final report of the project.

Task	Content of the Task	Laboratory
Task 1.4	Compilation of data	FIMR, CMR
Task 2.4	Revising of data by experts of FIMR	FIMR
Task 3.4	Writing of the final report	FIMR
Task 3.5	Writing of scientific publications	FIMR, CMR, IE/VU, IGG/VU, EELA

Abbreviations of the responsible institutions:

CMR – Center of Marine Research (Lithuania)
EELA – National Veterinary and Food Research Institute (Finland)
FGFRI – Finnish Game and Fisheries Research Institute (Finland)
FIMR – Finnish Institute of Marine Research (Finland)
IE/VU – Institute of Ecology/Vilnius University (Lithuania)
NERI – National Environment Research Institute (Denmark)
IGG/VU - Institute of Geology and Geography (Lithuania)

APPENDIX 2

SAMPLES AND RESULTS OF THE RESEARCH CRUISE ON 8-11 NOVEMBER 2005
ORIGINAL DATA

Table 1. Station data and hydrographic conditions 8-9 November 2005

Station	Latitude		Longitude		Date	Bottom	Time	Depth	Temperature	Salinity	Oxygen
	°	'	°	'	mm.dd.yyyy.	m	GMT	m	°C	‰	ml/l
6	**55**	**33.5**	**21**	**04.7**	**11.8.2005**	**13**	**10.47**	**1**	**9.66**	**7.13**	**7.59**
6	55	33.5	21	04.7	11.8.2005	13	10.47	5	9.65	7.16	
6	55	33.5	21	04.7	11.8.2005	13	10.47	10	9.43	7.16	7.63
N-5	**55**	**25.5**	**21**	**02.1**	**11.8.2005**	**13**	**12.31**	**1**	**10.05**	**7.19**	**7.45**
N-5	55	25.5	21	02.1	11.8.2005	13	12.32	5	10.03	7.19	
N-5	55	25.5	21	02.1	11.8.2005	13	12.32	10	9.78	7.19	7.55
N-9	**55**	**18.7**	**20**	**57.4**	**11.8.2005**	**14**	**14.57**	**1**	**10.59**	**7.23**	**7.31**
N-9	55	18.7	20	57.4	11.8.2005	14	14.57	5	10.59	7.23	
N-9	55	18.7	20	57.4	11.8.2005	14	14.58	10	10.56	7.22	7.36
N-9	55	18.7	20	57.4	11.8.2005	14	14.58	12	9.71	7.23	7.34
N-8	**55**	**21.7**	**20**	**49.5**	**11.8.2005**	**37**	**18.04**	**1**	**11.01**	**7.24**	**7.15**
N-8	55	21.7	20	49.5	11.8.2005	37	18.04	5	11.02	7.25	
N-8	55	21.7	20	49.5	11.8.2005	37	18.05	10	11.02	7.25	7.18
N-8	55	21.7	20	49.5	11.8.2005	37	18.05	20	11.02	7.25	7.24
N-8	55	21.7	20	49.5	11.8.2005	37	18.06	30	11.02	7.25	7.21
N-8	55	21.7	20	49.5	11.8.2005	37	18.06	34	11.02	7.24	7.13
N-6	**55**	**24.3**	**20**	**42.4**	**11.8.2005**	**36**	**21.19**	**1**	**11.00**	**7.24**	**7.2**
N-6	55	24.3	20	42.4	11.8.2005	36	21.19	5	11.00	7.24	
N-6	55	24.3	20	42.4	11.8.2005	36	21.20	10	11.00	7.24	7.21
N-6	55	24.3	20	42.4	11.8.2005	36	21.20	20	10.96	7.26	7.29
N-6	55	24.3	20	42.4	11.8.2005	36	21.21	30	10.94	7.25	7.27
N-6	55	24.3	20	42.4	11.8.2005	36	21.21	33	10.94	7.25	7.22
N-3	**55**	**28.0**	**20**	**32.0**	**11.8.2005**	**42**	**23.37**	**1**	**10.84**	**7.24**	**7.31**
N-3	55	28.0	20	32.0	11.8.2005	42	23.37	5	10.83	7.25	
N-3	55	28.0	20	32.0	11.8.2005	42	23.37	10	10.83	7.25	7.2
N-3	55	28.0	20	32.0	11.8.2005	42	23.38	20	10.76	7.25	7.23
N-3	55	28.0	20	32.0	11.8.2005	42	23.39	30	10.70	7.24	7.22
N-3	55	28.0	20	32.0	11.8.2005	42	23.39	40	5.44	7.55	6.38
N-2	**55**	**31.2**	**20**	**33.8**	**11.9.2005**	**65**	**1.47**	**1**	**10.56**	**7.22**	**7.31**
N-2	55	31.2	20	33.8	11.9.2005	65	1.47	5	10.57	7.23	
N-2	55	31.2	20	33.8	11.9.2005	65	1.47	10	10.57	7.23	7.33
N-2	55	31.2	20	33.8	11.9.2005	65	1.48	20	10.58	7.24	7.38
N-2	55	31.2	20	33.8	11.9.2005	65	1.49	30	10.58	7.24	7.58
N-2	55	31.2	20	33.8	11.9.2005	65	1.49	40	10.58	7.24	7.35
N-2	55	31.2	20	33.8	11.9.2005	65	1.50	50	3.84	7.84	5.71
N-2	55	31.2	20	33.8	11.9.2005	65	1.51	60	3.99	8.88	4.91
N-2	55	31.2	20	33.8	11.9.2005	65	1.51	62	4.03	8.94	2.7
N-1	**55**	**34.5**	**20**	**13.5**	**11.9.2005**	**70**	**6.12**	**1**	**10.71**	**7.22**	**7.36**
N-1	55	34.5	20	13.5	11.9.2005	70	6.12	5	10.70	7.23	
N-1	55	34.5	20	13.5	11.9.2005	70	6.13	10	10.70	7.23	7.37
N-1	55	34.5	20	13.5	11.9.2005	70	6.13	20	10.72	7.24	7.37
N-1	55	34.5	20	13.5	11.9.2005	70	6.14	30	10.74	7.25	7.34
N-1	55	34.5	20	13.5	11.9.2005	70	6.15	40	10.36	7.27	6.8
N-1	55	34.5	20	13.5	11.9.2005	70	6.15	50	4.00	7.61	5.76
N-1	55	34.5	20	13.5	11.9.2005	70	6.16	60	3.86	8.69	3.38
N-1	55	34.5	20	13.5	11.9.2005	70	6.16	67	4.41	9.38	2.34
65	**55**	**52.9**	**20**	**20.5**	**11.9.2005**	**47**	**10.38**	**1**	**10.72**	**7.23**	**7.25**
65	55	52.9	20	20.5	11.9.2005	47	10.38	5	10.74	7.24	7.22
65	55	52.9	20	20.5	11.9.2005	47	10.39	10	10.74	7.24	7.31
65	55	52.9	20	20.5	11.9.2005	47	10.39	15	10.74	7.24	7.26
65	55	52.9	20	20.5	11.9.2005	47	10.39	20	10.72	7.24	7.21
65	55	52.9	20	20.5	11.9.2005	47	10.40	30	10.72	7.26	7.28
65	55	52.9	20	20.5	11.9.2005	47	10.40	40	10.76	7.27	7.17
65	55	52.9	20	20.5	11.9.2005	47	10.41	44	10.72	7.28	7.13

Table 2. Phytoplankton species composition, abundance and biomass in November 2005

Station	Date	Algae class	Species	Abundance	%	Biomass	%
				10^3 cells/l		mg/l	
4	2005.11.09	Cyanophyceae	Coelomoron pusillum	1.8	0.12	0.00023	0.01
4	2005.11.09	Cyanophyceae	Merismopedia punctata	1.8	0.12	0.00041	0.01
4	2005.11.09	Cyanophyceae	Microcystis viridis	0.2	0.01	0.00105	0.02
4	2005.11.09	Cyanophyceae	Microcystis wesenbergii	0.3	0.02	0.00209	0.05
4	2005.11.09	Cyanophyceae	Planktothrix agardhii	14.6	0.98	0.02860	0.66
4	2005.11.09	Cyanophyceae	Pseudanabaena limnetica	5.5	0.37	0.00097	0.02
4	2005.11.09	Cyanophyceae	Snowella lacustris	1.8	0.12	0.00089	0.02
4	2005.11.09	Cyanophyceae	Woronichinia compacta	4.0	0.27	0.00294	0.07
4	2005.11.09	Cyanophyceae	***Total Cyanophyceae:***	**30.0**	**2.0**	**0.03719**	**0.9**
4	2005.11.09	Cryptophyceae	Katablepharis ovalis	3.6	0.24	0.00093	0.02
4	2005.11.09	Cryptophyceae	Leucocryptos marina	0.2	0.01	0.00002	0.0004
4	2005.11.09	Cryptophyceae	Plagioselmis prolonga	103.8	6.96	0.00564	0.13
4	2005.11.09	Cryptophyceae	Teleaulax amphioxeia	34.6	2.32	0.00777	0.18
4	2005.11.09	Cryptophyceae	***Total Cryptophyceae:***	**142.3**	**9.54**	**0.01436**	**0.33**
4	2005.11.09	Dinophyceae	Dinophysis acuminata	0.5	0.03	0.00237	0.05
4	2005.11.09	Dinophyceae	Dinophysis rotundata	0.2	0.01	0.00095	0.02
4	2005.11.09	Dinophyceae	Gymnodinium simplex	1.8	0.12	0.00049	0.01
4	2005.11.09	Dinophyceae	Heterocapsa rotundata	10.9	0.73	0.00367	0.08
4	2005.11.09	Dinophyceae	Heterocapsa triquetra	0.2	0.01	0.00028	0.01
4	2005.11.09	Dinophyceae	Prorocentrum minimum	1.8	0.12	0.00292	0.07
4	2005.11.09	Dinophyceae	Warnowia cf. rosea	1.8	0.12	0.01867	0.43
4	2005.11.09	Dinophyceae	***Total Dinophyceae:***	**17.2**	**1.15**	**0.02936**	**0.68**
4	2005.11.09	Prasinophyceae	Pyramimonas spp.	16.4	1.10	0.00197	0.05
4	2005.11.09	Prasinophyceae	***Total Prasinophyceae:***	**16.4**	**1.10**	**0.00197**	**0.05**
4	2005.11.09	Euglenophyceae	Eutreptiella spp.	5.5	0.37	0.00129	0.03
4	2005.11.09	Euglenophyceae	***Total Euglenophyceae:***	**5.5**	**0.37**	**0.00129**	**0.03**
4	2005.11.09	Craspedophyceae	Telonema antarctica	1.8	0.12	0.00031	0.01
4	2005.11.09	Craspedophyceae	***Total Craspedophyceae:***	**1.8**	**0.12**	**0.00031**	**0.01**
4	2005.11.09	Diatomophyceae	Actinocyclus normanii	6.2	0.42	0.07654	1.77
4	2005.11.09	Diatomophyceae	Actinocyclus octonarius v. octonarius	7.3	0.49	0.17043	3.94
4	2005.11.09	Diatomophyceae	Attheya septentrionalis	3.6	0.24	0.00037	0.01
4	2005.11.09	Diatomophyceae	Aulacoseira italica	31.0	2.08	0.08540	1.97
4	2005.11.09	Diatomophyceae	Cerataulina pelagica	14.6	0.98	0.04027	0.93
4	2005.11.09	Diatomophyceae	Cerataulina pelagica	49.2	3.30	0.21238	4.91
4	2005.11.09	Diatomophyceae	Cerataulina pelagica	52.8	3.54	0.41574	9.60
4	2005.11.09	Diatomophyceae	Cerataulina pelagica	41.9	2.81	0.50526	11.67
4	2005.11.09	Diatomophyceae	Chaetoceros brevis	61.9	4.15	0.18381	4.25
4	2005.11.09	Diatomophyceae	Chaetoceros brevis	98.4	6.60	0.48933	11.30
4	2005.11.09	Diatomophyceae	Chaetoceros danicus	5.5	0.37	0.00346	0.08
4	2005.11.09	Diatomophyceae	Chaetoceros ? socialis f. radians	16.4	1.10	0.03568	0.82
4	2005.11.09	Diatomophyceae	Dactyliosolen fragilissimus	32.8	2.20	0.21805	5.04
4	2005.11.09	Diatomophyceae	Dactyliosolen fragilissimus	98.4	6.60	0.80851	18.68
4	2005.11.09	Diatomophyceae	Dactyliosolen fragilissimus	45.5	3.05	0.69232	15.99
4	2005.11.09	Diatomophyceae	Diatoma tenuis	9.1	0.61	0.00875	0.20
4	2005.11.09	Diatomophyceae	Staurosira construens v. venter	32.8	2.20	0.01986	0.46
4	2005.11.09	Diatomophyceae	Fragilaria heidenii	16.4	1.10	0.01366	0.32
4	2005.11.09	Diatomophyceae	Fragilaria virescens	16.8	1.13	0.01327	0.31
4	2005.11.09	Diatomophyceae	Nitzschia acicularis	1.8	0.12	0.00051	0.01
4	2005.11.09	Diatomophyceae	Skeletonema costatum	5.5	0.37	0.00091	0.02

Station	Date	Algae class	Species	Abundance	%	Biomass	%
				10^3 cells/l		mg/l	
4	2005.11.09	Diatomophyceae	Skeletonema subsalsum	51.0	3.42	0.01999	0.46
4	2005.11.09	Diatomophyceae	Staurosira construens v. construens	34.6	2	0.01765	0.41
4	2005.11.09	Diatomophyceae	Stephanodiscus hantzschii	10.9	0.73	0.02480	0.57
4	2005.11.09	Diatomophyceae	Stephanodiscus rotula	1.8	0.12	0.02472	0.57
4	2005.11.09	Diatomophyceae	Surirella biseriata	0.1	0.01	0.04704	1.09
4	2005.11.09	Diatomophyceae	Thalassiosira baltica	0.1	0.01	0.00286	0.07
4	2005.11.09	Diatomophyceae	Thalassiosira sp.	7.3	0.49	0.00372	0.09
4	2005.11.09	Diatomophyceae	Thalassiosira spp.	10.9	0.73	0.00070	0.02
4	2005.11.09	Diatomophyceae	***Total Diatomophyceae:***	**764.7**	**51.3**	**4.13598**	**95.5**
4	2005.11.09	Chlorophyceae	Dictyosphaerium pulchellum	7.3	0.49	0.00644	0.15
4	2005.11.09	Chlorophyceae	Micractinium pusillum	1.8	0.12	0.00004	0.0009
4	2005.11.09	Chlorophyceae	Monoraphidium contortum	1.8	0.12	0.00004	0.0008
4	2005.11.09	Chlorophyceae	Pediastrum boryanum v. boryanum	3.6	0.24	0.03890	0.90
4	2005.11.09	Chlorophyceae	Pediastrum duplex v. duplex	0.1	0.01	0.00141	0.03
4	2005.11.09	Chlorophyceae	Planctonema lauterbornii	479.2	32.12	0.05818	1.34
4	2005.11.09	Chlorophyceae	Scenedesmus obliquus	3.6	0.24	0.00021	0.005
4	2005.11.09	Chlorophyceae	Scenedesmus obliquus	3.6	0.24	0.00065	0.02
4	2005.11.09	Chlorophyceae	Desmodesmus armatus v. armatus	7.3	0.49	0.00093	0.02
4	2005.11.09	Chlorophyceae	Desmodesmus armatus v. bicaudatus	1.8	0.12	0.00043	0.01
4	2005.11.09	Chlorophyceae	Desmodesmus maximus	3.6	0.24	0.00165	0.04
4	2005.11.09	Chlorophyceae	***Total Chlorophyceae:***	**513.9**	**34.45**	**0.10889**	**2.52**
4	2005.11.09		**Total:**	**1491.7**		**4.329**	
65	2005.11.09	Cryptophyceae	Leucocryptos marina	1.8	0.61	0.00021	0.01
65	2005.11.09	Cryptophyceae	Plagioselmis prolonga	91.1	30.28	0.00495	0.33
65	2005.11.09	Cryptophyceae	Teleaulax amphioxeia	40.1	13.32	0.00899	0.60
65	2005.11.09	Cryptophyceae	***Total Cryptophyceae:***	**133.0**	**44.21**	**0.01415**	**0.95**
65	2005.11.09	Dinophyceae	Dinophysis acuminata	0.2	0.05	0.00079	0.05
65	2005.11.09	Dinophyceae	Gymnodinium simplex	3.6	1.21	0.00096	0.06
65	2005.11.09	Dinophyceae	Gyrodinium sp.	7.9	2.62	0.00727	0.49
65	2005.11.09	Dinophyceae	Heterocapsa rotundata	2.4	0.81	0.00082	0.05
65	2005.11.09	Dinophyceae	Katodinium glaucum	2.7	0.91	0.00085	0.06
65	2005.11.09	Dinophyceae	Warnowia cf. rosea	1.2	0.40	0.01245	0.83
65	2005.11.09	Dinophyceae	***Total Dinophyceae:***	**18.1**	**6.01**	**0.02313**	**1.54**
65	2005.11.09	Prasinophyceae	Pyramimonas spp.	23.7	7.87	0.00284	0.19
65	2005.11.09	Prasinophyceae	***Total Prasinophyceae:***	**23.7**	**7.87**	**0.00284**	**0.19**
65	2005.11.09	Euglenophyceae	Eutreptiella spp.	2.4	0.81	0.00057	0.04
65	2005.11.09	Euglenophyceae	***Total Euglenophyceae:***	**2.4**	**0.81**	**0.00057**	**0.04**
65	2005.11.09	Craspedophyceae	Telonema antarctica	1.8	0.61	0.00031	0.02
65	2005.11.09	Craspedophyceae	***Total Craspedophyceae:***	**1.8**	**0.61**	**0.00031**	**0.02**
65	2005.11.09	Prymnesiophyceae	Chrysochromulina spp.	3.6	1.21	0.00048	0.03
65	2005.11.09	Prymnesiophyceae	***Total Prymnesiophyceae:***	**3.6**	**1.21**	**0.00048**	**0.03**
65	2005.11.09	Diatomophyceae	Actinocyclus octonarius v. octonarius	0.2	0.05	0.01087	0.73
65	2005.11.09	Diatomophyceae	Actinocyclus octonarius v. tenellus	0.2	0.08	0.00353	0.24
65	2005.11.09	Diatomophyceae	Cerataulina pelagica	0.3	0.11	0.00386	0.26
65	2005.11.09	Diatomophyceae	Chaetoceros brevis	1.1	0.37	0.00332	0.22
65	2005.11.09	Diatomophyceae	Chaetoceros brevis	0.6	0.19	0.00279	0.19
65	2005.11.09	Diatomophyceae	Chaetoceros danicus	0.2	0.08	0.00015	0.01
65	2005.11.09	Diatomophyceae	Coscinodiscus granii	1.7	0.56	1.36733	91.30
65	2005.11.09	Diatomophyceae	Cyclotella choctawhatcheeana	7.3	2.42	0.00062	0.04

Station	Date	Algae class	Species	Abundance	%	Biomass	%
				10^3 cells/l		mg/l	
65	2005.11.09	Diatomophyceae	Dactyliosolen fragilissimus	0.4	0.13	0.00266	0.18
65	2005.11.09	Diatomophyceae	Dactyliosolen fragilissimus	3.0	1.01	0.02498	1.67
65	2005.11.09	Diatomophyceae	Dactyliosolen fragilissimus	0.7	0.24	0.01094	0.73
65	2005.11.09	Diatomophyceae	Dactyliosolen fragilissimus	0.4	0.13	0.00681	0.45
65	2005.11.09	Diatomophyceae	Skeletonema costatum	3.6	1.21	0.00061	0.04
65	2005.11.09	Diatomophyceae	Thalassiosira sp.	14.6	4.84	0.00743	0.50
65	2005.11.09	Diatomophyceae	***Total Diatomophyceae:***	**34.4**	**11.4**	**1.44591**	**96.6**
65	2005.11.09	Chlorophyceae	Planctonema lauterbornii	83.8	27.86	0.01018	0.68
65	2005.11.09	Chlorophyceae	***Total Chlorophyceae:***	**83.8**	**27.86**	**0.01018**	**0.68**
65	2005.11.09		**Total:**	**300.8**		**1.49757**	
N-9	2005.11.08	Cyanophyceae	Aphanizomenon sp.	3.6	0.45	0.00458	0.58
N-9	2005.11.08	Cyanophyceae	***Total Cyanophyceae:***	**3.6**	**0.4**	**0.00458**	**0.58**
N-9	2005.11.08	Cryptophyceae	Leucocryptos marina	29.2	3.58	0.00340	0.43
N-9	2005.11.08	Cryptophyceae	Plagioselmis prolonga	342.5	42.04	0.01860	2.37
N-9	2005.11.08	Cryptophyceae	Teleaulax amphioxeia	82.0	10.06	0.01840	2.34
N-9	2005.11.08	Cryptophyceae	***Total Cryptophyceae:***	**453.7**	**55.68**	**0.04040**	**5.15**
N-9	2005.11.08	Dinophyceae	Dinophysis acuminata	0.1	0.01	0.00097	0.12
N-9	2005.11.08	Dinophyceae	Gyrodinium sp. 7	7.3	0.89	0.00052	0.07
N-9	2005.11.08	Dinophyceae	Heterocapsa rotundata	25.5	3.13	0.00856	1.09
N-9	2005.11.08	Dinophyceae	Katodinium glaucum	14.6	1.79	0.00452	0.58
N-9	2005.11.08	Dinophyceae	***Total Dinophyceae:***	**47.4**	**5.82**	**0.01458**	**1.86**
N-9	2005.11.08	Prasinophyceae	Pyramimonas spp.	94.7	11.63	0.01137	1.45
N-9	2005.11.08	Prasinophyceae	***Total Prasinophyceae:***	**94.7**	**11.63**	**0.01137**	**1.45**
N-9	2005.11.08	Euglenophyceae	Eutreptiella spp.	21.9	2.68	0.00516	0.66
N-9	2005.11.08	Euglenophyceae	***Total Euglenophyceae:***	**21.9**	**2.68**	**0.00516**	**0.66**
N-9	2005.11.08	Chrysophyceae	Pseudopedinella tricostata	7.3	0.89	0.00024	0.03
N-9	2005.11.08	Chrysophyceae	***Total Chrysophyceae:***	**7.3**	**0.89**	**0.00024**	**0.03**
N-9	2005.11.08	Diatomophyceae	Actinocyclus octonarius v. octonarius	0.1	0.01	0.00544	0.69
N-9	2005.11.08	Diatomophyceae	Actinocyclus octonarius v. tenellus	0.2	0.03	0.00353	0.45
N-9	2005.11.08	Diatomophyceae	Cerataulina pelagica	0.2	0.03	0.00104	0.13
N-9	2005.11.08	Diatomophyceae	Cerataulina pelagica	1.1	0.14	0.00881	1.12
N-9	2005.11.08	Diatomophyceae	Cerataulina pelagica	1.8	0.22	0.02122	2.70
N-9	2005.11.08	Diatomophyceae	Cerataulina pelagica	1.2	0.15	0.01884	2.40
N-9	2005.11.08	Diatomophyceae	Chaetoceros brevis	2.0	0.25	0.00593	0.76
N-9	2005.11.08	Diatomophyceae	Chaetoceros brevis	0.6	0.08	0.00318	0.41
N-9	2005.11.08	Diatomophyceae	Chaetoceros danicus	0.1	0.01	0.00005	0.01
N-9	2005.11.08	Diatomophyceae	Chaetoceros gracilis	0.1	0.01	0.00001	0.00
N-9	2005.11.08	Diatomophyceae	Chaetoceros impressus	0.9	0.11	0.00187	0.24
N-9	2005.11.08	Diatomophyceae	Coscinodiscus granii	0.7	0.09	0.58600	74.65
N-9	2005.11.08	Diatomophyceae	Dactyliosolen fragilissimus	0.2	0.03	0.00197	0.25
N-9	2005.11.08	Diatomophyceae	Dactyliosolen fragilissimus	1.1	0.14	0.01702	2.17
N-9	2005.11.08	Diatomophyceae	Dactyliosolen fragilissimus	0.8	0.10	0.01361	1.73
N-9	2005.11.08	Diatomophyceae	***Total Diatomophyceae:***	**11.2**	**1.4**	**0.68854**	**87.71**
N-9	2005.11.08	Chlorophyceae	Monoraphidium contortum	10.9	1.34	0.00022	0.03
N-9	2005.11.08	Chlorophyceae	Planctonema lauterbornii	164.0	20.13	0.01991	2.54
N-9	2005.11.08	Chlorophyceae	***Total Chlorophyceae:***	**174.9**	**21.47**	**0.02013**	**2.56**
N-9	2005.11.08		**Total:**	**814.7**		**0.785**	
6	2005.11.08	Cyanophyceae	Merismopedia punctata	3.6	0.18	0.00082	0.02
6	2005.11.08	Cyanophyceae	***Total Cyanophyceae:***	**3.6**	**0.18**	**0.00082**	**0.02**

Station	Date	Algae class	Species	Abundance	%	Biomass	%
				10^3 cells/l		mg/l	
6	2005.11.08	Cryptophyceae	Scenedesmus obliquus	76.5	3.84	0.00031	0.01
6	2005.11.08	Cryptophyceae	Katablepharis ovalis	3.6	0.18	0.00093	0.02
6	2005.11.08	Cryptophyceae	Leucocryptos marina	3.6	0.18	0.00040	0.01
6	2005.11.08	Cryptophyceae	Plagioselmis prolonga	120.2	6.03	0.02177	0.41
6	2005.11.08	Cryptophyceae	Teleaulax amphioxeia	74.7	3.75	0.02682	0.50
6	2005.11.08	Cryptophyceae	***Total Cryptophyceae:***	**278.8**	**13.98**	**0.05023**	**0.95**
6	2005.11.08	Dinophyceae	Dinophysis acuminata	0.2	0.01	0.00387	0.07
6	2005.11.08	Dinophyceae	Dinophysis norvegica	0.1	0.004	0.00117	0.02
6	2005.11.08	Dinophyceae	Gymnodiniales spp.	10.9	0.55	0.00982	0.18
6	2005.11.08	Dinophyceae	Gyrodinium sp. 7	7.3	0.37	0.00150	0.03
6	2005.11.08	Dinophyceae	Heterocapsa rotundata	10.9	0.55	0.00267	0.05
6	2005.11.08	Dinophyceae	Katodinium glaucum	5.5	0.27	0.00170	0.03
6	2005.11.08	Dinophyceae	***Total Dinophyceae:***	**34.9**	**1.75**	**0.02072**	**0.39**
6	2005.11.08	Chrysophyceae	Pseudopedinella tricostata	14.6	0.73	0.00095	0.02
6	2005.11.08	Chrysophyceae	***Total Chrysophyceae:***	**14.6**	**0.73**	**0.00095**	**0.02**
6	2005.11.08	Euglenophyceae	Eutreptiella spp.	7.3	0.37	0.00953	0.18
6	2005.11.08	Euglenophyceae	***Total Euglenophyceae:***	**7.3**	**0.366**	**0.00953**	**0.18**
6	2005.11.08	Craspedophyceae	Craspedophyceae spp.	29.2	1.46	0.00041	0.01
6	2005.11.08	Craspedophyceae	Telonema antarctica	3.6	0.18	0.00062	0.01
6	2005.11.08	Craspedophyceae	***Total Craspedophyceae:***	**32.8**	**1.65**	**0.00103**	**0.02**
6	2005.11.08	Prasinophyceae	Nephroselmis sp.	65.6	3.29	0.00110	0.02
6	2005.11.08	Prasinophyceae	Pyramimonas spp.	3.6	0.18	0.00033	0.01
6	2005.11.08	Prasinophyceae	***Total Prasinophyceae:***	**69.2**	**3.47**	**0.00143**	**0.03**
6	2005.11.08	Prymnesiophyceae	Chrysochromulina spp.	29.2	1.46	0.00041	0.01
6	2005.11.08	Prymnesiophyceae	***Total Prymnesiophyceae:***	**29.2**	**1.46**	**0.00041**	**0.01**
6	2005.11.08	Diatomophyceae	Actinocyclus octonarius v. tenellus	0.1	0.00	0.00040	0.01
6	2005.11.08	Diatomophyceae	Cerataulina pelagica	80.2	4.02	0.22029	4.15
6	2005.11.08	Diatomophyceae	Cerataulina pelagica	69.2	3.47	0.31307	5.89
6	2005.11.08	Diatomophyceae	Cerataulina pelagica	78.3	3.93	0.66912	12.60
6	2005.11.08	Diatomophyceae	Cerataulina pelagica	40.1	2.01	0.62928	11.85
6	2005.11.08	Diatomophyceae	Chaetoceros brevis	34.6	1.74	0.06847	1.29
6	2005.11.08	Diatomophyceae	Chaetoceros brevis	49.2	2.47	0.14595	2.75
6	2005.11.08	Diatomophyceae	Chaetoceros brevis	18.2	0.91	0.09062	1.71
6	2005.11.08	Diatomophyceae	Dactyliosolen fragilissimus	71.1	3.56	0.47244	8.89
6	2005.11.08	Diatomophyceae	Dactyliosolen fragilissimus	102.0	5.12	0.83845	15.78
6	2005.11.08	Diatomophyceae	Dactyliosolen fragilissimus	58.3	2.92	0.88617	16.68
6	2005.11.08	Diatomophyceae	Dactyliosolen fragilissimus	41.9	2.10	0.71295	13.42
6	2005.11.08	Diatomophyceae	Thalassiosira spp.	3.6	0.18	0.06200	1.17
6	2005.11.08	Diatomophyceae	Thalassiosira spp.	27.3	1.37	0.01394	0.26
6	2005.11.08	Diatomophyceae	***Total Diatomophyceae:***	**674.2**	**33.82**	**5.12314**	**96.43**
6	2005.11.08	Chlorophyceae	Monoraphidium contortum	7.3	0.37	0.00015	0.003
6	2005.11.08	Chlorophyceae	Planctonema lauterbornii	838.1	42.04	0.10175	1.92
6	2005.11.08	Chlorophyceae	***Total Chlorophyceae:***	**845.4**	**42.41**	**0.10190**	**1.92**
6	2005.11.08	Coccolithophoridae	Calyptosphaera sp.	1.8	0.09	0.00085	0.02
6	2005.11.08	Coccolithophoridae	Calyptosphaera sp.	1.8	0.09	0.00153	0.03
6	2005.11.08	Coccolithophoridae	***Total Coccolithophoridae:***	**3.6**	**0.18**	**0.00238**	**0.04**
6	2005.11.08		**Total:**	**1993.5**		**5.313**	
N-2	2005.11.09	Cyanophyceae	Aphanizomenon sp.	0.2	0.12	0.00030	0.01
N-2	2005.11.09	Cyanophyceae	***Total Cyanophyceae:***	**0.2**	**0.1**	**0.00030**	**0.01**

Station	Date	Algae class	Species	Abundance	%	Biomass	%
				10^3 cells/l		mg/l	
N-2	2005.11.09	Cryptophyceae	Katablepharis ovalis	5.5	2.68	0.00140	0.04
N-2	2005.11.09	Cryptophyceae	Leucocryptos marina	1.8	0.89	0.00021	0.01
N-2	2005.11.09	Cryptophyceae	Plagioselmis prolonga	85.6	42.03	0.00465	0.12
N-2	2005.11.09	Cryptophyceae	Teleaulax amphioxeia	49.2	24.15	0.01104	0.29
N-2	2005.11.09	Cryptophyceae	***Total Cryptophyceae:***	**142.1**	**69.76**	**0.01730**	**0.45**
N-2	2005.11.09	Dinophyceae	Amphidinium sphaenoides	0.2	0.08	0.00011	0.00
N-2	2005.11.09	Dinophyceae	Cladopyxis setifera	0.1	0.04	0.00014	0.00
N-2	2005.11.09	Dinophyceae	Gymnodinium simplex	5.5	2.68	0.00145	0.04
N-2	2005.11.09	Dinophyceae	Gymnodinium vestificii	0.2	0.08	0.00044	0.01
N-2	2005.11.09	Dinophyceae	Gyrodinium sp.	3.6	1.79	0.00336	0.09
N-2	2005.11.09	Dinophyceae	Gyrodinium sp. 7	1.8	0.89	0.00013	0.00
N-2	2005.11.09	Dinophyceae	Heterocapsa rotundata	1.8	0.89	0.00061	0.02
N-2	2005.11.09	Dinophyceae	Katodinium glaucum	16.4	8.05	0.00509	0.13
N-2	2005.11.09	Dinophyceae	Protoperidinium breve	0.1	0.04	0.00162	0.04
N-2	2005.11.09	Dinophyceae	Warnowia cf. rosea	0.2	0.12	0.00246	0.06
N-2	2005.11.09	Dinophyceae	***Total Dinophyceae:***	**29.9**	**14.66**	**0.01540**	**0.40**
N-2	2005.11.09	Prasinophyceae	Pyramimonas spp.	5.5	2.68	0.00066	0.02
N-2	2005.11.09	Prasinophyceae	***Total Prasinophyceae:***	**5.5**	**2.68**	**0.00066**	**0.02**
N-2	2005.11.09	Euglenophyceae	Eutreptiella spp.	7.3	3.58	0.00172	0.04
N-2	2005.11.09	Euglenophyceae	***Total Euglenophyceae:***	**7.3**	**3.58**	**0.00172**	**0.04**
N-2	2005.11.09	Chrysophyceae	Pseudopedinella tricostata	3.6	1.79	0.00012	0.00
N-2	2005.11.09	Chrysophyceae	***Total Chrysophyceae:***	**3.6**	**1.79**	**0.00012**	**0.00**
N-2	2005.11.09	Prymnesiophyceae	Chrysochromulina spp.	1.8	0.89	0.00012	0.00
N-2	2005.11.09	Prymnesiophyceae	***Total Prymnesiophyceae:***	**1.8**	**0.89**	**0.00012**	**0.00**
N-2	2005.11.09	Diatomophyceae	Actinocyclus octonarius v. tenellus	0.9	0.43	0.01295	0.34
N-2	2005.11.09	Diatomophyceae	Aulacoseira granulata v. granulata	0.2	0.08	0.00023	0.01
N-2	2005.11.09	Diatomophyceae	Chaetoceros danicus	0.2	0.08	0.00010	0.00
N-2	2005.11.09	Diatomophyceae	Coscinodiscus granii	0.5	0.24	0.07244	1.89
N-2	2005.11.09	Diatomophyceae	Coscinodiscus granii	1.1	0.55	0.31511	8.24
N-2	2005.11.09	Diatomophyceae	Coscinodiscus granii	4.0	1.96	2.19800	57.45
N-2	2005.11.09	Diatomophyceae	Coscinodiscus granii	1.0	0.51	0.84644	22.12
N-2	2005.11.09	Diatomophyceae	Coscinodiscus granii	0.3	0.16	0.34465	9.01
N-2	2005.11.09	Diatomophyceae	Cyclotella choctawhatcheeana	0.9	0.43	0.00007	0.002
N-2	2005.11.09	Diatomophyceae	***Total Diatomophyceae:***	**9.0**	**4.4**	**3.78998**	**99.1**
N-2	2005.11.09	Chlorophyceae	Planctonema lauterbornii	4.2	2.08	0.00051	0.01
N-2	2005.11.09	Chlorophyceae	***Total Chlorophyceae:***	**4.2**	**2.08**	**0.00051**	**0.01**
N-2	2005.11.09		**Total:**	**203.7**	**200**	**3.82612**	**200**
N-6	2005.11.08	Cryptophyceae	Cryptomonadales spp.	32.8	5.82	0.00013	0.01
N-6	2005.11.08	Cryptophyceae	Leucocryptos marina	3.6	0.65	0.00040	0.03
N-6	2005.11.08	Cryptophyceae	Plagioselmis prolonga	102.0	18.11	0.01847	1.26
N-6	2005.11.08	Cryptophyceae	Teleaulax amphioxeia	32.8	5.82	0.01177	0.81
N-6	2005.11.08	Cryptophyceae	***Total Cryptophyceae:***	**171.3**	**30.40**	**0.03078**	**2.11**
N-6	2005.11.08	Chrysophyceae	Pseudopedinella tricostata	7.3	1.29	0.00048	0.03
N-6	2005.11.08	Chrysophyceae	***Total Chrysophyceae:***	**7.3**	**1.29**	**0.00048**	**0.03**
N-6	2005.11.08	Euglenophyceae	Eutreptiella spp.	10.9	1.94	0.01430	0.98
N-6	2005.11.08	Euglenophyceae	***Total Euglenophyceae:***	**10.9**	**1.941**	**0.01430**	**0.98**
N-6	2005.11.08	Craspedophyceae	Craspedophyceae spp.	10.9	1.94	0.00015	0.01
N-6	2005.11.08	Craspedophyceae	***Total Craspedophyceae:***	**10.9**	**1.94**	**0.00015**	**0.01**
N-6	2005.11.08	Prasinophyceae	Nephroselmis sp.	18.2	3.23	0.00031	0.02
N-6	2005.11.08	Prasinophyceae	Pyramimonas spp.	21.9	3.88	0.00201	0.14
N-6	2005.11.08	Prasinophyceae	***Total Prasinophyceae:***	**40.1**	**7.12**	**0.00232**	**0.16**

Station	Date	Algae class	Species	Abundance	%	Biomass	%
N-6	2005.11.08	Prasinophyceae	Nephroselmis sp.	18.2	3.23	0.00031	0.02
N-6	2005.11.08	Prasinophyceae	Pyramimonas spp.	21.9	3.88	0.00201	0.14
N-6	2005.11.08	Prasinophyceae	***Total Prasinophyceae:***	**40.1**	**7.12**	**0.00232**	**0.16**
N-6	2005.11.08	Prymnesiophyceae	Chrysochromulina spp.	21.9	3.88	0.00031	0.02
N-6	2005.11.08	Prymnesiophyceae	***Total Prymnesiophyceae:***	**21.9**	**3.88**	**0.00031**	**0.02**
N-6	2005.11.08	Dinophyceae	Cladopyxis setifera	1.8	0.32	0.00095	0.07
N-6	2005.11.08	Dinophyceae	Gyrodinium spp.	9.1	1.62	0.00839	0.57
N-6	2005.11.08	Dinophyceae	Heterocapsa rotundata	14.6	2.59	0.00356	0.24
N-6	2005.11.08	Dinophyceae	Heterocapsa triquetra	3.6	0.65	0.00208	0.14
N-6	2005.11.08	Dinophyceae	Katodinium glaucum	3.6	0.65	0.00113	0.08
N-6	2005.11.08	Dinophyceae	Warnowia cf. rosea	0.2	0.03	0.00164	0.11
N-6	2005.11.08	Dinophyceae	***Total Dinophyceae:***	**33.0**	**5.85**	**0.01775**	**1.21**
N-6	2005.11.08	Diatomophyceae	Actinocyclus octonarius v. octonarius	0.2	0.04	0.01631	1.12
N-6	2005.11.08	Diatomophyceae	Actinocyclus octonarius v. tenellus	0.4	0.07	0.00199	0.14
N-6	2005.11.08	Diatomophyceae	Attheya septentrionalis	3.6	0.65	0.00036	0.02
N-6	2005.11.08	Diatomophyceae	Cerataulina pelagica	0.1	0.01	0.00022	0.02
N-6	2005.11.08	Diatomophyceae	Cerataulina pelagica	0.6	0.10	0.00242	0.17
N-6	2005.11.08	Diatomophyceae	Cerataulina pelagica	1.6	0.28	0.00724	0.49
N-6	2005.11.08	Diatomophyceae	Cerataulina pelagica	3.7	0.65	0.03143	2.15
N-6	2005.11.08	Diatomophyceae	Cerataulina pelagica	0.7	0.13	0.01130	0.77
N-6	2005.11.08	Diatomophyceae	Chaetoceros brevis	2.4	0.43	0.00712	0.49
N-6	2005.11.08	Diatomophyceae	Chaetoceros brevis	1.1	0.20	0.00661	0.45
N-6	2005.11.08	Diatomophyceae	Chaetoceros danicus	0.6	0.10	0.00115	0.08
N-6	2005.11.08	Diatomophyceae	Coscinodiscus granii	2.2	0.38	1.18692	81.20
N-6	2005.11.08	Diatomophyceae	Dactyliosolen fragilissimus	0.9	0.16	0.00723	0.49
N-6	2005.11.08	Diatomophyceae	Dactyliosolen fragilissimus	4.3	0.77	0.05112	3.50
N-6	2005.11.08	Diatomophyceae	Dactyliosolen fragilissimus	1.2	0.21	0.01824	1.25
N-6	2005.11.08	Diatomophyceae	Dactyliosolen fragilissimus	0.3	0.06	0.00544	0.37
N-6	2005.11.08	Diatomophyceae	Thalassiosira levanderii	7.3	1.29	0.00636	0.43
N-6	2005.11.08	Diatomophyceae	Thalassiosira spp.	7.3	1.29	0.00636	0.43
N-6	2005.11.08	Diatomophyceae	***Total Diatomophyceae:***	**38.5**	**6.83**	**1.36783**	**93.57**
N-6	2005.11.08	Chlorophyceae	Planctonema lauterbornii	229.6	40.75	0.02787	1.91
N-6	2005.11.08	Chlorophyceae	***Total Chlorophyceae:***	**229.6**	**40.75**	**0.02787**	**1.91**
N-6	2005.11.08		**Total:**	**563.3**		**1.462**	

Table 3. Chlorophyll a concentrations (mg/m3) in November 2005

Date	Station No	Depth	Chl a		Date	Station No	Depth	Chl a
		m	mg/m3				m	mg/m3
2005 11 08	6	1	7.86		2005 11 08	N-7	Int	2.8
2005 11 08	6	Int	2.14		2005 11 08	N-8	1	1.11
2005 11 08	N-9	1	3.89		2005 11 08	N-8	Int	0.95
2005 11 08	N-9	Int	1.14		2005 11 08	N-4	1	1.61
2005 11 09	4	1	8.51		2005 11 08	N-4	Int	1.48
2005 11 09	4	Int	8.63		2005 11 08	N-6	1	2.97
2005 11 09	65	1	1.41		2005 11 08	N-6	Int	2.99
2005 11 09	65	Int	2.54		2005 11 09	N-3	1	3.87
2005 11 09	N-2	1	3.34		2005 11 09	N-3	Int	3.92
2005 11 09	N-2	Int	1.06		2005 11 09	N-1	1	3.45
2005 11 08	N-5	1	1.65		2005 11 09	N-1	Int	3.08
2005 11 08	N-5	Int	1.96					
2005 11 08	N-7	1	3.97					

Table 4. Zooplankton species composition and abundance in November 2005

Date	Station	Cast (m)	Taxonomy group	Species	Sex	Abundance (ind/m3)	Abundance %
2005.11.09	N-1	67-0	CALANOIDA	*Acartia longiremis*	♂	268	5.34
			CALANOIDA	*Temora longicornis*	♀♂	758	15.13
			CALANOIDA	*Paracalanus parvus*	♀♂	297	5.93
			CALANOIDA	*Pseudocalanus m. elongatus*	♀♂	327	6.53
			CALANOIDA	*Calanus finmarchicus*	♀♂	193	3.86
			CLADOCERA	*Bosmina longirostris*	♂	15	0.29
			CLADOCERA	*Evadne nordmanni*	♀	30	0.59
			ROTATORIA	*Asplanchna priodonta*		208	4.15
			NAUPLII			1071	21.36
			LARVAE			1844	36.8
			TOTAL			**5011**	**99.98**
2005.11.09	N-2	62-0	CALANOIDA	*Acartia longiremis*	♀♂	203	3
			CALANOIDA	*Temora longicornis*	♀♂	1181	17.45
			CALANOIDA	*Eurytemora affinis*	♀	13	0.19
			CALANOIDA	*Paracalanus parvus*	♀	25	0.38
			CALANOIDA	*Pseudocalanus m. elongatus*	♀♂	508	7.5
			CALANOIDA	*Calanus finmarchicus*	♀♂	203	3
			HARPACTICOIDA	*Viguierella paludosa*	♂	25	0.38
			CLADOCERA	*Bosmina longirostris*	♀	13	0.19
			CLADOCERA	*Evadne nordmanni*	♂	38	0.56
			ROTATORIA	*Asplanchna priodonta*		152	2.25
			NAUPLII			1651	24.39
			LARVAE			2756	40.71
			TOTAL			**6768**	**100**
2005.11.09	N-3	39-0	CALANOIDA	*Acartia longiremis*	♂	277	2.31
			CALANOIDA	*Temora longicornis*	♀♂	1766	14.71
			CALANOIDA	*Eurytemora affinis*	♂	25	0.21
			CALANOIDA	*Paracalanus parvus*	♂	76	0.63
			CALANOIDA	*Pseudocalanus m. elongatus*	♂	76	0.63
			CALANOIDA	*Calanus finmarchicus*	♀	50	0.42
			CLADOCERA	*Bosmina longirostris*	♀	25	0.21
			CLADOCERA	*Evadne nordmanni*	♀	25	0.21
			ROTATORIA	*Asplanchna priodonta*		378	3.15
			ROTATORIA	*Conochilus unicornis*		555	4.62
			NAUPLII			3885	32.35
			LARVAE			4869	40.55
			TOTAL			**12007**	**100**
2005.11.08	N-4	30-0	CALANOIDA	*Acartia longiremis*	♀♂	213	3.23
			CALANOIDA	*Temora longicornis*	♀♂	705	10.67
			CLADOCERA	*Bosmina longirostris*	♀	16	0.25
			CLADOCERA	*Evadne nordmanni*	♀	16	0.25
			ROTATORIA	*Keratella quadrata*		16	0.25
			ROTATORIA	*Keratella cricifformis*		16	0.25
			ROTATORIA	*Synchaeta monopus*		623	9.43
			ROTATORIA	*Asplanchna priodonta*		591	8.93
			NAUPLII			5742	43.42
			LARVAE			3084	23.33
			TOTAL			**11022**	**100**

Date	Station	Cast (m)	Taxonomy group	Species	Sex	Abundance (ind/m3)	Abundance %
2005.11.08	N-5	10-0	CALANOIDA	*Acartia longiremis*	♀♂	1716	5.46
			CALANOIDA	*Temora longicornis*	♀♂	1961	6.24
			CALANOIDA	*Eurytemora affinis*	♀♂	147	0.47
			CLADOCERA	*Podon leuckarti*	♀	196	0.62
			CLADOCERA	*Evadne nordmanni*	♀	49	0.16
			ROTATORIA	*Asplanchna priodonta*		1716	5.46
			NAUPLII			15000	47.74
			LARVAE			10637	33.85
			TOTAL			**31422**	**100**
2005.11.08	N-6	33-0	CALANOIDA	*Acartia longiremis*	♀♂	268	3.04
			CALANOIDA	*Temora longicornis*	♀♂	656	7.43
			CALANOIDA	*Centropages hamatus*	♂	30	0.34
			CLADOCERA	*Evadne nordmanni*	♀♂	30	0.34
			ROTATORIA	*Keratella quadrata*		15	0.17
			ROTATORIA	*Synchaeta monopus*		612	6.93
			ROTATORIA	*Asplanchna priodonta*		865	9.8
			NAUPLII			8592	48.65
			LARVAE			4117	23.31
			TOTAL			**15185**	**100**
2005.11.08	N-7	13-0	CALANOIDA	*Acartia longiremis*	♀	90	0.51
			CALANOIDA	*Temora longicornis*	♀♂	392	2.19
			CALANOIDA	*Eurytemora affinis*	♀♂	60	0.34
			CLADOCERA	*Bosmina longirostris*	♂	30	0.17
			ROTATORIA	*Keratella quadrata*		30	0.17
			ROTATORIA	*Synchaeta monopus*		543	3.04
			ROTATORIA	*Asplanchna priodonta*		2142	11.97
			NAUPLII			10377	58.01
			LARVAE			4223	23.61
			TOTAL			**17887**	**100**
2005.11.08	N-8	34-0	CALANOIDA	*Acartia longiremis*	♀♂	317	1.47
			CALANOIDA	*Temora longicornis*	♀♂	1182	5.48
			CALANOIDA	*Centropages hamatus*	♀♂	58	0.27
			CALANOIDA	*Eurytemora affinis*	♂	29	0.13
			ROTATORIA	*Synchaeta monopus*		1153	5.35
			ROTATORIA	*Asplanchna priodonta*		894	4.14
			NAUPLII			10323	47.86
			LARVAE			7612	35.29
			TOTAL			**21568**	**100**
2005.11.08	N-9	12-0	CALANOIDA	*Acartia longiremis*	♀♂	229	0.72
			CALANOIDA	*Temora longicornis*	♀♂	1144	3.62
			CALANOIDA	*Centropages hamatus*	♀	65	0.21
			CALANOIDA	*Eurytemora affinis*	♀	33	0.1
			ROTATORIA	*Keratella cochlearis*		33	0.1
			ROTATORIA	*Synchaeta monopus*		1209	3.83
			ROTATORIA	*Asplanchna priodonta*		2941	9.32
			NAUPLII			18725	59.32
			LARVAE			7190	22.77
			TOTAL			**31569**	**100**
2005.11.08	6	10-0	CALANOIDA	*Acartia longiremis*	♀♂	74	0.91
			CALANOIDA	*Temora longicornis*	♀♂	196	2.43

Date	Station	Cast (m)	Taxonomy group	Species	Sex	Abundance (ind/m3)	Abundance %
			CLADOCERA	*Podon leuckarti*	♀	25	0.3
			ROTATORIA	*Keratella quadrata*		49	0.61
			ROTATORIA	*Synchaeta monopus*		1275	15.8
			ROTATORIA	*Asplanchna priodonta*		1495	18.5
			NAUPLII			3186	39.51
			LARVAE			1765	21.88
			TOTAL			**8065**	**100**
2005.11.09	4	12-0	CALANOIDA	*Acartia longiremis*	♀♂	147	3.32
			CALANOIDA	*Temora longicornis*	♀♂	163	3.69
			CALANOIDA	*Centropages hamatus*	♀	16	0.37
			CYCLOPOIDA	*Cyclops spp.*	♀♂	49	1.11
			CYCLOPOIDA	*Paracyclops spp.*	♂	33	0.74
			CLADOCERA	*Bosmina longirostris*	♂	16	0.37
			CLADOCERA	*Daphnia longispina*	♀	16	0.37
			CLADOCERA	*Podon leuckarti*	♀	49	1.11
			ROTATORIA	*Synchaeta monopus*		4118	23.25
			ROTATORIA	*Asplanchna priodonta*		3725	21.03
			NAUPLII			5817	32.84
			LARVAE			2092	11.81
			TOTAL			**16241**	**100**
2005.11.09	65	44-0	CALANOIDA	*Acartia longiremis*	♀♂	432	2.57
			CALANOIDA	*Temora longicornis*	♀♂	679	4.03
			CALANOIDA	*Centropages hamatus*	♀♂	165	0.98
			CALANOIDA	*Eurytemora affinis*	♀	21	0.12
			CALANOIDA	*Paracalanus parvus*	♂	21	0.12
			CLADOCERA	*Bosmina longirostris*	♂	21	0.12
			CLADOCERA	*Evadne nordmanni*	♂	21	0.12
			ROTATORIA	*Keratella quadrata*		21	0.12
			ROTATORIA	*Synchaeta monopus*		144	0.86
			ROTATORIA	*Asplanchna priodonta*		2716	16.14
			NAUPLII			8272	49.14
			LARVAE			4321	25.67
			TOTAL			**16834**	**100**

Table 5. Bacterial numbers and biomass, 8-9 November 2005.

Station	Date	Depth	Total number	Biomass	Saprophytic bacteria	Oil oxidizing bacteria
		m	cells/ml	mkg C/l	cells/ml	cells/ml
65	2005.11.09	1	642000	7.77	250	0
65	2005.11.09	44	418000	5.22	60	0
4	2005.11.09	1	1130000	16.96	250	600
4	2005.11.09	13	494000	6.18	600	60
N-2	2005.11.09	1	893000	11.12	250	25
N-2	2005.11.09	62	555000	6.39	60	60
N-6	2005.11.09	1	657000	8.23	250	60
N-6	2005.11.09	33	426000	5.20	600	25
N-9	2005.11.08	1	676000	8.35	250	25
N-9	2005.11.08	12	418000	4.92	130	60

Table 6. Macrozoobenthos species composition, abundance and biomass 8-9 November 2005

Station	Date	Depth	Sample	Taksonomy	Abundance	Biomass
		m			ind/m2	g/m2 ww
6	2005.11.08	13	1	Pygospio elegans	130	0.026
6	2005.11.08	13	1	Marenzelleria viridis	3230	1.615
6	2005.11.08	13	1	Nereis diversicolor	280	2.304
6	2005.11.08	13	1	Oligochaeta	540	0.09
6	2005.11.08	13	1	Hydrobia	230	0.0069
6	2005.11.08	13	1	Mya arenaria	850	1.105
6	2005.11.08	13	1	Macoma baltica	220	15.5615
6	2005.11.08	13	1	Cardium glaucum	70	12.842
6	**2005.11.08**	**13**	**1**	**Total**	**5550**	**33.5504**
6	2005.11.08	13	2	Pygospio elegans	350	0.07
6	2005.11.08	13	2	Nereis diversicolor	340	5.467
6	2005.11.08	13	2	Marenzelleria viridis	3600	1.3964
6	2005.11.08	13	2	Oligochaeta	1510	3.02
6	2005.11.08	13	2	Hydrobia	230	0.069
6	2005.11.08	13	2	Mya arenaria	790	3.7662
6	2005.11.08	13	2	Macoma baltica	310	133.126
6	2005.11.08	13	2	Cardium glaucum	50	9.102
6	**2005.11.08**	**13**	**2**	**Total**	**7180**	**156.0166**
N-5	2005.11.08	13	1	Marenzelleria viridis	3500	14
N-5	2005.11.08	13	1	Nereis diversicolor	290	0.4064
N-5	2005.11.08	13	1	Pygospio elegans	230	0.046
N-5	2005.11.08	13	1	Oligochaeta	540	0.108
N-5	2005.11.08	13	1	Hydrobia	50	0.015
N-5	2005.11.08	13	1	Cardium glaucum	50	3.83
N-5	2005.11.08	13	1	Mya arenaria	780	1.522
N-5	2005.11.08	13	1	Macoma baltica	280	89.944
N-5	**2005.11.08**	**13**	**1**	**Total**	**5720**	**109.8714**
N-5	2005.11.08	13	2	Marenzelleria viridis	3470	13.533
N-5	2005.11.08	13	2	Nereis diversicolor	290	4.234
N-5	2005.11.08	13	2	Pygospio elegans	70	0.014
N-5	2005.11.08	13	2	Oligochaeta	400	0.076
N-5	2005.11.08	13	2	Hydrobia	40	0.012
N-5	2005.11.08	13	2	Cardium glaucum	70	2.086
N-5	2005.11.08	13	2	Mya arenaria	960	1.642
N-5	2005.11.08	13	2	Macoma baltica	230	127.061
N-5	**2005.11.08**	**13**	**2**	**Total**	**5530**	**148.658**
N-9	2005.11.08	15	1	Marenzelleria viridis	4500	16.6311
N-9	2005.11.08	15	1	Pygospio elegans	120	0.024
N-9	2005.11.08	15	1	Nereis diversicolor	250	4.1014
N-9	2005.11.08	15	1	Oligochaeta	430	0.0817
N-9	2005.11.08	15	1	Hydrobia	20	0.107
N-9	2005.11.08	15	1	Mya arenaria	1890	3.534
N-9	2005.11.08	15	1	Macoma baltica	290	149.495
N-9	2005.11.08	15	1	Cardium glaucum	60	65.87
N-9	2005.11.08	15	1	Electra crustulenta		
N-9	**2005.11.08**	**15**	**1**	**Total**	**7560**	**239.8442**

N-2	2005.11.09	66	1	Halicryptus spinulosus	120	2.171
N-2	2005.11.09	66	1	Harmothoe sarsi	30	0.127
N-2	2005.11.09	66	1	Ostracoda	220	0.0088
N-2	2005.11.09	66	1	Mesidothea entomon	60	8.135
N-2	2005.11.09	66	1	Pontoporeia affinis	10	0.023
N-2	2005.11.09	66	1	Crangon crangon	10	0.315
N-2	2005.11.09	66	1	Macoma baltica	160	29.3831
N-2	**2005.11.09**	**66**	**1**	**Total**	**610**	**40.1629**
N-2	2005.11.09	66	2	Halicryptus spinulosus	80	0.7013
N-2	2005.11.09	66	2	Harmothoe sarsi	50	0.067
N-2	2005.11.09	66	2	Ostracoda	120	0.005
N-2	2005.11.09	66	2	Mesidothea entomon	30	0.646
N-2	2005.11.09	66	2	Macoma baltica	930	93.043
N-2	2005.11.09	66	2	Pontoporeia affinis	40	0.279
N-2	2005.11.09	66	2	Marenzelleria viridis	10	0.006
N-2	2005.11.09	66	2	Pontoporeia femorata	10	0.104
N-2	**2005.11.09**	**66**	**2**	**Total**	**1270**	**94.8513**
N-1	2005.11.09	70	1	Macoma baltica	10	2.971
N-1	**2005.11.09**	**70**	**1**	**Total**	**10**	**2.971**
N-1	**2005.11.09**	**70**	**2**	**Total**	**0**	**0**
65	2005.11.09	47	1	Marenzelleria viridis	380	1.359
65	2005.11.09	47	1	Pygospio elegans	60	0.012
65	2005.11.09	47	1	Halicryptus spinulosus	60	6.588
65	2005.11.09	47	1	Mesidothea entomon	40	56.536
65	2005.11.09	47	1	Pontoporeia femorata	10	0.052
65	2005.11.09	47	1	Pontoporeia affinis	330	0.1369
65	2005.11.09	47	1	Ostracoda	110	0.066
65	2005.11.09	47	1	Harmothoe sarsi	30	0.042
65	2005.11.09	47	1	Macoma baltica	490	77.574
65	**2005.11.09**	**47**	**1**	**Total**	**1510**	**142.3659**
65	2005.11.09	47	2	Marenzelleria viridis	360	1.26
65	2005.11.09	47	2	Pygospio elegans	30	0.006
65	2005.11.09	47	2	Halicryptus spinulosus	40	5.678
65	2005.11.09	47	2	Mesidothea entomon	20	38.964
65	2005.11.09	47	2	Pontoporeia affinis	290	0.1124
65	2005.11.09	47	2	Ostracoda	80	0.004
65	2005.11.09	47	2	Harmothoe sarsi	50	0.051
65	2005.11.09	47	2	Macoma baltica	510	71.253
65	**2005.11.09**	**47**	**2**	**Total**	**1380**	**117.3284**
4	2005.11.09	16	1	Nereis diversicolor	40	0.8687
4	2005.11.09	16	1	Marenzelleria viridis	1280	4.608
4	2005.11.09	16	1	Hydrobia	160	0.048
4	2005.11.09	16	1	Macoma baltica	170	1.198
4	**2005.11.09**	**16**	**1**	**Total**	**1650**	**6.7227**
4	2005.11.09	16	2	Nereis diversicolor	40	1.112
4	2005.11.09	16	2	Marenzelleria viridis	970	3.88
4	2005.11.09	16	2	Macoma baltica	120	0.987
4	2005.11.09	16	2	Hydrobia	130	0.039
4	**2005.11.09**	**16**	**2**	**Total**	**1260**	**6.018**

N-7	2005.11.08	16	1	Marenzelleria viridis	5250	22.153
N-7	2005.11.08	16	1	Nereis diversicolor	910	23.801
N-7	2005.11.08	16	1	Pygospio elegans	40	0.01
N-7	2005.11.08	16	1	Oligochaeta	1200	0.36
N-7	2005.11.08	16	1	Hydrobia	40	0.04
N-7	2005.11.08	16	1	Macoma baltica	390	265.383
N-7	2005.11.08	16	1	Mya arenaria	20	5.735
N-7	2005.11.08	16	1	Cardium glaucum	120	51.518
N-7	2005.11.08	16	1	Electra crustulenta	x	x
N-7	**2005.11.08**	**16**	**1**	**Total**	**7970**	**369**
N-4	2005.11.08	33	1	Marenzelleria viridis	260	0.52
N-4	2005.11.08	33	1	Macoma baltica	10	0.107
N-4	**2005.11.08**	**33**	**1**	**Total**	**270**	**0.627**
N-8	2005.11.08	37	1	Marenzelleria viridis	90	3.6
N-8	2005.11.08	37	1	Nereis diversicolor	20	4.26
N-8	2005.11.08	37	1	Oligochaeta	70	0.014
N-8	2005.11.08	37	1	Macoma baltica	100	58.11
N-8	2005.11.08	37	1	Electra crustulenta	x	x
N-8	**2005.11.08**	**37**	**1**	**Total**	**820**	**14.938**
N-6	2005.11.08	36	1	Nereis diversicolor	30	0.045
N-6	2005.11.08	36	1	Marenzelleria viridis	530	2.173
N-6	2005.11.08	36	1	Pygospio elegans	30	0.006
N-6	2005.11.08	36	1	Mesidothea entomon	30	9.56
N-6	2005.11.08	36	1	Gammarus sp.	30	0.082
N-6	2005.11.08	36	1	Macoma baltica	260	65.275
N-6	2005.11.08	36	1	Electra crustulenta	x	x
N-6	**2005.11.08**	**36**	**1**	**Total**	**910**	**77.141**
N-3	2005.11.09	42	1	Marenzelleria viridis	940	0.71
N-3	2005.11.09	42	1	Mesidothea entomon	20	7.87
N-3	2005.11.09	42	1	Macoma baltica	210	54.16
N-3	2005.11.09	42	1	Pontoporeia affinis	170	0.87
N-3	2005.11.09	42	1	Pygospio elegans	20	0.01
N-3	**2005.11.09**	**42**	**1**	**Total**	**1360**	**63.62**

Table 7. Nutrient and oxygen concentrations and pH 8-9 November 2005

Date	Station	Depth	NO3 µmol/l	NO2 µmol/l	NH4 µmol/l	TN µmol/l	PO4 µmol/l	TP µmol/l	SiO_4 µmol/l	O_2ml/l	pH
8.11.2005	**6**	**1**	0.78	0.082	10.13	12.76	0.092	0.95	9.22	7.59	8.26
8.11.2005	**6**	**10**	0.91	0.088	9.41	14.86	0.37	0.94	9.90	7.62	8.26
8.11.2005	**N-9**	**1**	2.01	0.18	12.49	19.20	0.87	0.97	9.01	7.31	8.25
8.11.2005	**N-9**	**10**	1.60	0.16	12.13	17.49	0.42	0.87	11.07	7.36	8.26
8.11.2005	**N-9**	**12**	1.52	0.15	12.85	22.27	0.42	1.00	11.75	7.34	8.25
9.11.2005	**N-2**	**1**	1.47	0.10	8.90	24.89	0.31	0.85	6.27	7.31	8.23
9.11.2005	**N-2**	**10**	1.14	0.10	8.62	24.02	0.31	0.74	4.22	7.33	8.25
9.11.2005	**N-2**	**20**	1.20	0.082	6.71	26.38	0.11	0.73	4.51	7.37	8.27
9.11.2005	**N-2**	**30**	1.17	0.16	2.92	21.30	0.28	0.95	4.31	7.57	8.22
9.11.2005	**N-2**	**40**	0.92	0.076	12.99	21.92	0.18	0.76	3.14	7.34	8.26
9.11.2005	**N-2**	**50**	5.35	0.082	14.98	22.91	1.71	1.95	24.02	5.71	7.87
9.11.2005	**N-2**	**60**	4.80	0.11	12.42	28.85	1.47	1.75	22.35	4.90	7.81
9.11.2005	**N-2**	**62**	8.12	0.12	7.69	23.53	2.71	2.86	37.06	2.70	7.79
9.11.2005	**65**	**1**	2.38	0.33	12.45	17.46	0.84	0.87	12.26	7.24	8.20
9.11.2005	**65**	**5**	2.56	0.34	6.77	21.92	0.70	0.90	10.59	7.22	8.19
9.11.2005	**65**	**10**	1.70	0.35	5.97	28.61	0.66	0.97	10.98	7.31	8.21
9.11.2005	**65**	**15**	1.65	0.35	11.93	23.78	0.55	0.95	10.69	7.26	8.18
9.11.2005	**65**	**20**	2.23	0.34	5.76	21.42	0.73	0.81	10.20	7.20	8.20
9.11.2005	**65**	**30**	2.17	0.31	0.83	26.13	0.40	0.88	9.12	7.27	8.20
9.11.2005	**65**	**40**	2.10	0.33	4.37	29.23	0.49	1.00	9.51	7.16	8.19
9.11.2005	**65**	**44**	1.64	0.32	9.96	25.39	0.52	1.08	9.71	7.12	8.18
9.11.2005	**4**	**1**	1.13	0.16	9.36	19.57	0.98	1.16	11.08	7.58	8.32
9.11.2005	**4**	**10**	0.92	0.076	10.41	13.00	0.46	0.81	10.10	7.56	8.32
9.11.2005	**4**	**13**	0.83	0.12	5.62	18.82	0.43	0.91	11.47	7.59	8.33
8.11.2005	**N-5**	**1**	1.51	0.11	5.46	20.31	0.40	0.97	10.88	7.45	8.19
8.11.2005	**N-5**	**10**	1.48	0.13	12.59	18.45	0.52	1.21	11.08	7.54	8.23
8.11.2005	**N-6**	**1**	1.28	0.24	12.70	16.35	0.28	0.90	10.88	7.20	8.25
8.11.2005	**N-6**	**10**	1.43	0.23	9.91	16.22	0.38	0.77	8.53	7.21	8.23
8.11.2005	**N-6**	**20**	1.36	0.21	12.19	15.60	0.32	0.90	7.94	7.29	8.22
8.11.2005	**N-6**	**30**	1.10	0.19	11.05	17.71	0.37	0.85	7.65	7.26	8.23
8.11.2005	**N-6**	**33**	1.34	0.19	13.27	49.91	0.08	0.80	8.04	7.22	8.24
8.11.2005	**N-7**	**1**	2.48	0.14	13.90	18.58	0.52	1.25	12.26	7.27	8.21
8.11.2005	**N-7**	**10**	1.53	0.14	12.33	17.96	0.46	1.11	11.57	7.50	8.26
8.11.2005	**N-7**	**13**	1.94	0.11	11.62	21.30	1.07	1.08	11.37	7.34	8.29

Table 7. (Cont.)

Date	Station	Depth	NO3 µmol/l	NO2 µmol/l	NH4 µmol/l	Nb µmol/l	PO4 µmol/l	Pb µmol/l	SiO_4 µmol/l	O_2ml/l	pH
8.11.2005	**N-8**	**1**	2.52	0.25	11.09	25.39	0.69	1.07	11.77	7.15	8.24
8.11.2005	**N-8**	**10**	2.16	0.22	9.99	18.45	0.43	0.98	11.57	7.17	8.27
8.11.2005	**N-8**	**20**	2.20	0.23	10.40	19.20	0.43	0.88	12.16	7.24	8.26
8.11.2005	**N-8**	**30**	1.84	0.20	11.04	23.90	0.35	0.90	11.47	7.21	8.25
8.11.2005	**N-8**	**34**	1.75	0.20	10.40	25.39	0.31	0.83	10.10	7.13	8.25
8.11.2005	**N-4**	**1**	1.82	0.22	12.48	19.32	0.49	0.76	9.80	7.20	8.25
8.11.2005	**N-4**	**10**	2.06	0.47	12.91	24.15	0.75	0.95	9.02	7.17	8.26
8.11.2005	**N-4**	**20**	1.69	0.26	11.71	25.14	0.23	0.90	8.43	7.24	8.26
8.11.2005	**N-4**	**30**	1.51	0.20	11.65	26.25	0.29	1.07	8.33	7.21	8.26
9.11.2005	**N-3**	**1**	1.76	0.10	13.93	21.80	0.38	0.90	5.78	7.31	8.23
9.11.2005	**N-3**	**10**	1.74	0.22	11.24	15.48	0.32	1.09	6.27	7.20	8.25
9.11.2005	**N-3**	**20**	1.38	0.10	13.66	26.01	0.15	0.81	5.59	7.23	8.26
9.11.2005	**N-3**	**30**	1.57	0.10	11.86	29.97	0.21	0.88	5.59	7.22	8.25
9.11.2005	**N-3**	**40**	3.66	0.11	12.33	21.42	1.01	1.43	15.29	6.38	7.94
9.11.2005	**N-1**	**1**	1.70	0.11	12.69	25.63	0.31	0.88	5.29	7.36	8.19
9.11.2005	**N-1**	**10**	1.56	0.11	7.46	23.28	0.50	0.76	5.69	7.36	8.21
9.11.2005	**N-1**	**20**	1.62	0.09	5.46	15.98	0.55	0.84	6.47	7.37	8.23
9.11.2005	**N-1**	**30**	0.94	0.11	6.16	16.84	0.47	0.78	5.00	7.34	8.23
9.11.2005	**N-1**	**40**	2.06	0.13	5.45	23.03	0.75	0.98	8.43	6.80	8.05
9.11.2005	**N-1**	**50**	5.78	0.023	13.35	21.92	1.87	1.91	20.78	5.75	7.91
9.11.2005	**N-1**	**60**	5.51	0.012	16.52	24.77	2.72	2.72	31.37	3.38	7.73
9.11.2005	**N-1**	**67**	7.46	0.018	11.68	20.93	2.89	2.89	34.80	2.34	7.61

Table 8. Oil hydrocarbons concentrations in water and sediments 8-11.2005 (CMR measurements)

Station	Latitude		Longitude		Date	Depth,	Time	Depth	Total hydrocarbons in water	Total hydrocarbons in sediments
					.	m	GMT	m	mg/l	mg/kg d.w.
6	55	33.5	21	04.7	11.8.05	13	10.47	1	<0.03	
6	55	33.5	21	04.7	11.8.05	13	10.47	10	<0.03	<5.1
N-5	55	25.5	21	02.1	11.8.05	13	12.31	1	<0.03	
N-5	55	25.5	21	02.1	11.8.05	13	12.32	10	<0.03	6.7
N-7	55	22.5	21	00.1	11.8.05	15	13.45	1	<0.03	
N-7	55	22.5	21	00.1	11.8.05	15	13.45	13	<0.03	5.2
N-9	55	18.7	20	57.4	11.8.05	14	14.57	1	<0.03	
N-9	55	18.7	20	57.4	11.8.05	14	14.58	12	<0.03	8.4
N-8	55	21.7	20	49.5	11.8.05	37	18.04	1	<0.03	
N-8	55	21.7	20	49.5	11.8.05	37	18.06	34	<0.03	5.8
N-4	55	27.0	20	48.0	11.8.05	33	19.58	1	<0.03	
N-4	55	27.0	20	48.0	11.8.05	33	20.00	30	<0.03	<5.1
N-6	55	24.3	20	42.4	11.8.05	36	21.19	1	<0.03	
N-6	55	24.3	20	42.4	11.8.05	36	21.21	33	<0.03	<5.1
N-3	55	28.0	20	32.0	11.8.05	42	23.37	1	<0.03	
N-3	55	28.0	20	32.0	11.8.05	42	23.39	40	0.09	6.7
N-2	55	31.2	20	33.8	11.9.05	65	1.47	1	0.09	
N-2	55	31.2	20	33.8	11.9.05	65	1.51	62	0.10	<5.1
N-1	55	34.5	20	13.5	11.9.05	70	6.12	1	0.17	
N-1	55	34.5	20	13.5	11.9.05	70	6.16	67	0.03	<5.1
65	55	52.9	20	20.5	11.9.05	47	10.38	1	0.11	
65	55	52.9	20	20.5	11.9.05	47	10.41	44	0.24	5.3
4	55	44.1	21	03.0	11.9.05	16	20.51	1	<0.03	
4	55	44.1	21	03.0	11.9.05	16	20.51	13	<0.03	<5.1

Table 9. Test report on measurements of organotin substances (NERI, Denmark)

DANAK
TEST Reg. nr. 411

Danmarks Miljøundersøgelser
Frederiksborgvej 399, 4000 Roskilde

Prøvningsrapport nr.140, 2006

Rekvirent:	Dr. Kari K. Lehtonen Department of Biological Oceanography Finnish Institute of Marine Research POB 2, FI-00561 Helsinki, FINLAND
J.nr.:	111/101-0006

Prøveopsamling/udtagning/indsamling:

Opsamlingssted:	Finland
Opsamlingsperiode:	ikke angivet
Prøvetype:	Sediment og *Macoma*
Opsamling udført af:	FIMR
Opsamlingsmetoder:	ikke angivet
Kontaktpersoner:	Kari Lehtonen

Analyser:

Prøvemodtagelse:	02-06-2006
Analysen udført af:	Danmarks Miljøundersøgelser Afdeling for Marin Økologi Frederiksborgvej 399 4000 Roskilde
Analysedato:	26-06-2006 – 28-07-2006
Analysemetoder:	Etylering, GC-PFPD
Måleusikkerhed:	De generelle usikkerheder fremgår af metodelisten og kan rekvireres fra Afdeling Marin Økologi, Danmarks Miljøundersøgelser

Underskrift

Bemærkning: *The result for sample N1 is only preliminary as there were too great interferences. This sample will be analysed again at a later occasion.

Kontaktpersoner: Ingela Dahllöf

Bilag:

Prøve sediment (DMU nummer)	TBT µg Sn/kg ww	DBT µg Sn/kg ww	MBT µg Sn/kg ww	Dry weight %	Loss on igition %
6 (2006-400)	<1	<1	<1	76.2	0.6
N5 (2006-401)	<1	<1	<1	76.3	0.7
N9 (2006-402)	<1	<1	<1	76.6	0.6
N8 (2006-403)	<1	<1	<1	86.3	0.4
N3 (2006-404)	<1	<1	<1	92.7	0.5
N2 (2006-405)	1.1	<1	<1	66.6	1.9
65 (2006-406)	1.6	<1	<1	65.2	2.2
N6 (2006-407)	<1	<1	<1	77.7	0.3
N1 (2006-408)*	180*	<1	<1	27.2	9.2

Prøve Macoma (DMU-nummer)	TBT µg Sn/kg ww	DBT µg Sn/kg ww	MBT µg Sn/kg ww
N9 (2006-409) (dobble analysis)	1.9 ± 0.11	0.5 ± 0.01	<0.3
N8 (2006-410)	2.2	0.5	<0.4
N3 (2006-411)	3.9	1.2	<0.5
N2 (2006-412)	5.1	0.6	<0.6
65 (2006-413)	4.3	1.3	1.3

Ansvarlig for prøvningsrapporten:

Dato: Roskilde den 28-07-2006

Underskrift:

Ingela Dahllöf
Stilling: Senior forsker/sektionsleder

Prøvningsresultaterne gælder udelukkende de prøver der er analyseret. Denne rapport må ikke gengives, undtagen i sin helhed, uden prøvningslaboratoriets skriftlige godkendelse.

Underskrift

NOTICE: Values for the station N-1 marked with * were preliminary. Corrected values are presented in the following table (Test report nr. 140, rev 1 2006, 17 January 2007)

Prøve sediment (DMU nimmer)	TBT µg Sn/kg	DBT µg Sn/kg	MTB µg Sn/kg	Dry weight %	Loss of ignition %
N 1 (2006-408)	90	2.4	< 2	27.2	9.2

Table 10. Test report on measurements of polycyclic aromatic hydrocarbons (PAH) in sediments (Nablabs)

Nab Labs Ympäristöanalytiikka Oy / Espoo
Otakaari 3, 02150 ESPOO

Lab. number: 3600875-3600887 **Customer:** Merentutkimuslaitos / Eila Lahdes
Sample(s) arrived: 10.3.2006 **Sample(s) prepared:** 12.4.2006

Method: J018S, PAH (polyaromatic hydrocarbons) from sediment samples.
Internal method (sample preparation modified from ISO 16703 method).

Lab no.:	3600875	3600876	3600877	3600878	3600879	3600880	3600881	3600882	3600881	3600882
Sample information:	S1. Sedimentti st. N-1	S2. Sedimentti st. N-2	S3. Sedimentti st. N-3	S4. Sedimentti st. N-6	S5. Sedimentti st. N-8	S6. Sedimentti st. N-9	S7. Sedimentti st. 6	S8. Sedimentti st. 65	S7. Sedimentti st. 6	S8. Sedimentti st. 65
Compound: \ Amount:	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)
Naphthalene	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Acenaphthylene	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Acenaphthene	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Fluorene	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Phenanthrene	11	<10	<10	<10	<10	<10	<10	<10	<10	<10
Anthracene	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Fluoranthene	110	37	<10	<10	<10	<10	<10	21	<10	21
Pyrene	40	13	<10	<10	<10	<10	<10	13	<10	13
Benzo(a)anthracene	25	<10	<10	<10	<10	<10	<10	<10	<10	<10
Chrysene	33	12	<10	<10	<10	<10	<10	12	<10	12
Benzo(b)fluoranthene	110	23	<10	<10	<10	<10	<10	16	<10	16
Benzo(k)fluoranthene	100	23	<10	<10	<10	<10	<10	17	<10	17
Benzo(a)pyrene	42	14	<10	<10	<10	<10	<10	11	<10	11
Indeno(1,2,3-cd)pyrene	150	31	<10	<10	<10	<10	<10	17	<10	17
Dibenzo(a,h)anthracene	25	<10	<10	<10	<10	<10	<10	<10	<10	<10
Benzo(g,h,i)perylene	130	24	<10	<10	<10	<10	<10	15	<10	15
PAH sum:	780	180	<10	<10	<10	<10	<10	120	<10	120

Determination limit was 10 µg/kg (dry matter).
PAH sum: sum of the compounds whose amount was over the determination limit.
This certificate can be copied only as a whole.
Results are valid only for the tested samples.

Reporter: Keijo Eilola, chemist
Date: 18.4.2006

Table 11. Test report on measurements of polycyclic aromatic hydrocarbons (PAH) in *Macoma balthica* (Nablabs)

Nab Labs Ympäristöanalytiikka Oy / Espoo
Otakaari 3, 02150 ESPOO

Lab. number:	3600875-3600887	**Customer:**	Merentutkimuslaitos / Eila Lahdes		
Sample(s) arrived:	10.3.2006		**Sample(s) prepared:**	21.4.2006	

Method: Internal method J018b, PAH (polyaromatic hydrocarbons) from biological samples.

Lab no.:	3600883	3600884	3600885	3600886	3600887	
	M1. Simpukka st. N-2	M2. Simpukka st. N-3	M3. Simpukka st. N-8	M4. Simpukka st. N-9	M5. Simpukka st. 65	
Compound: \ Amount:	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	
Naphthalene	21	35	36	32	19	
Acenaphthylene	<5	<5	<5	<5	<5	
Acenaphthene	7	<5	9	11	8	
Fluorene	<5	<5	<5	<5	<5	
Phenanthrene	<5	<5	<5	<5	6	
Anthracene	<5	<5	<5	<5	<5	
Fluoranthene	<5	<5	<5	<5	<5	
Pyrene	<5	<5	<5	<5	10	
Benzo(a)anthracene	<5	<5	<5	<5	<5	
Chrysene	<5	<5	<5	<5	<5	
Benzo(b)fluoranthene	7	5	<5	<5	8	
Benzo(k)fluoranthene	<5	<5	<5	<5	5	
Benzo(a)pyrene	17	23	18	15	16	
Indeno(1,2,3-cd)pyrene	<5	6	<5	7	9	
Dibenzo(a,h)anthracene	<5	<5	<5	<5	<5	
Benzo(g,h,i)perylene	<5	<5	<5	<5	<5	
PAH sum:	52	69	63	65	81	

Determination limit was 10 µg/kg (dry matter).
PAH sum: sum of the compounds whose amount was over the determination limit.
This certificate can be copied only as a whole.
Results are valid only for the tested samples.

Reporter: Keijo Eilola, chemist
Date: 18.4.2006

Table 12. Test report on measurements phenol compounds in sediments (Nablabs)

Nab Labs Ympäristöanalytiikka Oy / Espoo
Otakaari 3, 02150 ESPOO

Lab. number:	3600875-3600882	**Customer:**	Eila Lahdes / Merentutkimuslaitos
Sample(s) arrived:	10.3.2006	**Sample(s) prepared:**	12.4.2006

Method: Phenol determination from sediment samples. Internal method.

Reporter: Keijo Eilola, chemist
Date: 30.5.2006

Lab no.:	3600875	3600876	3600877	3600878	3600879	3600880	
Sample information:	S1. Sedimentti st. N-1	S2. Sedimentti st. N-2	S3. Sedimentti st. N-3	S4. Sedimentti st. N-6	S5. Sedimentti st. N-8	S6. Sedimentti st. N-9	
Compound: \ Amount:	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	Method uncertainty:
Phenol	<50	<50	<50	<50	<50	<50	-
o-cresol	<20	<20	<20	<20	<20	<20	-
m/p-cresol	<20	<20	<20	<20	<20	<20	-
2,4-/2,5-dimethylphenol	<10	<10	<10	<10	<10	<10	-
3,5-dimethylphenol	<20	<20	<20	<20	<20	<20	-
4-ethylphenol	<10	<10	<10	<10	<10	<10	-
2,3,5-trimethylphenol	<10	<10	<10	<10	<10	<10	-
2-n-propylphenol	<10	<10	<10	<10	<10	<10	-
4-n-propylphenol	<10	<10	<10	<10	<10	<10	-
2,4,6-trimethylphenol	<10	<10	<10	<10	<10	<10	-
4-tert-butylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
4-isopropyl-3-methylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
2-tert-butyl-4-methylphenol	<10	<10	<10	<10	<10	<10	-
4-n-butylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
4-tert-butyl-2-methylphenol	<20	<20	<20	<20	<20	<20	-
6-tert-butyl-2,4-dimethylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
2-tert-butyl-4-ethylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
2,5-diisopropylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
2,6-di-tert-butylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
4-n-pentylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
2,6-di-tert-butyl-4-methylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
4-tert-octylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
2-methyl-4-tert-octylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
4-n-heptylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
4-n-octylphenol	<5,0	<5,0	<5,0	<5,0	<5,0	<5,0	-
Phenols sum:	**<50**	**<50**	**<50**	**<50**	**<50**	**<50**	

Determination limit was 5-50 µg/kg (dry matter).
Phenols sum: sum of the compounds whose amount was over the determination limit.
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Results are valid only for the tested samples.

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Otakaari 3 • 02150 **Espoo** | Vuoksenniskantie 35 • 55800 **Imatra**
Harjutie 14 • PL 38 • 69601 **Kaustinen** | Sammonkatu 8 • PL 21 • 90570 **Oulu** | Raidetie 1 • 96910 **Rovaniemi**
Asiakaspalvelun puhelinnumerot: Ympäristöanalytiikka, maa puh. 02074 79102, Ympäristöanalytiikka, vesi puh. 02074 79106

Lab no.:	3600881	3600882					**Reporter:** Keijo Eilola, kemisti
Sample information:	S7. Sedimentti st. 6	S8. Sedimentti st. 65					**Date:** 30.5.2006
Compound: \ Amount:	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	(µg/kg)	Method uncertainty:
Phenol	<50	<50					-
o-cresol	<20	<20					-
m/p-cresol	<20	<20					-
2,4-/2,5-dimethylphenol	<10	<10					-
3,5-dimethylphenol	<20	<20					-
4-ethylphenol	<10	<10					-
2,3,5-trimethylphenol	<10	<10					-
2-n-propylphenol	<10	<10					-
4-n-propylphenol	<10	<10					-
2,4,6-trimethylphenol	<10	<10					-
4-tert-butylphenol	<5,0	<5,0					-
4-isopropyl-3-methylphenol	<5,0	<5,0					-
2-tert-butyl-4-methylphenol	<10	<10					-
4-n-butylphenol	<5,0	<5,0					-
4-tert-butyl-2-methylphenol	<20	<20					-
6-tert-butyl-2,4-dimethylphenol	<5,0	<5,0					-
2-tert-butyl-4-ethylphenol	<5,0	<5,0					-
2,5-diisopropylphenol	<5,0	<5,0					-
2,6-di-tert-butylphenol	<5,0	<5,0					-
4-n-pentylphenol	<5,0	<5,0					-
2,6-di-tert-butyl-4-methylphenol	<5,0	<5,0					-
4-tert-octylphenol	<5,0	<5,0					-
2-methyl-4-tert-octylphenol	<5,0	<5,0					-
4-n-heptylphenol	<5,0	<5,0					
4-n-octylphenol	<5,0	<5,0					
Phenols sum:	**<50**	**<50**					

Determination limit was 5-50 µg/kg (dry matter).
Phenols sum: sum of the compounds whose amount was over the determination limit.
This certificate can be copied only as a whole.

Results are valid only for the tested samples.

Table 13. Test report on measurements of total hydrocarbons in *Macoma balthica* (Nablabs)

Nab Labs Ympäristöanalytiikka Oy / Espoo
Otakaari 3, 02150 ESPOO

Lab. number:	3600875-3600887
Customer:	Merentutkimuslaitos / Eila Lahdes
Sample(s) arrived:	10.3.2006
Sample(s) prepared:	12.4.2006

Method: J018, Total hydrocarbons from biological samples with GC/MS instrument. Internal method (sample preparation modified from ISO 16703 method).

Lab no.	Sample information	Total amount (mg/kg)
3600883	M1. Simpukka st. N-2	<10
3600884	M2. Simpukka st. N-3	<10
3600885	M3. Simpukka st. N-8	<10
3600886	M4. Simpukka st. N-9	<10
3600887	M5. Simpukka st. 65	<10

Additional information:
Mineral oil fractions (mg/kg / % total amount)

Lab no.	C_6-C_{10}		C_{11}-C_{23}		C_{24}-C_{40}		Notices
	mg/kg	(%)	mg/kg	(%)	mg/kg	(%)	
-	-	-	-	-	-	-	-

Determination limit of the method is 10 mg/kg
This certificate can be copied only as a whole. Results are valid only for the tested samples.

Reporter: Keijo Eilola, chemist
2.5.2006

Table 14. Test report on measurements on total hydrocarbons in sediments (Nablabs)

Nab Labs Ympäristöanalytiikka Oy / Espoo
Otakaari 3, 02150 ESPOO

Lab. number:	3600875-3600887
Customer:	Merentutkimuslaitos / Eila Lahdes
Sample(s) arrived:	10.3.2006
Sample(s) prepared:	12.4.2006

Method: J018, Total hydrocarbons from sediment samples with GC/MS instrument. Internal method (sample preparation modified from ISO 16703 method).

Lab no.	Sample information	Total amount (mg/kg)
875	S1. Sedimentti st. N-1	<5
876	S2. Sedimentti st. N-2	<5
877	S3. Sedimentti st. N-3	<5
878	S4. Sedimentti st. N-6	<5
879	S5. Sedimentti st. N-8	<5
880	S6. Sedimentti st. N-9	<5
881	S7. Sedimentti st. 6	<5
882	S8. Sedimentti st. 65	<5

Additional information:
Mineral oil fractions (mg/kg / % total amount)

Lab no.	C_6-C_{10}		C_{11}-C_{23}		C_{24}-C_{40}		Notices
	mg/kg	(%)	mg/kg	(%)	mg/kg	(%)	
-	-	-	-	-	-	-	-

Determination limit of the method is 5 mg/kg (dry matter)
This certificate can be copied only as a whole. Results are valid only for the tested samples.
Reporter:

Reporter: Keijo Eilola, chemist
18.4.2006

Table 15. Test report on measurements of heavy metals in sediment and *Macoma balthica* (FIMR, Finland)

D6-project sediments
Measurements done by FIMR/ Jere Riikonen and Mirja Leivuori

Total digestion (HF/aqua regia/ boric acid) ICP-OES-instrument

Sample	DW	Al3961 dw	Al3961 ww	Ca3158 dw	Ca3158 ww	Cr2677A dw	Cr2677A ww	Cu3247-2 dw	Cu3247-2 ww	Fe2735 dw	Fe2735 ww	Mn2576A dw	Mn2576A ww	Ni2316 dw	Ni2316 ww
	%	%	%	%	%	µg/g	µg/g	µg/g	µg/g	%	%	µg/g	µg/g	µg/g	µg/g
N-1	25.3	6.2	1.6	0.44	0.11	93	24	41	10	4.4	1.1	275	70	40	10
N-3	89.1	2.2	1.9	0.50	0.45	6.0	5.3	<5		1.1	0.9	188	167	<5	
Sample	P_1782 ww	Ti3349 dw	Ti334 ww	V_2924A dw	V_2924A ww	Zn2138 dw	Zn2138 ww								
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g								
N-1	245	3854	976	115	29	153	39								
N-3	424	524	466	12	11	14	13								

Nitric acid digestion measured by ICP-OES-instrument other elements than As, Cd and Pb by GF-AAS

Sample	DW	Al3961 dw	Al3961 ww	Ca3158 dw	Ca3158 ww	Cr2677A dw	Cr2677A ww	Cu3247-2 dw	Cu3247-2 ww	Fe2735 dw	Fe2735 ww	Mn2576A dw	Mn2576A ww	Ni2316 dw	Ni2316 ww
	%	%	%	%	%	µg/g	µg/g	µg/g	µg/g	%	%	µg/g	µg/g	µg/g	µg/g
N-1	25.3	3.11	0.79	0.38	0.10	59	15	43	11	3.7	0.92	246	62	38	10
N-2	58.0	0.48	0.28	0.14	0.08	12	7.2	<5		0.61	0.35	41	24	<5	
N-3	89.1	0.18	0.16	0.27	0.24	<5		<5		0.67	0.60	122	108	<5	
N-5	76.4	0.40	0.31	0.66	0.50	13	10	<5		0.71	0.54	95	73	<5	
N-6	78.0	0.15	0.11	0.16	0.13	<5		<5		0.22	0.17	42	33	<5	
N-8	85.6	0.22	0.19	0.68	0.58	<5		<5		0.64	0.55	118	101	<5	
N-9	76.9	0.28	0.22	0.77	0.59	18	14	<5		0.77	0.59	127	97	<5	
6	77.1	0.26	0.20	0.54	0.41	17	13	<5		0.76	0.59	121	93	<5	
65	58.1	0.56	0.32	0.48	0.28	15	8.7	<5		0.89	0.52	95	55	<5	

Sample	P_1782 ww	Ti3349 dw	Ti3349 ww	V_2924A dw	V_2924A ww	Zn2138 dw	Zn2138 ww	As dw	As ww	Cd dw	Cd ww	Pb dw	Pb ww
	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
N-1	226	296	75	64	16	152	38	17	4.2	0.634	0.160	63	16
N-2	256	157	91	12	7.2	21	12	1.8	1.1	0.141	0.082	8.6	5.0
N-3	368	77	69	7.5	6.7	10	9.0	5.2	4.6	0.019	0.017	3.4	3.0
N-5	650	115	88	11	8.1	9.0	6.9	1.7	1.3	0.019	0.015	2.9	2.2
N-6	226	75	59	3.9	3.0	4.2	3.3	0.91	0.71	0.008	0.006	2.4	1.9
N-8	308	233	200	11	9.0	14	12	1.3	1.1	0.009	0.008	2.5	2.2
N-9	612	187	144	13	10	11	8.1	1.9	1.4	0.027	0.021	3.5	2.7
6	520	144	111	12	9.4	11	8.6	1.9	1.5	0.023	0.018	3.3	2.6
65	357	221	128	16	9.0	24	14	3.4	2.0	0.147	0.085	8.5	4.9

Reporter: Mirja Leivuori
26.8.2005

D6-project biota
Measurements done by FIMR/ Jere Riikonen and Mirja Leivuori

Sample	DW	Cd dw	Cd ww	Cu dw	Cu ww	Pb dw	Pb ww	Zn dm	Zn ww
	%	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g	µg/g
N-2	14.7	0.330	0.048	134.3	19.7	2.69	0.40	268.2	39.4
65	15.9	0.636	0.101	172.7	27.5	3.12	0.50	480.7	76.5
N-3	15.7	0.676	0.106	39.56	6.21	1.60	0.25	419.6	65.9
N-8	15.5	0.704	0.109	27.80	4.31	0.75	0.12	320.5	49.7
N-9	17.0	0.618	0.105	24.66	4.19	0.75	0.13	319.8	54.3

Reporter: Mirja Leivuori
2.8. 2005

Table 16. Liver histopathology in flounder. Samples colleted 9 December 2005

Liver histopathology in Baltic flounder (*Platichthys flesus*) from 4 sampling sites north of the D6 oil production site (Federal Research Centre for Fisheries, Institute for Fishery Ecology, Germany)

Station	ID	Sex	Length (cm)	Wet weight (g)	Weight without organs (g)	Liver weight (g)	Gonad weight (g)	Liver lesion	Lesion score
14	D6-1	f	29	348	282	10.3	33.0	hydropic vacuolation, granuloma	4
14	D6-2	f	30	317	249	6.7	34.7	necrosis	0,5
14	D6-3	f	33	519	413	4.1	51.4	lymphocytic/monocytic infiltration	0,5
14	D6-4	f	27	285	227	7.3	30.0	hepatocellular regeneration	0,5
14	D6-5	f	31	438	354	10.0	49.3	necrosis, granuloma	1
14	D6-6	f	33	463	369	17.7	51.3	-	0
14	D6-7	f	33	422	337	16.5	44.1	necrosis	0,5
14	D6-8	f	30	278	234	20.6	21.1	variable glycogen content	0,5
14	D6-9	f	33	487	394	9.2	46.7	necrosis	0,5
14	D6-10	f	35	575	458	11.8	74.0	-	0
14	D6-11	f	37	631	512	12.7	56.4	-	0
14	D6-12	f	35	509	394	5.0	55.9	hydropic vacuolation	4
14	D6-13	f	38	763	596	15.3	81.6	basophilic foci, lymphocytic/monocytic infiltration, granuloma	7
14	D6-14	f	29	345	284	19.7	34.1	hydropic vacuolation	4
14	D6-15	f	32	412	319	10.0	40.4	hydropic vacuolation	4
14	D6-16	f	40	846	654	9.5	114.6	basophilic foci, necrosis, increased macrophage aggregates	7
15	D6-17	f	38	741	578	9.6	86.4	necrosis	1
16	D6-18	f	31	436	347	4.4	52.9	-	0
17	D6-21	f	33	521	402	9.4	54.3	-	0
17	D6-22	f	34	619	432	8.0	96.4	vacuolated foci	8
17	D6-23	f	31	398	289	18.0	50.2	-	0
17	D6-24	f	28	276	220	9.1	20.7	eosinophilic foci	7
17	D6-25	f	22	151	106	11.8	15.4	regeneration	1
17	D6-26	f	26	218	160	11.9	30.3	regeneration	0,5
17	D6-27	f	31	350	283	15.8	24.7	necrosis, regeneration	1
17	D6-28	f	42	664	511	6.3	45.3	vacuolated foci, necrosis, increased macrophage aggregates, regeneration	7
17	D6-29	f	34	505	371	12.9	49.0	adenoma, vacuolated foci, increased macrophage aggregates, regeneration	11

Table 16. (cont.)

Station	ID	Sex	Length (cm)	Wet weight (g)	Weight without organs (g)	Liver weight (g)	Gonad weight (g)	Liver lesion	Lesion Score
17	D6-30	f	35	639	420	14.7	76.8	necrosis, increased macrophage aggregates, lymphocytic/monocytic infiltration	1
17	D6-31	f	30	335	264	18.3	27.6	necrosis, lymphocytic/monocytic infiltration	0,5
17	D6-32	f	31	441	341	14.7	52.4	-	0
17	D6-33	f	31	372	292	24.4	27.3	adenoma, eosinophilic foci, necrosis, increased macrophage aggregates, lymphocytic/ monocytic infiltration, regeneration	11
17	D6-34	f	27	244	201	8.3	21.4	necrosis, regeneration	1
17	D6-35	f	32	449	351	9.4	51.8	adenoma, necrosis, regeneration	10
17	D6-36	f	32	463	342	22.1	82.3	increased macrophage aggregates	0,5
17	D6-37	f	28	307	210	20.8	35.8	necrosis	1
17	D6-38	f	27	310	228	11.8	41.3	-	0
17	D6-39	f	30	325	256	16.2	33.8	necrosis, lymphocytic/monocytic infiltration	1
17	D6-40	f	24	216	171	26.9	21.9	lymphocytic/monocytic infiltration	0,5

APPENDIX 3

MEHODS USED IN SAMPLING AND ANALYSES FOR THE PROJECT

I Center of Marine Research (CMR), Klaipeda

- Salinity
- Oxygen and nutrients
- Phytoplankton
- Chlorophyll a
- Zooplankton
- Bacterioplankton
- Macrozoobenthos
- Heavy metals in sediments
- Hydrocarbons in water and sediments

II Institute of Geology and Geography (IGG), Vilnius

- Grain size distribution
- Total carbon
- Heavy metals

III Methods used in analyses in Finland and Denmark

- Heavy metals in sediments and *Macoma balthica*
- Biological effects studies (FIMR, IEVU, RKTL and EVIRA)

Consulting laboratory Nablabs

- Total hydrocarbons in sediments and Macoma balthica
- Polycyclic aromatic hydrocarbons in sediments and Macoma balthica
- Phenol compounds in sediments

Consulting laboratory National Environmental Research Institute (NERI), Denmark

- Organotin compounds

Abbreviations

CMR = Center of Marine Research, Klaipeda, Lithuania
EVIRA = Finnish Food Safety Authority, Helsinki, Finland
FIMR = Finnish Institute of Marine Research, Helsinki, Finland
IEVU = Institute of Ecology of Vilnius University, Vilnius, Lithuania
IGG = Institute of Geology and Geography, Vilnius, Lithuania
NERI = National Environmental Research Institute, Denmark
RKTL = Finnish Game and Fisheries Research Institute Helsinki, Finland

METHODS USED IN ANALYSES

I Center of Marine Research (CMR), Klaipeda

Salinity and temperature

Salinity and temperature were measured "in situ" with CTD ECO

Oxygen and nutrients

Samples were taken using a rosette sampler from different depths.

Oxygen concentrations of water samples were measured by Winkler titration (LST EN 25813:1999).

The determinations of nutrient concentrations were based on spectrophotometric methods: nitrate and silicate (Grasshoff et al. 1983), nitrite (LST EN 26777:1999), ammonia (LST ISO 7150-1:1998), phosphate and total phosphorus (LST EN ISO 6878:2004), total nitrogen (LST EN ISO 11905-1:2000). The concentration of dissolved inorganic nitrogen (DIN) was calculated as a sum of ammonia, nitrite and nitrate

Phytoplankton

The phytoplankton samples for this study were collected at 6 stations in the Lithuanian waters of the Baltic Sea in 1995-2005 in the framework of environmental monitoring programs performed by the Centre of Marine Research. Integrated samples were taken from the water surface layer (0-10 m) seasonally in January/February, May/June, July/August and October/November. An inverted microscope technique (Utermöhl, 1958) was applied for phytoplankton counting. The abundance was expressed in terms of cells, colonies, coenobiums, 100 μm filaments etc. according to HELCOM (1988). The cell volume was calculated from size measurements by using the appropriate stereo metric formula. It was converted to wet weight assuming that the density of plasma is equal to that water (~ 1 mg mm^{-3}).

Chlorophyll a measurements

Samples for chlorophyll a measurements were taken from surface and integrated surface layers (0-10 m). Determination of concentrations were done according HELCOM COMBINE manual (HELCOM, 2006) by spectrophotometric methods with ethanol as extraction solvent.

Zooplankton

Zooplankton sampling and analysis were done according requirements of marine monitoring methods of HELCOM COMBINE programme (HELCOM 2006). WP-2 (mesh size 108μm) net was used for sampling of zooplankton. Only taxonomic composition and abundances of species were estimated.

Bacterioplankton

Bacterioplankton data of the Baltic Sea were obtained during the period 1995-2005 at 4-6 ecological monitoring stations once a season from the surface and near-bottom layers. The

investigation included determination of numbers of total bacteria, saprophytic bacteria and oil-oxidizing bacteria.

The total abundance of micro-organisms was determined using the HELCOM methods (HELCOM 1988; Bergstrom et al.1986; Zimmerman & Meyer-Reil 1974). Membrane ultrafilters "*Osmonics*" (the Ø of the pore 0,20 μm) were used. The samples were fixed with 40% formaldehyde without sediments so that its concentration in the sample would be around 2%. After the filtration membranic filters were dyed with acridine orange. Bacteria were counted with fluorescence microscope "*Leitz Laborlux*" in 20 vision fields (magnification 1000x, using immersion oil).

The number of saprophytic bacteria in samples was determined using the method of repeated dilution in liquid ZoBell medium (ZoBell 1946; Tsyban 1988). Liquid mineral Mills medium with adding of diesel drops was used for oil-oxidizing bacteria growing and estimation (Mills et al. 1978).

Macrozoobenthos

Samples were taken by using Van-Veen grab (75 kg, 0.1m^2) and analyzed according HELCOM COMBINE monitoring programme (2006) The invertebrates retained by a sieve of 0.5mm/1 mm mesh size are included. The wet weight was estimated as biomass of macrozoobenthos species after 3 month fixation with formaldehyde.

Heavy metals in sediments

Sediment was collected by Van Veen grab. Surface layer (~ 1 cm) of sediments was taken for heavy metal analysis.

Dried and sieved sediment samples were digested with nitric acid in microwave oven (LST EN ISO 15587-2:2004 en) and analysed by electrothermal AAS for Cd, Cr, Cu, Pb (according to LST EN ISO 15586:2004 en) or flame atomic absorption spectrometry (flame-AAS) for Ni and Zn (according to LST ISO 8288:2002 lt).

Hydrocarbons

Water samples for oil hydrocarbons were taken from surface (1 m depth) and near-bottom water layers by plastic 5 liter water sampler (PWS). 1 liter of water was taken for the analysis. The extraction of samples was done onboard with carbon tetrachloride. In laboratory, after the purification with Al-oxide column, samples were analyzed by infrared spectrometry. As a calibration standard a mixture of benzene, hexadecane and iso-octane in carbon tetrachloride was used. A mixture of diesel fuel and lubricating oil was used for quality control. (LAND 49-2002).

Sediment samples were collected using a large Van Veen grab sampler (75 kg, with sampling area of 0.1 m^2). Sediments from the top ~ 1 cm were sub-sampled. For total oil hydrocarbon determination, sediment samples were extracted with 2 x acetone and 3 x methylene chloride, evaporated on a water bath and purified through Al-oxide column, dried and diluted with carbon tetrachloride. Concentrations of total oil hydrocarbons were determined by infrared spectrometry (Methodical recommendations 1979).

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All sampling locations were recorded with a global positioning system (GPS). The Van Veen grab sampler was used to take samples. Only the top of a grab samples (0-10 cm intervals) was used for our study. All superficial sediment samples were sub-sampled and subjected to grain and trace element analyses. All samples were stored at 4°C in plastic containers before analysis in the laboratory. Sediments were dried at 25-30 °C before analyses.

The grain-size composition of the sediments was determined following the sieving method for the gravely-sandy-silty sediment fractions. The sieving method provides 25 fractions ranging over a < 0.01 mm - > 10 mm particle sizes (Analizette 3 vibrator sieve shaker with 23 sieves).
For trace element analyses we used all fractions < 2 mm. A 4-acid digestion were used for metal analyzes. A 0.25 g split were heated in $HNO3$-$HClO_4$-HF to fuming and taken to dryness. The residue was dissolved in HCl. Solutions were analyzed by ICP-MS.

TC in sediments was measured by high-temperature catalytic oxidation at 910 °C by a liquid TOC analyzer. Detection is based on the IR-detector where combustion gases (carbon dioxide) are purged by synthetic air as carrier gas (Tiessen & Moir 1993)

III Methods used in analyses collected in November 2005 and analyzed in Finland and Denmark

Finnish Institute of Marine Research, Accredited laboratory SFS-EN ISO/IEC 17025, Helsinki (FIMR)

Heavy metals in sediment and Macoma balthica (FIMR)

Biological samples (FIMR)

Determination of cadmium, copper, lead and zinc concentration in tissues of Baltic clam, Macoma balthica.

Soft parts of *Macoma balthica* were freeze-dried and homogenized. Dry samples were digested in temperature controlled microwave oven with a mixture of 65 % nitrogen acid/MQ. After the digestion the concentration were analyzed by AAS spectrometry using AAS-graphite-oven for Cd, Cu and Pb, and Zn with AAS flame (standards SFS-5074 and -5502 for Cd, Cu & Pb; standards SFS-3044 &-3047 for Zn).

Sediment samples (FIMR)

Aluminium, calcium, chromium, copper, manganese, nickel, iron, titanium, vanadium and zinc are analyzed by ICP-OES-instrument, and arsenic, cadmium and lead by GF-AAS-instrument with flameless method.

Sediment samples were analyzed by two methods in order to be compared with the method used in CMR.

Sediment samples were freeze-dried, homogenised and sieved through 2 mm sieve. Both dry and wet weights of the sediment were determined.

1) Total digestion by *aqua regia*-hydrofluoric acid

Dried samples were digested in a laboratory microwave oven in teflon bombs with a mixture of *aqua regia* (65% HNO3 + 30% HCl; 1+3) and 40% hydrofluoric acid. 3% boric acid was added to the liquid phase and the samples was diluted to 50 ml with MQ. The samples were left over night to be stabilized before analysis by IPC-OES-instrument technique.

2) Digestion by nitric acid, HNO_3

Sediment samples were digested in Teflon-bombs with nitric acid (65%) using a laboratory microwave oven based on modification of the method EPA (1998). Samples were heated with pressure regulation at 120 psi for 45 minutes. Samples were then cooled, diluted up to the 50 ml mark in a volumetric plastic flask, transferred to plastic storage bottles, and stored at room temperature until analysis. Metal concentrations were measured by an ICP-OES instrument (ICP-OES based on FIMR's method) and an AAS-instrument with flameless method (modifications of SFS 5074 and SFS-EN ISO 15586)

Biological effects studies (FIMR, RKTL and EVIRA))

Ethoxyresorufin-O-deethylase activity

Liver samples of female flounder were measured for the CYP1A-dependent mono-oxygenase activity by fluorometric measurement of peroxisomal ethoxyresorufin-*O*-deethylase (EROD) activity with the technique presented by Stagg and McIntosh (1998), originally described by Burke and Mayer (1974). Prior to the assays, homogenisation of the samples was made in 100mM phosphate buffer (pH 8.0, 100mM KCl) 1/5 w/v with 150 to 300 mg liver tissues and stored at –80˚C. Otherwise the preparation of the S9 homogenates was made as it is described in context to GST and CAT assays. Activity of the S9 fraction was determined in RT in the presence of NADPH using 7-ethoxyresorufin as substrate. The excitation wavelength was adjusted to 544 nm and the emission wavelength to 590 nm for determination of the fluorescence on microplates with Cary Eclipse Fluorescence spectrophotometer (Varian). Calibration of fluorescence values was carried out with fresh resorufin standards.

Acyl-CoA oxidase activity

Peroxisomal Acyl-CoA oxidase (AOX) activity was measured as described by Small et al. (1985), on which the procedure by Orbea and Cajaraville (2006) is based on. In the method, palmitoyl-CoA as a fatty acyl-CoA is oxidised by the acyl-CoA oxidase (in the sample) producing hydrogen peroxide, which is still reduced to water-molecules with additional horseradish peroxidase. The latter reaction uses also leuco-dichlorofluorescein (leuco-DCF) as substrate, which is oxidized to DCF. The formation of DCF can be measured spectrophotometrically at 502 nm. The homogenisation of the *M. balthica* tissue (pools of 6 digestive glands) was made on 100mM TVBE-buffer (pH=7.6), the amount of horseradish peroxidase was 12U, the concentration of potassium phosphate was 0.01M and the amount of Triton X-100 was 0.01% from the total amount. 40mM sodium azide was used to inhibite catalase activity. The mass of tissue per sample varied from 125 to 550 mg and a volume of four times the mass of ice-cold TVBE-buffer was added. Activity was determined in RT spectrophotometrically using LAMBDA 2 - spectrophotometer (Perkin Elmer) with 5 s intervals between 60-300 s after the addition of

palmitoyl-CoA as the last reagent into the cuvette. Two parallel measurements for each sample were carried out and activity was determined towards a substrate blank.

Glutathione S-transferase and catalase activity

Prior to the enzymatic assays homogenisation of the samples was made in 100 mM phosphate buffer (pH 7.0) 1/3 w/v 25 to 250 mg *M. balthica* tissues (digestive gland) and 1/5 w/v (pH 7.5) with 170 to 320 mg flounder tissues (liver). Tissues were homogenised on ice in 1.5 ml Eppendorf tubes grinding the tissue 2 minutes with pellet pestles (Kimble/Kontes, Germany). Homogenates were centrifuged (10 000 g) and the S9-fractions were collected and used for assays. The peroxisomal GST activity was measured based on the reaction described by Habig et al. (1974), where a conjugation of xenobiotic 1-chloro-2,4-dinitrobenzene (CNDB) and glutathione (GSH) as a substrate is catalysed by GST. The rate of reaction is quantified spectrophotometrically at 340 nm. CAT activity was measured based on the method developed by Claiborne (1985), in which the rate of the degradation of hydrogen peroxide (H_2O_2) by CAT in the sample is measured spectrophotometrically at 240 nm.

Glutathione reductase activity

Peroxisomal glutathione reductase (GR) activity was measured based on the reaction described by Carlberg and Mannervik (1975), where a reduction of one glutathione disulfide molecule (GSSG) to two glutathione molecules (GSH) is catalysed by GR. The reaction is powered by oxidation of one NADPH molecule to $NADP^+$, which is quantified spectrophotometrically at 340 nm. *M. balthica* homogenates made for the AOX activity measurements and stored at –80˚C were used also for GR assays. Activity was determined spectrophotometrically in RT at 340 nm with BioRad microplate reader (model 550) with four parallel wells for each sample. The absorbance was measured with 8 s intervals between 0-500 s after the addition of NADPH as the last reagent into the wells.

Superoxide dismutase activity

Peroxisomal superoxide dismutase (SOD) activity was measured based on the reaction described by McCord and Fridovich (1969), where SOD catalyses the dismutation of reactive superoxide radicals to hydrogen peroxide. The reaction mixture consists of xanthine, xanthine oxidase and cytochrome *c*. Xanthine and xanthine oxidase are included in the reaction mixture, which serves a production of superoxide radicals. These radicals reduce the Fe^{3+} to Fe^{2+} in the heme group of cytochrome *c*. The reduction can be monitored spectrophotometrically at 550 nm. SOD activity is given in SOD units, where 1U equals to 50% inhibition of the reduction of cytochrome c. The same homogenates made for the AOX activity measurements and stored at –80˚C were also used as homogenates for SOD assays. Activity was determined in spectrophotometrically at 550nm using Lambda 2 UV spectrophotometer (Perkin-Elmer) at 5 s intervals between 80-120 s after the addition of xanthine oxidase as the final reagent into the cuvette. Two parallel measurements for each sample were carried out.

Acetylcholinesterase activity

Acetylcholinesterase (AChE) activity was measured with the technique presented in Bocquené and Galgani (1998), originally described by Ellman et al. (1961). The reaction bases on thiocholine, which is formed from the specific substrate acetylthiocholine iodide (ACTC) by AChE, and which further react with dithio*bis*nitrobenzoate (DTNB) ion forming a TNB-molecule. The formation of TNB changes the colour of the reaction mixture with absorption maximum at 412 nm and can be measured spectrophotometrically. The homogenisation in 0.02 M, 0.1% Triton

X-100, pH 7.0 phosphate buffer was made 1:2 *w/v* from 65 to 180 mg with *M. balthica* tissue (foot) and 1:4 *w/v* with 135 to 250 mg flounder tissues (muscle). *M. balthica* tissues were homogenised on ice for 3 x 20 s with Ultra-Turrax on ice and flounder tissues with Potter homogeniser (Braun)(5 strokes). Homogenates were centrifuged and the S9 fractions were collected and used for assays. Activity was determined spectrophotometrically in RT using 415nm filter in BioRad microplate reader (model 550) with four parallel wells for each sample.

Metallothionein content

Measurements of MT concentrations in the liver (flounder) and digestive gland (*M. balthica*) were performed according to the method developed by Viarengo et al. (1997). For flounder, pieces of liver were analysed from individual fish. For *M. balthica*, digestive glands of 6 individuals were pooled to obtain a sufficient amount of tissue for analysis (0.5 g wet wt) with 5 parallel samples per station. Briefly, tissues were homogenised in reducing conditions (0.05 M sucrose TRIS buffer, pH 8.6, containing 0.01 % β-mercaptoethanol). Homogenates were centrifuged at 30 000 × *g* for 20 min. Supernatants were collected, and ethanol/chloroform fractionation was used to obtain partially purified metalloprotein fraction. MT concentration was then measured by spectrophotometric determination of –SH groups using Ellman's reagent (DTNB).

Micronuclei frequency

After the sacrifice, small pieces of liver or cephalic kidney were dissected and directly smeared on slides, air-dried and fixed in methanol for 15 min. Slides were stained with 5% Giemsa solution for 10-20 min (Baršienė et al., 2004). In bivalves, two gill arches of each individual were placed in a big drop of 3:1 ethanol acetic acid (or methanol acetic acid) solution separately on two clean microscopic slides and gently nipped with tweezers for 2-3 min. Then the cell suspension was smeared on both slides. Dried slides were fixed in methanol for 10 min and stained with 4% Giemsa solution in phosphate buffer pH 6.8. The stained flounder and bivalve slides were analysed under the light microscope Olympus BX51 at a final magnification of 1000x. For each studied specimen of flounder, 5000 cells, for bivalve – 2000 cells with intact cytoplasm were scored. The blind scoring of MN was performed on coded slides without knowledge of the origin of samples. The frequency of MN was expressed as the number of MN per 1000 cells scored. Round or ovoid-shaped non-refractory particles with colour and structure similar to chromatin, with a diameter 1/3-1/20 of the main nucleus and clearly detached from it were interpreted as MN. In general, colour intensity of MN should be the same or less than of the main nuclei. Particles with colour intensity higher than of the main nuclei were not counted as MN. Mean of MN per 1000 studied cells, standard error and P values were calculated for each sampling group using PRISM statistical package. Non-parametric Mann-Whitney U-test was used to compare MN frequencies between sampling groups.

PAH metabolites in bile

PAH metabolites in bile samples were analysed as the total fluorescence (FAC) at the excitation/emission wavelengths 341/383 nm (FF method, Lin et al., 1996) and as individual hydroxy metabolites by high performance liquid chromatographic (HPLC) method with fluorescence detection as described in detail in (Vuontisjärvi et al., 2004). In brief, for the FF method bile was diluted 1:1000 with 48% ethanol and the diluted sample was measured by Hitachi F-4500 spectrofluorometer. For the HPLC method bile samples were hydrolysed enzymatically and concentrations of hydroxy metabolites of the five PAH compounds were determined by HPLC (Vuontisjärvi et al., 2004). For peak identification and quantitation 1-hydroxypyrene and 1-hydroxyphenanthrene were obtained from Dr. Ehrenstorfer GmbH

(Augsburg, Germany), 3-hydroxybenzo[a]pyrene and chrysene-1,2-diol from the National Cancer Institute Chemical Carcinogen Repository (Midwest Research Institute, Kansas City, MO, USA), and 2-hydroxynaphthalene from Sigma (Sigma-Aldrich Co., St. Louis, MO, USA). Standard solutions were prepared in acetonitrile, which was stabilized by adding vitamin C (J. T. Baker, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA). Validation of methods is described in Vuontisjärvi et al. (2004). All the samples were measured in duplicate and the means were used in calculations. For estimation of bile density absorbance at 380 nm for biliverdin (Doumas *et al*., 1987) was measured by Shimadzu UV-2401PC spectrophotometer in bile diluted 1:100 with 48% ethanol.

Protein concentration

The protein concentrations in the S9-homogenates were quantified with the method described by Bradford (1976). Bovine serum albumin (BSA) was used as the protein standard.

Examination for diseases in flounder

The inspection for externally visible diseases and parasites largely followed ICES guidelines (Bucke et al., 1996) and focused on the body surface including the spread-out fins and the gill and mouth chambers. Prior to inspection for diseases, the fish were cleaned in water, sexed, weighed and length-measured (total length to the cm below). For the inspection of flounder livers for nodules and parasites (again following ICES guidelines, Feist et al., 2004), the fish were anaesthetised by a blow on the head and killed by severing of the spinal cord. Methodologies applied (fish sampling, dissection, tissue sampling, fixation, histological processing, lesion diagnosis and classification, scoring system) were according to Lang et al. (2006).

Analyses of total hydrocarbons (THC) and polycyclic aromatic hydrocarbons (PAH) and phenolic compounds in sediment and biota (NABLABS)

NABLABS (T 111 accredited laboratory according to SFS-EN ISO 17025)

Sediment samples were taken by Van Veen crab during the RV Vejas cruise in November 2005. The surface sediment was collected and stored in glass jars in –20°C until freeze-drying. *Macoma batlhica* samples were collected by Van Veen crab and separated from sediments by sieving. Animals were dissected onboard and frozen in liquid nitrogen. During the transportation to Finland samples were stored in CO2-ice and in Helsinki at –80°C until analyses.

THC in sediments

Freeze-dried sediments (20 g sample) are extracted with a mixture of methanol-acetone-pentane and purified by silicagel. For analyses a GC/MS equipment and detected with MS SCAM-mode. Quantification was made with internal standards and calibration with a mixture of 1:1 light fuel oil and lubricating oil. Limit of detection 5 mg/kg dw.

TCH in Macoma balthica

Soft parts of the clam were homogenized and THC and analysis done as for THC in sediments. Limit of detection 10 mg/kg fresh weight

PAH's in sediments

Freeze-dried samples (20 g) are extracted with organic solvents and the extract is concentrated. Extract is injected to GC/MS-equipment and compounds identified according to the characteristic ions. Quantification was done by isotope-dilution technique for each compound with commercial calibration liquids. Limit of detection 10 µg/kg/compound dw.

PAH's in Macoma balthica

Frozen samples are homogenized and analyzed by the same technique as in sediments. Limit of detection was 5 µg/kg/compound fresh weight.

Phenols including alkylated phenols in sediments

Freeze-dried sediment (20 g) is extracted with organic solvents and the extract is concentrated. Phenolic compounds are then derivatized and injected to GC/MS equipment. Compounds are identified according to the characteristic ions. Quantification was done by isotope-dilution technique for each compound with commercial calibration liquids. Limit of detection 5-50 µg/kg/compound dw depending on the compound.

National Environmental Research Institute (NERI), Denmark

Organotins in sediment and Macoma balthica

Method is based on the ethylation *in situ* at pH ~ 5 using tripropyltin (TPrT) as internal standard, extraction into pentane and analysis by gas chromatography-pulsed flame-photometric detection (GC-PFPD) separation and detection (Jacobsen et al. 1997).

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APPENDIX 4

INTERCALIBRATION EXPERIMENTS

1. Metal analyses on sediments with nitric acid digestion. Intercalibration between FIMR and CMR

2. Metal analyses on sediments with total digestion method. Intercalibration between FIMR and IGG

3. Metal analyses in sediments with total digestion method: Comparison between total and nitric acid digestion methods on sediments (FIMR)

Abbreviations

CMR = Center of Marine Research, Klaipeda
FIMR = Finnish Institute of Marine Research, Helsinki
IGG = Institute of Geology and Geography, Vilnius

INTERCALIBRATION EXPERIMENTS

Considering the future monitoring activities intercalibration experiments were arranged in analyses of heavy and metals of bottom sediments. Experiments gave useful information about the efficiency and comparativeness of the methods. At the moment in the laboratory of Center of Marine Research only nitric acid digestion method for metal analyses is available for six metals, Cd, Cu, Cr, Ni, Pb and Zn. All those metals are harmful for biota if present in high concentrations. It is also important to notice the type of analysing method when values are compared with those taken from the literature.

The participating laboratories were;

1) Finnish Institute of Marine Research (FIMR), Helsinki
2) Center of Marine Research (CMR), Klaipeda
3) Institute of Geology and Geography (IGG), Vilnius

Three intercalibration experiments were arranged. Analyzing methods are described in Appendix

1. FIMR and CMR: Metal analyses on sediments with nitric acid digestion
2. FIMR and IGG: Metal analyses on sediments with total digestion method
3. FIMR: Comparison between total and nitric acid digestion methods on sediments

1. Intercalibration between FIMR and CMR

Table 1. Results obtained in analyses in the laboratories of FIMR and CMR. Differing values in bold.

	N-1	**N-2**	**N-3**	**N-5**	**N-6**	**N-8**	**N-9**	**6**	**65**
Cu/FIMR	42.7	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Cu/CMR	31.4	2.5	0.76	< 0.48	< 0.48	< 0.48	< 0.48	< 0.48	2.13
Cd/FIMR	0.634	0.141	0.019	0.019	0.008	0.009	0.027	0.023	0.147
Cd/CMR	**0.341**	**0.081**	0.014	0.011	<0.008	0.011	0.023	0.012	**0.081**
Cr/FIMR	59.1	12.5	< 5	13.4	< 5	< 5	17.7	17.1	15.1
Cr/CMR	39.9	12.0	4.02	7.96	2.21	2.72	13.1	9.27	17.2
Pb/FIMR	62.6	8.6	3.4	2.9	2.4	2.5	3.5	3.3	8.5
Pb/CMR	**<0.133**	6.48	**<0.133**	**<0.133**	**<0.133**	2.18	2.78	2.47	5.62
Ni/FIMR	38.1	< 5	< 5	< 5	< 5	< 5	< 5	< 5	< 5
Ni/CMR	48.3	6.89	<6.89	<6.89	<6.89	<6.89	<6.89	<6.89	<6.89
Zn/FIMR	151.7	20.0	10.2	9.0	4.2	13.7	10.6	11.1	24.0
Zn/CMR	131.0	16.1	11.7	6.2	< 5.2	**7.3**	8.2	8.2	17.4

Samples for both laboratories were collected with a Van Veen crab and surface layer (~ 1 cm) of sediments was taken for heavy metal analyses. Sediment samples were kept frozen until freeze-drying and sieving. Handling of sediment samples was made in FIMR and sub-samples were delivered to CMR for analyses.

In both laboratories sediments were digested with nitric acid (65%) in microwave oven.

Values in Table 1 show some differences between laboratories. In CMR the difference in Pb values is most probably due to an analytical error. There are also some single values, which show considerable difference, like Cd-concentration of the station N-1. FIMR laboratory did not give any numerical values for concentrations, which were under the detection limit although they were available.

Results show that the method used in CMR give a good estimate of the concentrations of measured harmful metals.

2. Intercalibration between FIMR and IGG

The results obtained from FIMR and IGG are in most cases comparable. FIMR values are higher for Cr (Table 2) In Pb results for the station N-1 are equal despite the less effective extraction method used in FIMR. For the station N-3 total digestion method used in IGG resulted 3.5 times higher Pb value than nitric acid extraction in FIMR which might demonstrate the effect of the digestion method.

3.Intercalibration between digestion methods in FIMR

It is well known that the nitric acid (65%) digestion is less effective that the digestion with the combination of 65% HNO3 + 30% HCl (1+3) + 40% hydrofluoric acid + 3% boric acid (referenssi). Therefore it was decided to apply both methods for both fine and coarse grained sediments. Stations N-1 and N-3 were selected. The grain size distribution is presented in Figure GS in the report.

Table 3 shows that considerable difference in Al, Cr, Ti and V values. If we take into account the Pb value obtained from IGG the difference caused by the extraction method for that metal is obvious, as well.

Table 2. Comparison of metal analyses between FIMR and IGG. Values are taken from Table X (FIMR) and XX (IGG) in Annex 2. Values marked with * are from Table XX, because total digestion method was not applied in As, Cd and Pb in the FIMR analyses. NA = not analyzed.

Sample	**Al**	**Ca**	**Fe**	**Cr**	**Cu**	**Mn**	**Ni**	**P**	**Ti**	**V**	**Zn**	**Pb**	**As**	**Cd**	**Li**	**Co**	**Hg**
	% dw			µg/g dw													ng/g dw
N-1/FIMR	6.2	0.44	4.4	**93.2**	40.7	275.2	40.0	966.7	3854.0	115.4	152.5	62.6*	16.5*	0.634*	NA	NA	NA
N-1/IGG	6.6	NA	NA-	**65.5**	39.5	NA -	40.3	NA	NA	118.0	166.0	64.1	15.0	0.500	49.7	11.9	99
N-3/FIMR	2.2	0.50	1.1	**6.0**	<5	187.7	<5	476.5	523.5	12.0	14.4	**3.4***	5.2*	0.019*	NA	NA	NA
N-3/IGG	2.9	NA	NA	**1.6**	2.4	NA	2.0	NA	NA	13.0	16.0	**12.2**	4.0	<0.1	5.6	1.4	<10

Table 3. Comparison between metal analyses of sediments between laboratories of FIMR and IGG. Values taken from Table X in Annx 2. Values marked with ** are from Table XX in the same appendix originating from result of IGG

Digestion	**Cr**	**Cu**	**Mn**	**Ni**	**P**	**Ti**	**V**	**Zn**	**Pb**	**As**	**Cd**	**Fe**	**Al**	**Ca**
	µg/kg dw											% dw	% dw	% dw
N-1/Tot.	**93.2**	40.7	275.2	40.0	966.7	**3854.0**	**115.4**	152.5	63.1**	14.0**	0.500**	4.4	**6.2**	0.442
N-1/HNO_3	**59.1**	42.7	245.8	38.1	891.6	**296.0**	**63.7**	151.7	63.6	16.5	0.634	3.7	**3.1**	0.380
N-3/Tot	**6.0**	<5	187.7	<5	476.5	**523.5**	**12.0**	14.4	**12.2****	4.0**	<0.1**	1.1	**2.2**	0.503
N-3/HNO_3	**<5**	<5	121.7	<5	413.0	**77.2**	**7.5**	10.2	**3.4**	5.2	0.019	0.67	**0.18**	0.270