

## Profiling four brackish-water harbours: zoobenthic composition and invasion status

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*Received 21 Dec. 2006, accepted 31 May 2007 (Editor in charge of this article: Johanna Mattila)*

Paavola, M., Laine, A. O., Helavuori, M. & Kraufvelin, P. 2008: Profiling four brackish-water harbours: zoobenthic composition and invasion status. *Boreal Env. Res.* 13: 159–175.

Many studies worldwide have emphasized the need to map the potential of commercial ports to function as receiver and donor areas for nonindigenous species (NIS). In this study, the macroinvertebrate composition of hard and soft sea bottoms, hydrographical conditions, and traffic connections of four Finnish coastal harbours (Naantali, Koverhar, Porvoo, Hamina) were investigated and compared with 'natural' reference areas lacking anthropogenic constructions. The harbours hosted macroinvertebrate communities fairly similar to those found in adjacent reference areas, but sediment community diversity was slightly higher in harbour basins relative to in surrounding areas. A total of eight NIS were registered in the study. With regard to hydrography, NIS that tolerate low salinities, low temperatures and eutrophic conditions are most likely to survive local conditions and hence pose a potential risk for larger areas of the Baltic Sea. A ship traffic analysis revealed that the most important donor and recipient areas of ballast water to and from the study harbours are coastal harbours of other parts of the Baltic Sea and of the North Sea. Some suggestions are made for future harbour surveys along the Finnish coast.

### Introduction

The unintentional introduction of aquatic species into new areas by shipping, in ballast water and through hull fouling, has become a rapidly expanding research arena during the past decade. Globally, some three to five billion tonnes of ballast water and an unknown amount of ballast sediment are transported per year, transferring some 3000 species at any time (or 10 000 or more each week) between harbours (Carlton 1999). In addition to planktonic taxa and life stages, macroinvertebrates and fish are also transported in ballast-water tanks. Aquatic nonindigenous

species (NIS), sometimes referred to as biological pollution (e.g. Elliott 2003), are not only a concern for the diversity and functioning of native ecosystems, but also for society, in terms of health threats to humans and socio-economic costs. When a NIS establishes in a new area and becomes invasive, i.e. begins to play a disproportionately large role in a system, it may be harmful to, for example, fisheries, recreation and aquaculture. This is why an emphasis in policy and decision-making should be put on preventative measures against the introduction of alien species, including the development of proper ballast water treatment methods, and risk assessments

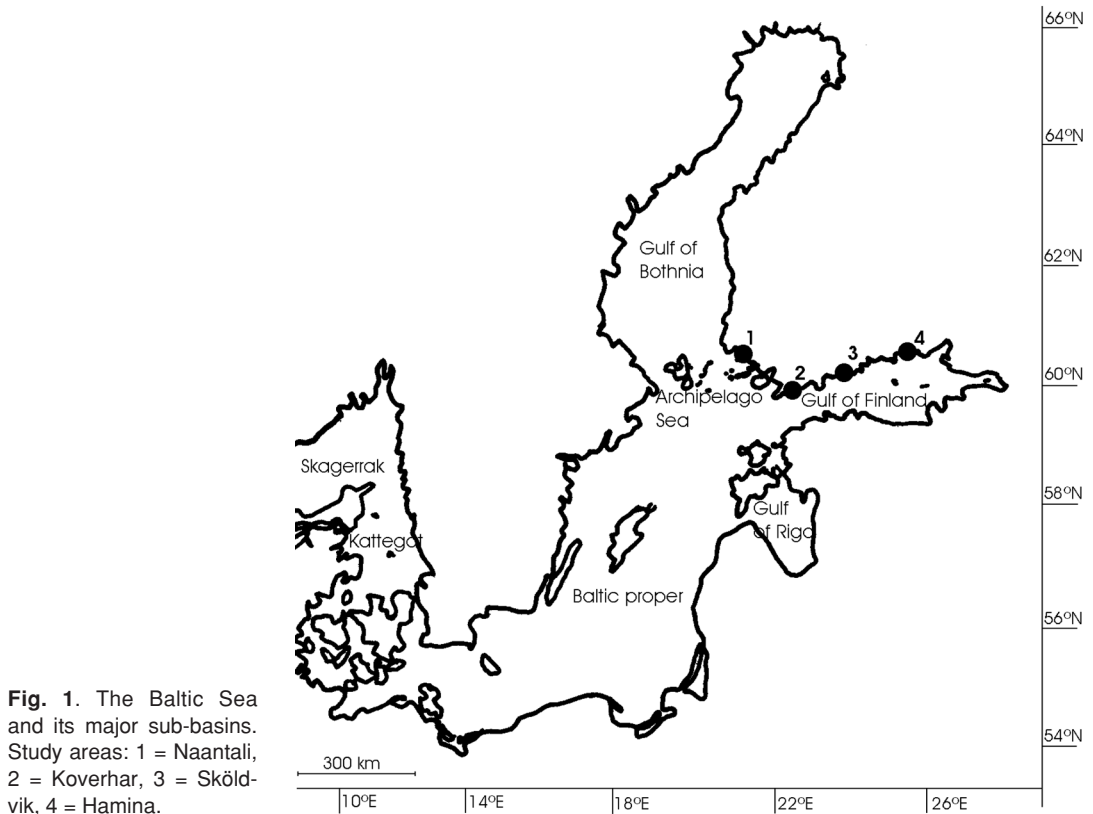
of future invasions (e.g. Hicks 2004, IMO 2004). A prerequisite for controlling the introduction of NIS is determining the current invasion status of an area (Hewitt and Martin 2001). Knowledge of ship traffic patterns and the biological state and structure of the recipient area are also central to risk assessments of NIS. Recent port studies have taken these aspects into consideration. For example, the GEF/UNDP/IMO Global Ballast Water Programme (Globallast) has funded port survey studies in developing countries to help them begin to tackle the ballast water problem. Port surveys have also been conducted in Australia (e.g. Australian Museum Business Services 2002) and in the USA (e.g. Cohen *et al.* 2005).

Brackish waters are considered to be important bridgeheads for NIS, since many harbours worldwide are located at river mouths or in coastal inlets with reduced salinity. A low native species richness and diversity of functions (i.e. empty niches available) are also factors facilitating successful introductions of NIS into brackish waters (e.g. Wolff 1999, Paavola *et al.* 2005). In the Baltic Sea, which is a semi-enclosed non-tidal brackish water sea (25–0 psu), approximately 70 NIS have established, of which 20 are considered harmful. The most important vector of these species has been ballast water (Leppäkoski 2002). The Baltic Sea has few areas exceeding 200 m in depth that are more than 50 nautical miles from the nearest shore. Therefore, the exchange of ballast water according to Regulation B-4 of the International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO 2004), is not possible. Eventually ballast water will have to be treated according to the Ballast Water Performance standard (IMO 2004), but until then, ballast water exchange zones might have to be designated, taking into account the risk assessments required in the Guidelines on the Designation of Areas for Ballast Water Exchange (G14) (IMO 2006).

The Finnish coast of the Baltic Sea (which encompasses the Gulf of Finland, the Archipelago Sea and the Gulf of Bothnia) constitutes a challenging area for aquatic bioinvasion studies. The coast is characterized by salinity gradients decreasing eastwards and northwards, and there are also large-scale gradients in insolation, air and water temperature, ice cover, fresh-

water inflow and nutrient concentrations (e.g. Leppäkoski and Bonsdorff 1989, Alenius *et al.* 1998). The majority of Finnish coastal waters are clearly eutrophic as a result of land-based runoff, their basic geomorphology, a low water exchange rate and poor mixing with open waters (Kauppila and Bäck 2001). Although wastewater treatment has improved over the past few decades, the effects of eutrophication are evident, for example, in vast late-summer algal blooms and in the zoobenthos. Especially in the Gulf of Finland and in the Archipelago Sea, the naturally low zoobenthic diversity has further decreased and species assemblages are now often indicative of poor oxygen conditions and pollution (Laine and Kangas 2004). Many of these factors interact in a manner that opens up colonization opportunities for an array of potential NIS (Occhipinti-Ambrogi and Savini 2003). In addition, both ship traffic and the amount of ballast water transported are steadily increasing in the northern Baltic Sea (Rytkönen *et al.* 2002), especially due to the opening of several new oil terminals in the eastern Gulf of Finland. So far, around 20 NIS have established along the Finnish coast, mostly during the last 100 years, with ship traffic (ballast water, hull fouling) as their main transport vector (<http://www.corpi.ku.lt/nemo/>). Species such as *Balanus improvisus*, *Cercopagis pengoi*, *Dreissena polymorpha* and *Marenzelleria cf. viridis* have caused economic harm or ecological changes, e.g. by introducing novel functions (Olenin and Leppäkoski 1999).

In this study, we describe the invasion status and 'biological profile' of four Finnish coastal harbours based on benthic macrofaunal samples and water quality data. We carried out a literature review on the species encountered, to examine which have life stages that make them successful ballast tank travellers. We also compared the harbour sediment and littoral faunas with those of surrounding more 'natural', non-constructed areas to see whether differences in species abundance and diversity exist. In addition, the main ship traffic patterns of the ports are described. These harbour profiles provide the basis for more extensive risk assessments, where species, pathway and vector analyses are included. They will further offer data on the northern Baltic Sea as a potential donor area of NIS. The aim is also



**Fig. 1.** The Baltic Sea and its major sub-basins. Study areas: 1 = Naantali, 2 = Koverhar, 3 = Sköldvik, 4 = Hamina.

to clarify practical methods for the initial risk assessment of NIS and to provide authorities and environmental agencies a framework for performing these assessments in environmentally similar areas. Though important in harbour surveys, plankton sampling was excluded from this study due to limitations of time and expertise. However, ballast harbour sediment sampling for dinoflagellates was conducted at the same time and is reported in Pertola *et al.* (2006).

## Material and methods

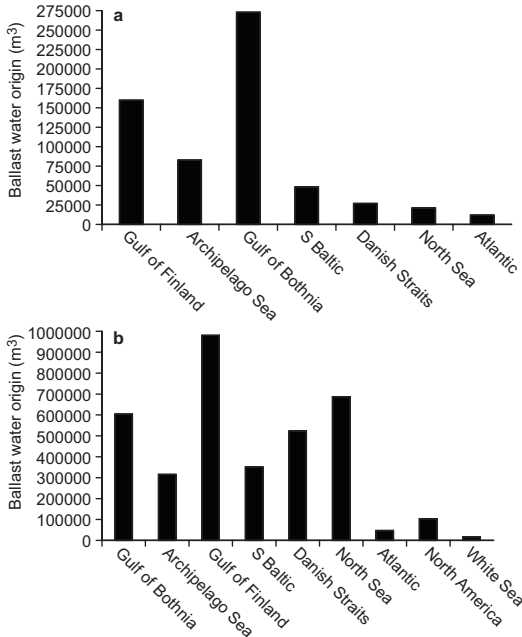
### Study areas

Four Finnish coastal harbours were investigated in August 2003. The harbours were the oil terminals in Naantali and Porvoo, Koverhar steel harbour in Hanko and the Port of Hamina (Fig. 1). These harbours were chosen to sample a gradient along the coast of southern Finland. All four har-

bours have regular international ship traffic, carrying large amounts of ballast water, and trade mainly with ports along the coasts of the Baltic Sea and the North Sea (FMA 2007).

Naantali oil terminal (60°27.21'N, 22°04.10'E) is located on the coast of the innermost Archipelago Sea, 80 km north of the entrance to the Gulf of Finland (GoF). About 350 ships (four Mt cargo), mainly tankers, arrive at the oil terminal in Naantali per year (Fortum Shipping Oy pers. comm.) (Fig. 2a). These ships mainly call at other ports in the northern Baltic Sea. In the vicinity of this site are the ports of Naantali, Turku and Pansio. In a previous study by Gollasch and Leppäkoski (1999), the Port of Naantali was estimated to have an intermediate likelihood of receiving NIS in the near future.

Koverhar steel harbour (59°52.72'N, 23°13.68'E) is located on the eastern part of the Hanko Peninsula at the entrance of the GoF and is the most marine- and wave-exposed of the four harbours in this study. Koverhar receives



**Fig. 2.** Ship traffic statistics from Naantali (Fortum) and Sköldvik. Source port of ships calling the ports with no or little cargo on board, i.e. potential origin of ballast water transported to (a) Naantali and (b) Sköldvik (Fortum Shipping Oy pers. comm.).

around 200 ships per year (1.4 Mt cargo), most of which arrive from and depart to areas within the Baltic Sea or the North Sea (Koverhar Shipping Oy pers. comm.). These are mainly cargo ships, and more ballast water is transported out of the Koverhar area than is released into the harbour. Koverhar is the smallest of the four ports in this study.

Sköldvik oil terminal (60°18.39'N, 25°33.08'E), with ca. 1100 arrivals per year, is on the southwestern shore of the Porvoo inlet, 20 km west of Helsinki. Sköldvik is the largest port in Finland according to the tonnage handled per year (in total 16.1 Mt cargo) (FMA 2007). The ports of origin of ships calling at Sköldvik are mainly located in the Baltic Sea and the North Sea (Fig. 2b), although there are also some connections with the Atlantic coast of North America and the North American Great Lakes (Fortum Shipping Oy pers. comm.).

The Port of Hamina (60°31.51'N, 27°10.14'E) in the eastern GoF receives around 1300 ships

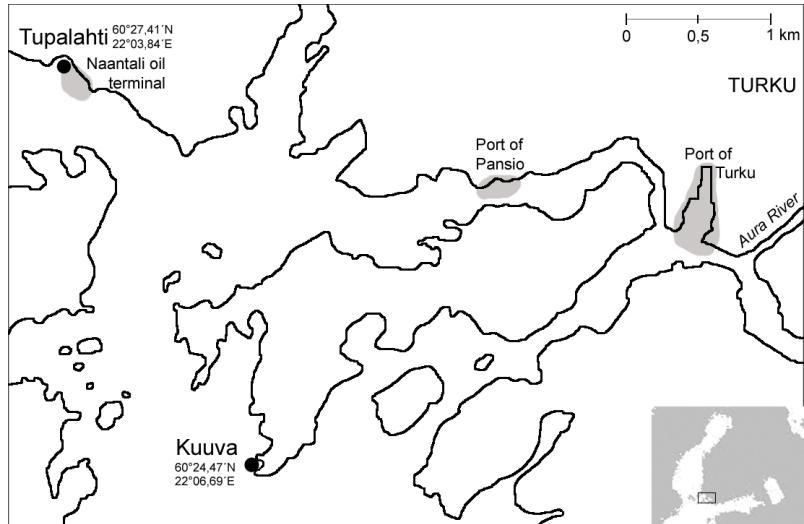
each year and is the fifth largest common port in Finland (4.7 Mt cargo per year) (FMA 2007). Ship traffic from the Baltic Sea, North Sea and eastern Atlantic ports is frequent (once to three times weekly) (Port of Hamina pers. comm.). The Port of Kotka, third largest in Finland (9.3 Mt per year) (FMA 2007), is located 15 km west of Hamina, at the mouth of the Kymijoki.

## Sampling methods

Three different types of samples for faunal studies were taken: littoral net samples, scrape samples, and benthic soft-sediment samples. Temperature and salinity were measured at each sampling location with a YSI 63 sound. Monitoring data from stations within a 2 km radius of the study harbours were used as a complement to our material (Finnish environmental administration database HERTTA for water quality; local monitoring agencies for zoobenthos).

Littoral net samples were collected with a custom-made fine-meshed (< 0.5 mm) hand net from five natural locations in the vicinity of each harbour (0.2–1 km from the harbour basin). At each location, three samples were taken, summing to a total of 15 littoral samples from each harbour. For each individual sample, the hand net was pulled along the sea bottom at approximately 0.5 m water depth for a distance of about 1.5 m. Each sample was collected independently of the previous ones. In Naantali, littoral sampling was conducted throughout the ice-free part of the year at one of the five stations (Tupalahti) to monitor seasonal variation in the littoral biota. For comparison, a natural shore located 3 km from the Naantali harbour area (Kuuva) was also sampled simultaneously (Fig. 3).

Scrape samples were also collected with a fine-meshed hand net, but this time the net was equipped with a sharper, partly curved edge to loosen sessile organisms from their substrate. Four different types of substrates were sampled, i.e. harbour basin edges of stone or concrete, wooden poles, metal constructions and plastic spar buoys. Two to eight samples were taken from each substrate, depending on its availability and work safety. The total number of scrape



**Fig. 3.** Location of the all-season littoral sampling stations in Naantali.

samples from each harbour was between seven and twelve. Each sample was obtained by scraping the substrate over an area of approximately 0.5 m<sup>2</sup> three times, from 1-m depth up towards the surface.

Benthic sediment samples were taken with an Ekman grab (area 256 cm<sup>2</sup>). In each harbour, three independent samples (from three different points) were taken close to the piers (50–100 m). Additional data on the macrozoobenthos within a 2-km radius of the study harbours was gathered from published monitoring reports in order to compare the species composition of harbour basin communities with that of nearby more natural areas (unaltered substrates) (Table 1).

All samples were sieved through a 0.5-mm mesh and fixed in the field with 4% buffered formaldehyde. In the laboratory, the animals were identified to the lowest possible taxonomic level and counted. Abundance and biomass (wet weight) were registered for sediment macrofauna.

### Statistical analysis

The littoral and scrape samples were analysed with non-parametric multivariate statistical tests due to the qualitative character of the samples and problems with the multi-way normality of large species lists (Clarke 1993). Differences in community structure between harbours were tested with the two-way nested analysis of similarity (ANOSIM), separately for the littoral samples (shores nested in harbours) and scrape samples (substrates nested in harbours). Data from both types of samples were analysed untransformed, but were standardised prior to analysis to account for differences in sampling effort. After hypothesis testing, non-metric multidimensional scaling (NMDS) was used to produce a graphical overview of between and within group Bray-Curtis similarities. Finally, the similarity percentage breakdown procedure (SIMPER) was used to identify the species that contributed most

**Table 1.** Monitoring stations in the vicinity (within 2 km radius) of the four harbours for comparison with more 'natural' areas.

| Harbour  | Depth interval | Bottom type           | Reference                  |
|----------|----------------|-----------------------|----------------------------|
| Naantali | 7–35 m         | clay, mud             | Turkki 2001                |
| Koverhar | 13–35 m        | silt, sand, clay, mud | Mettinen 2004              |
| Sköldvik | 18–35 m        | clay, mud             | Erkkilä <i>et al.</i> 2002 |
| Hamina   | 9.5–15 m       | mud, clay, gravel     | Anttila-Huhtinen 2005      |

to the observed dissimilarities between harbours (Clarke 1993).

The sediment samples were analysed with both univariate and multivariate tests. Differences in animal total abundance, total biomass, species richness, and Shannon-Wiener diversity amongst the four regions (harbour areas) as well as between treatments, i.e. 'harbour impacted' versus 'natural' sites, were examined using the univariate two-way factorial ANOVA. The factors were: T = treatment (two levels, i.e. harbour basin and adjacent control area, fixed and orthogonal) and R = Region (four levels, the harbours, fixed and orthogonal). The model describing the data was:  $X_{ijk} = \mu + T_i + R_j + TR_{ij} + e_{k(ij)}$ . The factor Region was considered fixed, since along the southern Finnish coast, these harbours were among the few that receive ships with large amounts of ballast water and for which simultaneous monitoring data from surrounding areas were available (the four harbours could not be chosen randomly from a high number of possible alternatives). For significant overall differences, the Student-Newman-Keul (SNK) corrected *a posteriori* test was run to determine between which Regions and Treatments the differences were actually present. Non-parametric multivariate tests were also applied to both the species abundance and species biomass data. To obtain a balance between dominant and rare species, all sediment data were square-root transformed. Differences in sediment faunal community structure (abundance and biomass data) among the regions and treatments (harbour and control areas) were also tested for with the two-way crossed ANOSIM. NMDS was further used to visualize

similarities in species composition among samples and SIMPER was used to list the species contributing most to observed dissimilarities.

The univariate statistical analyses were run on GMAV5, whereas all multivariate analyses were run on PRIMER 5.2.2. Before running parametric tests, data normality was checked with the Kolmogorov-Smirnov test and homogeneity of variances with Cochran's C-test. After finalising the analyses, the table-wise sequential Bonferroni test (Hochberg 1988) was used to adjust the overall significance levels to the number of tests performed.

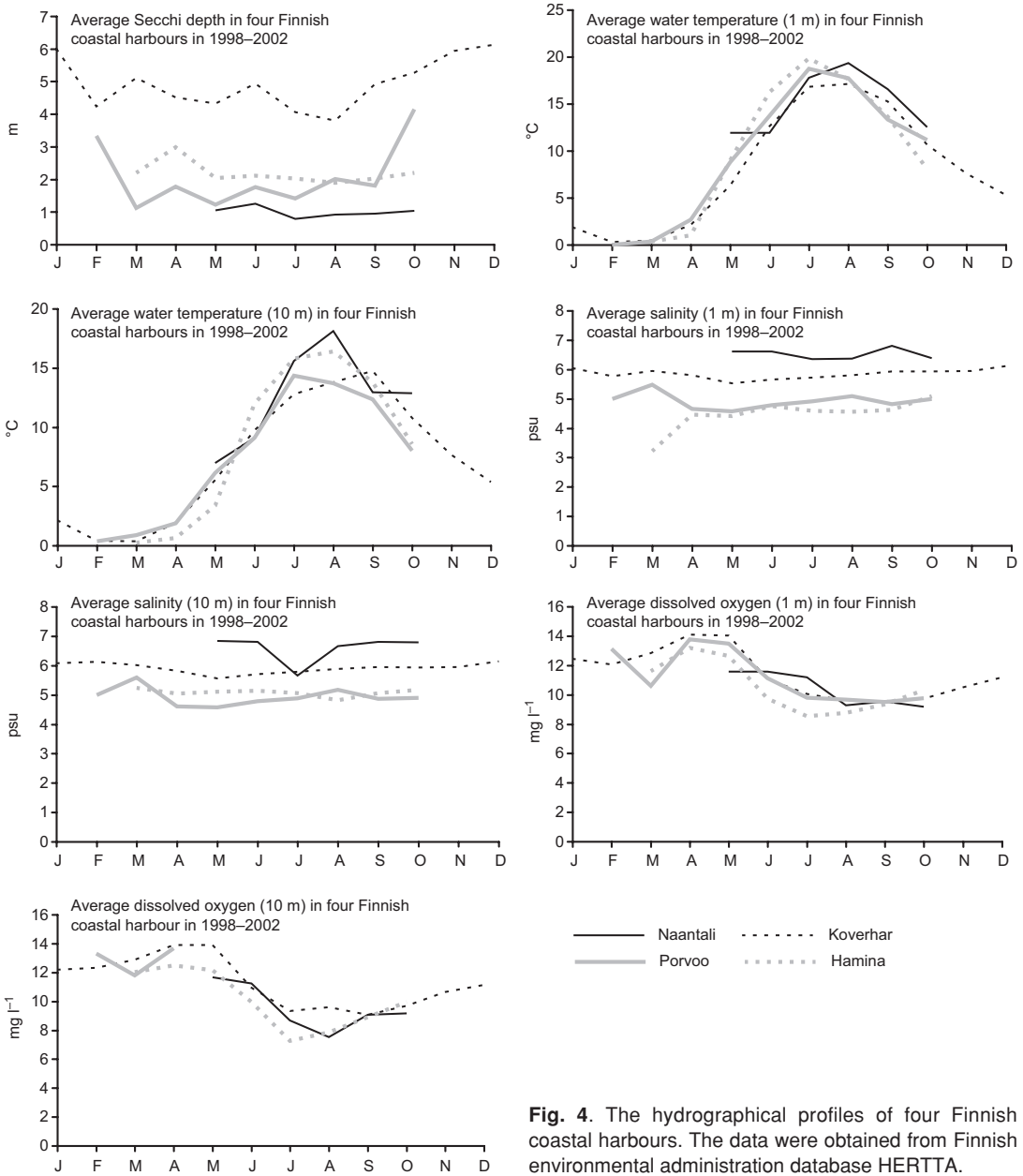
## Results

Water data from the harbours and surrounding areas show a salinity gradient that is typical for the GoF, being stable 6–7 psu at the entrance to the Gulf and 3–4 psu in the easternmost Finnish waters. Temperature varies with seasons, reaching maximum values in July and August. Secchi depth and dissolved oxygen content also vary with seasons, being lowest in late summer, while pH is stable at around eight in the whole sea area. Typical annual hydrographical profiles of each harbour are given in Fig. 4.

The predominant bottom substrate in the harbour basins comprised either soft mud or coarser mixed sediments (sand or clay) covered by mud. A total of eight NIS were recorded in our samples. All of these are well established in most areas of the Baltic Sea (<http://www.corpi.ku.lt/nemo/>) (Table 2), although *Gammarus tigrinus* is new to Finnish waters (Pienimäki et al. 2004)

**Table 2.** Nonindigenous species found in the four Finnish coastal harbours in 2003. Harbour: N = Naantali, K = Koverhar, S = Sköldvik, H = Hamina. Sample: B = Benthos, L = Littoral, S = Scratch. Species data from <http://www.corpi.ku.lt/nemo/> and references therein.\* zooplankton not sampled, specimen noticed in macrofaunal samples.

| Taxon      | NIS                             | Harbour    | Sample  | Origin        | 1st finding in the Baltic |
|------------|---------------------------------|------------|---------|---------------|---------------------------|
| Hydrozoa   | <i>Cordylophora caspia</i>      | N, S, H    | L, S    | Ponto-Caspian | 1803                      |
| Polychaeta | <i>Boccardia redeki</i>         | N, K       | B, L    | North Sea     | 1960                      |
| Polychaeta | <i>Marenzelleria viridis</i>    | N, K, S, H | B       | North America | 1985                      |
| Mollusca   | <i>Mya arenaria</i>             | N, K       | B       | North America | 1245                      |
| Mollusca   | <i>Potamopyrgus antipodarum</i> | N, K, S    | B, L    | New Zealand   | 1887                      |
| Cirripedia | <i>Balanus improvisus</i>       | N, K, S, H | B, L, S | North America | 1844                      |
| Cladocera  | <i>Cercopagis pengoi</i>        | N, K, S, H | L, S*   | Ponto-Caspian | 1992                      |
| Amphipoda  | <i>Gammarus tigrinus</i>        | N, H       | L       | North America | 1975                      |



**Fig. 4.** The hydrographical profiles of four Finnish coastal harbours. The data were obtained from Finnish environmental administration database HERTTA.

and was actually discovered for the first time in connection with this study. All of these NIS contribute some new ecosystem function not supported by the previous community members (Olenin and Leppäkoski 1999), but their broader impacts on native species and ecosystem functioning are still unknown.

The four harbours hosted invertebrate com-

munities broadly similar to those found in adjacent reference areas. In all, 46 species (including two taxa not determined to species level) were recorded during the single-occasion sampling in August (Table 3). Of these species, 40% have pelagic larvae (mainly polychaetes and bivalves) and 54% have a mobile adult stages (mainly polychaetes and crustaceans) with swimming

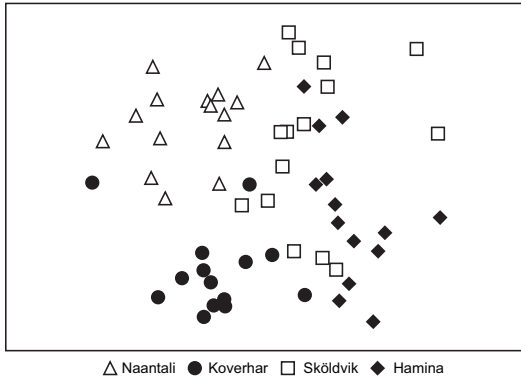
behaviour. Sessile adult stages were recorded for 26% (mainly molluscs) of the species, and two species had some kind of a resting, extra tolerant life stage (both parasites). The number of species in all sample types decreased with salinity, being

lowest in Hamina. Long-term littoral sampling in Tupalahti revealed eleven additional species, and the monitoring reports on sediment macrofauna revealed four more than those sampled in August in the four harbours.

**Table 3.** List of invertebrate species that occurred in littoral, scratch and sediment samples in the four harbours (only from the one-occasion sampling in August). N = Naantali, K = Koverhar, S = Sköldvik, H = Hamina, PL = Planktonic larval stage, MA = Mobile adult stage (swimming behaviour), SA = Sessile (on hard substrate) adult stage, RS = Resting stage. Planktonic species, meiofauna and algae that occurred in samples are not included. \*One of two hydrobia (*H. ulvae*) species in Finland has a planktonic larval stage.

| Taxon          | Species                         | Littoral |    |    |    | Scratch |    |   |   | Sediment |    |   |   | PL | MA | SA | RS                  |   |
|----------------|---------------------------------|----------|----|----|----|---------|----|---|---|----------|----|---|---|----|----|----|---------------------|---|
|                |                                 | N        | K  | S  | H  | N       | K  | S | H | N        | K  | S | H |    |    |    |                     |   |
| HYDROZOA       | <i>Cordylophora caspia</i>      |          |    | x  | x  | x       |    | x | x |          |    |   |   | x  |    |    | x                   |   |
| HIRUDINEA      | <i>Piscicola geometra</i>       | x        |    | x  | x  |         |    |   |   |          |    |   |   |    | x  |    |                     | x |
| OLIGOCHAETA    | <i>Chaetogaster</i> sp.         | x        | x  | x  | x  |         |    | x |   |          |    |   |   |    |    |    | x                   |   |
|                | <i>Stylaria lacustris</i>       | x        | x  | x  | x  | x       | x  |   |   |          |    |   |   |    |    |    | x                   |   |
|                | <i>Slavina appendiculata</i>    |          |    |    | x  | x       |    |   | x | x        |    |   |   |    |    |    |                     |   |
|                | Indetermined                    |          |    |    |    |         |    |   | x | x        | x  |   | x |    |    |    | various life cycles |   |
| POLYCHAETA     | <i>Boccardia redeki</i>         | x        |    |    |    |         |    |   |   | x        | x  |   |   | x  |    | x  |                     |   |
|                | <i>Bylgides sarsi</i>           |          |    |    |    |         |    |   |   | x        | x  | x |   |    |    | x  | x                   |   |
|                | <i>Hediste diversicolor</i>     | x        | x  |    |    |         |    |   |   | x        | x  |   |   |    | x  |    | x                   |   |
|                | <i>Marenzelleria viridis</i>    |          |    |    |    |         |    |   |   | x        | x  | x | x |    | x  |    | x                   |   |
|                | <i>Pygospio elegans</i>         |          |    |    |    |         |    |   |   |          | x  |   |   |    | x  |    |                     |   |
|                | <i>Manauynkia aestuarina</i>    |          |    |    |    |         |    |   |   | x        | x  |   |   |    |    |    |                     |   |
| TRICLADIDA     | <i>Polycelis tenuis</i>         |          |    |    | x  |         |    |   |   |          |    |   |   |    |    | x  |                     |   |
| PULMONATA      | <i>Lymnaea peregra</i>          | x        |    |    |    |         |    |   |   |          |    |   |   |    |    |    | x                   |   |
| PROSOBRANCHIA  | <i>Viviparus</i> sp.            |          |    |    | x  |         |    |   |   |          |    |   |   |    |    |    |                     | x |
|                | <i>Hydrobia</i> spp.            |          |    | x  |    |         | x  | x |   |          | x  | x |   |    | x* |    |                     | x |
|                | <i>Bithynia tentaculata</i>     | x        | x  | x  | x  |         |    |   |   |          |    |   |   |    |    |    |                     | x |
|                | <i>Potamopyrgus antipodarum</i> | x        | x  |    |    |         |    |   |   |          | x  | x | x |    |    |    |                     | x |
|                | <i>Theodoxus fluviatilis</i>    | x        | x  | x  | x  |         |    |   | x |          |    |   |   |    |    |    |                     | x |
|                | <i>Valvata</i> sp.              |          |    |    |    |         |    |   |   |          |    |   |   |    |    |    |                     | x |
| OPISTHOBANCHIA | <i>Limapontia capitata</i>      | x        |    | x  | x  | x       | x  |   |   |          |    |   |   | x  |    |    | x                   |   |
| BIVALVIA       | <i>Cerastoderma</i> sp.         | x        | x  | x  |    | x       | x  | x |   |          | x  |   |   |    | x  |    |                     |   |
|                | <i>Macoma balthica</i>          | x        | x  | x  |    | x       |    |   |   | x        | x  | x | x |    | x  |    |                     |   |
|                | <i>Mytilus edulis</i>           | x        |    |    |    | x       | x  |   |   |          |    |   |   |    | x  |    |                     | x |
|                | <i>Mya arenaria</i>             |          |    |    |    |         |    |   |   | x        | x  |   |   |    | x  |    |                     |   |
| NEMERTINEA     | <i>Cyanophthalma obscura</i>    | x        | x  | x  | x  |         |    |   |   |          | x  | x | x | x  |    |    |                     |   |
| BRANCHIURA     | <i>Argulus</i> sp.              | x        | x  |    |    |         |    |   |   |          |    |   |   |    | x  | x  |                     | x |
| CIRRIPEDIA     | <i>Balanus improvisus</i>       | x        | x  | x  | x  | x       | x  | x | x |          |    |   |   |    | x  |    |                     | x |
| AMPHIPODA      | <i>Calliopius laeviusculus</i>  | x        |    |    |    |         |    |   |   |          |    |   |   |    |    |    |                     | x |
| AMPHIPODA      | <i>Gammarus zaddachi</i>        |          |    | x  | x  |         |    |   | x |          |    |   |   |    |    |    |                     | x |
|                | <i>Gammarus duebeni</i>         |          |    |    |    | x       |    |   |   |          |    |   |   |    |    |    |                     | x |
|                | <i>Gammarus salinus</i>         | x        | x  |    |    | x       |    |   |   |          |    |   |   |    |    |    |                     | x |
|                | <i>Gammarus locusta</i>         |          |    |    | x  |         |    |   |   |          |    |   |   |    |    |    |                     | x |
|                | <i>Gammarus oceanicus</i>       | x        | x  |    |    |         |    |   |   |          |    |   |   |    |    |    |                     | x |
|                | <i>Gammarus tigrinus</i>        |          |    |    |    | x       |    |   |   |          |    |   |   |    |    |    |                     | x |
|                | <i>Corophium volutator</i>      |          |    |    | x  |         |    | x |   |          | x  |   | x | x  |    |    |                     | x |
|                | <i>Monoporeia affinis</i>       |          |    |    |    |         |    |   |   | x        | x  | x |   |    |    |    |                     | x |
|                | <i>Jaera</i> sp.                | x        | x  | x  | x  | x       | x  | x | x |          |    |   |   |    |    |    |                     |   |
| MALACOSTRACA   | <i>Idotea chelipes</i>          | x        | x  | x  |    |         |    |   |   |          |    |   |   |    |    |    |                     | x |
|                | <i>Saduria entomon</i>          |          |    |    |    |         |    |   |   |          | x  |   |   |    |    |    |                     | x |
|                | <i>Neomysis integer</i>         | x        | x  | x  | x  |         | x  |   |   | x        | x  |   |   |    |    |    |                     | x |
|                | <i>Praunus flexuosus</i>        | x        |    |    |    |         |    |   |   |          |    |   |   |    |    |    |                     | x |
|                | <i>Praunus neglectus</i>        |          |    |    |    |         |    | x |   |          |    |   |   |    |    |    |                     | x |
| DECAPODA       | <i>Palaemon adspersus</i>       |          |    |    | x  | x       |    |   |   |          |    |   |   |    | x  |    |                     | x |
| INSECTA        | Chironomidae                    | x        | x  | x  | x  | x       | x  | x | x | x        | x  | x | x |    |    |    | various life cycles |   |
| ECTOPOCTA      | <i>Electra crustulenta</i>      | x        | x  | x  |    | x       | x  |   |   | x        | x  | x |   |    |    |    |                     | x |
| Total          | 45                              | 24       | 20 | 20 | 19 | 12      | 12 | 8 | 7 | 17       | 15 | 9 | 6 | 17 | 25 | 11 | 2                   |   |



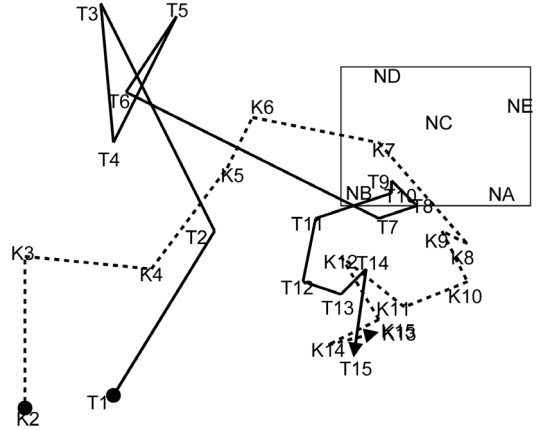


**Fig. 5.** NMDS ordination of untransformed but standardised littoral macrofauna abundance data from the four harbours. Stress 0.23.

### Littoral macrofauna

Littoral macrofaunal composition differed significantly between the harbour shores ( $p < 0.001$ ) and the four coastal areas outside the harbours (overall  $p < 0.001$ , pair-wise  $p$  values amongst harbours always  $< 0.05$ ) in the two-way nested ANOSIM. Regional differences in species composition are illustrated in Fig. 5. The SIMPER analysis showed that the highly abundant *Macoma balthica* in littoral samples from Naantali and *Cerastoderma* sp. at Koverhar were the most important discriminators between the harbours. Other important discriminating littoral taxa were *Stylaria lacustris* (very abundant in Naantali) and *Gammarus* spp. (very abundant in Hamina). Average SIMPER dissimilarities showed that Naantali–Koverhar and Naantali–Hamina were the pairs most different from each other, whereas Sköldvik–Hamina was the most similar harbour pair.

The community data from the one-occasion samples collected in Naantali in August matched those from the all-season sampling scheme (Tupalahti and Kuuva), i.e. the one-occasion sampling data clustered with August samples T7–T8 and K7–K8 in the ordination diagram (Fig. 6). Sampling in August–September also yielded the highest species richness, and probably also the most representative data for each area, since most species were more common during late summer and autumn (exceptions were *Neomysis integer*, *Manayunkia aestua-*

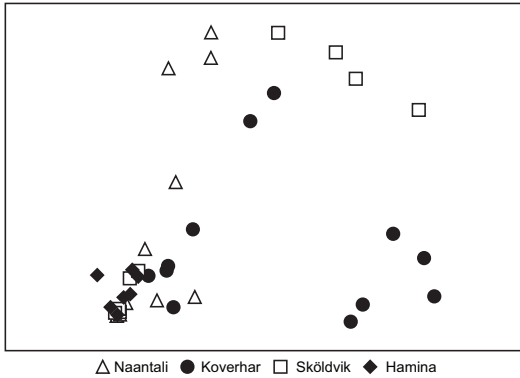


**Fig. 6.** NMDS describing changes in littoral fauna over time (arrow tail = May, arrowhead = December 2003) at Tupalahti (T1–T15, bold arrow) and at Kuuva (K2–K15, dashed arrow). The rectangle indicates the distribution of the five Naantali samples (NA–NE) from August 2003. Stress 0.18.

*rina*, *Mya arenaria*, which were more commonly found in spring and early summer). With regard to species richness, the Naantali/Tupalahti littoral all-season samples, with  $17.53 \pm 1.32$  (mean  $\pm$  SE) species, did not differ significantly from those from Kuuva, where on average  $18.43 \pm 1.18$  species were found (one-way ANOVA:  $F_{1,29} = 0.253$ ,  $p = 0.619$ ). Significant differences between Naantali/Tupalahti and Kuuva in animal community structure over time, however, were present (one-way ANOSIM:  $R = 0.12$  and  $p = 0.020$ ). The species contributing most to these differences, based on the SIMPER analysis, were *Neomysis integer*, *Cerastoderma* sp., *Limapontia capitata*, *Boccardia redeki*, *Stylaria lacustris*, *Cyanophthalma obscura*, *Gammarus zaddachi*, *Jaera* sp., *Electra crustulenta*, *Macoma balthica* and *Mytilus edulis* (all more common at Kuuva) as well as *Balanus improvisus*, Copepoda and *Gammarus salinus*, which were more common at Tupalahti/Naantali.

### Scrape macrofauna

For scrape samples, only overall differences in the animal community structures amongst harbours were significant in the two-way nested ANOSIM. There were no significant differences



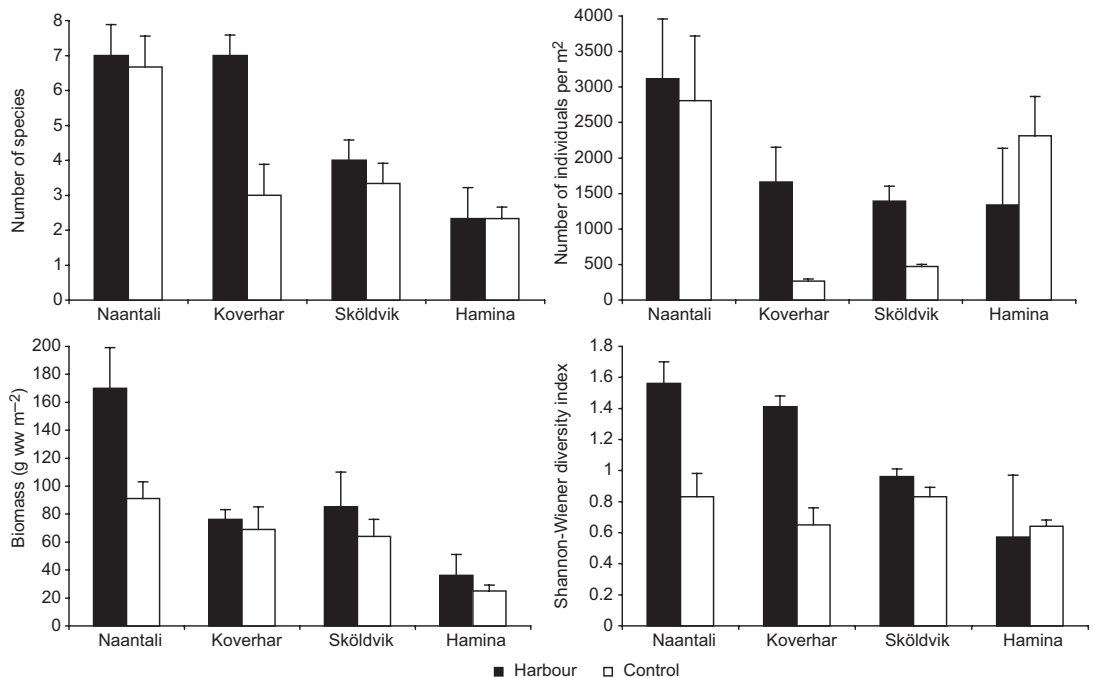
**Fig. 7.** NMDS of untransformed but standardised scrape macrofauna abundance data from the four harbours. Stress 0.09.

amongst the various substrata, nor any pair-wise differences amongst harbours (after Bonferroni correction). This could also be anticipated from the NMDS, where samples from all four harbours, including all the Hamina samples, are represented in the largest cluster to the left in Fig. 7. With regard to the SIMPER analysis, the average dissimilarities amongst harbours in scrape samples were lower throughout than the cor-

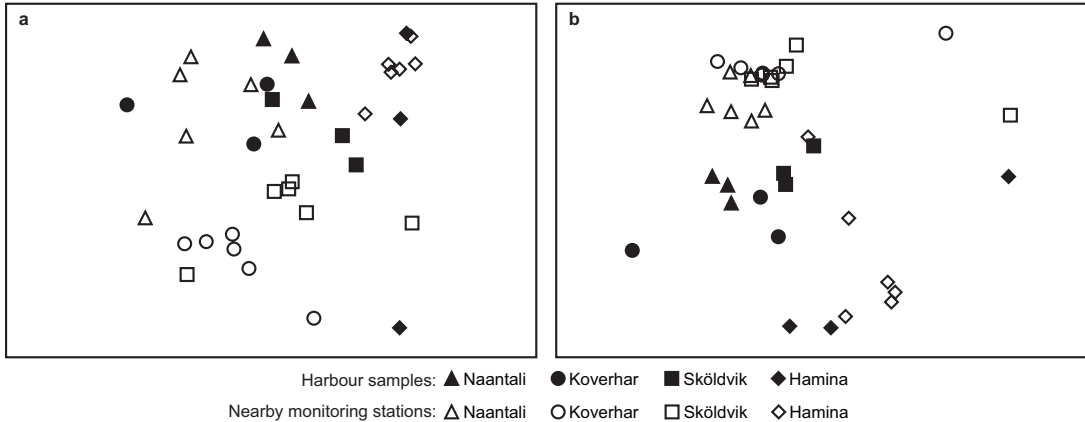
responding values for littoral samples. *Balanus improvisus* was by far the most abundant species at all sites and was the most important discriminator, followed by *Gammarus* spp. (especially abundant in Naantali) and *Cerastoderma* sp. (especially abundant in Koverhar).

### Sediment macrofauna

Differences in sediment fauna amongst harbour regions as well as between 'treatments' (harbour basins and adjacent control areas) in the species richness, total abundance, total biomass and Shannon-Wiener diversity ( $\pm$  SE) are shown in Fig. 8. For species richness, there was a significant interaction between region and treatment ( $p = 0.035$ ) (Table 4). The *a posteriori* SNK test revealed that this was because there were significantly more species in the Koverhar harbour area than in adjacent control areas. The SNK test also showed that there were significantly more species in Naantali and Koverhar than in Sköldvik or Hamina. For total abundance and total biomass, the only significant differences were between



**Fig. 8.** The sediment fauna of the four Finnish coastal harbours; differences in (a) species richness, (b) total abundance, (c) total biomass, and (d) Shannon-Wiener diversity ( $\pm$  SE) between harbour basins (black) and adjacent control areas (white).



**Fig. 9.** NMDS of square root transformed (a) macrofauna abundance (stress 0.16), and (b) biomass data (stress 0.15) from the four harbour regions.

harbour regions (higher total abundances in Naantali). The Shannon-Wiener diversity, on the other hand, was clearly higher in harbour basins than in control areas ( $p = 0.005$ ) and was also higher in Naantali and Koverhar than in the two other harbours (Table 3).

The multivariate two-way crossed ANOSIM, with which we examined the whole community structure at once, was more sensitive to differences in zoobenthic community structure than were the univariate analyses. Significant differences for both abundance and biomass data were evident. These differences between treatments (harbour basins and adjacent control areas) and between harbour regions are also

evident from the NMDS (Fig. 9). The SIMPER analysis showed that the same four taxa, in the same order, were responsible for about 70% of differences between harbour and control areas in both the abundance and biomass data. Oligochaetes, chironomids and *Marenzelleria cf. viridis* were more common in harbour samples, whereas *Macoma balthica* was more common in control samples (Table 5, only abundance data shown). With regard to regional differences, *M. balthica* (most common in Naantali followed by Koverhar and Sköldvik, rare in Hamina), *M. viridis* (most common in Naantali) and chironomids and oligochaetes (both most common in Hamina) were again the most important discriminators.

**Table 4.** Sediment macrofauna: two-way ANOVA on differences in community variables between harbour basins and adjacent control areas for the four harbours (df = 1,16 for treatment, 3,16 for region and 3,16 for interaction).  $P$  values that are still significant at the 0.05 level after correction for the number of tests with Hochberg's sequential Bonferroni are set in boldface.

| Source                   | MS               | $F$   | $p$            | Source                          | MS   | $F$   | $p$            |
|--------------------------|------------------|-------|----------------|---------------------------------|------|-------|----------------|
| <b>Total abundance</b>   |                  |       |                | <b>Total biomass</b>            |      |       |                |
| Treatment                | $1.0 \times 106$ | 0.98  | 0.338          | Treatment                       | 5133 | 5.60  | 0.031          |
| Region                   | $5.5 \times 106$ | 5.25  | <b>0.010</b>   | Region                          | 9995 | 10.89 | < <b>0.001</b> |
| T × R                    | $1.6 \times 106$ | 1.51  | 0.25           | T × R                           | 1702 | 4.52  | 0.178          |
| Residual                 | $1.0 \times 106$ |       |                | Residual                        | 917  |       |                |
| <b>Number of species</b> |                  |       |                | <b>Shannon-Wiener diversity</b> |      |       |                |
| Treatment                | 9.38             | 6.62  | <b>0.020</b>   | Treatment                       | 0.90 | 10.60 | <b>0.005</b>   |
| Region                   | 22.15            | 15.64 | < <b>0.001</b> | Region                          | 0.37 | 4.36  | <b>0.020</b>   |
| T × R                    | 5.15             | 3.64  | <b>0.036</b>   | T × R                           | 0.27 | 3.20  | 0.052          |
| Residual                 | 1.42             |       |                | Residual                        | 0.08 |       |                |

## Discussion

### Port profiles

With the exception of salinity, the hydrographical profiles of the four harbours were similar. Salinity seems to be the most important factor controlling species colonisation of brackish water seas, but other variables, such as temperature, community structure and functions, and stress levels, may also affect the invasion success of species (Cognetti and Maltagliati 2000, Occhipinti-Ambrogi and Savini 2003, Paavola et al. 2005). Judging from the hydrographical data from the harbour areas, however, NIS tolerating low salinities, low temperatures and eutrophic conditions are most likely to be established in coastal areas of Finland. Although soft sediments are the most common habitats along the Finnish coast, artificial constructions such as harbour piers and buoys may facilitate the establishment of species requiring hard substrates. Areas with similar hydrographical and habitat profiles elsewhere have the potential to exchange species transported in ballast water with Finnish coastal

harbours. The most likely donor and recipient areas are the coastal and coastally connected harbours of the Baltic Sea, North Sea and north-eastern Atlantic. Sköldvik has some connections with ports in the North American coastal areas and in the Great Lakes region. A deeper analysis of ship traffic patterns is needed for further assessment of the most important donor harbours of NIS to the Gulf of Finland and of where, for example, ballast water treatment methods should be applied to optimize the prevention of bioinvasions.

The littoral fauna of the harbours was diverse, and species composition varied significantly with location. Local differences in habitat complexity, salinity conditions, water exchange rate, pollution and degree of wave exposure may cause most of this variation. Salinity may be the most important reason for the decreasing number of species from west to east (Table 3). The physical profiles of the harbours were similar, however, and visually the littoral zones were reminiscent of each other. The all-season sampling scheme revealed that the highest species richnesses and abundances occurred in late summer, which

**Table 5.** Each species' contribution ( $\delta$ ) to the average dissimilarities between harbour sediment samples ( $n = 12$ ) and control samples ( $n = 24$ ) in the four harbour regions. Square-root transformed macrofauna abundance data was used. Total  $\delta\% = 64.66\%$ .

| Taxonomic group                  | Average abundance harbours | Average abundance control | Average $\delta_i$ | Dis./SD ratio | Percentage | Cumulative percentage |
|----------------------------------|----------------------------|---------------------------|--------------------|---------------|------------|-----------------------|
| <i>Macoma balthica</i>           | 517.6                      | 588.5                     | 13.7               | 1.3           | 21.2       | 21.2                  |
| Oligochaeta                      | 504.8                      | 329.6                     | 13.0               | 1.1           | 20.1       | 41.2                  |
| Chironomidae                     | 299.0                      | 200.1                     | 11.5               | 1.2           | 17.7       | 59.0                  |
| <i>Marenzelleria cf. viridis</i> | 257.2                      | 60.4                      | 8.4                | 1.2           | 13.0       | 72.0                  |
| <i>Potamopyrgus antipodarum</i>  | 45.0                       | 3.9                       | 2.6                | 0.6           | 4.1        | 76.0                  |
| <i>Monoporeia affinis</i>        | 35.4                       | 16.7                      | 2.5                | 0.6           | 3.9        | 79.9                  |
| <i>Hydrobia</i> spp.             | 83.6                       | 4.4                       | 2.2                | 0.4           | 3.3        | 83.3                  |
| <i>Cyanophthalma obscura</i>     | 16.1                       | 2.8                       | 1.7                | 0.5           | 2.6        | 85.8                  |
| <i>Corophium volutator</i>       | 38.6                       | 0                         | 1.6                | 0.5           | 2.5        | 88.3                  |
| <i>Boccardia redeki</i>          | 32.2                       | 0                         | 1.3                | 0.4           | 2.0        | 90.4                  |
| <i>Manayunkia aestuarina</i>     | 12.9                       | 0                         | 1.2                | 0.5           | 1.8        | 92.2                  |
| <i>Mya arenaria</i>              | 12.9                       | 0.3                       | 1.1                | 0.5           | 1.6        | 93.8                  |
| <i>Hediste diversicolor</i>      | 9.7                        | 0.5                       | 1.0                | 0.5           | 1.6        | 95.4                  |
| <i>Bylgides sarsi</i>            | 3.2                        | 13.5                      | 0.9                | 0.4           | 1.4        | 96.8                  |
| <i>Pygospio elegans</i>          | 6.4                        | 0                         | 0.8                | 0.4           | 1.2        | 97.9                  |
| <i>Neomysis integer</i>          | 0                          | 6.2                       | 0.7                | 0.4           | 1.0        | 98.9                  |
| <i>Halicryptus spinulosus</i>    | 0                          | 2.2                       | 0.5                | 0.3           | 0.8        | 99.7                  |
| <i>Cerastoderma</i> sp.          | 0                          | 0.5                       | 0.1                | 0.2           | 0.2        | 99.9                  |
| <i>Saduria entomon</i>           | 0                          | 0.5                       | 0.1                | 0.2           | 0.1        | 100.0                 |

means that one-occasion biological harbour surveys might be conducted during this period to maximize their efficiency. However, the seasonality of species' life-stages, and consequent fluctuations in the risk that organisms are taken up with ballast water, is of major importance in risk assessment of these harbours as donor areas. To cover a broader spectrum of the harbour biota, harbour surveys should therefore preferably be made over a longer period (at least over one ice-free season in northern waters) and sampling for planktonic species should be included. In addition, the diurnal activity patterns of the fauna should be considered. For example, *Gammarus* spp. and *Idotea* spp. are nekto-benthic, hiding on the bottom during the day and swimming in the pelagial during the night (Jansson and Källander 1968, Christie and Kraufvelin 2004). Other groups with similar habits include mysids, the brown shrimp *Crangon crangon*, the prawn *Palaemon adspersus*, the polychaetes *Hediste diversicolor* and *Marenzelleria* cf. *viridis*, and naidid oligochaetes (Jansson and Källander 1968, Panelius 1973, Huhta 1986, Bochert *et al.* 1996). Additional sampling during the night would therefore provide a broader view of the structure of these animal communities.

The species composition of sessile scrape biota was more or less the same across substrates and harbours. The scrape samples contained a sessile fauna typical of fouling communities and natural hard substrates in the northern Baltic Sea. Artificial constructions such as piers and buoys offer additional substrate for these species and seem to attract animals just as well as natural materials (e.g. Thompson *et al.* 2002, Glasby *et al.* 2007). The less diverse faunal composition of scrape samples relative to littoral samples (Table 3) may relate to differences in exposure and habitat complexity, whereby more diverse (three-dimensional) and sheltered substrates in the littoral zone, such as perennial and annual macroalgae, angiosperms and natural stones, are capable of supporting more diverse animal communities. Artificial substrates (scraped areas) are also often vertical planes with less sedimentation and less food for grazers and deposit feeders than natural ones.

Comparisons of the sediment macrofauna of harbour basins with that of adjacent control areas

offered an opportunity to assess the current biological profile of the four harbours. The macrozoobenthos of the harbour basins was apparently no more affected by pollution than were zoobenthic assemblages of the adjacent areas. The situation was rather the reverse, with a higher Shannon-Wiener species diversity in harbour basins than in surrounding control areas. The three most important discriminator taxa between harbour areas and surrounding control areas were oligochaetes, chironomids and *Marenzelleria* cf. *viridis*, all of which were more common in harbours. Both oligochaetes and chironomids are known as indicators of impoverished environmental conditions (Leppäkoski 1975) and *M. viridis* is also tolerant of environmental disturbance and moderate hypoxia (Kube and Powilleit 1997, Schiedek 1997). The main reasons for their success in harbours may either be their opportunistic life cycles or that they are favoured by the traffic itself, which may cause increased sediment turnover rates and enhanced nutritive conditions, due to improved nutrient recirculation (e.g. Lindholm *et al.* 2001).

Littoral, scrape and sediment samples are all needed to depict the macrofaunal composition of harbours and for the determination of the potential of organisms to be taken up with ballast water. All species may sometimes be found in the pelagial as tycho plankton, due to the resuspension of sediments (Hayes and Hewitt 1998), but many species also have a specific pelagic life-stage. Decisive factors for the occurrence of species in the pelagial are the time of day, the season and the current life-stage of the organism. The larvae of most species can be found in the pelagial during the summer. The duration of the pelagic stage varies between species. In general, larvae settle within a period of some weeks. Examples of common littoral or sessile species along the Finnish coast with a pelagic larval stage are *Crangon crangon*, *Balanus improvisus*, *Electra crustulenta*, *Marenzelleria* cf. *viridis* and *Boccardia redeki* (Eliason and Hahtela 1969, Panelius 1973, Bochert *et al.* 1996). Additional sampling of plankton, in particular, but also of nekton, algae and plants would provide a more representative harbour profile.

The basic methods for port survey studies according to Hewitt and Martin (2001) have

been applied in an increasing number of studies (e.g. Australian Museum Business Services 2002, Cohen *et al.* 2005), most carried out at the times of our study. The extent of sampling in these studies varied, mainly according to the financial and technical resources available. Sampling techniques varied, depending on the target species. Common biological survey methods applied included the use of divers, grab samplers for sediments, scrape and sledge samplers for sessile biota, and a variety of trap designs for mobile species. The methods used in our study are comparable to those in other studies.

### Invasion status

The soft-shelled clam *Mya arenaria* probably arrived in Scandinavian waters from North America with the Vikings in the 13th century (Petersen *et al.* 1992). It is now a common species of soft sea bottoms throughout the Baltic as far as the northern Archipelago Sea. Other 'historical' NIS are the barnacle *Balanus improvisus* and the mud snail *Potamopyrgus antipodarum*. The hydroid *Cordylophora caspia* was recorded in the Baltic in the early 1800s. More recent newcomers are the spionid polychaetes *Boccardia redeki* and *Marenzelleria cf. viridis*, and the fish-hook water flea *Cercopagis pengoi*. Of these, at least *M. viridis* has been shown to have some negative impact on the abundance of native macrofaunal species (Kotta *et al.* 2001, Kotta and Ólafsson 2003, Neideman *et al.* 2003). Of the eight NIS recorded in this study, negative socio-economic impacts in the Baltic Sea have been recorded for *C. caspia*, *C. pengoi* and *B. improvisus* (Leppäkoski 2002).

### Port profiles and risk assessments — future implications

The introduction and establishment potentials of NIS into new areas can be evaluated in risk assessment by environmental matching in combination with pathway, vector and species analyses (Hayes and Hewitt 1998, Gollasch and Leppäkoski 1999). Harbour profiles serve as baseline data in shedding light on the status of coastal

areas as recipients and donors of NIS. Once the characteristics of harbours and their present ecosystem are known, one can seek potential 'stowaway' species that are adapted to similar environmental conditions and have the possibility to be transported into the area. Species presence data from harbours can be used for this. Species information will also be important for granting exceptions to ballast water exchange or treatment according to the International Convention for the Control and Management of Ships' Ballast Water and Sediments (IMO 2004). Not only local assessors, but also assessors in hydrographically similar areas concerned about new species invasions may benefit from harbour profiles, since the environmental matching approach requires data from all the areas concerned.

Recommendations for future harbour surveys include:

- Long-term monitoring data from e.g. water protection agencies (both water data and biological monitoring) should be incorporated, especially if the environmental status of a harbour is good and comparable to surrounding areas.
- Different kinds of samples should be collected to obtain a representative profile of the harbour biota. In addition to sediment, littoral and scrape samples, plankton samples should also be included as a minimum requirement, since many potentially harmful NIS are planktonic (e.g. some toxic microalgal species). Life stage and seasonality analyses of the species concerned would clarify which ones are susceptible to uptake and transport with ballast water or sediment (e.g. those with planktonic life stages). Additional sampling of nekton, algae and plants would provide a more representative harbour profile for all groups of organisms.
- Littoral zones are normally not included in long-term monitoring agendas. Many littoral species with pelagic or free-swimming life stages, i.e. potential ballast water travellers, may therefore remain unnoticed. For instance, the exact date and place of arrival of *Gammarus tigrinus* is very difficult to predict due to the low sampling frequency of the Finnish littoral zone in time and space.

Sampling of littoral habitats must thus be encouraged.

- Extensive sampling may not be needed for accurate profiling, but seasonal variations in water quality and biota, and diurnal patterns of native species, should definitely be considered in designing a sampling scheme. These variations may reveal when the risks are the highest for successful NIS introductions and/or for native species to be taken up with ballast water. Some taxa or species' life stages might only be detected by very frequent sampling.
- Other aspects of the study design should also receive careful attention. The design of studies capable of investigating causality issues is especially important (Kraufvelin *et al.* 2001).
- The hydrographical and physical-chemical profiles of harbours can be used for mapping potential donor/recipient areas with similar habitats.
- Adequate taxonomic knowledge is needed when executing field studies. Gammaridean amphipods are, for example, often not determined to species level, and are only listed as *Gammarus* spp. An improved knowledge of native species' ecology and functions are also needed to better our understanding of the consequences and management needs of NIS.
- Suggested risk assessments in relevant IMO regulations and guidelines should, if possible, be taken into account when designing studies.

Regarding NIS, an additional concern for the future may be increased seawater temperatures caused by global warming. This development will allow southern species, tolerant of brackish waters, from both nearby and distant areas to penetrate further northwards either naturally or with the help of man-mediated vectors (e.g. Slynko *et al.* 2002). And, if water quality of coastal areas is improved, this will also mean more opportunities for the survival of newcomers now restricted by polluted and/or eutrophic conditions. These facts make the list of potential future invaders to northern seas even longer and the importance of risk assessment greater.

Further studies on both native and alien spe-

cies and their ecosystems and interactions along the coasts of the Baltic Sea will help to clarify its invasion status and the magnitude of NIS threat to the biological integrity of the sea. Then, not only crucial physiological requirements such as the temperature and salinity tolerance of species, but also other niche dimensions, such as their feeding habits and substrate availability, should be annexed with the harbour profile information, to provide a more refined risk assessment with less uncertainty. Well-performed risk assessments will allow risk managers to allocate resources more efficiently (for example, to certain sea areas, seasons, or species life stages) in order to obtain a realistic picture of potential NIS invasions and identify where prevention methods are most urgently needed.

*Acknowledgements:* The port authorities of the Port of Hamina, Fortum Shipping Oy and Koverhar Shipping Oy are warmly acknowledged for their co-operation. The Regional Water Protection agencies that provided the monitoring data were also a great help. Financial support for this study was obtained from the Academy of Finland (Baltic Sea Research Programme BIREME), the Ministry of Transport and Communications, Finland, and the Finnish Graduate School in Environmental Sciences and Technology (EnSTe). Professor Erkki Leppäkoski (Åbo Akademi University) gave valuable comments on the manuscript and PhD Mirkka Jones (University of Turku) greatly improved the language quality.

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