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Bacterial freshwater species successfully immigrate to the brackish water environment in the northern Baltic

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Abstract

We studied the distribution and seasonal dynamics of five species from the genus *Flavobacterium* and one species from the genus *Marinomonas* over the course of a year along a northern Baltic Sea river–marine transect. All of the species had been previously demonstrated as important consumers of riverine dissolved organic carbon. Quantitative DNA–DNA hybridization data showed that two of the *Flavobacterium* spp. and the *Marinomonas* sp. had highest abundance in the river water (maximum 20,000 cells ml^{-1}), with maximum relative abundance of 0.5–2.5% of the bacterial community. These species declined in abundance from the river to the estuary and the offshore site. Abundance and dynamics in the estuarine environment suggested successful immigration of freshwater bacteria, accompanied by growth in the brackish water environment. Two of the three abundant species showed high cell numbers also during late autumn to early spring in the estuary, indicating a selective advantage when riverine dissolved organic carbon was the main carbon source. The remaining three species showed more episodic abundance close to the detection limit of the method, providing weaker evidence of occurrence in the freshwater environment. Some bacterioplankton consuming riverine organic carbon in the brackish water environment in the northern Baltic are therefore freshwater species, with a selective advantage during winter.

Riverine carbon can contribute significant carbon and energy to coastal food webs and thereby force carbon flow toward the heterotrophic and microbial part of the food web (Findlay et al. 1991; Sandberg et al. 2004). Most of the riverine dissolved organic carbon (DOC) is of humic nature and known to be refractory (Pettersson et al. 1997). Improved knowledge of bacteria that consume riverine DOC is therefore directed toward understanding how bacteria mobilize carbon from humic matter, contribute to coastal carbon cycling, and influence food web structure and function.

We recently demonstrated that five *Flavobacterium* spp. and one *Marinomonas* sp. constitute important consumers of riverine dissolved organic matter (DOM) in the northern Baltic (Kisand et al. 2002; Kisand and Wikner 2003). However, studies of the spatial and temporal distribution of *Flavobacterium* or *Marinomonas* at the species level in a river–marine transect have not been previously reported to our knowledge.

A fundamental question for the biogeography of coastal bacterioplankton is the geographic origin of species. Taxa identified in the coastal zone could have immigrated from the limnic environment, be indigenous to the coastal zone, or have immigrated from the offshore environment. Whether a gradual replacement of important taxa occurs or whether there are drastic shifts from freshwater to marine water is not clear and might be dependent partly on specific hydrographic conditions.

Changes in microbial community composition have been reported in an upwelling region over several kilometers in Monterey Bay, California (Suzuki et al. 2001), the Skagerakk–Kattegat Front in the North Sea (Pinhassi et al. 2003), and the estuarine gradient in Eastern Australia (Hewson and Fuhrman 2004). This suggests that the relatively small-scale shifts in environmental factors between limnic, brackish, and

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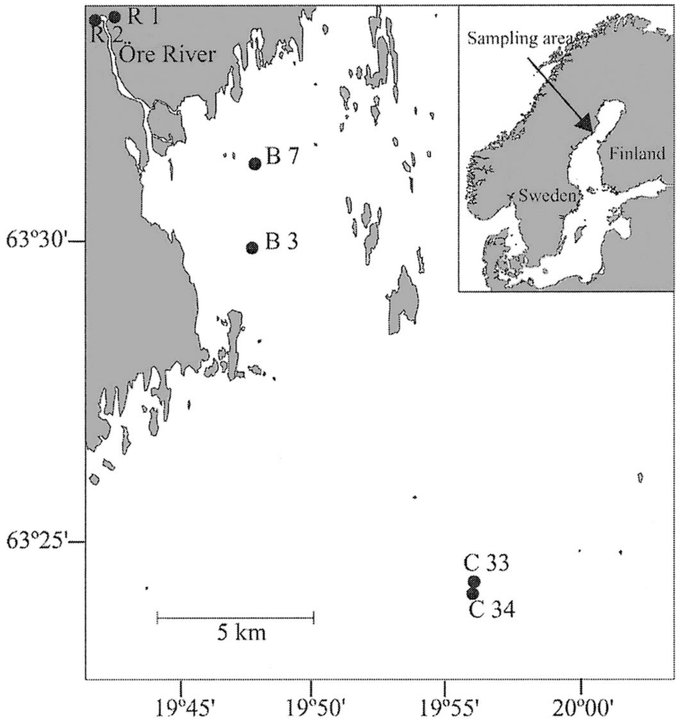

Fig. 1. Study area in the Öre River watershed in the northern Baltic Sea. The locations of the duplicate stations in the three different environments are indicated. Coordinates are given in the WGS84 system (dd°mm).

marine ecosystems is of marked importance to aquatic bacterial community structure.

Hagström et al. (2000) speculated that because of the low-salinity brackish water, Baltic Sea isolates often form separate lineages. This would explain the lack of 'marine' (salt-tolerant) genera in this brackish water and why members of the genera *Pseudomonas*, *Shewanella*, and *Sphingomonas* are common in the Baltic Sea. This is in contrast to oceanic environments, in which salt-tolerant species from the genera *Roseobacter*, *Vibrio*, and *Alteromonas* dominate. On the basis of this insight, it can be hypothesized that the *Flavobacterium* spp. and *Marinomonas* sp. that consume riverine DOC in the estuary are indigenous to the brackish water environment (Kisand et al. 2002).

The seasonal occurrence of bacteria-consuming riverine DOC provides information on environmental conditions promoting transformation of this carbon source. The Bothnian Sea coastal bacterioplankton growth rate is higher than in the offshore environment, especially during periods with low phytoplankton production (Wikner and Hagström 1999). Riverine DOC of refractory nature constitutes a potential carbon and energy source for this production. We therefore hypothesize that bacterial species consuming riverine DOC would primarily occur at the coast and during winter. Consequently, a significant part of riverine DOC might be respired during the cold season in the marine environment and explain the sustained bacterial growth also during low photosynthetic carbon fixation.

Quantitative estimates of organism abundance are required to study their biogeography. DNA–DNA hybridization has been successfully applied to identify and quantify culturable bacteria within complex environmental communities (Voordouw et al. 1993; Pinhassi et al. 1997). This method allows species-specific detection even at the low abundances typically found in natural environments, below the detection limit of direct microscopy (i.e., ~10,000 cells ml^{-1}). Error rates from cross-hybridization have been shown to be low, suggesting DNA–DNA hybridization is sufficiently accurate to record bacteria at the species level (Pinhassi et al. 1997; Telang et al. 1997; Kisand et al. 2002). DNA–DNA hybridization is easily applied and economical, thus allowing replication at various sampling levels, comparable to that applied in ecological studies of macroorganisms.

In this study, we investigated the distribution and temporal dynamics of six bacterial species constituting important consumers of riverine DOC in the same river–marine transect. We also addressed the question of whether the species originated in the freshwater environment or were indigenous to the brackish water environment. Finally, we elucidated whether consumption of riverine DOC has a selective advantage during low photosynthetic production for species, and whether their abundance differed between surface and deep waters in the marine environment.

Table 1. Specification of sampling sites in the northern Baltic Sea. Marine sampling sites are given as coordinates in the WGS84 system (dd°mm.mm).

Area	Station name	Latitude (N)	Longitude (E)	Maximum depth (m)
River	R1	—*	—*	4
River	R2	—†	—†	5
Estuary	B3	63°30.47	19°47.84	25
Estuary	B7	63°31.50	19°48.49	21
Offshore	C33	63°24.20	19°56.00	66
Offshore	C34	63°24.07	19°56.01	64

* Located in the middle of the river furrow, 100 m downstream from the Strömsör bridge.
† Located in the middle of the river furrow, 100 m downstream of R1.

Materials and methods

Study site—Three areas in the Öre River watershed were selected to represent a riverine, an estuarine, and an offshore environment (Fig. 1). The study area was located in the Swedish coastal temperate zone in the northern Baltic Sea.

The riverine site was located 2 km upstream of the river mouth just downstream of the Strömsör bridge (Table 1). The Öre River is not regulated, has a natural seasonal flow pattern, and lacks important sources of industrial and municipal wastewater. Dissolved total organic carbon concentrations have been measured up to 800 μmol L^{-1}, of which 80% is composed of humic substances leached from coniferous forest and mires in the watershed (Ivarsson and Jansson 1994; Pettersson et al. 1997).

The Öre River estuary has a residence time of 10 d, a mean depth of 16 m, and a water volume of 1 km^3. Salinity varies between 2 and 6, and the estuary is typically covered in ice during January–April (Forsgren and Jansson 1992). Sampling sites were the Swedish national monitoring sta-

Table 2. Bacterial species investigated in this study. The local strain code and known taxon are shown. Strains were originally isolated from enrichment cultures with riverine DOC inoculated with a pooled sample (4 and 20 m depth) from the Öre estuary in 1998 (Kisand et al. 2002). Their importance as consumers of riverine DOC relative the total community was quantified by DNA—DNA hybridization at three different seasons.

Strain code	Taxon	Isolation date	GenBank Accession No.
GOBB3-C101	*Flavobacterium* sp.	26 Mar 98	AF321008
GOBB3-C103-3	*Flavobacterium* sp.	26 Mar 98	AF321019
GOBB3-209	*Flavobacterium* sp.	25 May 98	AF321038
GOBB3-309	*Flavobacterium* sp.	18 Nov 98	AF321037
GOBB3-317-2	*Flavobacterium* sp.	18 Nov 98	AF321014
GOBB3-320	*Marinomonas* sp.	18 Nov 98	AF321017

tions B3 and B7 (coordinates in Table 1), situated in the central estuary 2 km apart.

Offshore sampling sites were C33 and C34 situated outside the Snöan archipelago in the open sea, with a maximum depth of 70 m, situated 240 m apart.

Sampling—For all samples, two stations in each environment were sampled at two depths each (in duplicate) to allow an analysis of variance of these factors. The river samples were taken at 1 and 2 m depth with a Ruttner sampler. Samples were collected in 2.5-liter polyethylene bottles. All plastic- and glassware used for sampling was washed in 10% HCl and carefully rinsed twice with sample water before collection of the sample.

Temperature was recorded during river sampling. Other river variables were derived from databases of river monitoring programs. Estuarine and marine samples were collected in 5-liter Niskin bottles attached to a rosette sampler carrying a conductivity, temperature, and depth (CTD) probe. Samples in the estuary were taken at 5 and 15 m, whereas samples from the offshore site were taken at 5 and 50 m. Chemical and hydrographical background data were obtained from the national monitoring program at stations B3 and B7. Hydrographical data were derived from the CTD profiles at the offshore stations C33 and C34. Samples were maintained close to the sampling temperature until processed. Filtration of samples for bacterial species was typically carried out within 3 h of sampling.

Quantitative DNA–DNA hybridization—The abundance of six bacterial species was estimated by quantitative DNA–DNA hybridization (QDH; Table 2). These bacteria were selected on the basis of their occurrence as dominant bacterial species in enrichment cultures with riverine DOC as the main carbon source (Kisand et al. 2002). Thus, the selected species probably represent specialized bacterial consumers of imported riverine dissolved carbon in the marine environment.

Bacteria in the samples (10 ml) were collected on a positively charged hybridization membrane (Hybond-N+, Amersham) with a blotting apparatus (Gibco BRL with 6-mm-diameter dots) and a specifically developed lid containing 12 wells allowing 15-ml sample volumes. The cells were lysed in the blotting apparatus by covering the slots with 100 μl of 0.5 mol L^{-1} NaOH and incubating for 5 min. The whole sample was filtered, and the slot was covered with 100 μl of 1 mol L^{-1} Tris-HCl (pH 7.4) for 5 min. The sample was then filtered through the membrane. Finally, the dot was briefly covered with 100 μl of 1.5 mol L^{-1} NaCl and 0.5 mol L^{-1} Tris-HCl (pH 7.4) and filtered through the membrane. The adsorbed DNA was linked to the membrane by optimal cross-linking (1,200 mJ cm^{-2}, 14 s).

Standard dilution series for each of the studied strains were prepared from cultivated strains (Kisand et al. 2002). Because the hybridization signal was found to be growth dependent, for each strain, duplicate or triplicate dilution series were prepared from different batch culture growth stages. Their average value was used for calibration to cell numbers and variation included in the uncertainty estimates. Dilutions ranged from 1×10^4 to 7×10^6 cells per dot. All samples and standard series associated for each strain were hybridized simultaneously in the same hybridization tube.

A pure culture of each species was incubated in liquid ZoBell medium at 15°C for 2–10 d. ZoBell medium (ZoBell and Upham 1944) was made from 800 ml of 0.2-μm-filtered (Durapore, Millipore) seawater and 200 ml of MilliQ water. This was amended with 5 g of pancreatic digest of casein (Trypton, Difco), 1 g of yeast extract (Difco), and 0.01 g of $FePO_4 \times 2H_2O$ (Aldrich).

From each culture, 10 ml was centrifuged (1,000 $\times$ g for 15 min). The pellet was resuspended in 10 mmol L^{-1} Tris base and 1 mmol L^{-1} disodium ethylenediaminetetraacetic acid (EDTA) adjusted to pH 8.0 with HCl (TE buffer), and the cells were lysed with a final concentration of 1 mg ml^{-1} lysozyme for 1 h at 37°C, followed by 100 μg proteinase K ml^{-1} (final concentration) for 1 h at 37°C, and finally 1% cetyltrimethylammonium bromide (CTAB)-NaCl treatment and chloroform-phenol extraction. DNA was precipitated in ethanol and resuspended in TE buffer (pH 8.0) or MilliQ ultrapure water (Wilson 1994). Prepared DNA was labeled with 50 μCi [α-32P]-dATP (3,000 Ci [mmol $L^{-1}]^{-1}$, Amersham Pharmacia Biotech) with the use of the High Prime DNA Labeling Kit (Roche Diagnostics) according to the manufacturer's instructions. Labeled DNA was purified from free nucleotides with spin columns (MicroSpin S-300 HR, Amersham Pharmacia Biotech) and used as a probe.

The filtration membranes were prehybridized in a solution consisting of 10% dextran sulfate, 1% sodium dodecyl sulfate (SDS), and 100 salmon sperm DNA μg ml^{-1} for at least 2 h at 69°C in a hybridization incubator (model 400, Robbins Scientific). The denatured probe (10 min at 95°C) was added to the hybridization tube (Robbins Scientific). The membranes with community DNA and standard curve of each isolate were hybridized in the same hybridization tube overnight at 69°C. Membranes were washed with 5$\times$ sodium chloride–sodium citrate buffer (SSC)–0.1% SDS solution at 74°C for 45 min and in 2$\times$ SSC at room temperature for 45 min. Washed and dried membranes were exposed in a PhosphorImager (Molecular Dynamics) for detection of radiation from the hybridized ^{32}P isotope. Cell numbers were calculated from the standard curves obtained from the radioactive signal dilution series from the respective studied strain. Coefficients of the standard curve were estimated by taking the mean value of all standard curve replicates for each strain.

Quantitative DNA–DNA hybridization values were corrected for background hybridization with probes from the acidophilic sulfur bacterium *Acidithiobacillus caldus* and the pathogen *Borrelia burgdorferi.* These microorganisms were assumed not to be present in detectable numbers in the environmental water, thereby representing unspecific binding of the probes of the investigated species. Background values were estimated by applying samples from the three environments on dot-blot membranes as described previously. Probe preparation and hybridization with *A. caldus* and *B. burgdorferi* was as described for the six studied isolates. Calibration curves for background bacteria were derived from samples of pure cultures filtered onto membranes at an abundance of 1×10^4 to 7×10^6 cells per dot, similar to the studied species. Cell concentrations from the background signal were calculated and subtracted from the sample values.

Bacterial abundance—Formaldehyde-preserved samples (1–5 ml, 4% final concentration) were filtered within 3–4 h after preservation onto 0.2-μm black polycarbonate filters, stained with acridine orange, mounted on microscopy slides with paraffin oil, and frozen at -20°C until counting (Wikner and Hagström 1999). To estimate bacterial abundance, at least 400 cells per filter (or at least 30 fields when cell concentration was low) were counted with the use of an epifluorescence microscope at 1,250-fold magnification. Cell numbers were determined by an automated image analysis system (Blackburn et al. 1998). Briefly, for each filter, five pictures were photographed with a cooled, slow-scan, charge-coupled device (CCD) camera (C4742 Hamamatsu). Images were calibrated in the camera controller and transmitted to a digital frame grabber program (IQBase ver. 1.20). Image analysis was then performed with the software LabView (ver. 2.0, National Instruments).

Nutrients—Nutrient data were derived from Swedish national monitoring data of rivers (Department of Environmental Analysis, Swedish University of Agricultural Sciences, Uppsala) and the sea (Swedish Meteorological and Hydrological Institute, Gothenburg).

Statistics—Analysis of significant effects from the environment, depth, and time were carried out by applying a general linear model (GLM) in SAS/STAT software (ver. 6 for Windows, SAS Inc.). Specific test problems were solved by tailor-made ESTIMATE and CONTRAST clauses. Before analysis, data were transformed by taking a binary logarithm of species abundances. After this transformation, the residual distribution of variables was close to a normal distribution. Sampling site location (levels: river [RIV], estuary [EST], offshore sea [SEA]) and sampling depth (levels: surface [Surf], deeper layer [Deep]) were treated as fixed factors. The effect of sampling day within year was represented by a fourth-order polynomial, and seasonality was tested as nonconstancy of this polynomial. A significance level of 0.05 was used consistently. The standard variation for replicates within depth was estimated by the residuals of a one-way analysis of variance. The standard deviation for each species was presented as the average over the sampling period.

Results

Hydrography and chemistry of the environments—Discharge from the Öre River during the period under investigation was highly variable (Fig. 2A). The summer and autumn periods of year 2000 were characterized by unusual flood events, such that the spring flood was not markedly different in magnitude from the summer and autumn discharge (150–155 $m^3 s^{-1}$). In winter, water discharge was at a typically low level (20–30 $m^3 s^{-1}$). During winter, the inorganic nutrient concentration in the river water was low (e.g., PO_4-P, 0.03–0.2 μmol L^{-1}).

According to CTD measurements, the water column in the Öre River estuary was stratified (5-m vs. 15-m layers) from the beginning of June 2000 until the river flooded at the end of August. A similarly strong density difference was re-established from December 2000 to March 2001 because of low surface temperatures and high salinity in the bottom water. The water layers at the Bothnian Sea stations C33 and C34 (5 m vs. 50 m) never completely mixed during the sampling period. The seasonal temperature dynamics were similar in the surface layers of the Öre River estuary and the Bothnian Sea, with both environments showing a 4–6-week lag relative to the Öre River (Fig. 2B). Highest temperatures were recorded in July in the Öre River and in August–September at both marine environments. The deeper layers of the Öre River estuary and the Bothnian Sea had maximum temperatures at the beginning of September (~13°C) and the middle of October (~11°C), respectively.

Total phosphorus (P-TOT) varied from 0.16 to 0.46 μmol L^{-1} in the Öre River estuary (stations B3 and B7) and inorganic phosphorus (P-INORG) from 0.02 to 0.26 μmol L^{-1}. P-INORG was highest in December–March (~50% of P-TOT). Total nitrogen (N-TOT) fluctuated from 15.0 to 21.3 μmol L^{-1}. Up to ~30% of N-TOT in November–March was the most abundant inorganic nitrogen species, NO_3^-. Chlorophyll *a* (Chl *a*) concentration was highest during the spring bloom in May 2000 (2.56 μg Chl *a* L^{-1}). In summer, Chl *a* fluctuated around 1.5 μg Chl *a* L^{-1}, and the lowest values were recorded before the next spring bloom on 27 March 2001 (0.25 μg Chl *a* L^{-1}; Fig. 2A).

Abundance and distribution of the total bacterioplankton community—Total bacterial numbers (TBN) were highest and most variable in the Öre River and lowest in the Bothnian Sea (Fig. 2). In the Öre River estuary and Bothnian Sea, the deep-water layers (15 and 50 m, respectively) had statistically lower TBN compared with surface layers (5 m) of the same environment (GLM; $p > 0.008$ and 0.0001, respectively). This was primarily because of clear stratification during the summer, whereas abundances were similar during the rest of the year.

TBN in the river showed a weak correlation to water flow in the river ($r = 0.46$, $p = 0.039$).

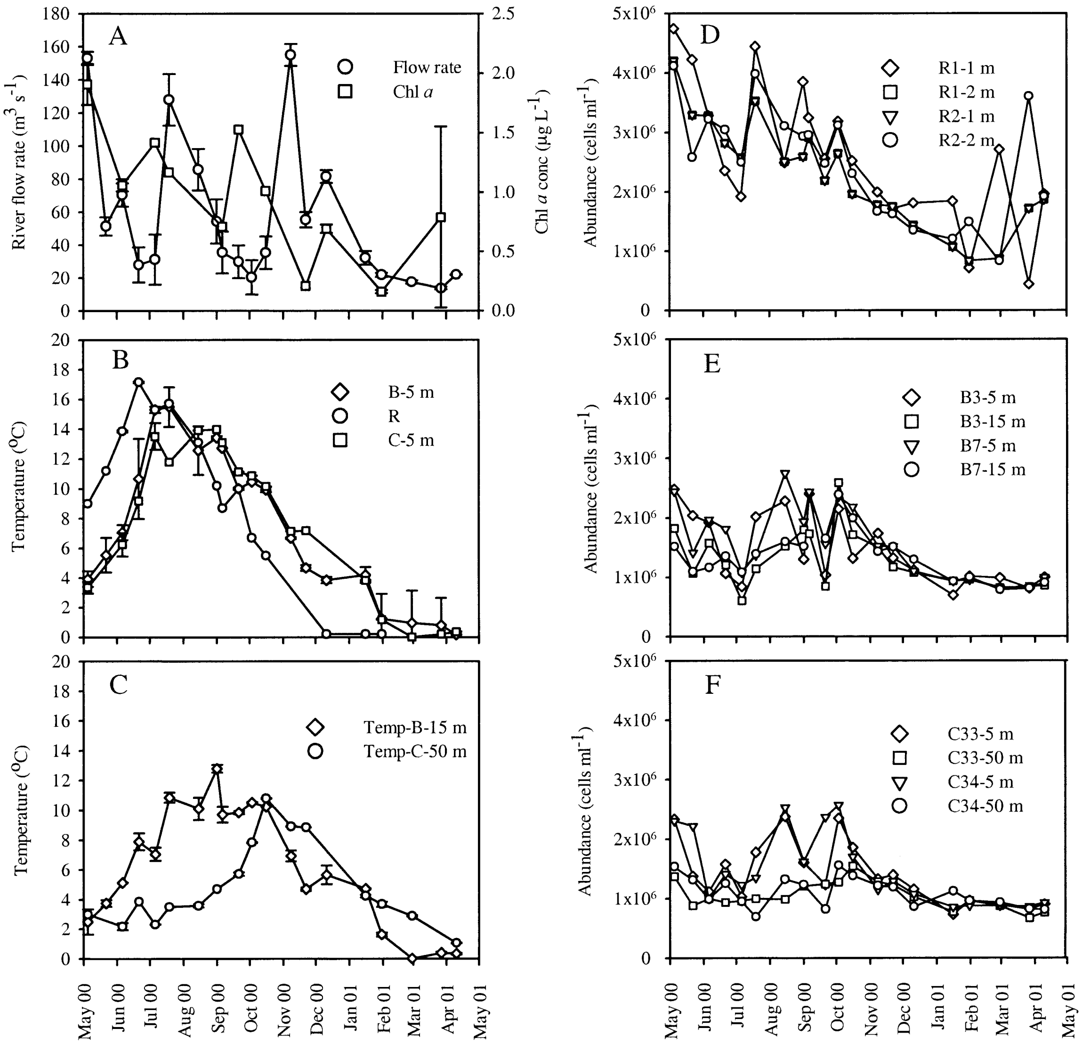

Fig. 2. (A) Flow rate in the Öre River and Chl *a* concentration for the Öre River estuary (B stations 5 m). (B, C) Temperature in the Öre River, Öre River estuary (B stations 5 and 15 m), and northern Bothnian Sea (C stations 5 and 50 m). (D) The total number of bacteria in the Öre River (1 and 2 m). (E) The Öre estuary (5 and 15 m) and (F) the northern Bothnian Sea (5 and 50 m). Error bars are ±SD.

Abundance and distribution of bacterial species—The temporal and spatial distributions of six bacterial species (Table 2) during a full year are presented in Figs. 3 and 4. The interpretation of the initial peaks should be viewed with caution because the calibration of hybridization signal and cell numbers showed higher variability than in other parts of the study. As a rough guide to interpreting Figs. 3 and 4, significantly different values would be expected when cases differed by >700 cells ml^{-1} for *Flavobacterium* spp. GOBB3-C101, GOBB3-317-2, and GOBB3-309 and *Marinomonas* sp. GOBB3-320. For *Flavobacterium* spp. GOBB3-C103-3 and GOBB3-209, differences of 3,000 and 1,600 cells ml^{-1} would have been required. However, these latter species showed markedly higher abundances than the former species.

Difference in abundance between environments—The three most abundant species (*Flavobacterium* spp. GOBB3-C103-3 and GOBB3-209 and *Marinomonas* sp. GOBB3-320) were most numerous in the Öre River (Fig. 3). For *Flavobacterium* spp. GOBB3-209 and GOBB3-C103-3, the estuary had significantly higher values than in the open sea, whereas no significant difference could be demonstrated between the river and estuary (Table 3). This suggested that these species were seeded from the river and were better fitted for the estuary than the open sea. The lack of a significant difference between the river and estuary might be attributed partly to the high variability in the former environment, as seen in higher average values in the river than the estuary (Fig. 3).

Flavobacterium spp. GOBB3-209 and GOBB3-C103-3 were the most abundant bacteria, with average abundances >10,000 cells ml^{-1}, primarily in the riverine environment (Fig. 3). Both species were relatively abundant in the estuary and offshore environments, for which several samples reached abundances close to 5,000 cells ml^{-1}.

Differences between stations—Stations in the same environment covaried and did not show significant differences

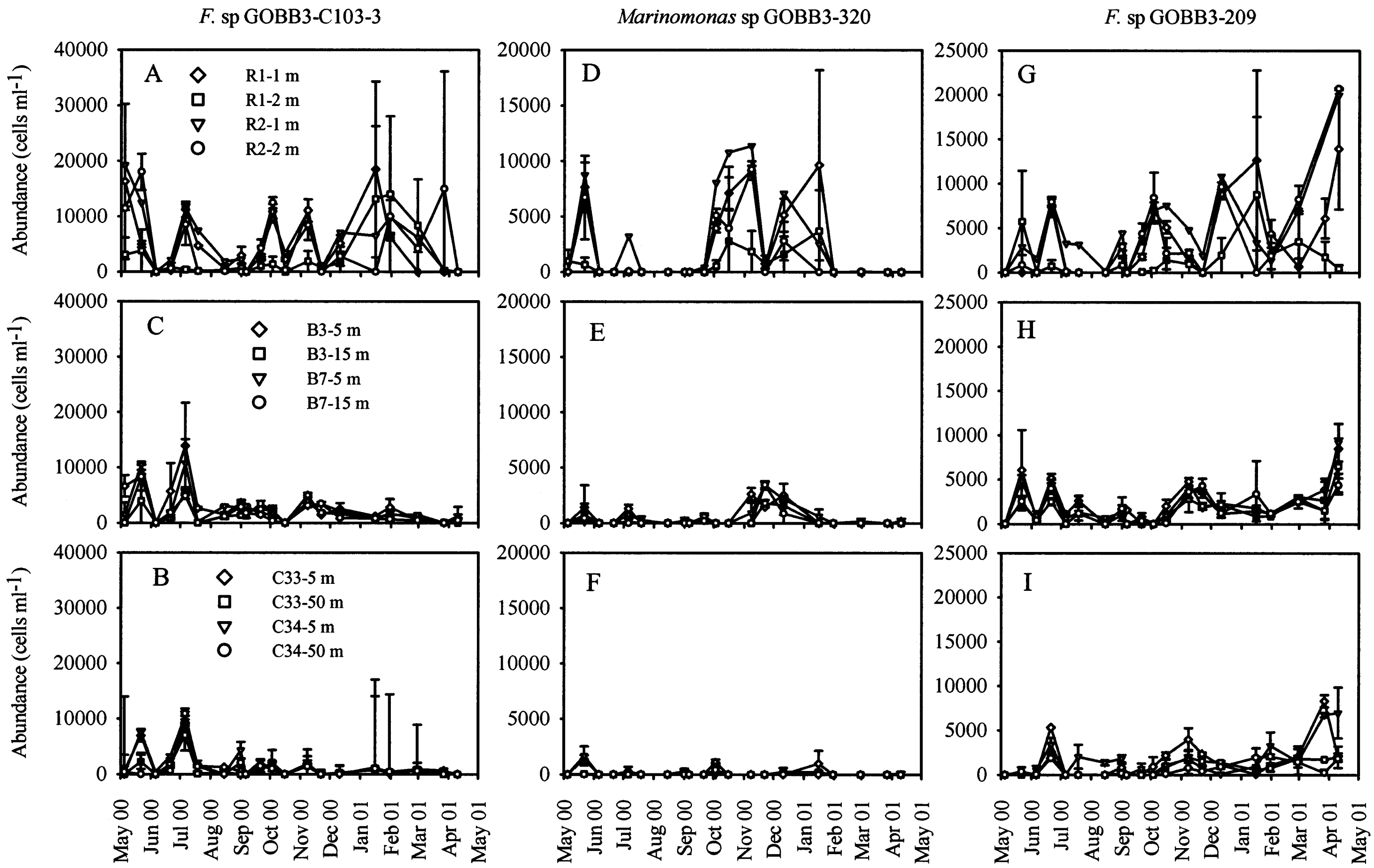

Fig. 3. Abundance of *Flavobacterium* sp. GOBB3-C103-3, *Marinomonas* sp. GOBB3-320, and *Flavobacterium* sp. GOBB3-209 in the (A, D, G) Öre River (stations R1 and R2), (B, E, H) Öre River estuary (stations B3 and B7), and (C, F, I) northern Bothnian Sea (stations C33 and C34). Error bars are ±SD. Symbols are the same in each row of panels.

at a given depth despite distances between sampling stations of 100 m to 2 km (Fig. 1; mixed GLM procedure, $p > 0.05$).

Differences between water layers—In several samples, the difference in bacterial abundance between surface and deeper layers of the marine environments (estuary and open sea) were observed for the three most abundant species (Table 3). *Flavobacterium* sp. GOBB3-209 showed a significantly higher abundance in surface than deep waters in both marine environments (Table 3, see Surf/Deep differences). *Marinomonas* sp. GOBB3-320 and *Flavobacterium* sp. GOBB3-C103-3 only showed a significantly higher abundance in the Öre River estuary surface water.

Abundance during autumn and winter—The increase in *Flavobacterium* sp. GOBB3-209 and *Marinomonas* sp. GOBB3-320 concentration in the river from September 2000 was followed by their increase in the estuary and sea during October (Fig. 3). A similar sequence of events might have occurred in May–July 2000. *Flavobacterium* sp. GOBB3-209 showed similar coupled dynamics between the riverine and marine environments in April 2001. Significant abundance of *Flavobacterium* sp. GOBB3-209 and *Marinomonas* sp. GOBB3-320 was observed during the winter months from December 2000 to February 2001, primarily in the river and estuary.

Relative abundance compared with the total community—The relatively abundant *Flavobacterium* sp. GOBB3-209 composed ~0.5% of the Öre River winter TBN, increasing to 2.5% at the beginning of April 2001 (Fig. 5). In the estuary and in the open sea, its highest relative importance in the community occurred from January to April, although abundance remained <0.5% of TBN over the whole year in these environments. *Flavobacterium* sp. GOBB3-C103-3 was most abundant in the Öre River, with a peak relative abundance of up to 1% of winter TBN (January–April), whereas occasional peaks were observed at the end of May and in July in the estuary and offshore environments, respectively. Its relative abundance decreased steadily along the river–open sea transect. A similar biogeography in terms of relative abundance was observed for *Marinomonas* sp. GOBB3-320.

Successive occurrence between environments—A successive occurrence between the river and estuarine environments was observed for *Marinomonas* sp. GOBB3-320 (Fig. 3). A important rise in cell numbers during October 2000 was followed by a smaller peak in the estuary after approximately a 1-month lag. A slight increase in abundance was also recorded in the open sea surface layer at the end of January. However, *Marinomonas* sp. GOBB3-320 did not appear to persist in the open sea (stations C33 and C34),

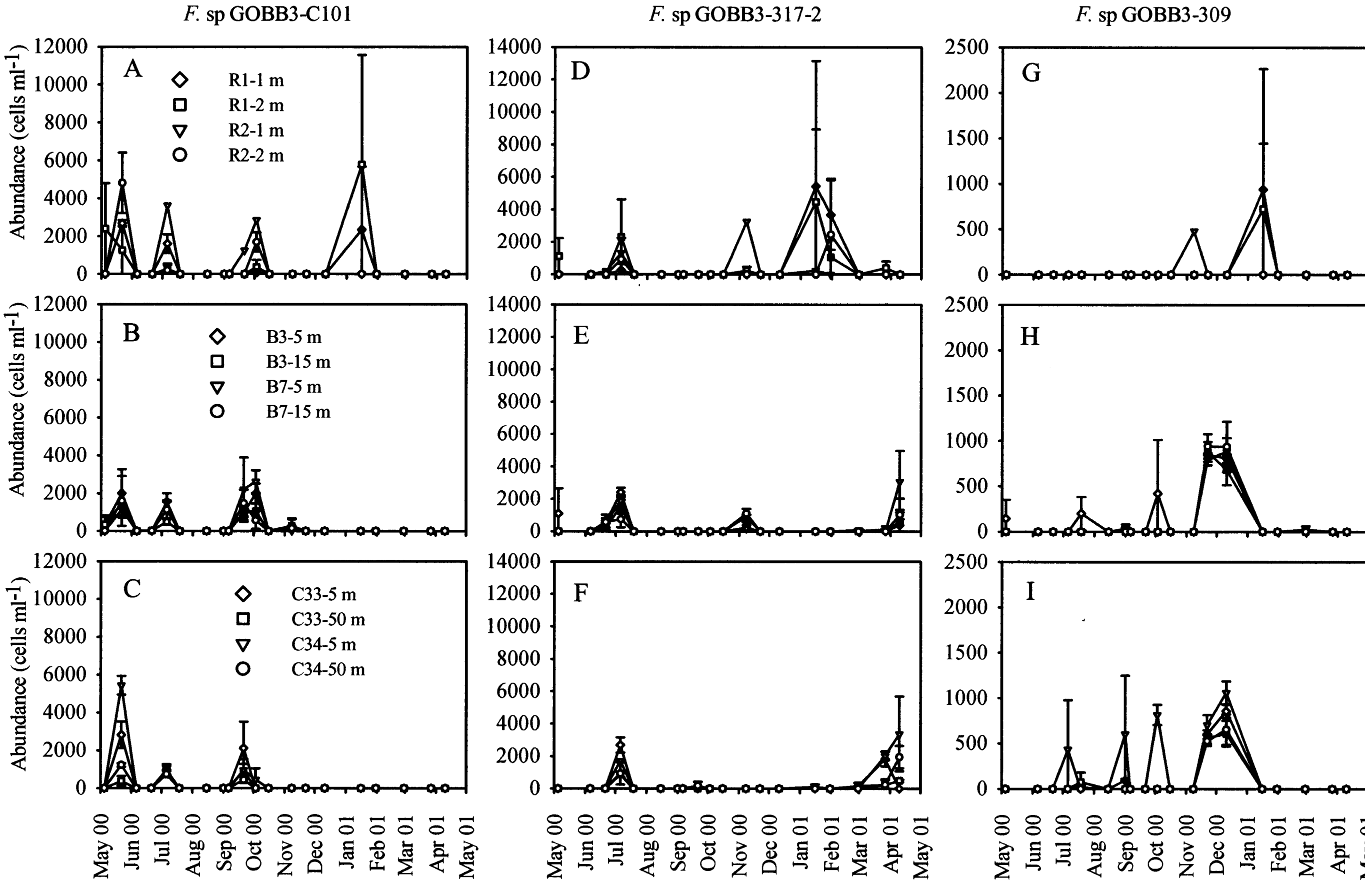

Fig. 4. Abundance of *Flavobacterium* spp. GOBB3-C101, GOBB3-317-2, and GOBB3-309 in the Öre River, Öre River estuary, and northern Bothnian Sea. Station names, error bars, and symbols are as in Fig. 3.

Table 3. Test of significant differences between abundance of bacterial species in time and space. Seasonality shows differences between sampling dates within an environment and station for a given species. Systematic differences between the environments were tested for the river and estuary (RIV-EST), estuary and sea (EST-SEA), and river and sea (RIV-SEA). Differences between the surface (Surf) and deep water (Deep) were also tested within each environment. The significance level of 0.05 was used consistently for the Type 1 error, and values in italics were significant.

Species	Seasonality	Statistic	RIV-EST	EST-SEA	RIV-SEA	Surf/Deep/RIV	Surf/Deep/EST	Surf/Deep/SEA
Marinomonas sp.								
GOBB3-320	<0.0001	Estimate	1.4453	1.3123	2.7575	0.8807	1.3394	0.4559
		p	*0.0003*	*0.0011*	*<0.0001*	0.1211	*0.0186*	0.4219
Flavobacterium sp.								
GOBB3-209	<0.0001	Estimate	0.0201	1.5406	1.5607	1.239	1.3923	2.333
		p	0.9652	*0.0009*	*0.0008*	0.0576	*0.033*	*0.0004*
Flavobacterium sp.								
GOBB3-C103-3	<0.0001	Estimate	0.1585	2.6765	2.835	1.7609	1.835	1.2411
		p	0.79	*<0.0001*	*<0.0001*	*0.0369*	*0.0297*	0.1408
Flavobacterium sp.								
GOBB3-C101	<0.0001	Estimate	−0.2026	0.4611	0.2585	0.04365	0.06282	0.2869
		p	0.4417	0.0803	0.3264	0.9067	0.866	0.4411
Flavobacterium sp.								
GOBB3-309	<0.0001	Estimate	−0.3353	0.05568	−0.391	0.188	0.7634	0.5316
		p	0.2	0.8313	0.1352	0.611	*0.0394*	0.1508
Flavobacterium sp.								
GOBB4-317-2	<0.0001	Estimate	−0.2121	0.1048	−0.1074	0.1219	0.05534	0.1543
		p	0.3757	0.6617	0.6538	0.7188	0.8701	0.6486

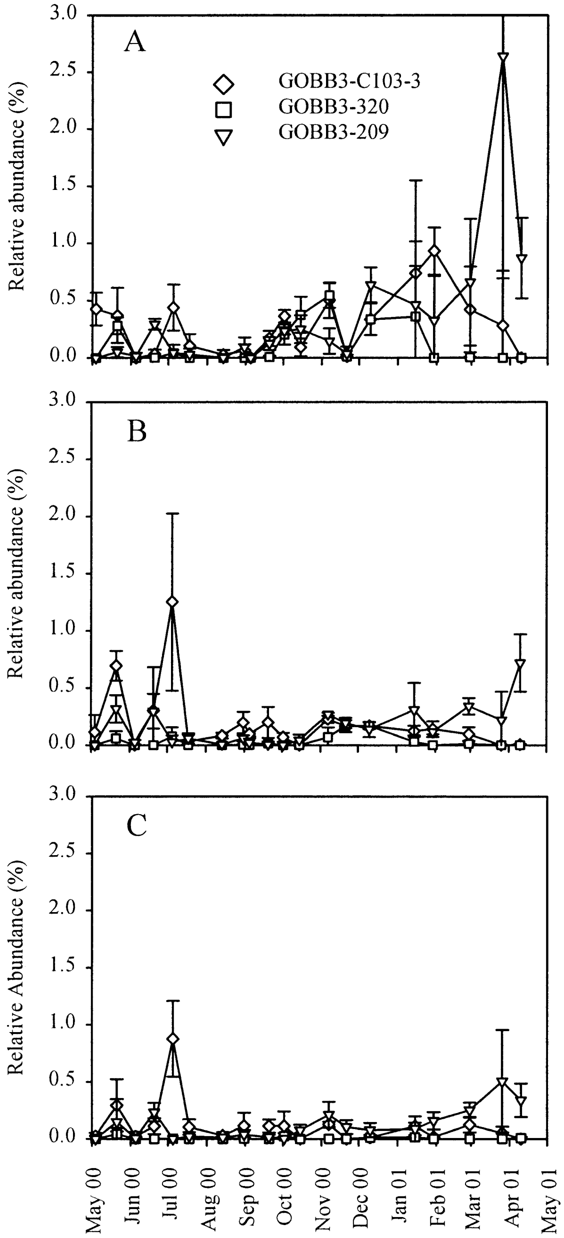

Fig. 5. Abundance of *Flavobacterium* sp. GOBB3-C103-3, *Marinomonas* sp. GOBB3-320, and *Flavobacterium* sp. GOBB3-209 relative to TBN (% of TBN). Values were averaged over both depths and stations ($\pm$SD bars) in each environment: (A) river, (B) estuary, (C) offshore sea. Species codes are given in the figure legends.

and low abundances were measured for most of the year. Similar successive occurrences for the other abundant species were not as clear because of their more general occurrence over the year.

Species with low abundance—Flavobacterium species GOBB3-309, GOBB3-317-2, and GOBB3-C101 were present in lower abundances than the species described previously (Fig. 4). For the majority of the samples, the counts were below the detection limit of the method. *Flavobacterium* sp. GOBB3-C101 occasionally peaked, for example, during May, coinciding with the phytoplankton spring bloom. All peaks occurred simultaneously in all environments. *Flavobacterium* sp. GOBB3-317-2 peaked in July in all three environments. In winter 2001, a rise in numbers of this bacterium was, with some delay, followed by an increase in numbers in the estuary and open sea stations, coinciding with the start of the spring flood. *Flavobacterium* sp. GOBB3-309 showed a different pattern, with a clear peak occurring simultaneously in both marine environments during winter (December–January). This peak was preceded by scattered occurrences in time in the surface waters of both marine sites.

Background correction of quantitative DNA–DNA hybridization—The background hybridization signal typically corresponded to 11,842 $\pm$ 10,147, 1,880 $\pm$ 6,692, and 1,902 $\pm$ 733 cells ml^{-1} in the river, estuary, and offshore sea, respectively. After subtraction of the background hybridization signal from samples, the remaining abundance was assumed to represent an accurate estimation of the species studied. Binding of DNA probes from *A. caldus* and *B. burgdorferi* was linearly correlated with the volume of sample filtered onto the membranes (tested range 2–12 ml), suggesting that objects or material in the sample rather than the filter per se were the cause of the background. This unspecific labeling was on average three times higher in the river samples than marine samples. Nonspecific labeling of prefiltered river water (0.2-μm filter) with both above-mentioned DNA probes was on average five times lower (tested range 2–20 ml) compared with unfiltered samples. Prefiltered samples of river, estuary, and offshore sea had comparable levels of nonspecific labeling. Therefore, particles larger than 0.2 μm (primarily occurring in freshwater) constituted the source of the background.

Discussion

Consumers of riverine DOC were freshwater bacteria—The six investigated bacterioplankton species belong to the most important specialist in catabolism of riverine DOC in the northern Baltic (Kisand et al. 2002). The selected bacteria are shown to occur and are able to grow on riverine DOC at estuarine conditions, whereas their distribution in the natural environment remained unknown.

Three of the investigated species were found to be most abundant in the riverine environment, which strongly suggests that these bacterioplankton were of lacustrine (i.e., freshwater) origin (Figs. 3, 4). The more abundant *Flavobacterium* and *Marinomonas* species could therefore be viewed as immigrants from the river to the estuarine environment, contradicting our previous hypothesis that the important consumers of riverine organic matter are of brackish water origin. It is not likely that cell populations with the same genotype, but of different origin (i.e., freshwater vs. estuarine) would form separate ‘‘species’’ in the estuary because a plausible factor for such selection is lacking.

Our study contributes to other recent evidence for the importance of bacterioplankton taxa moving across ecosystem boundaries. Crump et al. (2004) showed that riverine and marine bacterial assemblages significantly influence the phylogenetic composition of estuarine bacterial assemblages. Lindström and Bergström (2004) similarly showed that bacteria from creeks might significantly influence bacterial community composition in lakes.

The remaining three species showed more episodic abundance close to the detection limit of the method and less clear occurrence in the freshwater environment (Fig. 4). We suggest that the rare species either occupied narrow and poor niches in the pelagic environment or mainly preferred other habitats, such as the sediment surface. *Flavobacterium* sp. GOBB3-309 only showed convincing occurrence in marine environments during winter and could represent a rare marine species.

The estimates in the riverine environment for all species in January showed high variability, likely because of extensive ice formation in the river at this time and sampling of an ice slurry rather than water.

Enhancement of the brackish water ecosystem productivity at moderate salinity levels (3–10) compared with adjacent freshwater ecosystems has been demonstrated (Ducklow and Carlson 1992; Wikner et al. 1999; Cunha et al. 2001). Our data suggested that freshwater bacterial taxa such as *Flavobacterium* and *Marinomonas* also might contribute to the consumption of allochthonous carbon in the estuary.

The biogeography and seasonal dynamics of the selected bacteria were clearly different between species and environments, despite some species belonging to the same genus. This suggested that generalization regarding biogeography and dynamics over a genus was not possible. It also indicated that cross-hybridization was not interfering significantly with the estimates of individual species.

Flavobacterium *is a common freshwater genus*—The conclusion that the studied species originated from the freshwater environment is in accordance with earlier reports of the occurrence of *Flavobacterium* but is contrary to current knowledge for *Marinomonas.* Species affiliated with Bacteroidetes (former Cytophaga-Flavobacteria-Bacteroides [CFB] group) are found to be abundant in coastal ecosystems and rivers, with most taxa identified as belonging to the *Cytophaga* (Crump et al. 1999). Bacteroidetes seem to be abundant in both free-living and particulate detritus assemblages. According to fluorescent in situ hybridization (FISH) counts with Cytophaga–Flavobacterium (CF) probes, CF-like bacteria were abundant over a 200-km-long transect (salinities 0.1–29) in the Delaware River estuary (Manz et al. 1996). CF-like bacteria dominated at lower salinities (0.1–9) and were similar to those in this study (Cottrell and Kirchman 2003). Bacteria affiliated with the Bacteroidetes are also abundant during the phytoplankton spring bloom in the Öre River estuary (Pinhassi and Hagström 2000). Interestingly, Bacteroidetes are more abundant in rivers (and river–reservoir systems) than lake systems (*see* references in Kirchman 2002). However, none of the previously reported species were closely related (>97% 16S rDNA sequence similarity) to the species studied here.

Marinomonas spp. are reported to be open ocean organisms, and have not been observed previously in rivers and coastal zones (Crump et al. 1999). However, these species have been found in sea ice (Romanenko et al. 2003) and Antarctic lakes (closest sequence, direct submission to GenBank, Accession AY092071). Our data suggest that *Marinomonas* spp. might inhabit lacustrine as well as coastal environments. An alternative explanation is that the 16S rRNA gene sequence was insufficient to discriminate strain GOBB3-320 from the *Marinomonas* genus. However, physiologic properties of strain GOBB3-320 were similar to other *Marinomonas* spp., supporting affiliation with this genus (unpubl. data).

Successive occurrences between environments—A successive occurrence between the river and estuarine environment could be inferred for *Marinomonas* sp. GOBB3-320 (Fig. 3). An increase in abundance in the riverine environment was followed by a smaller increase in the estuary with about a 1-month lag. *Flavobacterium* sp. GOBB3-209 and *Flavobacterium* sp. GOBB3-C103-3 also had some peaks with coupled dynamics between environments, but also consistently higher abundance, making any couplings less clear (Fig. 3). The response at the offshore stations was weaker and better correlated in time with the estuarine peaks. This was especially clear for *Flavobacterium* sp. GOBB3-309, showing an extended peak in both marine environments during winter. Successive occurrence between environments could be due primarily to the dynamics of organic substrate moving from the riverine environment to the marine environment. As discussed later, growth on the organic substrate by the species in each environment was required to explain the observed population dynamics. Therefore, passive cell import could not explain bacterial dynamics alone; consequently, a direct coupling to river flow is not expected.

Growth of freshwater bacteria in brackish environments—On the basis of increasing cell numbers of several bacterial species and establishment of significant populations for periods of weeks to months, our data suggest that several of the freshwater bacteria were capable of growing in the brackish water estuarine environment (Figs. 3, 4). Import of nongrowing cells could not alone explain the increase in bacterial numbers, even for the three strains clearly abundant in the river (Figs. 3, 4). This is because the retention time of the estuary is estimated to be 10 days, and even at the highest flow rates, the daily freshwater discharge (150 m^3 s^{-1}) only amounted to $\sim$1.7% of the water volume of the estuary (total volume $\sim$1.0 km^3). This converts to an approximate 50-fold dilution, neglecting the even greater water exchange with the Bothnian Sea. The peaks in the estuary typically corresponded to as much as 20% of the abundance in the river and extended for periods of weeks to months. The probable occurrence of mortality from grazing and viruses would further support necessity of substantial cell growth for population increase and abundance. We therefore infer that primarily growth and mortality, rather than river flow, determined marine distribution and seasonal dynamics of the bacteria investigated. The cells imported to the estuary instead provided a recurring seeding of freshwater genotypes to the marine environment.

The lack of coupling between flood events and species-specific dynamics within the study period provided further support for growth of the freshwater species in the marine environment (Fig. 2). No clear species-specific correlation with these flood events and bacterial abundance in the estuary was found, although TBN in the river showed a weak correlation with the discharge pattern ($r = 0.46$, $p = 0.039$; Figs. 3, 4). A numerical increase of *Marinomonas* sp. GOBB3-320 and *Flavobacterium* sp. GOBB3-209 in October also coincided with a flow minimum, rather than a maximum. Finally, high flow rates further reduced the ''bloom'' of *Flavobacterium* sp. GOBB3-209 and *Marinomonas* sp. GOBB3-320 at the end of November–December, rather than promoting it.

Selective advantage during low photosynthetic production—Apart from short-lived peaks in June and July, the periods of highest abundance for many of the consumers of riverine DOC occurred during the autumn and winter when photosynthetic production is at its lowest level and the TBN decreases (Figs. 2, 3). This suggested that some consumers of riverine DOC had a selective advantage, especially during periods of low algal and phytoplankton production of substrates. During autumn and winter, *Flavobacterium* spp. GOBB3-209 and GOBB3-C103-3 and *Marinomonas* sp. GOBB3-320 might increase their relative abundance in the community on the basis of allochthonous carbon and energy from river water (Fig. 5). Dissolved compounds of a refractory nature discharged at a low rate by river flow is one of the few important sources of carbon during winter, as photosynthetic organic production is below the detection limit for several months (Chl *a* in Fig. 2; Andersson et al. 1996). Consequently, these bacteria likely contribute to the elevated numbers and growth rate of bacterioplankton during winter that is observed in the coastal zone of the northern Baltic Sea (Wikner and Hagström 1999).

The peaks in abundance observed during May and July by several species and in several environments suggested that substrates used by these species were also present during summer (Figs. 3, 4). No clear source of these bursts of growth could be identified, partly because of the short-lived peaks. The occurrence of *Flavobacteria* during summer was in accordance with the elevated abundance of Bacteroidetes bacteria during the spring bloom, found by Pinhassi and Hagström (2000) at the same site.

Salinity as a structuring factor—Our results suggest that there is no sharp physiological limit between growth in freshwater and growth in brackish water with salinities from $\sim$0 to 4. Freshwater and marine ecosystems are considered to have dissimilar bacterial communities, mainly because of strong differences in important environmental factors (Nold and Zwart 1998; Zwart et al. 2002). With the use of DGGE analysis, Troussellier et al. (2002) found that very few freshwater bacterial species ($n < 5$) from the Rhone River could survive under marine conditions (salinity > 35). In other studies with comparisons of DGGE profiles FISH or ARISA the bacterial communities in freshwater, brackish water, and marine environments differed clearly from each other (Hollibaugh et al. 2000; Bouvier and del Giorgio 2002; Selje and Simon 2003; Hewson and Fuhrman 2004). Two important physical factors believed to determine survival of bacterial species in alien environments are salinity and pH. However, most stringent marine bacteria seem to be associated with high salinities ($>$34–36). In the salinity range between 0 and 35, a greater similarity in the occurrence of bacterial species is observed (Troussellier et al. 2002). A potential role of freshwater bacteria in estuaries is also supported by Langenheder et al. (2004), demonstrating that some of the species in this study could grow at similar rates at both riverine and estuarine salinity.

Consequently, our finding that riverine bacterioplankton successfully immigrated and grew in seawater could be largely explained by the low salinities in the adjacent estuarine environment. Salinity increased from $\sim$0.1 in freshwater to 4 in the estuary. Therefore, the osmotic pressure increased by only 0.15 atm in absolute terms, even if the fivefold relative increase can be considered important (20 mOsm in freshwater vs. 110 mOsm in the estuary at salinity 4). It has been reported that such moderate salinity changes (up to salinity 20) do not sufficiently increase the mortality of freshwater bacteria (Painchaud et al. 1995). The activity of riverine bacterioplankton should decline more rapidly when rivers discharge directly into oceanic waters.

The observation that bacterial diversity altered the greatest at the turbidity maximum (Crump et al. 1999) was not applicable in the environment of this study. Because of relatively low salinity where river water enters the brackish water environment ($\sim$4), there is no marked estuarine turbidity maximum in the northern Baltic.

Typical abundance of individual species in the bacterial community—Three species (two *Flavobacterium* spp. and the *Marinomonas* sp.) had relatively high abundance (Fig. 3), whereas the other three *Flavobacterium* strains were only occasionally detectable by the QDH method (Fig. 4). The species with the highest abundance in our study were still 10-fold lower in abundance compared with the dominating species from the same site during summer (Pinhassi and Hagström 2000). Each individual species constituted a relatively small percentage (typically $<$1%) of the TBN (Fig. 5), perhaps because of the refractory nature of riverine DOM that is 80% humic material with high aromatic content and an unfavorable carbon to nitrogen and phosphorus relationship, resulting in a low growth yield (Pettersson et al. 1997; Wikner et al. 1999).

However, considering the appreciable total diversity of bacterial species in the northern Baltic ($\sim$150 taxa; Pinhassi et al. 1997; Pinhassi and Hagström 2000; Kisand and Wikner 2003), the abundance of the riverine DOC–consuming species (0.1–2.5% of total direct counts) could still be considered typical for a single species. Making the hypothetical assumption of an equal share of resources for all 150 species found, they would each contribute 0.7% to the total community. We also recognize that the fraction of the studied species relative to the active bacteria should be 27% (summer) to 400% (autumn/spring) higher according to previous estimates of active bacteria in the estuary (Pinhassi and Hagström 2000). The importance of the studied species relative to all active bacteria is clearly higher during the cold season. This is because the fraction of active bacteria in the community is lower during winter than summer.

The relative numbers found in this study were comparable to relative abundances of single species reported from other coastal zones and estuaries. In the estuary of the Choptank River (Maryland), the abundance of several species ranged from 0.1% to 4% (Heidelberg et al. 2002). In a similar study in the Ogeechee River (Georgia), relative abundances of bacterioplankton species ranged from 0.4% to 4.9% (Leff 2000). However to date, there is limited information regarding abundances of single species inhabiting aquatic ecosystems, making more general comparisons premature.

QDH methodology—We consider that sufficiently accurate estimates of the individual species were obtained for application during field conditions by quantitative DNA–DNA hybridization. DNA–DNA similarity is a well-founded criterion for discriminating between bacterial species, constituting the basis for the prevailing convention that individuals within a species show >70% similarity on the DNA level (Wayne et al. 1987; Stackebrandt et al. 2002). All the studied *Flavobacterium* spp. possess genomic DNA similarities below the species boundary to qualify as separate species (<70% similarity), despite high similarity on the 16S rRNA gene sequence level (>1,300–base pair–long fragments had similarities of between 94.44% and 99.49%).

The low, and in many samples not detected, signals from applied probes supported the unlikelihood that cross-hybridization would be a significant contributor to a systematic overestimation of population abundances (Figs. 3, 4). Empirical estimates suggest that the level of cross-hybridization is ~2.5% for bacteria in general (Pinhassi et al. 1997; Telang et al. 1997), although it might be higher within a genus (16S rDNA similarity of 95–97%; Kisand et al. 2002). As shown by Greene and Voordouw (2003), the influence of cross-hybridization can vary, being less in limited diversity (e.g., enrichment cultures) than in very complex communities (~1,000 species). They suggested that dominant members (>5%) of complex communities (100 species) represent the lower limit of resolution of the DNA–DNA hybridization with whole genomes.

The application of a background subtraction in our study should have improved the detection limit further, accounting for many types of unspecific binding of probe DNA to both nontarget DNA and particulate and dissolved matter. The background measurements showed that unspecific binding was important and mainly associated with riverine particulate matter (>0.2-μm fraction). However, we recognize that cross-hybridization with closely related species to the target organism will not be fully accounted for by this background correction. Therefore, we cannot exclude a cross-hybridization contribution to the hybridization signal for a given target genome.

This study demonstrated a clear variation in biogeography and seasonal dynamics of even some closely related species over the land–sea interface. The distribution of *Flavobacterium* spp. and a *Marinomonas* sp. presented new insights into the environmental factors influencing their distribution in the environment. Some of these species appeared to be freshwater taxa that also were competitive in the estuarine environment and during the cold season.

References

ANDERSSON, A., S. HAJDU, P. HAECKY, J. KUPARINEN, AND J. WIKNER. 1996. Succession and growth limitation of phytoplankton in the Gulf of Bothnia (Baltic Sea). Mar. Biol. **126:** 791–801.

BLACKBURN, N., A. HAGSTRÖM, J. WIKNER, R. CUADROS-HANSSON, AND P. K. BJORNSEN. 1998. Rapid determination of bacterial abundance, biovolume, morphology, and growth by neural network-based image analysis. Appl. Environ. Microbiol. **64:** 3246–3255.

BOUVIER, T. C., AND P. A. DEL GIORGIO. 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol. Oceanogr. **47:** 453–470.

COTTRELL, M. T., AND D. L. KIRCHMAN. 2003. Contribution of major bacterial groups to bacterial biomass production (thymidine and leucine incorporation) in the Delaware estuary. Limnol. Oceanogr. **48:** 168–178.

CRUMP, B. C., E. V. ARMBRUST, AND J. A. BAROSS. 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia river, its estuary, and the adjacent coastal ocean. Appl. Environ. Microbiol. **65:** 3192–3204.

———, C. S. HOPKINSON, M. L. SOGIN, AND J. E. HOBBIE. 2004. Microbial biogeography along an estuarine salinity gradient: Combined influences of bacterial growth and residence time. Appl. Environ. Microbiol. **70:** 1494–1505.

CUNHA, M. A., M. A. ALMEIDA, AND F. ALCANTARA. 2001. Short-term responses of the natural planktonic bacterial community to the changing water properties in an estuarine environment: Ectoenzymatic activity, glucose incorporation, and biomass production. Microb. Ecol. **42:** 69–79.

DUCKLOW, H. W., AND C. A. CARLSON. 1992. Oceanic bacterial production. Microb. Ecol. **12:** 113–181.

FINDLAY, S., M. L. PACE, D. LINTS, J. J. COLE, N. F. CARACO, AND B. PEIERLS. 1991. Weak coupling of bacterial and algal production in a heterotrophic ecosystem: The Hudson river estuary. Limnol. Oceanogr. **36:** 268–278.

FORSGREN, G., AND M. JANSSON. 1992. The turnover of river-transported iron, phosphorus and organic carbon in the Öre estuary, northern Sweden. Hydrobiologia **235/236:** 585–596.

GREENE, E. A., AND G. VOORDOUW. 2003. Analysis of environmental microbial communities by reverse sample genome probing. J. Microbiol. Methods **53:** 211–219.

HAGSTRÖM, Å., J. PINHASSI, AND U. L. ZWEIFEL. 2000. Biogeographical diversity among marine bacterioplankton. Aquat. Microb. Ecol. **21:** 231–244.

HEIDELBERG, J. F., K. B. HEIDELBERG, AND R. R. COLWELL. 2002. Seasonality of Chesapeake Bay bacterioplankton species. Appl. Environ. Microbiol. **68:** 5488–5497.

HEWSON, I., J. A. FUHRMAN. 2004. Richness and diversity of bacterioplankton species along an estuarine gradient in Moreton Bay, Australia. Appl. Environ. Microbiol. **70:** 3425–3433.

HOLLIBAUGH, J. T., P. S. WONG, AND M. C. MURRELL. 2000. Similarity of particle-associated and free-living bacterial communities in northern San Francisco Bay, California. Aquat. Microb. Ecol. **21:** 103–114.

IVARSSON, H., AND M. JANSSON. 1994. Temporal variation of organic carbon in the river Öre, northern Sweden. Verh. Int. Verein. Limnol. **35:** 1522–1525.

KIRCHMAN, D. L. 2002. The ecology of Cytophaga-Flavobacteria in aquatic environments. FEMS Microbiol. Ecol. **39:** 91–100.

KISAND, V., R. CUADROS, AND J. WIKNER. 2002. Phylogeny of culturable estuarine bacteria catabolizing riverine organic matter in the N Baltic. Appl. Environ. Microbiol. **68:** 379–388.

———, AND J. WIKNER. 2003. Combining culture-dependent and -independent methodologies for estimation of richness of estuarine bacterioplankton consuming riverine dissolved organic matter. Appl. Environ. Microbiol. **69:** 3607–3616.

Langenheder, S., V. Kisand, E. Lindström, J. Wikner, and L. Tranvik. 2004. Growth dynamics within bacterial communities in riverine and estuarine batch cultures. Aquat. Microb. Ecol. **37:** 137–148.

Leff, L. G. 2000. Longitudinal changes in microbial assemblages of the Ogeechee River. Freshw. Biol. **43:** 605–615.

Lindström, E. S., and A. K. Bergström. 2004. Influence of inlet bacteria on bacteria assemblage composition in lakes of different hydraulic retention time. Limnol. Oceanogr. **49:** 125–136.

Manz, W., R. Amann, W. Ludwig, M. Vancanneyt, and K. H. Schleifer. 1996. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. Microbiology **142:** 1097–1106.

Nold, S., and G. Zwart. 1998. Patterns and governing forces in aquatic microbial communities. Aquat. Ecol. **32:** 17–35.

Painchaud, J., J. C. Therriault, and L. Legendre. 1995. Assessment of salinity-related mortality of freshwater bacteria in the Saint Lawrence estuary. Appl. Environ. Microbiol. **61:** 205–208.

Pettersson, C., B. Allard, and H. Boren. 1997. River discharge of humic substances and humic-bound metals to the Gulf of Bothnia. Estuar. Coast. Shelf Sci. **44:** 533–541.

Pinhassi, J., and Å. Hagström. 2000. Seasonal succession in marine bacterioplankton. Aquat. Microb. Ecol. **21:** 245–256.

———, A. Winding, S. J. Binnerup, U. L. Zweifel, B. Riemann, and Å. Hagström. 2003. Spatial variability in bacterioplankton community composition at the Skagerrak–Kattegat Front. Mar. Ecol. Prog. Ser. **255:** 1–13.

———, U. L. Zweifel, and Å. Hagström. 1997. Dominant marine bacterioplankton species found among colony-forming bacteria. Appl. Environ. Microbiol. **63:** 3359–3366.

Romanenko, L. A., M. Uchino, V. V. Mikhailov, N. V. Zhukova, and T. Uchimura. 2003. *Marinomonas primoryensis* sp. nov., a novel psychrophile isolated from coastal sea-ice in the Sea of Japan. Int. J. Syst. Evol. Microbiol. **53:** 829–832.

Sandberg, J., A. Andersson, S. Johansson, and J. Wikner. 2004. Pelagic food web structure and carbon budget in three brackish water environments: Potential importance of terrigenous carbon. Mar. Ecol. Prog. Ser. **268:** 13–29.

Selje, N., and M. Simon. 2003. Composition and dynamics of particle-associated and free-living bacterial communities in the Weser estuary, Germany. Aquat. Microb. Ecol. **30:** 221–237.

Stackebrandt, E., and others. 2002. Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. Int. J. Syst. Evol. Microbiol. **52:** 1043–1047.

Suzuki, M. T., C. M. Preston, F. P. Chavez, and E. F. DeLong. 2001. Quantitative mapping of bacterioplankton populations in seawater: field tests across an upwelling plume in Monterey Bay. Aquat. Microb. Ecol. **24:** 117–127.

Telang, A., S. Ebert, J. M. Foght, D. W. S. Westlake, G. E. Jenneman, D. Gevertz, and G. Voordouw. 1997. Effect of nitrate injection on the microbial community in an oil field as monitored by reverse sample genome probing. Appl. Environ. Microbiol. **63:** 1785–1793.

Troussellier, M., and others. 2002. Bacterial activity and genetic richness along an estuarine gradient (Rhone River plume, France). Aquat. Microb. Ecol. **28:** 13–24.

Voordouw, G., Y. Shen, C. S. Harrington, A. J. Telang, T. R. Jack, and D. W. S. Westlake. 1993. Quantitative reverse sample genome probing of microbial communities and its application to oil-field production waters. Appl. Environ. Microbiol. **59:** 4101–4114.

Wayne, L. G., and others. 1987. Report of the Ad-Hoc-Committee On Reconciliation of approaches to bacterial systematics. Int. J. Syst. Bacteriol. **37:** 463–464.

Wikner, J., R. Cuadros, and M. Jansson. 1999. Differences in consumption of allochthonous DOC between a lake and an estuary in a temperate watershed. Aquat. Microb. Ecol. **17:** 289–299.

———, and Å. Hagström. 1999. Bacterioplankton intra-annual variability: Importance of hydrography and competition. Aquat. Microb. Ecol. **20:** 245–260.

Wilson, F. 1994. Preparation of genomic DNA from bacteria, p. 2.4.1–2.4.2. *In* F. Ausubel, and others [eds.], Current protocols in molecular biology. Greene Publishing Associates.

ZoBell, C., and H. Upham. 1944. A list of marine bacteria including description of sixty new species. Bull. Scripps Inst. Oceanogr. **5:** 239–292.

Zwart, G., B. C. Crump, M. P. Kamst-van Agterveld, F. Hagen, and S. K. Han. 2002. Typical freshwater bacteria: An analysis of available 16S rRNA gene sequences from plankton of lakes and rivers. Aquat. Microb. Ecol. **28:** 141–155.

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