Non-indigenous species field study report

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Field studies on non-indigenous species

Summary

The aim of the two case studies on alien species performed by the GES-REG project partner institutes was to improve the knowledge on the effects of the non-indigenous species on the environment and gather new information about methodological aspects on alien species monitoring.

The objective of the Latvian pilot study was two-fold: 1) to increase knowledge about the impacts of the invasive fish species, the round goby *Neogobius melanostomus*, on the environment and 2) to support development of new indicators for non-indigenous species. For these purposes, an extensive sampling programme was designed and executed in the open part of the Baltic Sea along the Latvian coast. Information was obtained on round goby distribution and feeding aspects as well as on the state of the benthic habitat before and after the round goby invasion. In addition, the first study in the Baltic Sea on the hematology of the round goby was conducted. Due to the limited amount of data on hematotoly this is a preliminary observation in need of further analysis. The obtained data gave substantial input to HELCOM CORESET indicator development "Abundance and distribution of Round goby (*Neogobius melanostomus*)" and in cooperation with the LIFE+ project MARMONI ("Innovative Approaches for Marine Biodiversity Monitoring and Assessment of Conservation Status of Nature Values in the Baltic Sea") served as data source for the development of a new indicator, "Abundance and impact of non-native fish development (round goby example)".

The objective of the Estonian pilot study was to test, develop further, and assist in finalising the HELCOM port survey guidelines. For this purpose, phytoplankton, zooplankton, benthic biota and fish were sampled in one of the largest ports in the Baltic Sea, the port of Tallinn. The obtained results are meant to serve as a reliable basis of data to be used in the A-4 risk assessments in granting ballast water management exemptions according to "The International Convention for the Control and Management of Ships' Ballast Water and Sediments" of the International Maritime Organization (IMO 2004).

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Latvian case study on non-indigenous species

The impact of the round goby (*Neogobius melanostomus*) on the benthic environment

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History of the round goby invasion in the Baltic Sea

The round goby (*Neogobius melanostomus* Pallas 1811) is a fish species that has been able to successfully invade different marine habitats in both Europe and in North America. Round gobies were first observed in the Baltic Sea in 1990, in the Gulf of Gdansk (Skóra and Stolarski 1993, 1995). The same year, they were also first found in the Great Lakes in North America (Crossman *et al.* 1992, Jude *et al.* 1992). Since then, the round goby population has established itself in Polish waters (Sapota 2004) and has expanded to neighboring Baltic Sea areas. In 1999, this species was reported from Germany waters around the Rugia Island (H. Winkler, University of Rostock, unpubl. data cited in Corkum *et al.* 2004); in 2003 the round goby was reported from several locations along the north coast of Germany and west of Rostock (K. Skora, University of Gdansk, unpubl. data cited in Corkum *et al.* 2004).

In Polish waters, the round goby has spread and established strong populations in three shallow areas of the Gulf of Gdansk, as well as in the Vistula lagoon and in the Vistula river up to 40 km upstream (Sapota 2004). The total area colonized by the round goby in the Gulf of Gdansk is estimated to be around 400 km^2; this is considered the greatest possible distribution in the area due to a lack of further suitable habitats for the species (Sapota 2005). Within the colonized territory, the round goby is the dominant species both by total numbers and biomass (Sapota 2004, Sapota and Skora 2005).

In 2002, the round goby was first discovered in Lithuania near the Klaipeda strait and in the Curonian lagoon (Rakauskas *et al.* 2008). The same year it was discovered in Estonian waters in the Pärnu Bay, the Gulf of Riga and in the Muuga Bay; in 2005 it was found in the Gulf of

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Finland (Ojaveer 2006). The round goby has been also found in the southern Swedish waters of the Baltic Sea (Björklund and Almqvist 2010).

Arrival of the round goby in Latvian waters

In Latvian waters, the round goby was first observed in catches of coastal fishermen near Liepaja on the open Baltic Sea coast in 2004, and in the southern Gulf of Riga in 2005. Since then, the species has established a population in the vicinity of Liepaja harbor from where it is spreading to adjacent hard bottom habitats. Information obtained from coastal fishermen and coastal fisheries statistics indicate that the round goby is colonizing areas around Ventspils harbor on the open Baltic Sea coast, in Roja harbor and Engure in the western part of the Gulf of Riga; however the abundance of the fish in thise areas has not yet been estimated. A few round goby specimens are frequently found in catches along the whole Latvian open Baltic Sea coast, however the species is rare in the Irbe strait and in the southern and eastern Gulf of Riga (A. Minde, unpubl. data).

Within the framework of Latvian annual coastal fish surveys (the so-called coastal net series), the first round goby individuals were caught in 2006 in Liepaja (Table 1). It is evident that the numbers of round gobies have increased rapidly following almost an exponential increment pattern.

Table 1. Total number of round goby in scientific surveys using gillnets (coastal net series) (unpubl data by BIOR (Institute of Food Safety, Animal Health and Environment) Fish research departament)

Sampling location	2006	2007	2008	2009	2010	2011	2012	Total
Bernati					5	110		115
Liepaja	8	53	280	254	1323	1125	2381	5424
Pape					46	195	539	780
Total	8	53	280	254	1374	1430	2920	6319

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Round goby habitat

Within their natural distribution range, round gobies occupy up to 20 m deep shallow coastal zones with rock, gravel and sandy bottoms (Miller 1986). They are known to occupy brackish and freshwater areas, estuaries and the lower and middle reaches of rivers (Miller 1986, Kornis *et al*. 2012). Adult individuals prefer sandy and stony substrates, mussel beds and piers, while juveniles occur on muddy, sandy, humus-containing bottoms overgrown with benthic flora (Skora and Stolarski 1996Ray and Corkum 2001). Even though round gobies prefers stony bottoms or other hard underwater structures as nesting sites, they can occupy almost any type of bottom habitat (Sapota and Skora 2005). Round gobies have a small home range and their migrations do not exceed 100 m, with the exception of seasonal migrations during late autumn and spring when part of the population migrates to deeper waters for the winter (Miller 1986).

Round goby biology

Round gobies belong to the family Gobiidae, in the order Perciformes. Although round gobies have a short lifespan, it can differ between areas. In their natural distribution area in the Ponto-Caspian region as well as in most invaded regions they reach a maximum age of 4 years and a total length (TL) of 180 mm. The Gulf of Gdansk, where the largest and oldest round gobies are reported to reach an age of 6 years and 250mm TL, is an exception (Solokovska and Fey 2011). It is possible that the lifespan of round gobies in the Gulf of Gdansk has changed with time, because in the beginning of the colonization their lifespan was similar to that in their native area and in the Great Lakes (Sapota 2004, 2005). Growth and length-at-age is sex-dependent as males of round goby grow faster at any given age than females; in the Baltic Sea males and females reach the maximum length of 25 cm and 19 cm, respectively, in 4–6 years (Sapota 2005, Solokovska and Fey 2011). Females reach sexual maturity in their second year and males in their third year (Nikolskii 1954). The spawning season lasts from May to September, because round gobies are multi-spawners. The spawning period can differ between geographical regions. Males usually protect the nest where one or several females lay eggs (Miller 1986). After the spawning and nest-defense period the males die, whereas some females can spawn for two seasons (Charlebois et al. 1997). Round goby spawning strategy is still unclear and often in the population can be found in specimens of different ages. It is possible that males eat eggs during nesting and thus gain enough energy to survive after reproduction (Marsden *et al*. 2007).

In the Great Lakes the round goby has two male reproduction strategies – the dark parental male and the light sneaker male morph strategies. Dark males are regular males reaching a large size

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and protecting the nests, while the light sneaker males are probably mimicking female round gobies and covertly taking part in egg fertilization (Marentette *et al*. 2009).

Round goby feeding and competition

Benthic invertebrates – crustaceans and molluscs – are the most common food items of the round gobies, however also polychaetes, small fish and fish eggs, as well as chironomid larvae can be included in the diet (Miller 1986, Rakauskas *et al.* 2008). In the Baltic Sea bivalves, especially *Mytilus edulis*, are the most important food source for adult round gobies (Karlson *et al.* 2007).

To date, there is no firm evidence of the round goby being the main factor behind the changes in native fish community structure in the colonized areas in Baltic Sea, because it is possible that the appearance of the species coincided with ecological or anthropogenic changes in the habitats. Such changes or habitat degradation could have created favorable conditions for successful adaptation and colonization by the newly introduced species (Sapota and Skora 2005).

However, little is still known about how round gobies interact with potential competitors, predators and prey. Several studies in the Great Lakes revealed that competition between the round goby and local fish species was evident (Corkum *et al.* 2004), and that round gobies are very aggressive and territorial (Dubs and Corkum 1996). Round goby predation on early stage native fish has been linked to the decline of native benthic fish populations (French and Jude 2001); and whatever the reason, a negative correlation between the abundances of round goby and flounder (*Platichthys flesus*) has been documented in the Gulf of Gdansk (Karlson *et al.* 2007). Studies in the Great Lakes show that round gobies have a potential to alter the size structure of zebra mussel (*Dreissena polymorpha*) populations (Ray and Corkum 1997). Interactions between round gobies and benthic invertebrates change food web dynamics by directly and indirectly affecting the energy flow of benthic communities in nearshore areas (Kuhns and Berg 1999).

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Materials and methods

Study area and fish collection

The study area was located in the Latvian coastal waters in the eastern Baltic Proper. Fish were collected at three stations of different depth (5, 10, 15 m) during July (25 – 27) and August (4 – 6) 2012. The samples were caught using Nordic Coastal survey nets (45 m long, 1.8 m deep, divided into nine sections with bar mesh with the mesh sizes 10, 12, 15, 19, 24, 30, 38, 48 and 60 mm). Additionally, coastal gillnets with the bar mesh sizes 17, 22 and 25 mm, each 30 m long and 1.8m deep, were attached to Nordic Coastal survey nets to get higher number of round goby specimens for the digestive tract analysis. The nets were set diurnal and fish were collected with three hours interval. For the digestive tract and hematological analysis analysis of benthivorous fish, specimens were randomly selected.

For all fish, total length was measured to the nearest millimeter and body weight to the nearest 0.1 g. For further data analysis, all caught round gobies were divided into 15 length classes: first class was 3,4-7,9 cm, the next 13 classes were separated by 1 cm intervals (starting at 8 cm); the 15^{th} class by a 2,5 cm interval (ending at 23,5 cm).

Soft bottom macrofauna sampling

Soft bottom macrofauna samples were collected during July (25 – 27) 2012 at three selected depths (2, 4, 6 m). At each station three replicate samples of sediments were collected using the Ponar Type Grab sampler (sample area 0.023 m^2). The sediments were sieved through a 0.5 mm nylon sieve and fixed in a buffered 4% formaldehyde solution. From each sample all macrofauna organisms are sorted, identified and counted to species or forms level using a stereo microscope at 16x magnification. The biomass (formalin wet weight) was determined, after gently blotting the invertebrate on filter paper, by weighing on an electronic balance. Before weighing, the specimens of *Macoma balthica* and *Mytilus trossulus* were opened to remove excess formalin from the mantle cavity. The number of individuals and their weight were expressed as ind./m^2 and g/m^2.

Hard bottom macrofauna and flora sampling

Hard bottom macrofauna and flora samples were collected by SCUBA divers. Samples from 11, 13, 14, 15 m depths were selected and at each station three replicate samples were collected. Organisms were scrapped from the surface of stones using a metal frame (20 x 20 cm) into a mesh bag with 0.5 mm mesh size. The samples were preserved and treated in the same way as the soft bottom macrofauna samples.

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Round goby counting by SCUBA diver

To estimate local round goby density (ind./m^2), a SCUBA diver swam along seven 50 m transects where fish nets were placed (1 m above the bottom) while holding a one-meter pole at arm's length perpendicularly to each transect and parallel to the bottom. All gobies that passed under the pole were counted (cf. Barton *et al.* 2005). At each transect the area of 100 m^2 was observed. Further fish counting was done according method published by Kipp e*t al.* (2012).

Visual observation of round goby densities using transect counts may result in underestimates of densities because of the cryptic nature of round gobies (Ray and Corkum, 2001) and whereas Johnson *et al.* (2005) note that they may overestimate abundances if divers stir up sediments, owing to the curiosity of the fish.

Estimation of macrobenthos species distribution and coverage using a drop video camera

Video analysis is useful for the determination of sediment substrates and the coverage of macrobenthos. Total sampling for the study area included 30 video stations classified into following sediment size classes: rock; boulder (with size > 600 mm); stones (100–600 mm); stones (60–100 mm); gravel (2–60 mm); sand (0.06–2 mm); silt (0.002–0.06 mm).

In the laboratory the video records of each transect were viewed and all macroalgae and fauna were identified to the lowest possible taxonomical level. Coverage of the animals and perennial macrophyte species (or groups) was visually estimated on a 10%-step scale.

Food content of the round goby

Most fish were gutted immediately after sampling and the entire digestive tracts (esophagus to anus) were fixed in 70% ethanol for later analysis. Some fish were however instantly frozen and gutted in laboratory.

Food items were identified to the lowest possible taxonomic level, counted (for partial food items, head capsules were counted) and weighed (wet weight, 0.001 g precision), and their proportion in the total gut content was assessed. Because of fragmentation of the shells of bivalves, we counted the hinge ligaments and measured the maximum length of intact shells.

Gravel and other unknown items were excluded from the gut contents and weighed as unknown food items. For data analysis, all food items were classified in eight groups: *Macoma balthica*, *Mytilus trossulus*, polychaetes, amphipods, mysids+decapods, fish, zooplankton, and other.

The *frequency of occurrence of food items* was calculated by dividing the number of individuals in whose stomachs a particular prey item was found by the total number of individuals studied, and multiplying the result by 100; this was done for all seven investigated fish species. The *relative proportion of different prey items* was calculated by dividing the weight of a particular prey item by the total weight of the gut contents, and then multiplying the result by 100, again, for the seven fish species separately. Finally, the *average fullness index* was calculated by dividing the average stomach fullness (in g) of each fish age group by the highest stomach fullness (in g) of this group, and then multiplying the result by 100.

Age determination of the round goby

The age of round gobies was determined from otoliths. The sagittal otoliths were extracted from each fish and age was estimated by counting the annual rings using a stereo microscope. The age reading was performed twice by the same person on different occasions. If the ages obtained from the two readings were different, the otolith was re-examined again.

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Results

Macrofauna species distribution and coverage using drop video camera

In the pilot study area, the first extensive drop video camera mapping of dominant benthic species (the macrofauna baseline studies) was performed in 2006 within the LIFE project "Marine Protected Areas in the Eastern Baltic Sea (Baltic MPA)" (2005–2009). On the basis of the obtained results, the marine protected area "Nida-Pērkone" was established due to the discovery of dense colonies of *Mytilus trossulus*. Consequently the video data in 2012 were collected at the same locations and the aim was to detect any potential changes in the area.

The bottom substrate of the shallow coastal zone (2–7 m depth) comprises fine and rough sands, while the bottom of the deeper part (8–20 m depth) is covered by boulders, stones, pebbles and rough sands. Comparing the results of the present pilot study with the baseline studies, we found that considerable changes in the coverage of *M. trossulus* had occurred. These changes varied between the substrate size classes. On large boulders (>600 mm), the coverage of *M. trossulus* was practically the same (30–50%) as in 2006, whereas considerable differences in *M. trossulus* coverage were discovered on large stones (100–600 mm) and small stones (60–100 mm). In 2006 average coverage of *M. trossulus* was between 20% and 55% while in 2012 it reached only 10% – 20%. Thus currently the average percentage of *Mytilus trossulus* coverage in the study area in 2012 was less than half of what it was six years previously.

Analysis of video data showed differences also in the macroalgal communities; both species diversity and occurrence frequency varied between the investigated years. In 2006, four algal taxa were found, while only one species (the red algae *Coccotylus truncatus*) was observed in 2012.

A significant decrease in the coverage of macroalgal species occurred between 2006 and 2012. The coverage of *C. truncatus* was estimated up to 10% and *Pylaiella sp.* up to 20% in 2006, while in the current pilot study the coverage of *C. truncatus* did not exceed a few percent and *Pylaella sp.* had disappeared completely.

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Structure and distribution of soft and hard bottom macrofauna

In the study area altogether 32 macrozoobenthos taxa were observed – Mollusca (6 species), Polychaeta (7 species), Crustacea (12 species), as well as representatives of Oligochaeta, Hirudinea, Chironomidae, Halacaridae, and Turbellaria. The macrozoobenthos assemblage was characterized by the dominance of Vermes (average proportion of total macrozoobenthos density and average proportion of total macrozoobenthos biomass 74% and 59%, respectively). *Marenzelleria neglecta*, *Pygospio elegans* and *Manayunkia aestuarina* were the dominant taxa forming 71% and 53% of the total macrozoobenthos density and biomass. Mollusca and Crustacea, both groups showed similar density and biomass values (14% and 19%; 12% and 21%). The average density and biomass of total macrozoobenthos were 20059 ind/m^2 and 524 g/m^2, respectively.

The number of macrozoobenthos species as well as their density and biomass increased with depth. At the depth of 2 m six species were determined. The average abundance of benthic organisms at this depth was 5763 ind/m^2 (max. 6725 ind/m^2) and average biomass was approx. 1.6 g/m^2 (max. 1.7 g/m^2). Nine species were determined at the depth of 4 m. The average abundance of zoobenthos was 13077 ind/m^2 (max. 20522 ind/m^2) and the average biomass there was 2.7 g/m^2 (maximum 4.1 g/$m^{2)}$. Moving to greater depth, at 6 m ten benthic species were determined. At this depth the abundance of macrofauna was 22473 ind./m^2 with maximum 44072 ind./m^2 and the biomass 3.8 g/m^2 (max. 7.3 g/m^2). No significant difference was found between the benthic communities at the depth zones 4 and 6 m (Figs. 1–2). A total of 24 species and groups of macrobenthos were determined in the depth >10 m. The average abundance of macrobenthos at this depth was 34206 ind./m^2 (max. 97475 ind./m^2) and average biomass was approx. 1231 g/m^2 with a maximum of 4812 g/m^2.

The nectobenthos species were not enumerated. Species of the *Mysidae* group (*Mysis mixta*, *Neomysis integer*) were present in the samples but were not included in the estimations as the used grab is not an efficient device for collecting them. Two *Palaemon* species, *Palaemon adspersus* and *Palaemon elegans*, as well as the grey shrimp (*Crangon crangon*) were found in the Pape area.

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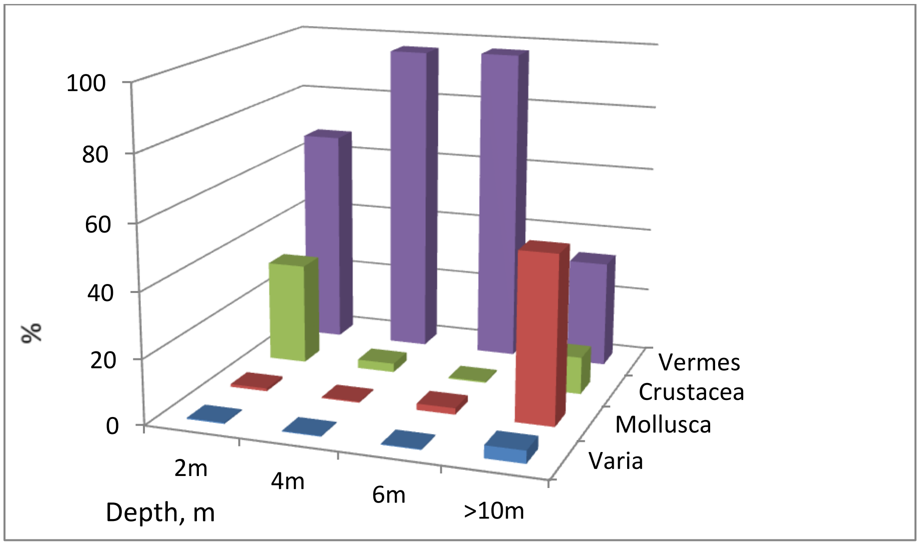

Figure 1. Relative average proportion of total macrozoobenthos density of the main groups of soft bottom macrofauna at different depths in the study area in July 2012.

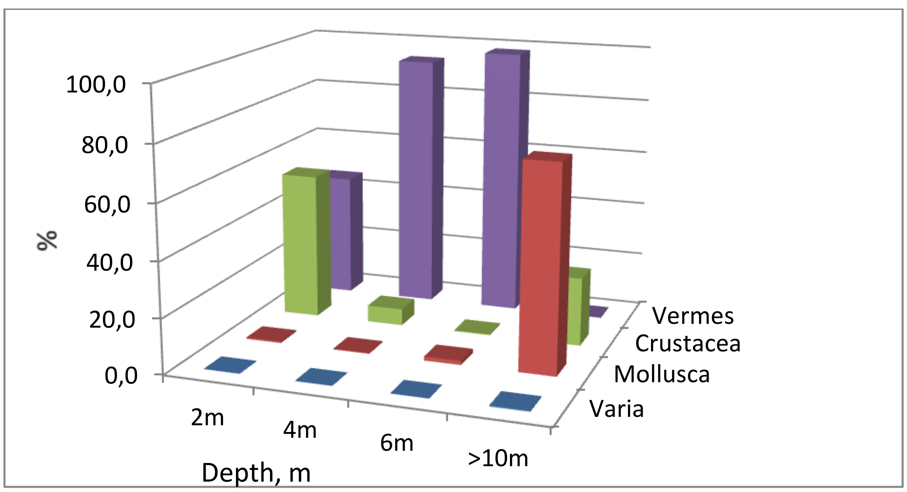

Figure 2. Relative average proportion of total mecrozoobenthos biomass of the main groups of soft bottom macrofauna at different depths in the study area in July 2012.

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The number of round gobies caught by Nordic coastal nets compared with the number of observed fish in diving transects shows that the first length class (3.4-7.9 cm) round gobies were the most common in the diving transects at 5 and 10 m depth. The number of length classes 8-15.9 cm round gobies increases with sampling site depth in the diving transects (Table 2). Contrary to the data from diving transects, larger (length classes 8-15.9 and 16-23.5) round gobies collected by Nordic coastal nets were found at all depths. Most round gobies were caught at 10 m depth (Fig. 3).

Table 2. Number of round goby, observed by SCUBA divers in the study area, summer 2012

Date	Transect Nr.	Depth,m	Time	3.4-7.9 cm	8-15.9 cm
26.07.12	1	14.5	11:00	47	33
26.07.12	2	14.0	13:00	42	22
26.07.12	3	16.6	15:00	51	10
26.07.12	1	14.5	23:50	32	40
27.07.12	2	14.0	0:30	9	8
05.08.12	4	16.3	17:25	40	8
05.08.12	5	9.4	18:37	91	0
05.08.12	6	5.9	19:25	331	0
05.08.12	7	2.8	20:09	0	0

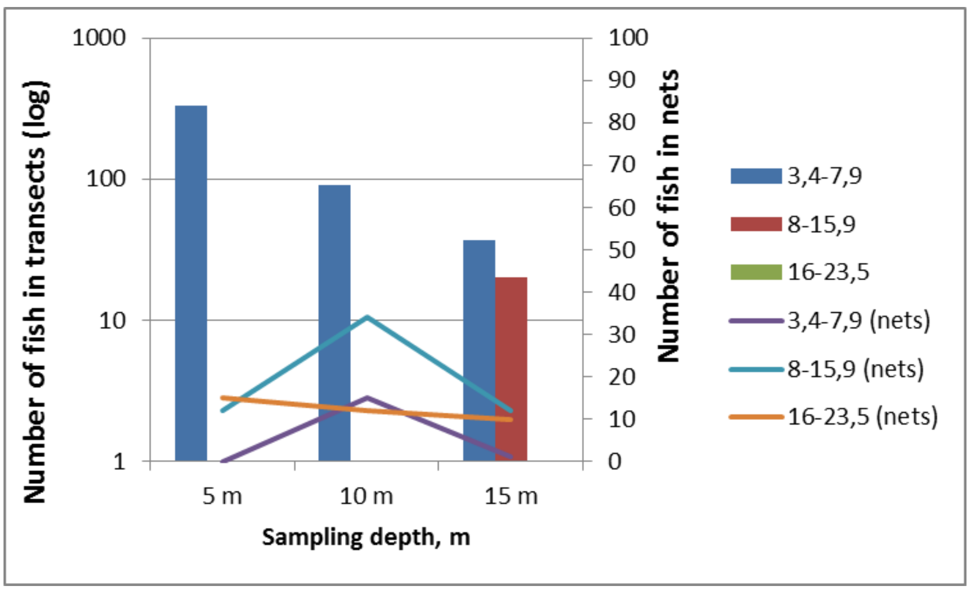

Figure 3. Number of round gobies in SCUBA diving transects and Nordic coastal gillnets in the Pape littoral in late July and early August 2012.

Abundance and distribution of the round goby

In this pilot study, a total of 687 fish representing 16 species were collected by Nordic coastal survey nets (Fig. 6). A further 220 round gobies were sampled by coastal gillnets for digestive tract analyses. Considering the three sampled depths together, round goby was the third most abundant fish species (after flounder and perch), in Pape in late July and early August (Fig. 4).

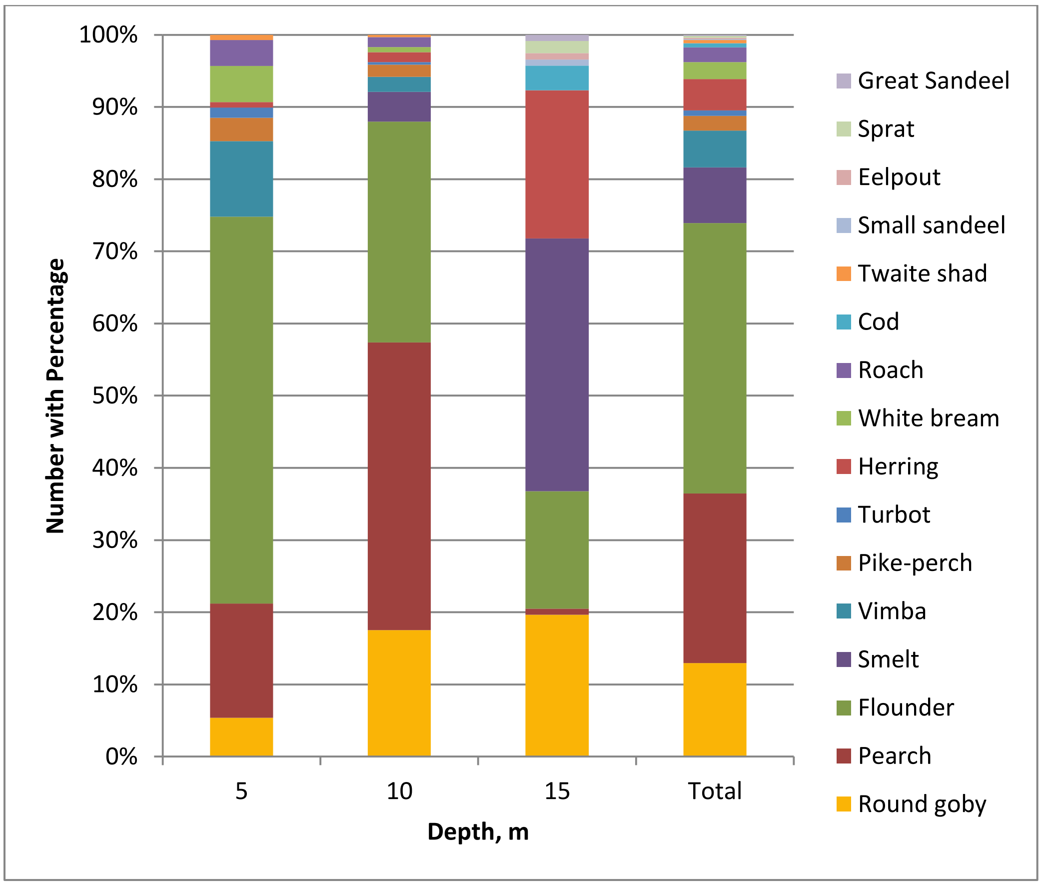

Figure 4. Percentage of fish caught by Nordic coastal survey nets at 5, 10 and 15 meter depth in the Pape littoral in late July and early August 2012.

The fish community composition varied with depth (Fig. 4). At all three depths, the four most abundant species constituted approx. 90% of encountered fish individuals. Flounder was the most abundant fish species at 5 m and its abundance decreased with depth. The highest number of round gobies was found at 10 m. Perch was most abundant species at 10 m, smelt at 15 m.

Species such as pike-perch, vimba, turbot, white bream, roach and twaite shad were found only at 5 and 10 m, whereas cod, small sandeel, eelpout, sprat and great sandeel were only found at 15 m.

Age structure of the round goby population

Age was determined for 212 round gobies. The most abundant round gobies were medium-sized, more than three (3+) years old individuals. Excepting the extremes, the length of 2+ round gobies was 8–12,9 cm, whereas 3+ round gobies measured 12–19 cm, 4+ round gobies 17–23.5 cm, and 5+ round gobies 18–23.5 cm in length.

Feeding and food selectivity

A total of 309 round gobies, 114 flounders, 106 perch, 50 smelts, 35 vimbas, 14 pike-perch and 6 turbots were collected for digestive tract analysis. 24 different prey items were identified in the fish stomachs. The most recurrent prey items in the round goby diet were *Mytilus trossulus* and *Macoma balthica*, whereas the most common prey of flounder were polychaetes and the amphipod *Corophium volutator*. Also in the diet of vimbas polychaetes were the most common prey. Perch, smelt, turbot and pike-perch are piscivores and their most common prey items were fish and fish larvae.

The relative proportions (by weight) of eight prey item groups were compared in the three competing benthic fish species, the round goby, flounder, and vimba (Fig. 5). *Mytilus trossulus* and *Macoma balthica* formed the biggest proportion of the round goby diet, while flounder fed almost exclusively on *M. balthica*. *Macoma balthica*, polychaetes and amphipods composed the greatest relative proportion in the vimba diet (Fig. 5).

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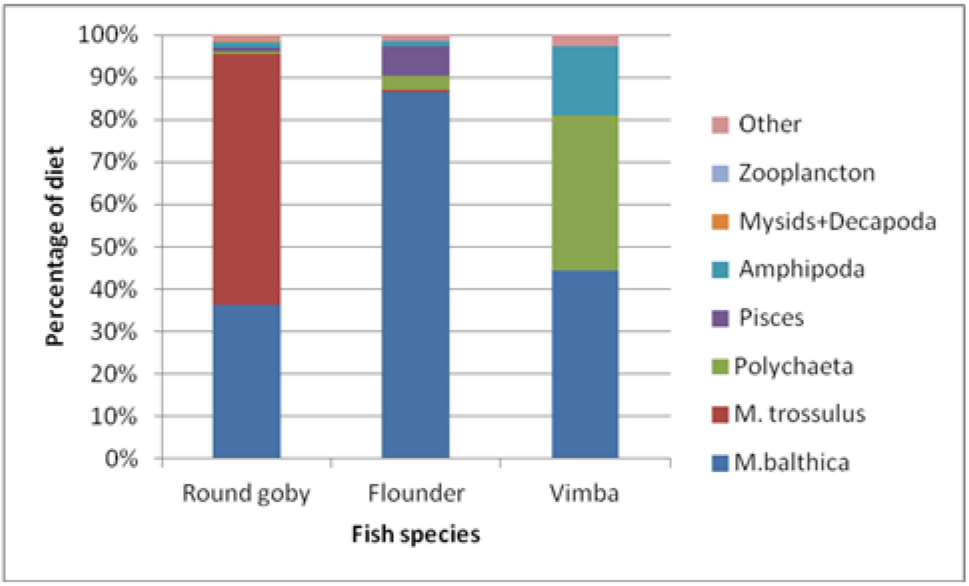

Figure 5. Relative proportion (by weight) of different prey items in the stomachs of the round goby, flounder, and vimba in the Pape littoral in late July and early August 2012.

The stomachs of round gobies were half empty. 5+ year old fish had the highest average fullness index value (59%) and 2+ years old fish the lowest (22%), with the age groups 3+ and 4+ situated in between (36% and 35%, respectively). This means that the largest round gobies are more satiated compared to the smallest.

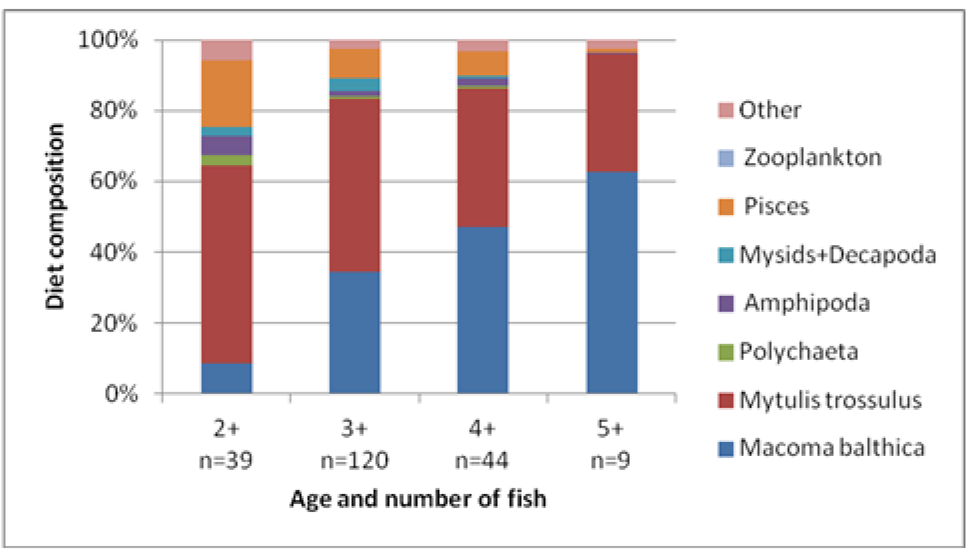

Figure 6. Diet composition (by weight) of 2+, 3+, 4+ and 5+ year-classes of round gobies in the Pape littoral in late July and early August 2012. n – number of investigated fish.

Mytilus trossulus composed the largest part of diet of the 2+ age group and with increasing fish age, the proportion of *M. trossulus* in the diet of round gobies decreased. Also the proportion of fish larva decreased with the age in round gobies. Conversely, the proportion of *Macoma balthica* increased, so that in the 5+ age group it composed the largest part of the diet (Fig. 6).

The most common prey items found in the round goby digestive tracts are presented in Fig. 7. Round gobies were divided into 15 length groups. Zooplankton was common food only for the smallest round goby size class (3,4 – 7,9 cm). In addition to zooplankton, a considerable part of the diet of the smallest round gobies consisted of amphipods. With increasing fish size, the proportion of bivalves (*Mytilus trossulus* and *Macoma balthica*) in the diet increased. *Mytilus trossulus* was the dominant prey item in the medium round goby length groups, while the proportion of *M. balthica* increased in the diets of large (15–23.5 cm) round gobies.

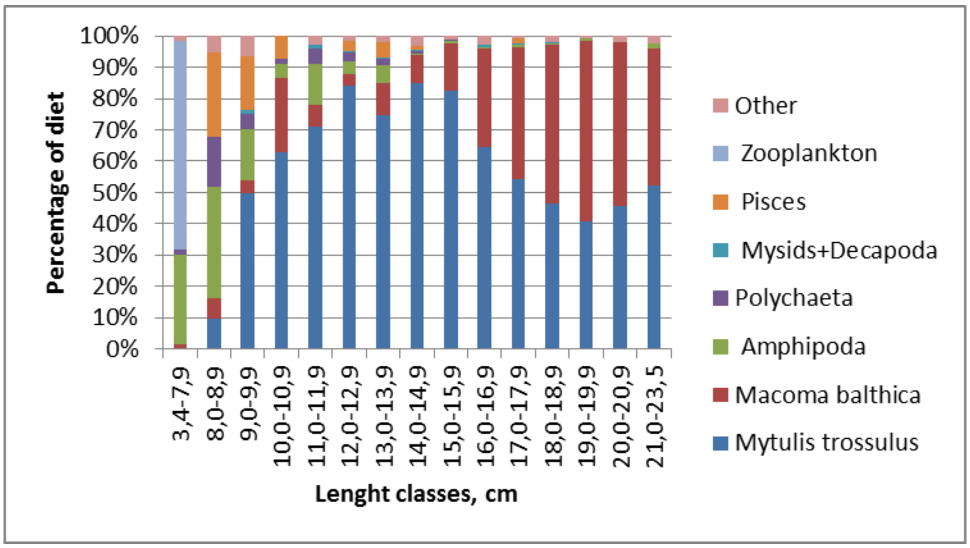

Figure 7. Relative proportion (by weight) of different prey items in the digestive tracts of round gobies in different length classes.

In general, the most common food items of round gobies were *Mytilus trossulus* and *Macoma balthica*. The prey item size preference seems to shift to proportionally bigger prey items (the afore mentioned *Mytilus trossulus* and *Macoma balthica*) as the fish grow in size (Fig. 8).

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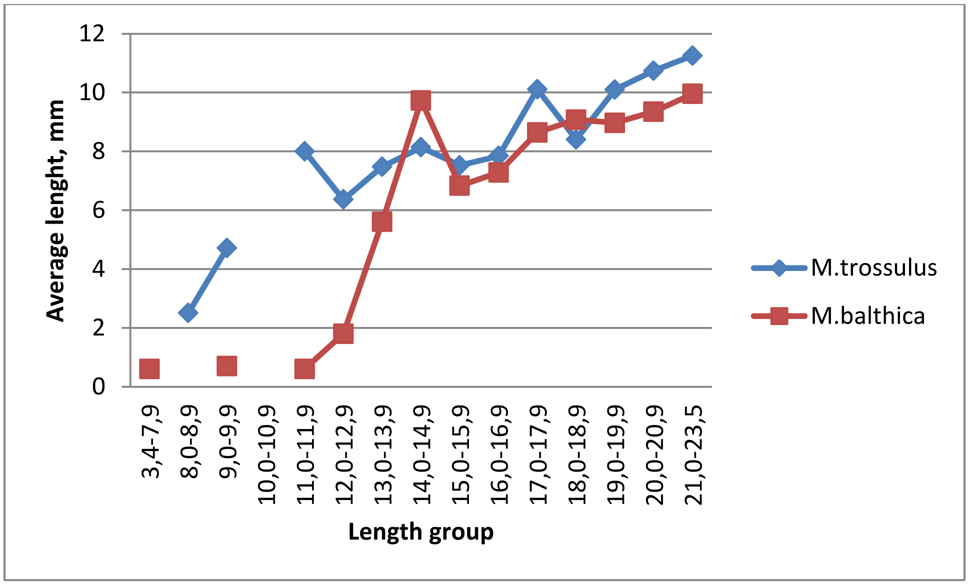

Figure 8. Size of consumed Macoma balthica *and* Mytilus trossulus *(on Y-axis) for different length groups of round gobies (on X-axis).*

The ratio of *M. trossulus* in round goby stomachs increased with depth. Polychaeta, fish larvae, Amphipoda, Decapoda and zooplankton are rife in stomachs of round gobies sampled at 5 m. *Macoma balthica* was consumed in similar proportions at all depths.

Discussion

Soft and hard bottom macrofauna

The macrozoobenthos at the Baltic Sea eastern coast between Klaipeda and Venspils has been studied on several occasions previously, in 1977–1981 (Lagzdinsh 1987), in 1980–1992 (Olenin 1997) and in 1986 (Appolov 1987). Benthos grabs were used for sampling in all these studies. In 1976 and 1977 in the Pape–Ventspils region the numbers of macrofauna were low, having an average abundance between 93 and 1454 ind./m^2, and a biomass between 2.9 and 57 g/m^2 (Kostrichkina and Kaleja 1980); however, Lagzdinsh *et al*. (1987) recorded quite differing densities in the area in 1976–1981. At the depth of 5–20m the average abundance of zoobenthos varied between 1960 and 8650 ind./m^2 and biomass was between 36 and 1160 g/m^2. The maximum biomass reached 5.8 kg/m^2 (Lagzdinsh *et al*. 1987). Hence different data obtained from the same area and almost at the same time show how difficult it is to obtain credible results from hard bottoms using grabs. Therefore, in the present study we consider the hard bottom data collected by divers more representative than that collected with grabs.

Appolov (1987) studied *Mytilus edulis* populations in the Klaipeda–Akmenrags area in 1986. According to Appolov (1987) the density of *M. edulis* in this area was greater than 2000 ind./m^2 and biomass reached 2–2.5 kg/m^2. The average abundance of *M. edulis* in the Palanga–Sventoja area in 1980–1992 was 40000 ind./m^2 and the biomass 1.6 kg/m^2 (Olenin, 1997). According to our investigations density of *M. edulis* was even 33000 ind./m^2 and biomass 3.5 kg/m^2.

Quite intensive macrofauna studies were performed after the tanker ”Globe Assimi” accident at the port of Klaipeda in 1981. It was found that the number of species, the biomass and abundance increased together with depth at the coastal zone in 1982–1983. Eight taxa were determined at the depth of 2.5 m. The average abundance of macrobenthos there was 1010 ind./m^2 (max. 3880 ind./m^2; min. 40 ind./m^2) and average biomass approx. 0.004 kg/m^2 (max. 0.002 kg/m^2). Eighteen species were determined at the depth of 5 m. The average abundance of benthic organisms at this depth was 4754 ind./m^2 (max. 12100 ind./m^2; min. 80 ind./m^2) and average biomass was approx. 0.039 kg/m^2 (max. 0.113 kg/m^2; min. 0.0005 kg/m^2) (Lagzdinsh 1990, Olenin 1990).

The abundance of *Mytilus* on hard bottoms varied between 1300 and 7400 ind./m^2 and biomass between 180 and 2400 g/m^2 (Lagzdinsh 1990, Olenin 1990). Altogether 30 taxa were identified (Lagzdinsh 1990, Olenin 1990). Our results are thus similar to the findings in 1981–1998.

Despite the increasing pressure of new predators (our study on round goby) on benthic invertebrates, benthic macrofauna still has the same species richness and also the abundance and biomass of macrofauna is close to values estimated in earlier studies. Our data indicate a decrease in *Mytilus* coverage in the study area. It is possible that after appearance of the round goby in the area the size structure of *Mytilus* populations has changed.

The occurrence of the round goby

Regardless that the round goby was first found in the Pape area during fish monitoring as recently as in 2010 (A. Minde, unpubl. data), it was the third most abundant species in the coastal waters of Pape in 2012 when this study was conducted. Studies from the North American Great Lakes have shown that the round goby is territorial and aggressive (Dubs and Corkum 1996, Balshine *et al.* 2005) and it quickly adapts to new environments (Sapota 2004), thereby it succeeds in reproducing rapidly and can affect the prevalence of other fish species such as flounder (Karlson *et al.* 2007).

The species composition and abundance of caught fish varied markedly between different depths. Neither drop video or SCUBA diving were considered to produce representative results regarding the distribution and occurrence of the round goby, wherefore the presented results are based on net sampling. The discrepancy in the numbers of round gobies observed in the diving transects and caught in Nordic coastal gillnets is due to the fact that both active and passive fish were seen and counted in diving transects whereas only active and mobile fish were caught in Nordic coastal gillnets. For the most part, the species composition and abundance of fish at different depths is influenced by water temperature, prey item availability and the bottom substrate of the coastal zone (Vetemaa 2006). The increase in round goby numbers with depth could be explained by changes in the bottom substrate type: up to 7 m depth there was mainly sand, but below 8 m the bottom is composed of boulders, shingles and gravel. The round goby (adult) prefers greater depths because their typical habitat is characterized as rocky and pebbly (Miller 1986); furthermore, these boulders and shingles are covered with *M. trossulus*, a prey item of round goby (Karlson *et al.* 2007, the present study). On the contrary, flounder typical habitat is sand where to sit and hunt on (Summers 1979), which explains the abundance of flounder down to 10 m depth. The abundance of freshwater fish species like perch, vimba, silver bream, roach and sander is promoted by increased water temperature (Repečka 2003, Bērziņš and Minde 2007). These species were mostly present up to 10 m depth which may be explained by the drop in water temperature between 5 and 15 m of 2.35 °C (LIAE, unpubl. data).

Age structure of the round goby population

The longest and oldest round gobies observed in the Baltic Sea region have been 25 cm and 6+ years, respectively (Sokolowska and Fey 2011). In this study the longest and oldest round gobies reached 23.5 cm and 5+ years of age. The observed length and age relationships of round gobies

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(Table 6) is in compliance with the characteristic length-age correlation for round gobies in the Baltic Sea (Sokolowska and Fey 2011). When comparing the age structure results of this pilot study with results from other areas it appears that relatively younger individuals (3+ years) are more common in Latvian coastal waters near Pape, while relatively older round gobies (4+ years) are more frequent in the Polish Gulf of Gdansk (Sokolowska and Fey 2011). This could be explained by the differences in the time of round goby introduction to the areas: the spreading to Gdansk Bay occurred much earlier (in 1990; Skóra and Stolarski 1993, 1995) than to the Pape area (2010; A. Minde, unpubl. data). Therefore, the population growth spurt has by now slowed down in the Gdansk Bay, whereas a rapid invasion phase currently takes place in the coastal waters of Latvia (Sapota 2004).

Feeding and food selectivity in the round goby

The juveniles of round gobies were too small and hid under stones, making them difficult to catch with the Nordic coastal nets and coastal gillnets. However, round gobies of a size corresponding with juveniles were counted in the diving transects. Despite the fact that juveniles were not found in the Nordic coastal gillnets samples their numbers in diving transect samples (5 and 10 m depths) were remarkable. There were approximately 2,0–3,0 cm long round gobies found at a rate of 327 individuals per 100 m^2 at 5 m depth, and 79 individuals per 100 m^2 at 10 m depth (there were no round gobies of this length at 15 m depth). The prevalence of 0+ and 1+ round gobies down to 10 m could be explained by availability of food items. Young round gobies are known to mostly feed on Chironomidae, Ostracoda and Amphipoda (Rakauskas *et al.* 2007, Raby *et al*. 2010), and these benthic invertebrates inhabit soft gravel substrates, which occur in shallow waters in the area.

Because prey items differ in diurnal activity, round gobies were caught at different times of day in order to better assess the diversity of their diet. Data on average stomach fullness showed that the stomachs of round gobies were half-empty the most time, furthermore, stomachs were fuller in larger fish compared to smaller ones (judging by length). A similar tendency was observed in study on feeding habits of round gobies by Raby *et al.* (2010). The half-empty stomachs were most probably related to water temperature which is known to have a direct impact on the activity and metabolism of fish (Linlokken and Haugen 2006, Kellnreitner *et al.* 2011). Prey items are digested more quickly during summer months (July and August) when water temperature is the highest. Similarly, metabolism rates differ depending on the age of fish – they are higher in younger individuals (Fonds *et al.* 1992) – which could explain the stomachs being emptier in younger round gobies when compared to older individuals.

The round goby is a benthic species wherefore it would seem likely that it competes for prey items with other benthic fish such as flounder and vimba in the coastal waters of Pape. When comparing the frequency of occurrence of prey items in the stomachs of those three species it was found that *Mytulis trossulus* and *Macoma balthica* were most common in round gobies' stomachs whereas *Polychaeta* and *Amphypoda* more often in stomachs of flounder and vimba. But such comparison doesn't allow to judge about the proportion of each prey item in the stomach. Hence by comparing the relative proportion of different prey items in competing benthic fish stomachs we found that the keenest competition is for *Macoma balthica*. The study conducted by Karlson et.al. (2007) also demonstrates high competition for *Macoma balthica* between round goby and flounder. The results of our study show that *Mytulis trossulus* constitutes the most part of round goby diet, followed by *M. balthica. M. trossulus* is found in flounder stomachs as well, but its relative amount is negligible. Taking into account above mentioned, our results allows to state that round goby competes with the natives species (flounder and vimba), since considerable shares of the diets of all three species consist of *M. balthica*. Currently the competition for *Macoma* not of great consequence for any of the three species since the alien, unlike the natives, consumes considerable amounts of *Mytilus* also. Altogether these facts suggest that round goby has found an unoccupied feeding niche in coastal area of Pape, and it is not threatened by competition for prey items with other species. On the other hand, the conclusion is not unambiguous because during some stages of development round goby's range of prey items overlaps with those of other species. As mentioned previously, other studies on feeding habits of round gobies (Lederer et.al 2006, Rakauskas et al. 2007, Raby et. al 2010) demonstrate that the diet of young fish (0+, 1+) is composed mainly of *Amphipoda*, *Chironomidae* and other small organisms inhabiting soft ground and that the dietary proportion of *Bivalves* increases when fish grows older. This study, too, showed that *Amphipoda*, *Polychaeta*, fish larva and Zooplankton are the main components in round gobies with length up to 10 cm, but the proportion of Bivalves increases sharply with age.

Small-sized *M. trossulus* and *M. balthica* are already fed on by round gobies in length groups of 3.4–7.9 cm and 8.0–8.9 cm, but their relative proportion is negligible. The share of bivalves in the round goby diet increases when fish comes greater length and the gape height increases as well. (Ray and Corkum 1997, Barton *et al.* 2005).

Chotkowski and Marsden (1999) and Steinhart *et al.* (2004) showed that round gobies feeds on fish spawn and larvae. This was established also in our study, where fish larvae occurred in the diets of round gobies as young as 2+ years. Although the biomass of *M. trossulus* and fish larvae in the diet does not markedly decrease with round goby age, their proportion decreases as the proportion of *M. balthica* in the diet increases.

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Conclusions

In the study area round goby was the third most abundant fish species. Analysis of fish population age structure shows that population dominated by 3+ years old round gobies.

This study shows that benthic fauna has changed. Comparing the results of the present study with the baseline studies done in the same study area 2006, we found that considerable changes in the coverage of *M. trossulus* had occurred. In 2006 average coverage of *M. trossulus* was between 20% and 55% while in 2012 it reached only 10% – 20%. Thus currently the average percentage of *M. trossulus* coverage in the study area in 2012 was less than half of what it was six years previously.

The most common food items of round gobies were *Mytilus trossulus* and *Macoma balthica*. With increasing fish size, the proportion of bivalves (*M. trossulus* and *M. balthica*) in the diet increased. *M. trossulus* was the dominant prey item in the medium round goby length groups, while the proportion of *M. balthica* increased in the diets of large (15–23.5 cm) round gobies. Zooplankton and amphipods were common food only for the smallest round goby size class (3,4 – 7,9 cm).

Round goby competes with the natives species (flounder and vimba), since considerable shares of the diets of all three species consist of *M. balthica*. Currently the competition for *Macoma* not of great consequence for any of the three species since the alien, unlike the natives, consumes considerable amounts of *Mytilus* also. However, this observation need of further analysis.

Results of this study suggest that changes in the benthic fauna a large extend can be attributed to the round goby invasion and provides further evidence that the expansion of the round goby to new areas has the potential to change the trophic interactions of benthic communities. A series of complex interactions between the round goby, *Mytilus trossulus*, and other benthic invertebrates can directly and indirectly affect food resources and energy flows, thereby altering the structure and functioning of the littoral zone communities in Latvian coastal waters.

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Annexes

Annex 1. Hematology of round goby (*Neogobius melanostomus*)

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Introduction

Hematology, i.e. the study of blood, the blood-forming organs, and blood diseases, can be a useful tool in monitoring the welfare and health status of fish, in detecting potential illness and in following the progress of disease (Tavares Dias *et al*. 2008). The physical and chemical components of fish blood are very sensitive to environmental changes, pathogens and diseases. This is the first study on the hematology of the round goby and possible health status of fish.

The components of blood

Blood is one of components of the internal environment and it keeps relatively constant the water, salt, protein and glucose levels and the acid-base concentration in a fish body. Fish blood is composed of plasma (approx. 50–70%) and blood cells (approx. 30–50%): erythrocytes (red blood cells; RBC), leukocytes (white blood cells; WBC), and platelets (Jemeļjanovs *et al.* 2007, Noga 1996, Stoskopf 1993).

RBC are the most common type of blood cells in vertebrates. RBC deliver oxygen to body tissues, constitute a body's active response to pH and maintain ion balance by taking part in the exchange water and salts. In addition, red blood cells are able to absorb toxins (Jemeļjanovs *et al.* 2007, Noga 1996, Stoskopf 1993). Low amounts of RBC have been noted in stormiiforms and alepocephalids. Elasmobrannchs in general show lower RBC amount than teleosts (0.1–0.4 10^{12} µL) (Hoar *et al.* 1992).

Hematocrit is the volume percentage (%) of red blood cells in blood (Jemeļjanovs *et al.* 2007). Hematocrit values of fish range from 0 to 50%, but most of cases it is between 20 and 40 %. The hematocrit value is relatively constant within species, but varies between species (Hoar *et al.* 1992).

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Hemoglobin (Hgb) is the iron-containing oxygen-transport protein in the RBC of all vertebrates. Hemoglobin in the blood carries oxygen from the gills to the body tissues (Jemeļjanovs e*t al.* 2007, Noga 1996, Stoskopf 1993).

WBC play a vital role in the fish immune system. Fish WBC can be both larger and smaller than RBC. WBC are divided in two large groups: granulocytes and agranulocytes. Granulocytes are divided into neutrophils, eosinophils and basophils; agranulocytes into lymphocytes and monocytes.

Granulocytes are the largest cells in the blood system. Their cytoplasm is usually light blue, but their nucleus has varying shapes. In most fish species the nucleus of granulocytes is oval or kidney-shaped, in some fish species it is lobed into segments. Granulocytes constitute 4.5–18% of the blood of teleosts (Hoar *et al.* 1992).

Neutrophils are the predominating, and sometimes the only existing, type of granulocytes in a teleost (Hoar *et al.* 1992). Neutrophils are the first to respond when an invasion of foreign bacteria or fungi occurs. Eosinophils phagocytize mostly metazoon and other parasites. The primary function of basophils is to release a chemical known as histamine in response to an infection.

Lymphocytes are relatively small cells with a round or oval nucleus. The cytoplasm is not granulated (Hoar *et al.* 1992); hence the term agranulocyte. Fish lymphocytes can be divided into large and small lymphocytes (some authors divide them into three groups) (Hrubec and Smith 2010, Hoar *et al.* 1992).

The last type of WBC is actually a modified monocyte. Monocytes are the largest agranulocytes that transforms to macrophages when it moves from blood toconnective tissue behind a blood vessel wall (Jemeļjanovs *et al.* 2007, Stoskopf 1993).

Materials and methods

This research was conducted in the Baltic Sea near Liepaja. Blood samples of 31 round gobies caught for the actual report in the coastal zone were examined. Fish of both sexes (10 males, 21 females) and of the different size and age were analyzed. Blood sampling and hematological analyses were performed in the summer of 2012. Blood was sampled from the caudal vein of fish. EDTA was used to avoid hemocoagulation. The blood was kept on ice and analysed in four hours . The following indices of blood structure were established: concentration of RBC in peripheral blood, concentration of Hgb, and hematocrit. Following RBC indices were calculated: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Blood smears were prepared to establish the percentage of various types of WBC.

The concentration of RBC was determined by photometric turbidity analysis using Gowers' solution. 10 μL of well-mixed blood was transferred into 2.5 mL of Gowers' solution. The solution was gently mixed by inversion and measured after exactly 5 min. The optical density was determined using a MP_plus 25 photometer at a wave length of 546 nm against a blank of Gowers' solution (Fig.1). The absorbance was converted to erythrocyte concentration (cells • 10^{12} L^{-1}) after calculating the "specific species factor".

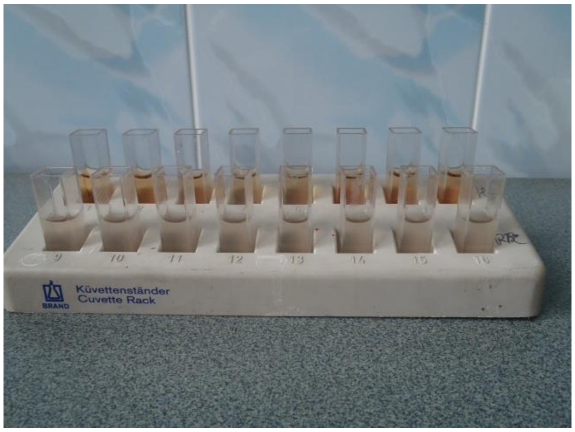

Figure 1. Round goby blood samples prepared for the analysis of the hemoglobin level and the red blood cells count.

Hemoglobin (g dL^{-1}) was measured by the cyanmethemoglobin method using a hemoglobin transformation solution. 10 μL of blood was placed into 2.5 mL of transformation solution. The solution was gently mixed by inversion and left still for 5 min for full conversion. The optical density was determined using a MP_plus 25 photometer at a wavelength of 546 nm against a blank of transformation solution. This reading was converted to hemoglobin concentration (g dL^{-1}) by using the "specific species factor". Hematocrit was measured by collecting blood in non-anticoagulated microhematocrit tubes which were centrifuged for 6 min at 12500 rpm and read immediately.

Differential white blood cells (WBC) or leukocytes count was performed with blood smears stained with Dip Quick Stain J_322 solution. Smears were examined by light microscopy under oil immersion at 1000 × magnification. In order to determine the percentage of various types of white blood cells, 200 leucocytes per slide were counted (Conroy and Conroy 2006). Leucocytes

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in the round goby were divided into basophils, eosinophils, monocytes and lymphocytes (large and small).

Results and discussion

The weight of the investigated round gobies was 61.5±42.8 g, length 16.5±2.4 cm. This is the first study on the hematology of the round goby; no information on the blood parameters of this species can be found in the literature. However, in the Baltic Sea hematological studies have been performed on two other demersal species, eelpout (*Zoarces viviparus*) and flounder (*Platichthys flesus*) (Medne and Balode 2012), and our results will be discussed in relation to these.

The hemoglobin was low in the round goby (3.5–8.8 g dL^{-1}; for mean values for the sexes separately see Table 1). Similar values have been obtained for eelpout (Medne and Balode 2012). The mean number of erythrocytes was 1.09 • 10^{12} L^{-1} in female round gobies and 1.10 • 10^{12} L^{-1} in males (Table 1) which is higher than in eelpouts (0.67–0.92 • 10^{12} L^{-1}), but lower than in flounders 1.71–1.75 • 10^{12} L^{-1}) (Medne and Balode 2012). Hematocrit (36.8–42.7%) was very high in round goby compared with both eelpouts (13.6–15.1%) and flounders (23.7–28.4%) (Medne and Balode, 2012).

Table 1. The hematology of the round goby

Indices	**Females (n=21)**	**Males (n=10)**
	Mean ± STDEV	Mean ± STDEV
Hemoglobin (g dL^{-1})	3.35±1.3	3.91±1.2
RBC (10^{12} L^{-1})	1.09±0.14	1.10±0.09
Hematocrit (%)	42.7±14.5	36.8±8.95
MCV (fL)	376.2±197.7	296.1±120.3
MCH (pg/cell)	19.3±9.2	20.4±4.7
MCHC (g/L)	66.2±10.1	65.0±5.2
Differential white blood cell count (%)		
Basophils	10.1±6.3	6.3±4.5
Eosinophils	8.1±7.9	3.3±2.5
Monocytes	1.6±2.3	0.7±1.4
Lymphocytes (total)	76.2±22.3	89.7±5.6
Large lymphocytes	10.2±9.0	7.0±5.5
Small lymphocytes	69.9±18.4	82.7±7.5

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Annex 2. Stable isotope analysis

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Introduction

Stable isotopes are a classical way to trace food sources of aquatic animals (Peterson & Fry 1987). In the present study we use natural stable-isotope signatures, to investigate the relative importance of *Mytilus trossulus* as food for round gobies.

Material and methods

A piece of the dorsal muscles of the round goby (*Neogobius melanostomus*) and abundant native benthopelagic fish species (Table 1.), as well as the muscles of the blue mussel (*Mytilus trossulus*) were collected for stable isotope analysis. Muscle samples were oven-dried at 60ºC for 24 h, ground to a fine powder using a mortar and pestle, weighed to within approx. 0.7 mg and stored in clean tin cups for isotope analysis. Carbon and nitrogen isotope analysis was undertaken by Elemental Analysis - Isotope Ratio Mass Spectrometry (EA-IRMS). Stable isotope ratios were expressed in δ units using the following equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where X is either ^{13}C or ^{15}N and R is the ratio of the heavy to the light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Pee Dee Belemnite and atmospheric N_2 were used as carbon and nitrogen standards, respectively.

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Results

Table 1 and Figure 1 summarize the $\delta^{13}C$ and $\delta^{15}N$ values for all examined fish species and *M. trossulus*. The average $\delta^{13}C$ ratio of fish ranged from -21.9‰ for round goby to -23.2‰ for *Vimba vimba*. The average $\delta^{13}C$ ratio of *M. trossulus* was 23.6‰.

Table 1. $\delta^{13}C$ and $\delta^{15}N$ values (average ± SD) for fish species and M. edulis. n – sample size.

Species (n)	**$\delta^{13}C$**	**$\delta^{15}N$**
Neogobius melanostomus (10)	-22.0 ± 0.8	12.6 ± 0.6
Perca fluviatilis(5)	-22.2 ± 1.4	14.4 ± 0.8
Platichthis flesus (5)	-22.8 ± 0.5	13.6 ± 0.2
Psetta maxima (5)	-22.2 ± 0.6	14.7 ± 0.5
Osmerus eperlanus (2)	-22.2 ± 0.2	13.8 ± 0.01
Vimba vimba (2)	-23.2 ± 0.9	13.7 ± 0.1
Mytilus trossulus (3)	-23.6 ± 0.4	8,4 ± 0.3

Stable nitrogen ratios, which are indicative of trophic level, varied from 12.6‰ for the omnivorous *N. melanostomus* to 14.7‰ for the piscivorous *Psetta maxima* (Table 1). A relatively large variation in isotopic signatures of the individuals of round gobies was found. More depleted $\delta^{13}C$ values and higher $\delta^{15}N$ values were found in smaller individuals (Fig. 2).

Discussion

The stable isotope analyses results of all the analyzed fish species and *M. trossulus* support the insights gained by gut content analysis. As expected from the literature (e.g. Nordström *et al.* 2010) the piscivorous fish species, namely *P. maxima* and *P. fluviatilis*, had the highest $\delta^{15}N$ values indicating their being positioned highest in the coastal food web.

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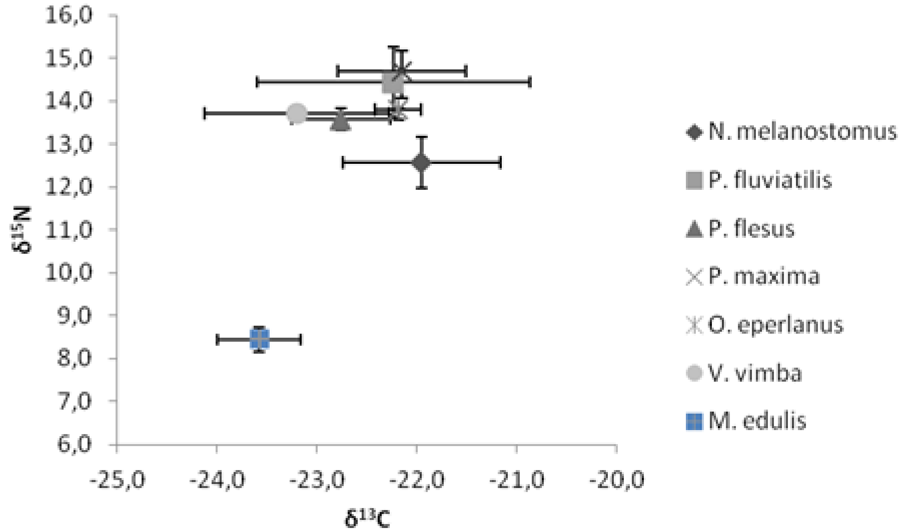

Figure 1. Mean δ^{13}C and δ^{15}N (±SD) values of fish species and M. trossulus.

An explanation to the smaller individuals (8.6 and 9 cm SL) of round gobies having more depleted carbon isotopic values can be found when looking at our gut content data. The data showed that the fish of smaller size classes were consuming mostly *M. trossulus* but larger fish switched gradually to *Macoma balthica* dominated diet. Our stable isotope analysis of *M. trossulus* showed that their average δ^{13}C value was -23.6 ‰, close to that of small round gobies (-22.9 ‰). This indicates a greater trophic reliance of smaller gobies on blue mussels and a size specific dietary shift.

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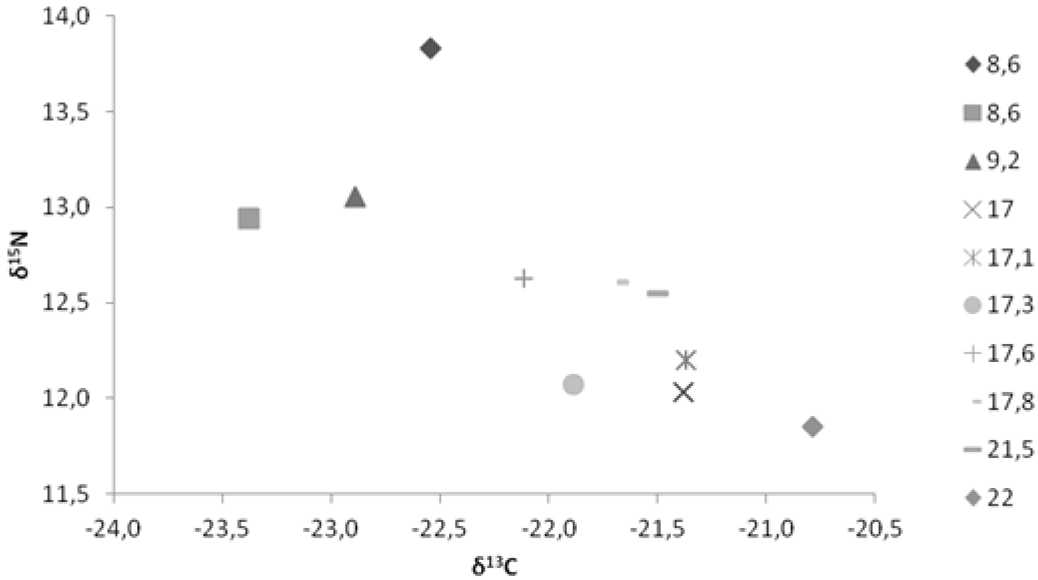

Figure 2. $\delta^{13}C$ and $\delta^{15}N$ values of individual N. melanostomus. Symbols indicate individuals SL's (mm).

The similar carbon isotopic values indicate a potential competition for resources between flounder and round goby. This is supported by the overlapping diet compositions. Furthermore, several authors have indicated that the above mentioned species compete for resources (Karlson *et al.* 2007). It has to be noted that due to the limited amount of data on the food web structure this is a preliminary observation in need of further analysis.

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Estonian case study on non-indigenous species

Port biological survey in Muuga harbour, port of Tallinn

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Summary

The objective of the Estonian pilot study was to test, develop further, and assist in finalising the HELCOM port survey guidelines. For this purpose phytoplankton, zooplankton, benthic biota and fish were sampled in one of the largest ports in the Baltic Sea, the port of Tallinn. The obtained results are meant to serve as a reliable basis of data to be used in A-4 risk assessments in granting ballast water management exemptions according to "The International Convention for the Control and Management of Ships' Ballast Water and Sediments" of the International Maritime Organisation (IMO 2004).

Introduction

"The International Convention for the Control and Management of Ships' Ballast Water and Sediments", BWMC, was adopted in 2004 (IMO 2004). In the HELCOM Baltic Sea Action Plan, BSAP, the Contracting Parties have agreed to ratify BWMC no later than in 2013. According to the BWMC, ships are required to implement ballast water management unless an exemption has been granted following a risk assessment of whether a ship poses a high or low risk of spreading alien species.

In the Road map towards harmonized implementation and ratification of the BWMC, adopted as part of the BSAP, it was agreed to develop a unified Baltic Sea exemption system. IMO provides Guidelines for Risk Assessment under Regulation A-4 (G7) as an appendix of the BWMC resolution. Amongst others, it defines the requirements for granting exemptions, starting with data quality requirements to risk assessment procedures. Availability of data on alien species and environmental conditions in ports are a pre-requisite for carrying out reliable A-4 risk assessments. However, the availability of port data for the purpose of the risk assessments were identified by HELCOM as topics requiring further development. For this purpose, the HELCOM

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Aliens 2 project was established with one of the three targets aiming at "*establishing a protocol to be used in collecting information from ports in order to conduct risk assessments"* (HELCOM 2012).

Material and methods

Biological sampling was performed in the Muuga harbour, port of Tallinn, to characterise the composition and biomass/abundance of the local community. The survey, the aim of which was to test, develop further, and assist in finalising the HELCOM port survey guidelines (HELCOM 2012), was performed at the three terminals – Ro-Ro and container terminal, grainterminal and oil terminal (Fig. 1) – on September 17th, 2012. In addition to employing HELCOM routine marine monitoring guidelines to sample phytoplankton, zooplankton and macrozoobenthos, additional specific sampling gear were applied in collecting epifauna and fouling communities. These were crab traps, fish traps, and a scraping tool (Figure 2). Two crab traps and three fish traps were employed for 48 hours in all terminals. Fouling communities were sampled from as many different substrates as possible according to HELCOM guidelines (HELCOM 2012). Riikka Puntila from HELCOM kindly assisted in the sampling to ensure sampling consistency and result comparability with other ports tested with the same methodology.

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Figure 1. Locations of port biological sampling in Muuga harbour, port of Tallinn (from left to right): oil terminal, grainterminal, and Ro-Ro and container terminal. Sampling locations of Chinese crab (C) and Minnow fish (M) traps.

Figure 2. Sampling equipment used in the alien species survey (from left to right): Chinese crab trap, Minnow fish trap and scraping tool (HELCOM 2012).

Results and discussion

Here we present the sampling results by major organism groups (phytoplankton, zooplankton, benthic biota and fish) in detail, by incuding both native and alien/cryptogenic species.

Phytoplankton

The most abundant phytoplankton taxa, showing also fairly high biomasses, were *Eutreptiella gymnastica* and species from the genus *Teleaulax*, but also *Plagioselmis prolonga* (Table 1). This situation is characteristic for the nearby areas in Muuga Bay and Tallinn Bay in September (Anon. 2013). Thus we did not observe any substantial differences in the phytoplankton communities in the port area and at the monitoring stations in nearby small bays on the southern coast of the Gulf of Finland.

Table 1. Abundance and biomass of phytoplankton taxa in different terminals in the Muuga harbour, port of Tallinn, on September 17th, 2012.

Taxa	**Ro-Ro and container terminal**		**Grain terminal**		**Oil terminal**	
	Abundance (ind./l)	**Biomass (µg/l)**	**Abundance (ind./l)**	**Biomass (µg/l)**	**Abundance (ind./l)**	**Biomass (µg/l)**
Aphanocapsa	2845	0,4	2845	0,3	0	
Chroococcales	5121	0,7	3983	0,3	5580	1
Aphanothece			6828	0,4	1674	1
Woronichinia	0		0		0	
Planktolyngbya	2845	0,6	0			
Pseudanabaena	12518	2,8	0		12276	2,3
Hemiselmis virescens	0		0		0	
Plagioselmis prolonga	168975	3,8	298549	6	523869	10,7
Teleaulax	75100	10,5	95761	12,7	191522	29
Glenodium					0	
Gymnodiniales	7510	4,9	0			
Gymnodinium					11824	1,3
Heterocapsa rotundata					0	
Heterocapsa triquetra					0	
Heterocapsa triquetra					1674	2
Peridiniales	0		0		0	
Chrysochromulina	37550	0,7	0		0	

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Pseudopedinella	135180	5,1	84495	3,8	157724	6,2
Actinocyclus octonarius var. octonarius			0			
Coscinodiscus granii	0				0	
Cyclotella choctawhatcheeana	41305	2,8	0		0	
Cylindrotheca closterium			0		0	
Nitzschia acicularis var. acicularis			0		0	
Eutreptiella	33795	8,7	0		157724	44,5
Pyramimonas	7510	0,6	28165	2,5	39431	4
Chlorococcales					0	
Monoraphidium minutum	0		0		0	
Monoraphidium contortum	0		0		0	
Oocystis	0				0	
Planctonema lauterbornii	0		0		0	
Mesodinium rubrum	0		1707	7,5	6696	38,4
Flagellates	22530	1	0		45064	2,6
Amphidinium crassum					0	
Gymnodiniales	0		0			
Katablepharis					0	
Ebria tripartita	0				1674	4

Zooplankton

The highest abundance of mesozooplankton was recorded in the vicinity of the oil terminal, where the abundance of the rotifer *Keratella quadrata* exceeded 43000 ind. m^{-3} (Table 2). Similarly, the highest biomass was observed nearby the same terminal with strong domination of *K. quadrata* and *Acartia bifilosa*. The latter species dominated in all mesozooplankton samples while other copepods (*Eurytemora affinis*, *Centropages hamatus*, *Temora longicornis*) where substantially less abundant, and cladoceran densities were also very low. In all samples, larvae of the cryptogenic cirriped *Balanus improvisus* were present.

Table 2. Abundance and biomass of zooplankton taxa in different terminals in the Muuga harbour, port of Tallinn, on September 17th, 2012.

Species	Comments	Container terminal		Grain terminal		Oil terminal	
		Ind/m^3	Wet wt. mg/m^3	Ind/m^3	Wet wt. mg/m^3	Ind/m^3	Wet wt. mg/m^3
Rotifers							
Keratella cochlearis		800	0,8	1550	1,6	5050	5
Keratella cruciformis		400	0,4	450	0,5	1313	1,3
Keratella quadrata		6760	6,8	12150	12,2	43430	43,4
Synchaeta baltica		1280	6,4	1000	5	2626	13,1
Synchaeta monopus		400	1,2	350	1,1	808	2,4
Cladocerans							
Bosmina coregoni	small	260	2,6	102	1	1050	10,5
Bosmina coregoni	medium	20	0,3			150	1,9
Evadne nordmanni	small	400	12	51	1,5	200	6
Evadne nordmanni	medium			34	1,4		
Pleopsis polyphemoides	small	120	1,2	221	2,2	400	4
Pleopsis polyphemoides	medium			17	0,3	150	3
Podon intermedius		20	0,6	1	0	2	0,1
Copepods							
Acartia bifilosa	Copepodite stage I	1080	5,4	1800	9	4646	23,2
Acartia bifilosa	Copepodite stage II	1840	16,6	1750	15,8	6262	56,4
Acartia bifilosa	Copepodite stage III	2560	30,7	2550	30,6	3535	42,4
Acartia bifilosa	Copepodite stage IV	1820	23,7	1598	20,8	2050	26,6
Acartia bifilosa	Copepodite stage V	860	17,2	1020	20,4	1100	22
Acartia bifilosa	female	360	9,4	289	7,5	50	1,3
Acartia bifilosa	male	280	7	187	4,7	100	2,5
Centropages hamatus	Copepodite stage III			17	0,2		
Eurytemora affinis	Copepodite stage I	60	0,3	102	0,5	150	0,8
Eurytemora affinis	Copepodite stage II	80	0,7	187	1,7	100	0,9
Eurytemora affinis	Copepodite stage III	240	2,6	153	1,7	200	2,2
Eurytemora affinis	Copepodite stage IV	120	1,6	170	2,2		
Eurytemora affinis	Copepodite stage V	80	1,6	68	1,4	100	2
Eurytemora affinis	female	20	0,6				
Eurytemora affinis	male			17	0,4		
Temora longicornis	Copepodite stage I	120	0,6	51	0,3	50	0,3
Temora longicornis	Copepodite stage II			34	0,3		
Temora longicornis	Copepodite stage III	20	0,2				
Temora longicornis	Copepodite stage IV	20	0,3	34	0,4		
Temora longicornis	Copepodite stage V			51	2		
Copepod nauplii		7920	23,8	1400	42	14847	44,5
Mysids							
Mysids		1	0				
Meroplankton							
Balanus improvisus nauplii	small	680	3,4	1250	6,3	909	4,5
Balanus improvisus nauplii	bid					101	1
Bivalvia larvae				50	0,3		
Gastropoda		40	0,4				

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Benthic biota

Benthic biota were sampled with both the Ekman-Birge sampler and the scraping tool (Fig. 2). As the sampling devices substantially differ in terms of their characteristics, results are presented separately for the two different samplers (Table 3). In total, five benthic invertebrate and three algal species were identified in the samples collected with the Ekman-Birge sampler. *Macoma balthica* was the dominat species; it occurred in seven samples out of nine and with the highest biomass (5.6 g m^{-2}). The following algae were found in samples: *Pilayella littoralis*, *Cladophora glomerata* and *Ulva intestinalis*. Cryptogenic species were represented by the cirriped *Balanus improvisus* only.

In samples collected by the scraping tool, *Balanus improvisus* dominated and *Macoma balthica* was not observed at all. While all three algal species found using the Ekman-Birge sampler were present also in the samples collected with the scraping tool, there were some differences in the fauna. Using the scraping tool several gammarids and two nectobenthic species (*Praunus flexuosus* and *Palaemon adspersus*) were caught; these were not present in the Ekman-Birge samples. Thus, different sampling devices are useful to employ in parallel to investigate and characterise species composition of benthic biota in port areas.

Tabel 3. Abundance and/or biomass of benthic species in different terminals in the Muuga harbour, port of Tallinn, on September 17th, 2012.

Sampling device: Ekman-Birge				
Terminal	**dominant substrate**	**Species**	**Biomass (g/m²)**	**Abundance (ind./m²)**
Grain terminal	mud	*Macoma balthica*	0,1269	47
		Chironomidae l.	0,0094	94
	clay	*Macoma balthica*	0,2209	47
		Corophium volutator	0,0423	47
		*Balanus improvisus**	1,8612	47
	sand	*Macoma balthica*	1,0058	423
Ro-Ro and container terminal	mud	*Hediste diversicolor*	0,0376	47
	pebble	*Hediste diversicolor*	0,0282	47
		Pilayella littoralis	0,047	
		Cladophora glomerata	0,0094	
Ro-Ro and container terminal	mud	*Macoma balthica*	1,8048	141
		Hediste diversicolor	0,5781	94
Oil terminal	clay	*Macoma balthica*	0,141	47
		Chironomidae l.	0,0094	94

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Oil terminal	caly	*Macoma balthica*	5,6212	47
		Hediste diversicolor	0,0611	47
Oil terminal	clay	*Macoma balthica*	0,4089	47
		Chironomidae l.	0,0094	47
		Ulva intestinalis	0,0517	
Sampling device: scraper				
Terminal	**substrate**	**Species**	**Weight (g)**	**Number of individuals**
Oil terminal	rubber	*Balanus improvisus**	0,1154	106
		Chironomidae l.	0,0007	4
		Praunus flexuosus	0,004	1
		Palaemon adspersus	0,0038	1
		Ulva intestinalis	0,9556	
		Cladophora glomerata	1,4098	
		Pilayella littoralis	0,1653	
Oil terminal	metal	*Balanus improvisus**	0,0761	25
		Ulva intestinalis	0,0059	
Oil terminal	concrete	*Balanus improvisus**	0,4598	264
		Gammarus juv.	0,0061	15
		Ulva intestinalis	0,348	
		Cladophora glomerata	0,0024	
		Gammarus salinus	0,0046	5
Oil terminal	natural rock	*Balanus improvisus**	0,0411	21
		Gammarus zaddachi	0,0059	2
		Palaemon adspersus	0,1258	2
		Cerastoderma glaucum	0,0026	1
		Ulva intestinalis	0,3438	
		Pilayella littoralis	0,0313	
		Cladophora glomerata	0,0629	

Fish

In total nine fish individuals, all of them being the round goby *Neogobius melanostomus*, were found in the crab and fish traps. The sampling details were: Ro-Ro and container terminal locality C1 – 3 individuals, locality C2 – 2 individuals and F1 – 1 individual; Oil terminal locality F2 – 2 individuals; Grain terminal locality F3 – 1 individual. No other fish species nor any Chinese mitten crab individuals were present in traps.

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