Effect of seawater–freshwater cross-transplantations on viral dynamics and bacterial diversity and production

Osana Bonilla-Findji[1,2], Emma Rochelle-Newall[1,2,3,*], Markus G. Weinbauer[1,2], Marie-Dominique Pizay[1,2], Marie-Emmanuelle Kerros[1,2], Jean-Pierre Gattuso[1,2]

[1]CNRS, Laboratoire d'Océanographie de Villefranche, BP 28, 06234 Villefranche-sur-Mer Cedex, France
[2]Université Pierre et Marie Curie-Paris 6, Laboratoire d'Océanographie de Villefranche, 06230 Villefranche-sur-Mer, France

[3]*Present address:* UR 103 Camelia, UMR 5119 ECOLAG, Université Montpellier II, Case 093, 34095 Montpellier, France

ABSTRACT: Dilution experiments were carried out to investigate the community composition and the metabolic response of seawater and freshwater bacteria to cross-transplantation, and the effects of non-indigenous bacterial hosts on viral dynamics. Changes in viral and bacterial abundance and production, as well as bacterial respiration, carbon demand and diversity were regularly monitored over a 6 d period. Bacterial production in the transplanted seawater (SB-t) and freshwater (FB-t) bacteria treatments was stimulated up to 256 and 221%, respectively, compared to controls. The stimulation of bacterial production and carbon demand was accompanied by a decrease in bacterial richness. Net viral production was stimulated by 81% in SB-t and repressed by 75% in FB-t. Transplantation increased the virus-induced mortality of marine bacteria, but decreased it for freshwater bacteria. These results suggest that (1) marine bacteria can readily oxidize freshwater dissolved organic matter, and (2) freshwater viruses might be able to infect marine hosts, thus highlighting their potential role in fueling bacterial growth under resource stress or nutrient-depleted conditions.

KEY WORDS: Transplantation · Bacterial production · Bacterial richness · Virus

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INTRODUCTION

Substrate availability has been extensively studied as a potentially important factor controlling prokaryotic activity. It is well known that the quality and quantity of inorganic and organic nutrients can exert significant control on prokaryotic structure and function (e.g. Azam & Malfatti 2007). In coastal areas, and particularly in estuarine systems, large variations in nutrient and organic carbon concentration can occur over relatively small distances. For example, in Randers Fjord, a small estuary (27 km long) in northern Denmark, concentrations of inorganic and organic nitrogen increase by 1 to 3 orders of magnitude between the seawater and freshwater end-members, respectively (Veuger et al. 2004). Similarly, dissolved organic carbon (DOC) concentrations also vary along estuarine gradients, with generally higher concentrations in the freshwater end-members and lower concentrations in the seawater end-members (Fisher et al. 1998, Abril et al. 2002). Superimposed upon these gradients, biological processes both modify and are modified by geochemical processes.

The dynamic nature of estuaries means that solutes and organic matter from the freshwater and seawater end-members become mixed along the estuarine gradient. The manner in which these inputs mix is related to the relative proportions of each input, as well as to other physical properties (tidal regime, winds, etc.). Therefore, communities of bacteria and other planktonic organisms are exposed to strong physicochemical gradients and constantly varying environmental condi-

tions along the length of the estuary. Moreover, in estuaries where freshwater inputs are relatively low compared to that of seawater inputs, such as in the Scheldt estuary (SW Netherlands and NE Belgium), bacteria and other organisms experience a situation where salinity changes are reduced but relatively large changes in dissolved organic matter (DOM) and other solutes can occur. This results in the exposure of seawater bacteria to freshwater DOM. Conversely, in systems with high seawater to freshwater inputs, freshwater bacteria will be subject to the dual problems of changing salinity and DOM through mixing with seawater. Estuaries are therefore attractive systems to address the question of substrate availability as a controlling factor of prokaryotic activity (Jones et al. 2007).

Strong compositional and metabolic changes have been shown to occur in bacterial communities between the freshwater and saltwater portions of estuaries (Bouvier & del Giorgio 2002, Kirchman et al. 2004), and several authors have proposed the existence of a unique estuarine community that is found in the mixing zone (e.g. Crump et al. 2004). Indeed, the domination of a unique estuarine community vs. a mixing community in the middle estuary largely depends on water residence time (Bouvier & del Giorgio 2002, Crump et al. 2004, Kan et al. 2006). However, regardless of water residence time, the instability of the environment is accompanied by changes in bacterial metabolism, with bacterial cells more dedicated to physiological maintenance than cell production (del Giorgio & Bouvier 2002). Moreover, a recent meta-analysis of bacterial diversity data has shown that salinity plays an important role in structuring bacterial communities (Lozupone & Knight 2007); it also seems to be a determining factor in carbon substrate utilization in estuaries (Thottathil et al. 2008).

Although several studies have recently investigated bacterial responses to changes in the supply of organic matter in estuaries (Stepanauskas et al. 1999, Findlay et al. 2003, Langenheder et al. 2004), few have considered the impact of the associated variation of the coexisting viral communities, another key factor controlling bacterial dynamics. It is now well established that virioplankton exerts a 'top-down' pressure and is a significant mortality agent for heterotrophic bacteria. Virioplankton also plays a significant role in shaping the composition and controlling the diversity of its hosts (Thingstad 2000, Wommack & Colwell 2000), and up to 50% of bacterial mortality can be due to viral activity (Fuhrman & Schwalbach 2003, Weinbauer 2004, Bouvier & del Giorgio 2007). Viral activity therefore has important effects on bacterial processes in aquatic systems. Over and above the direct effect of viruses on infected prokaryotic cells, viral lysis can enhance the activity of non-infected prokaryotic cells, which benefit from the release of organic matter by cell lysis (Middelboe et al. 1996). Recent work has also highlighted the complexity of the interactions between viral and bacterial activity and has shown that viral lysis does not always result in increased bacterial growth efficiency (BGE) (Bonilla-Findji et al. 2008). These authors reported that bacterial respiration (BR) was stimulated by up to 113% in the presence of active viruses, whereas bacterial production (BP) and BGE were reduced by up to 51%, and suggested that viruses enhance the role of bacteria as oxidizers of organic matter and as producers of CO_2.

Similar to that observed for bacteria, spatiotemporal changes in the virioplankton composition and structure have been observed in estuaries (Wommack et al. 1999) and different viral infection rates are known to occur along salinity gradients (Almeida et al. 2001). Burst size, and, thus, viral production, tends to increase as a function of increasing DOM and nutrient concentration (Bettarel et al. 2004, Parada et al. 2006). It is therefore probable that changes in DOM in terms of quality and quantity play a role in determining viral activity in estuarine systems.

The present study was carried out in the Scheldt estuary (SW Netherlands and NE Belgium), one of the most eutrophic estuaries in Europe (Wollast 1988), in order to extend previous results on the functional response of a seawater bacterial community to freshwater DOM (Rochelle-Newall et al. 2004). Two objectives were addressed: (1) to determine the structural and metabolic response of seawater and freshwater bacterial communities to cross-transplantation, and (2) to investigate how viral dynamics change during transplantation relative to changes in DOM concentration and host diversity.

MATERIALS AND METHODS

Experimental setup. In order to assess the response of bacteria and viruses to mixing along an estuarine salinity gradient, water samples were collected in April 2003 from the seawater (Stn SW) and freshwater (Stn FW) end-members of the Scheldt estuary (Fig. 1). The response of seawater bacteria to freshwater DOM and viruses and, conversely, the response of freshwater bacteria to seawater DOM and viruses were investigated using dilution experiments. This transplant method was chosen to experimentally mimic the dynamic mixing of water masses and hence bacterial and viral communities and DOM along the estuarine salinity gradient of the Scheldt estuary.

Bacteria and viruses from the 2 end-members of the estuary were inoculated in filtered water from the other site in a 1:18 volume ratio (Fig. 2). At each station, 18 l of <0.2 μm filtrate water were distributed into two 20 l, acid-washed and Milli-Q rinsed poly-
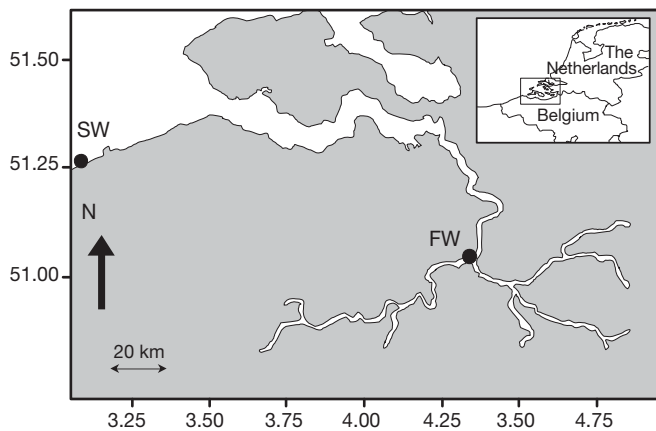
Fig. 1. The freshwater and seawater sample sites (Stns FW and SW, respectively) in the Scheldt estuary

carbonate carboys and then inoculated with 1 l of either unfiltered seawater or unfiltered freshwater to obtain 4 treatments: transplanted seawater bacteria (SB-t), seawater control (SB-c), transplanted freshwater bacteria (FB-t) and freshwater control (FB-c). The salinity of the freshwater filtrate was adjusted to 30 with an artificial seawater (ASW) (Guillard 1975) mix to minimize any effect of the salinity on the seawater bacterial community.

The 4 treatments were incubated for 6 d in the dark and at *in situ* temperature (7.6°C) and subsamples

were collected for bacteria and viral counts, BP and bacterial diversity at 6 time points (0, 17, 43, 91, 139, and 157 h). Samples for determination of DOC were taken at the beginning and end of the experiment.

Sample analyses. Viral abundance was measured in duplicate 1 ml samples fixed with glutaraldehyde (0.5% final concentration, EM-grade; Merck) for 30 min at 4°C in the dark, flash frozen in liquid nitrogen and stored at −80°C pending analysis by flow cytometry as described by Brussaard (2004).

Viral counts were performed with the CellQuest-Pro software (Becton Dickinson) after staining with SYBR GREEN I (S7567; Invitrogen) that was pre-diluted in 0.2 µm filtered, autoclaved Milli-Q (final dilution of 5×10^{-5} the commercial stock). Three viral groups were discriminated from scatter plots of side scatter (SSC) vs. green fluorescence (FL1). These groups were labeled according to their increasing fluorescence signal: V1 (low), V2 (medium) and V3 (high). Previous comparisons (including the studied environment) of the total counts obtained by this method with viral abundances determined by microscopy were very similar and differences were generally less than 20% (O. Bonilla-Findji unpubl. data). Viral production and decay were calculated from the net changes in viral abundance between each time point (Bratbak et al. 1990). The values obtained should therefore be considered as conservative estimations of viral production and decay.
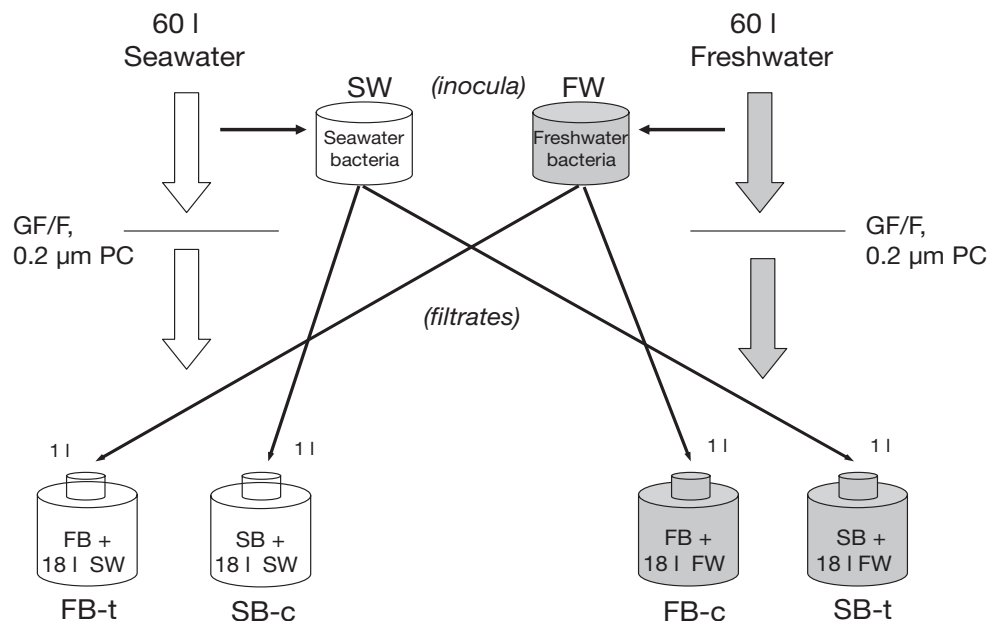

Fig. 2. Experimental setup. Seawater (SW) or freshwater (FW) (60 l) was filtered sequentially through GF/F (Whatman) filters and 0.2 µm Durapore (Millipore) cartridge filters; 18 l of each filtrate was dispatched into four 20 l polycarbonate carboys (PC); 1 l of unfiltered freshwater or seawater was added to each incubation to provide the natural bacterial inoculum. Two transplants and 2 controls were used (SB-c: seawater + seawater bacteria; SB-t: freshwater + seawater bacteria; FB-c: freshwater + freshwater bacteria; FB-t: seawater + freshwater bacteria)

Bacterial abundance was measured by a direct count method using epifluorescence microscopy and DAPI-stained samples (Porter & Feig 1980). Inspection of filters during enumeration did not reveal that flagellates were present in significant numbers. BP was estimated from ^{3}H-leucine incorporation in accordance with the method of Kirchman (1992) and as previously described by Rochelle-Newall et al. (2004). BR was calculated based on standard dark-bottle O_2 consumption rates. Replicate biological oxygen demand (BOD) bottles were incubated in the dark following the Joint Global Ocean Flux Study (JGOFS) protocol (Knap et al. 1996), and at distinct time points, oxygen concentration was titrated using an automated Winkler titration technique with a potentiometric end-point detection (Anderson et al. 1992). The respiration rate at each time point T and its SE were determined by regressing O_2 concentration against time during the intervals of incubation T to $T + 1$.

Due to the difference in the time scales of the measurement of BP (1 h) and BR (17 to 48 h), bacterial carbon demand (= BP + BR) was calculated using BR, expressed in carbon units assuming a respiratory quotient of 1. Mean BP was determined during the same time interval as used to measure the rate of respiration using $[(BP_{T1} + BP_{T2})/2]$, where BP_{T1} and BP_{T2} are BP at the start and end of the respiration measurement, respectively.

Samples (10 ml) for determination of DOC were filtered through combusted (450°C, overnight) glass fiber filters (Whatman GF/F) and sealed in pre-combusted (450°C for 4 h) glass ampoules after adding 12 µl of 85% v/v phosphoric acid (H_3PO_4). Samples were stored in the dark at 4°C pending analysis. DOC concentration was determined as previously described (Rochelle-Newall et al. 2007) by the high temperature combustion method using a Shimadzu TOC-5000 analyzer. Certified reference materials (Hansell Laboratory, University of Miami) were also used to assess the performance of the instrument on and between measurement days (Sharp 2002).

For extraction of DNA from prokaryotic cells, 50 to 150 ml samples were recovered on a 0.2 µm pore-size polycarbonate filter (47 mm diameter; Whatman) and kept frozen at –80°C pending analysis. Nucleic acids were extracted from the filters and purified as described elsewhere (Winter et al. 2001, 2004b). In contrast to the phenol-chloroform extraction step from the original protocol, nucleic acids were extracted with 4.5 M NaCl and chloroform. This modified procedure avoids manipulation of a toxic chemical and yields fingerprints identical to those obtained by the original method (data not shown).

Polymerase chain reaction (PCR) conditions and chemicals were as described in Schäfer et al. (2001). Briefly, 1 to 4 µl of the nucleic acid extracts were quantified on an agarose gel using a DNA mass ladder (EasyLadder I; Bioline, #BIO-33045). When sufficient DNA was detected it was used in 50 µl PCR reactions (1.5 mmol l^{-1} MgCl$_2$, 0.25 µmol l^{-1} of each primer and 2.5 U Taq polymerase; Sigma, #D 5930) together with a positive and a negative control. A fragment of the 16S rRNA gene was amplified using the bacterial primer pairs 341F-GC/907R (Schäfer et al. 2001).

When sufficient PCR products were obtained, denaturing gradient gel electrophoresis (DGGE) was carried out as described by Schäfer et al. (2001). PCR products (500 ng) were separated into bands by electrophoresis for 18 h at 100 V on acrylamide/bis-acrylamide (8%) gels prepared using a denaturing gradient from 30 to 70% (urea and formamide). DGGE gels were photographed with a gel documentation system GelDoc EQ (Bio-Rad) after 15 min staining with a 10X SYBR Gold solution (pre-diluted in 0.2 µm filtered, autoclaved Milli-Q; Molecular Probes, #S11494). Analysis of band patterns between lanes of the same gel was performed with Quantity One Software (Bio-Rad). Apparent bacterial richness and band intensity (measured as peak area) is considered as the number of detectable bands on the DGGE gels.

The Statgraphics Centurion XV software package (Statpoint) was used to test the relationships between the treatments and respective controls. After verifying that assumptions were met (Shapiro-Wilks' test), t-tests were used to determine if the effect of transplantation was significant relative to the appropriate control. Significance is given at the p < 0.05 level.

RESULTS

The *in situ* physicochemical and biological characteristics of the 2 sampling stations differed considerably. The freshwater (FW) station exhibited higher bacterial abundance, BP and richness, viral abundance and DOC concentration than the seawater (SW) station (Table 1).

Bacterial dynamics

The initial bacterial abundance in the incubations was 5.2 and 8.3×10^5 ml^{-1} in the seawater and the freshwater bacterial treatments, respectively (Fig. 3a). This was in comparison to an *in situ* abundance of 7.4×10^6 and 3.1×10^6 ml^{-1} for the freshwater and seawater stations, respectively (Table 1). Cell numbers continuously increased during the experiment and reached values 2 to 3 times higher at the end of the incubation. This resulted in maximum abundances that were always less than the *in situ* values by a factor

Table 1. Physicochemical and biological characteristics of surface water sampled at the freshwater (FW) and seawater (SW) ends of the Scheldt estuary. DOC: dissolved organic carbon (±SD); BP: bacterial production (±SD). DGGE: denaturing gradient gel electrophoresis

	Stn FW	Stn SW
Temperature (°C)	11.04	7.73
Salinity	1.20	30.4
DOC (μmol l^{-1})	427 ± 1.9	195.0 ± 1.6
Virus ($\times 10^7$ ml^{-1})	11.1	1.3
Bacteria ($\times 10^6$ ml^{-1})	7.4	3.1
BP (μmol C l^{-1} h^{-1})	0.255 ± 0.006	0.070 ± 0.002
Bacterial richness (no. DGGE bands)	40	37

of 2 to 3 (Table 1, Fig. 3a). The only exception was in treatment SB-c, where bacterial abundance declined to initial values at the endpoint.

BP increased throughout the incubation, although controls and transplanted treatments showed different patterns (Fig. 3b). While a lag period occurred in the trans-
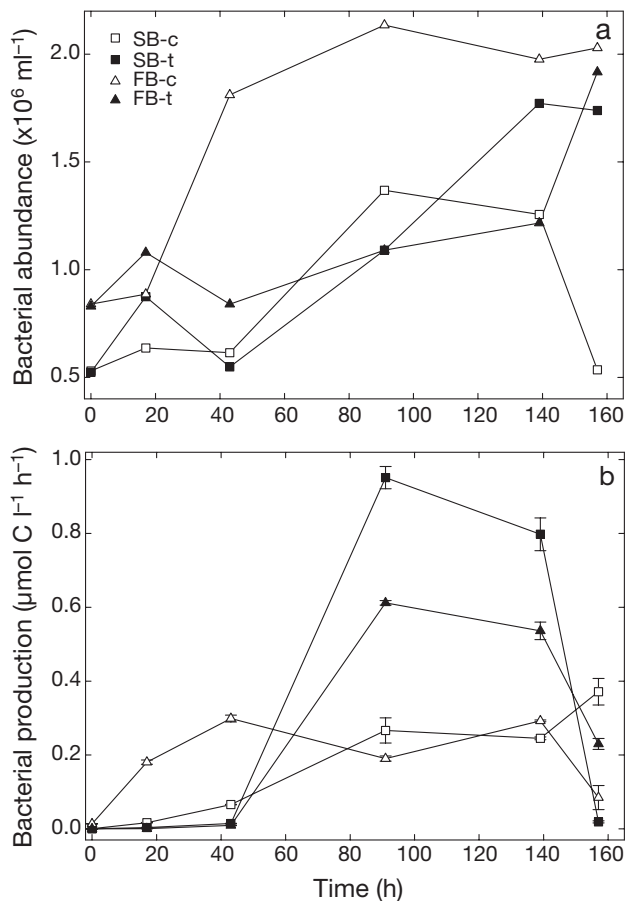
planted treatments, it greatly increased in the controls during the first 36 h. Thereafter, BP strongly increased and was up to 3-fold higher in the transplanted treatments than in the controls. Thus, although BP was low in the transplantations in the short term (<36 h), a 2-fold stimulation was observed after 48 h in treatments SB-t and FB-t. At the end of the experiment, the stimulation of BP was nearly 3-fold higher in SB-t than in FB-t.

During the first 91 h of the incubation, BR was significantly lower in the transplanted treatments relative to their respective controls (t-test, p < 0.05). During the second part of the incubation (>91 h), respiration generally decreased as a function of time in all treatments (Fig. 4a). Across the 7 d incubation period, respiration rates were statistically significantly lower in FB-t than in FB-c (p < 0.05). This is in contrast to the seawater bacterial transplantation (SB-t) and control (SB-c), where no significant difference (p > 0.05) in respiration was observed over the 7 d incubation period.

Fig. 3. Bacterial (a) abundance and (b) production in the 4 different treatments (SB-c, SB-t, FB-c and FB-t). Values in (b) represent means ± SD
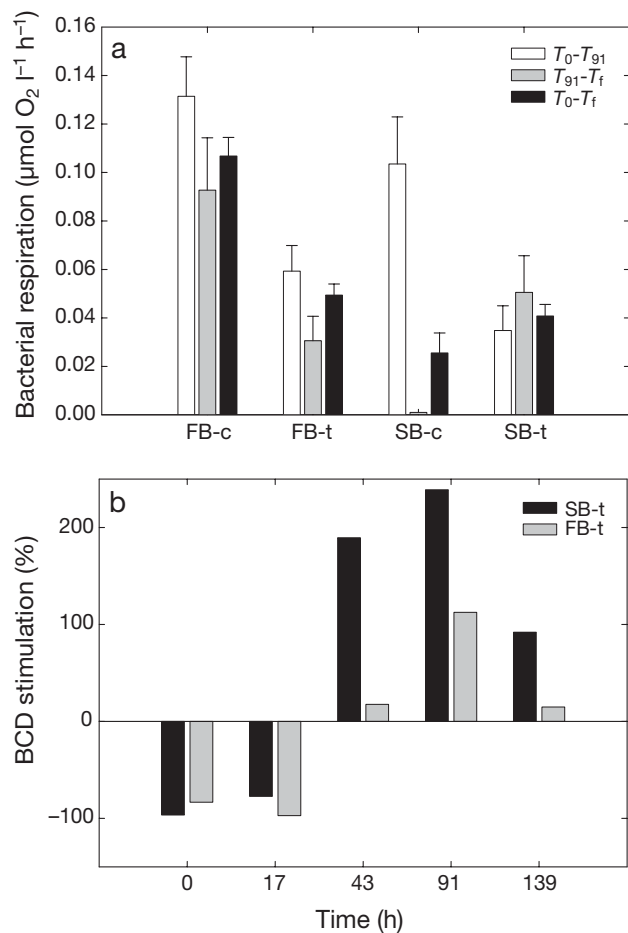

Fig. 4. (a) Bacterial respiration in the 4 treatments during the different phases of the experiment. (b) Transplantation effect on bacterial carbon demand (BCD) across the incubation period in transplanted treatments (SB-t and FB-t) relative to their corresponding controls. T_0, T_{91} and T_f: start, first 91 h and end of incubation, respectively

Bacterial carbon demand was relatively stable throughout the control incubations (ranging from 0.10 to 0.48 µmol C l^{-1} h^{-1}) and increased slightly at the end of the experiment (data not shown). In contrast, in the transplanted treatments, it was initially close to zero (0.01 µmol C l^{-1} h^{-1}) but greatly increased after 2 d (up to 0.88 µmol C l^{-1} h^{-1}) until the end of the experiment (data not shown). The stimulation of bacterial carbon demand was stronger in SB-t (up to 239%) than in FB-t (up to 112%) (Fig. 4b).

The relative amount of DOC utilized during the incubation differed between the treatments (data not shown). There was a trend of higher consumption in the freshwater and seawater controls (22 and 13% of the initial DOC concentration, respectively) than in the corresponding transplanted treatments (9 and 3.5%, respectively).

The apparent bacterial richness (i.e. the number of bands on DGGE gels) during the incubation decreased in all treatments and this effect was detectable after 43 h or less (Fig. 5). There were significant differences between the transplanted treatments and their respective controls (t-test, p < 0.05). Both transplanted treatments showed a continuous decrease in apparent richness, whereas it stabilized and even slightly increased towards the end in both control incubations. The largest loss in apparent richness occurred in SB-t, where a decrease of 16 bands (41%) at the endpoint of the experiment was observed, compared to 13 bands in FB-t and only 6 in both controls.

Transplantation effects, whether negative or positive, were always larger for SB-t than for FB-t. The relative intensity of bands differed strongly during the confinement but varied also between treatments (Fig. 6). For example, band 1 decreased from around 10% of the total at the start of the experiment to less than 5% towards the end of the experiment in most treatments. The relative abundance of bands 21, 23 and 27 was typically less than 5% at the start of the experiment, and increased over the course of the experiment, with band 21 showing large increases (up to almost 30%). Interestingly, the intensity of these 3 bands in the FB-c remained comparatively stable throughout the experiment.

Viral dynamics

The initial viral abundance was higher in the freshwater-diluted treatments (FB-c and SB-t) than in the seawater treatments (SB-c and FB-t), accounting for 10×10^7 and 1.5×10^7 particles ml^{-1}, respectively (Fig. 7a). This is in comparison to *in situ* values of 11.1 and 1.3×10^7 particles ml^{-1} for Stns FW and SW, respectively (Table 1). Contrasting viral dynamics were observed between the treatments diluted with freshwater filtrate (FB-c and SB-t) and those diluted in seawater filtrate (SB-c and FB-t). The abundance of freshwater viruses in FB-c and SB-t varied more over time than the abundance of seawater viruses in SB-c and FB-t (Fig. 7a), and strongly decreased during the first day of incubation (by 33 and 42% in FB-c and SB-t, respectively). At the end of the experiment, the viral abundance in FB-c and SB-t was slightly lower than at time T_0 (15%). In FB-t, viral abundance at the endpoint was similar to T_0, while in SB-c it was 60% higher than the initial values.

Although viral production exhibited similar patterns during the control incubations, it was 43 to 235% higher in FB-c than in SB-c. Total viral production over the entire experiment was lower than
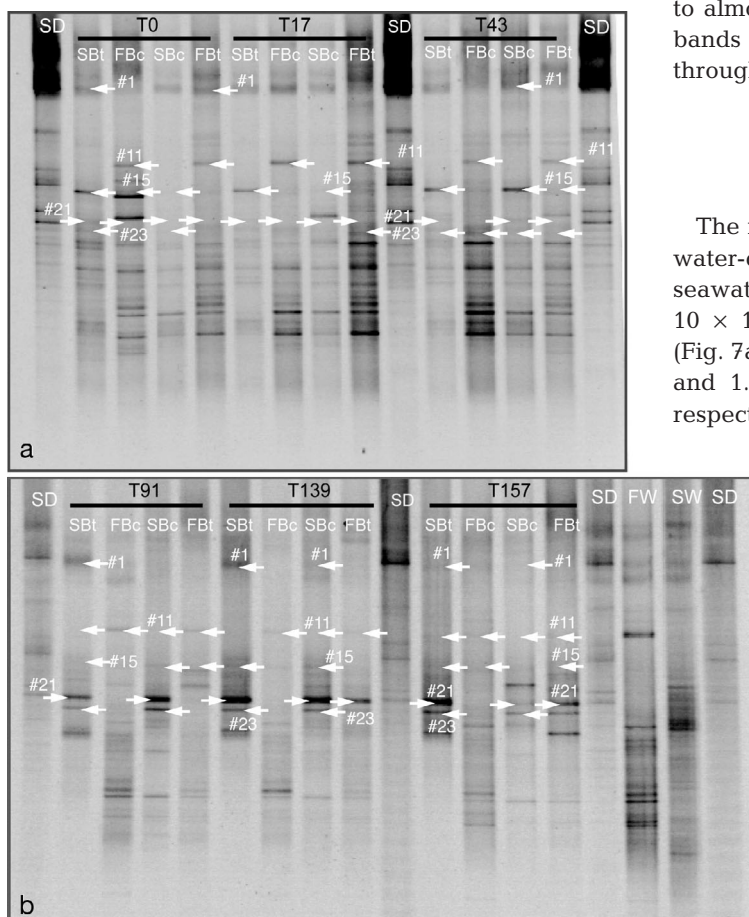

Fig. 5. DGGE gels from the incubations. Each lane is labeled with the incubation and the time point (in hours); bands discussed in the text are also noted. (a) Time points T_0 to T_{43}; (b) T_{91} to T_{157}. SD: standard; FW: freshwater; SW: seawater
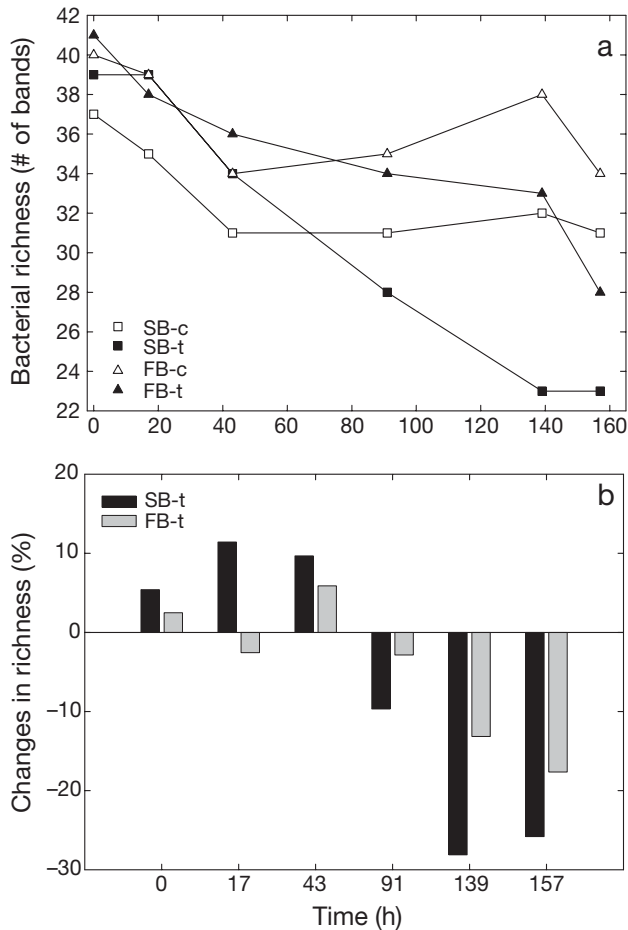
Fig. 6. (a) Bacterial richness and (b) changes in richness in the 4 treatments

decay in FB-c, FB-t and SB-t, and viral production significantly exceeded the decay rate in SB-c. By comparing the viral dynamics in FB-t and SB-t relative to the SB-c and FB-c, respectively, it is possible to evaluate the response of freshwater and marine viruses to the presence of a non-indigenous bacterial community (Fig. 7b). Despite initially high viral production values, viral production was repressed by up to 190% in the FB-t incubation relative to SB-c. In contrast, the transplantation of a seawater bacterial community (SB-t) into a freshwater viral community resulted in a strong stimulation of viral production (up to 840% after 91 h).

The percentage of bacterial cells lysed per day can be estimated from the net increases in viral abundance in the incubations using the assumption of a burst size of 50 viruses, which represents high values from the North Sea (Winter et al. 2004a). Bacterial mortality due to viruses was higher in FB-c than in SB-c (23 and 12% cells lysed d^{-1}, respectively), and while transplantation of seawater bacteria increased virus-induced mortality to 52% d^{-1}, the transplan-
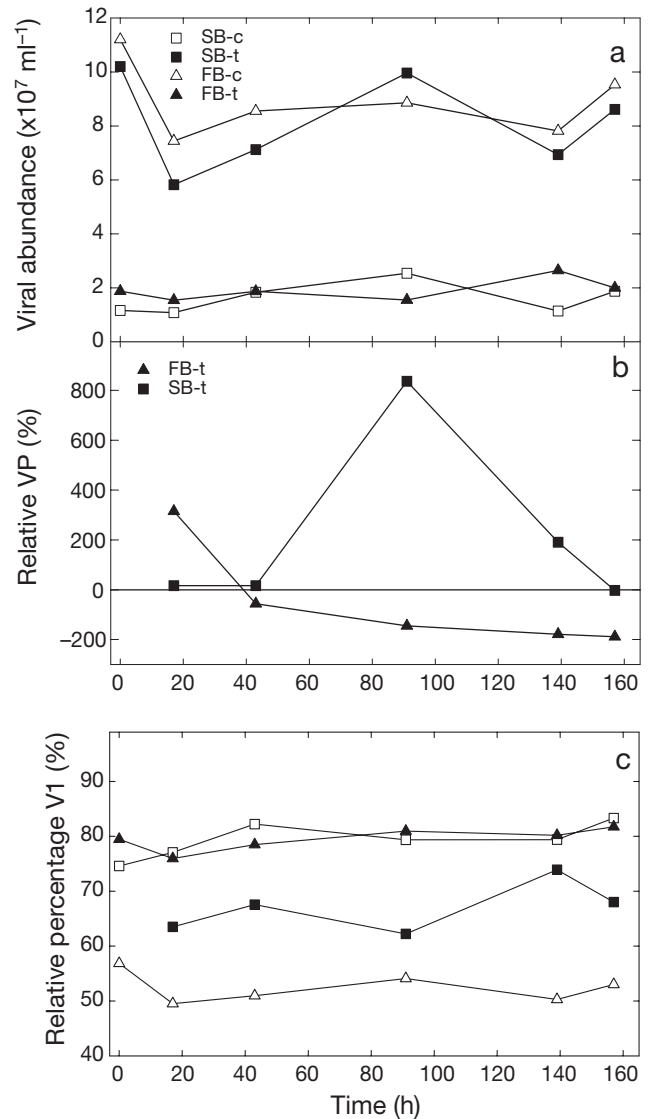

Fig. 7. (a) Viral abundance, (b) relative viral production (VP) and (c) relative percentage of V1 (low fluorescence group) in the different treatments across the experiment. Relative VP is expressed as a percentage of the respective viral control (SB-c for FB-t, FB-c for SB-t); relative percentage of V1 is expressed as the percent total abundance

tation of freshwater bacteria decreased mortality to 10% d^{-1}.

Most viruses (50 to 83%) were from the low fluorescence group (V1). However, the relative contribution of V1 to the total abundance differed between treatments (Fig. 7c). Over the course of the experiment, the contribution of the V1 group varied little in the SB-c and FB-t treatments. This is in contrast to the SB-t incubation, where the proportion of the V1 group was between that of FB-c (virus source) and SB-c (bacteria source), and exhibited an increasing trend towards the end of the incubation.

DISCUSSION

Metabolic and structural response of bacterial communities to transplantation

The present study investigates how the metabolism and diversity of estuarine and riverine bacteria respond to transplantation and exposure to non-indigenous virus communities. Results show that transplantation increased both BP and bacterial carbon demand in seawater as well as freshwater bacterial communities. This response is concomitant with a decrease in apparent bacterial richness, and suggests that bacteria were able to grow under allochthonous sources of DOM, supporting the conclusions of a previous study carried out in a Danish estuary (Rochelle-Newall et al. 2004). The data also show that transplantation stimulated total virus production and virus-induced mortality in SB-t but repressed it in FB-t.

It is well established that incubations can significantly affect bacterial community composition as well as activity parameters (Massana et al. 2001, Gattuso et al. 2002, Winter et al. 2004b); however, incubations are often the only possible approach to investigate ecological and biogeochemical issues. In the present study, the decrease in bacterial richness in the control incubations reflects both a confinement effect and the impact of the initial dilution. The decline of richness was similar in the 2 controls, where 6 bands were lost by the end of the experiment. In contrast, band loss was more than twice as high in the transplanted communities (13 and 16 bands for the FB-t and SB-t communities, respectively). This implies that the negative effects of transplantation on bacterial richness were higher than those of confinement alone. The lower apparent richness is probably related to the higher dominance of a few favored, fast growing phylotypes in the transplanted treatments relative to the controls. Furthermore, the loss of some phylotypes may well have helped to enhance the dominance of faster growing ones, such as can be observed in the SB-t incubation with the loss of band 15 and the increase in band 23 intensity throughout the experiment (Fig. 6).

These opportunistic species may have taken advantage of the nutrient amendment resulting from the dilution, thus out-competing other members of the assemblage (Massana et al. 2001, Gattuso et al. 2002, Winter et al. 2004b). Also, exposure of seawater bacteria to freshwater DOM could have activated specific ectoenzymes or favored certain members of the community capable of expressing them for hydrolyzing allochthonous DOM (Kirchman et al. 2004). This could explain the decrease in richness and the changes in community composition (Pinhassi et al. 1999).

The increase in BP, BR and bacterial carbon demand in the transplantations indicates that seawater bacteria could readily oxidize freshwater DOC, despite the fact that it has been reported as relatively less labile than marine DOC (del Giorgio & Davis 2003). This argument, combined with the relatively long residence time in the Scheldt estuary (1 to 3 mo; Wollast 1988), suggests that it is unlikely that there was an export of a labile freshwater DOC fraction that had not been completely taken up by the riverine bacteria as observed in other areas (Kirchman et al. 2004).

The higher metabolic activity and larger decrease in bacterial richness observed in SB-t compared to FB-t may also result from the different virus-induced bacterial mortality. Indeed, virus-induced mortality was almost 5 times higher in the SB-t than in the FB-t. Moreover, although the change in salinity experienced by the FB-t treatment could have had a negative effect on the bacterial communities, there was no salinity effect for the seawater bacteria transplanted into freshwater DOM as salinity was adjusted.

Viral dynamics

The experimental setup (dilution of bacterial communities with <0.2 µm filtered water) artificially increased the initial virus to bacteria ratio; it was 5 to 9 times higher in the controls than in the corresponding inoculum increasing by a factor of 9 in SB-t, but being 5 times lower in FB-t compared to FB-c. The FB-t bacteria therefore experienced reduced viral pressures relative to FB-c, whereas SB-t bacteria experienced a stronger viral pressure than SB-c.

It has been shown that viral communities from different parts of an estuary exhibit pronounced differences in their genotypic composition (Wommack et al. 1999). Thus, the viral communities in the freshwater and seawater parts of the estuary should differ; this is potentially supported by the difference in the proportions of viral groups, as measured by fluorescence, between the 2 environments. Differences between the FB-c and SB-c treatments potentially suggest the presence of different viral communities in seawater and freshwater. These differences in relative proportion of low fluorescence viruses were also evident in the FB-t and SB-t incubations, reflecting the seawater and freshwater viral sources, respectively. The transplantation of seawater bacteria into a freshwater viral community resulted in large changes in the relative proportion of the low fluorescence virus group, which may suggest that the seawater bacterial community produced viruses different from those produced by the indigenous freshwater bacterial community. However, this remains to be clarified in a more rigorous manner.

Nevertheless, this hypothesis is also supported by the fact that the SB-t transplantation resulted in an elevated viral production rate (Fig. 7b). Thus, it is possible that the transplanted seawater bacteria were infected by viruses of the freshwater community. Similar observations have been made by Sano et al. (2004).

Interestingly, this pattern did not seem to hold for the seawater viruses in the FB-t incubation. Although viral production was high in the first hours, viral production was much lower in the transplanted incubation than in the control. This is suggestive of increased survival of transplanted bacteria relative to the control, and points towards the hypothesis that the freshwater bacteria did not act as hosts for the seawater viruses. Although the mechanisms for the lower viral production of the freshwater bacteria are not clear, the salinity effects of transplanting freshwater bacteria into virus-containing seawater may play a non-negligible role in altering the virus–bacteria encounter and infection rates.

It should be noted that we compared treatments with similar initial abundances (FB-t with SB-c and SB-t with FB-c). Within these comparisons, total contact rate between viruses was 60% higher in the treatment with freshwater bacteria, since initial bacterial abundance was higher in freshwater and the same % dilution was used for freshwater and seawater bacteria in the experiments. This could result in higher viral production rates by the freshwater bacteria (Murray & Jackson 1992). However, we found the opposite trend, i.e. transplantation of seawater bacteria into freshwater resulted in higher net viral production than for the freshwater bacteria, whereas transplantation of freshwater bacteria into seawater resulted in lower net viral production than the seawater control (Fig. 7b).

Viral production could be detected in the transplanted freshwater and the transplanted seawater bacterial communities (Fig. 7a). Several non-mutually exclusive mechanisms could explain the production of viruses in transplanted bacterial communities. Firstly, the host range could be broader than previously assumed, allowing viruses to infect hosts from different environments. For example, Jensen et al. (1998) have argued that the concept, derived from isolated virus–host systems, that viruses do not trespass the genus barrier (Ackerman & Dubow 1987) is an isolation artifact. This is also supported by Chiura (1997), who has shown that marine viruses can infect *Escherichia coli*. Secondly, transplantation into a different environment (e.g. with a large change in salinity) could cause induction of lysogenic cells (Jiang & Paul 1996). The enhanced growth rates (Figs. 3 & 4) could have acted as inducing agent (Weinbauer 2004) and caused the virus production observed. Finally, it is possible that cosmopolitan bacterial phylotypes that can grow and produce viruses under both freshwater and marine conditions exist. Indeed, several identical bands were found in both environments and all treatments. Although there is no definitive evidence to support the hypothesis that freshwater viruses can infect seawater bacteria and vice versa, it is clear that transplanted bacteria were able to produce viruses in the new environment, at least in the freshwater virus incubations. Moreover, as we found a strong increase in viral production, it is clear that these viruses came from either the transplanted community (by induction) or by new infection from the original viral community. Obviously, our estimates of viral production and decay can only be considered as net changes and so must be viewed as conservative estimates. Nevertheless, it is clear that these estimates were different between the different incubations (e.g. strong stimulation in SB-t and strong repression in FB-t) and so it is probable that there were real differences in viral production and decay between the different treatments. To conclude, although the freshwater viral community seems to be able to infect seawater hosts, the converse did not appear to occur.

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