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

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29 **Abstract**

30

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

44 **Introduction**

45 Substrate availability has been extensively studied as a potentially important factor
46 controlling prokaryotic activity. It is well known that the quality and quantity of inorganic and
47 organic nutrients can exert significant control on prokaryotic structure and function (e.g. Azam
48 and Malfatti (2007)). In coastal areas, and particularly in estuarine systems, large variations in
49 nutrient and organic carbon concentration can occur over relatively small distances. For
50 example, in Randers Fjord, a small estuary (27 km long) in Northern Denmark, concentrations in
51 inorganic and organic nitrogen increase by 1 to 3 orders of magnitude between the seawater and
52 freshwater end-members, respectively (Veuger et al. 2004). Similarly, dissolved organic carbon
53 concentrations also vary along estuarine gradients with generally higher concentrations in the
54 freshwater end-members and lower concentrations in the seawater end-members (Fisher et al.
55 1998, Abril et al. 2002). Superimposed upon these gradients, biological processes both modify
56 and are modified by geochemical processes.

57 The dynamic nature of estuaries means that solutes and organic matter from the
58 freshwater and seawater end-members become mixed along the estuarine gradient. The manner
59 in which these inputs mix is related to the relative proportions of each input, as well as to other
60 physical properties (tidal regime, winds, etc). Therefore, communities of bacteria and other
61 planktonic organisms are exposed to strong physicochemical gradients and constantly varying
62 environmental conditions along the length of the estuary. Moreover, in estuaries where
63 freshwater inputs are relatively low compared to that of seawater inputs, such as in the Scheldt
64 estuary, bacteria and other organisms experience a situation where salinity changes are reduced
65 but where relatively large changes in dissolved organic matter (DOM) and other solutes can
66 occur. This results in the exposure of seawater bacteria to freshwater DOM. The reverse side of
67 this, of course, is that in these systems with high seawater to freshwater inputs, freshwater
68 bacteria will be subject to the dual problems of changing salinity and DOM through mixing with

69 seawater. Estuaries are therefore attractive systems to address the question of substrate
70 availability as a controlling factor of prokaryotic activity (Jones et al. 2007).

71 Strong compositional and metabolic changes have been shown to occur in bacterial
72 communities between the freshwater and saltwater portions of estuaries (Bouvier & del Giorgio
73 2002, Kirchman et al. 2004) and several authors have proposed the existence of a unique
74 estuarine community that is found in the mixing zone (e.g. Crump et al. 2004). Indeed, the
75 domination of a unique estuarine community *vs.* a mixing community in the middle estuary
76 largely depends on the water residence time (Bouvier & del Giorgio 2002, Crump et al. 2004,
77 Kan et al. 2006). However, regardless of water residence time, the instability of the environment
78 is accompanied by changes in bacterial metabolism with bacterial cells more dedicated to
79 physiological maintenance than cell production (del Giorgio & Bouvier 2002). Moreover, a
80 recent meta-analysis of bacterial diversity data has shown that salinity plays an important role in
81 structuring bacterial communities (Lozupone & Knight 2007) and it also seems to be a
82 determining factor in carbon substrate utilisation in estuaries (Thottathil et al. 2008).

83 Although several studies have recently investigated bacterial responses to changes in the
84 supply of organic matter in estuaries (Stepanauskas et al. 1999, Findlay et al. 2003, Langenheder
85 et al. 2004), few have considered the impact of the associated variation of the coexisting viral
86 communities, another key factor controlling bacterial dynamics. It is now well established that
87 virioplankton exerts a “top-down” pressure and is a significant mortality agent for heterotrophic
88 bacteria. Virioplankton also plays a significant role in shaping the composition and controlling
89 the diversity of its hosts (Thingstad 2000, Wommack & Colwell 2000) and up to 50% of
90 bacterial mortality can be due to viral activity (Fuhrman & Schwalbach 2003, Weinbauer 2004,
91 Bouvier & del Giorgio 2007). Viral activity therefore has important effects on bacterial
92 processes in aquatic systems. Over and above the direct effect of viruses on infected prokaryotic
93 cells, viral lysis can enhance the activity of non-infected prokaryotic cells, which benefit from

94 the release of organic matter by cell lysis (Middelboe et al. 1996). Recent work also highlighted
95 the complexity of the interactions between viral and bacterial activity and showed that viral lysis
96 does not always result in increased bacterial growth efficiency (Bonilla-Findji et al. 2008). These
97 authors reported that BR was stimulated (up to 113%) in the presence of active viruses whereas
98 BP and BGE were reduced (up to 51%) and suggested that viruses enhance the role of bacteria as
99 oxidizers of organic matter and as producers of CO₂.

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

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

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118 **Methods**

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

127 Bacteria and viruses from the two end-members of the estuary were inoculated in filtered
128 water from the other site in a 1:18 volume ratio (Fig. 2). At each station, 18 L of $< 0.2 \mu\text{m}$ filtrate
129 water were distributed into two 20 L, acid washed and Milli-Q rinsed polycarbonate carboys and
130 then inoculated with 1 L of either unfiltered seawater or unfiltered freshwater in order to obtain
131 four treatments: transplanted seawater bacteria (SB-t), seawater control (SB-c); transplanted
132 freshwater bacteria (FB-t) and freshwater control (FB-c). The salinity of the freshwater filtrate
133 was adjusted to 30 with an artificial seawater (ASW; Guillard 1975) mix to minimize any effect
134 of the salinity on the seawater bacterial community.

135 The four treatments were incubated for 6 d in the dark and at *in situ* temperature (7.6°C)
136 and sub-samples were collected for bacteria and viral counts, bacterial production and diversity
137 at six time points (0h; 17h; 43h; 91h; 139h; 157h). Samples for determination of dissolved
138 organic carbon (DOC) were taken at the beginning and end of the experiment.

139
140 *Sample analyses.* Viral abundance was measured in duplicate, 1 ml samples fixed with
141 glutaraldehyde (0.5% final concentration, EM-grade; Merck) for 30 min at 4°C in the dark, flash

142 frozen in liquid nitrogen and stored at -80°C pending analysis by flow cytometry as described by
143 Brussaard (2004).

144 Viral counts were performed with the CellQuest-Pro software (Becton Dickinson and
145 Company) after staining with SYBR GREEN I (Invitrogen: S-7567) that was pre-diluted in 0.2
146 μm filtered, autoclaved Milli-Q (final dilution of 5×10^{-5} the commercial stock). Three viral
147 groups were discriminated from scatter plots of side scatter (SSC) versus green fluorescence
148 (FL1). These groups were labeled according to their increasing fluorescence signal: V1 (low),
149 V2 (medium) and V3 (high). Previous comparisons (including the studied environment) of the
150 total counts obtained by this method with viral abundances determined by microscopy were very
151 similar and differences were generally less than 20% (O. Bonilla-Findji, unpublished). Viral
152 production and decay was calculated from the net changes in viral abundance between each time
153 point (Bratbak et al. 1990). The values obtained should therefore be considered as conservative
154 estimations of viral production and decay.

155 Bacterial abundance was measured by a direct count method using epifluorescence
156 microscopy and DAPI-stained samples (Porter & Feig 1980). Inspection of filters during
157 enumeration did not reveal that flagellates were present in significant numbers. Bacterial
158 production was estimated from ^3H -leucine incorporation in accordance with the method of
159 Kirchman (1992) and as previously described elsewhere (Rochelle-Newall et al. 2004). Bacterial
160 respiration was calculated based on standard dark-bottle O_2 consumption rates. Replicate BOD
161 bottles were incubated in the dark following the JGOFS protocol (Knap et al. 1996) and at
162 distinct time points, oxygen concentration was titrated using an automated Winkler titration
163 technique with a potentiometric end-point detection (Anderson et al. 1992). The respiration rate
164 at each time point T and its standard error were determined by regressing O_2 concentration
165 against time during the intervals of incubation T to $T+I$.

166 Due to the difference in the time scales of the measurement of bacterial production (1h) and

167 bacterial respiration (17 to 48 h), bacterial carbon demand (= bacterial production + bacterial
168 respiration) was calculated using bacterial respiration, expressed in carbon units assuming a
169 respiratory quotient of 1. Mean bacterial production was determined during the same time
170 interval as used to measure the rate of respiration using $((BP_{T1} + BP_{T2})/2)$, BP_{T1} and BP_{T2} are
171 bacterial production at the start and end of the respiration measurement, respectively.

172 Samples (10ml) for determination of dissolved organic carbon were filtered through
173 combusted (450°C, overnight) glass fiber filters (Whatman GF/F) and sealed in pre-combusted
174 (450°C for 4h) glass ampoules after adding 12 μ l of 85% v/v phosphoric acid (H_3PO_4). Samples
175 were stored in the dark at 4°C pending analysis. DOC concentration was determined as
176 previously described (Rochelle-Newall et al. 2007) by the high temperature combustion method
177 using a Shimadzu TOC-5000 analyzer. Certified reference materials (Hansell Laboratory,
178 University of Miami) were also used to assess the performance of the instrument on and between
179 measurement days (Sharp 2002).

180 For extraction of DNA from prokaryotic cells 50 to 150 ml samples were recovered on a
181 0.2 μ m pore-size polycarbonate filter (47 mm diameter; Whatman) and kept frozen at $-80^\circ C$
182 pending analysis. Nucleic acids were extracted from the filters and purified as described
183 elsewhere (Winter et al. 2001, Winter et al. 2004b). In contrast to the phenol-chloroform
184 extraction step from the original protocol, nucleic acids were extracted with 4.5 M NaCl and
185 chloroform. This modified procedure avoids manipulation of a toxic chemical and yields
186 fingerprints identical to those obtained by the original method (data not shown).

187 PCR conditions and chemicals were as described in Schäfer et al. (2001). Briefly, 1 to 4 μ l
188 of the nucleic acid extracts were quantified on an agarose gel using a DNA mass ladder
189 (EasyLadder I; Bioline #BIO-33045). When sufficient DNA was detected it was used in 50 μ l
190 PCR reactions (1.5 mmol L⁻¹ MgCl₂, 0.25 μ mol L⁻¹ of each primer and 2.5U *Taq* polymerase;

191 Sigma; #D 5930) together with a positive and a negative control. A fragment of the 16S rRNA
192 gene was amplified using the bacterial primer pairs 341F-GC/ 907R (Schäfer et al. 2001).

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

202 The Statgraphics Centurion XV software package (Statpoint Inc, USA) was used to test
203 the relationships between the treatments and respective controls. After verifying that
204 assumptions were met (Shapiro-Wilks' test), t-tests were used to determine if the effect of
205 transplantation was significant relative to the appropriate control. Significance is given at the $p <$
206 0.05 level.

207

208 **Results**

209 The *in situ* physico-chemical and biological characteristics of the two sampling stations
210 differed considerably. The freshwater (FW) station exhibited higher bacterial abundance,
211 production, and richness, viral abundance and dissolved organic carbon concentration than in the
212 seawater (SW) station (Table 1).

213

214 **Bacterial dynamics.** The initial bacterial abundance in the incubations was 5.2 and 8.3 x
215 10^5 ml^{-1} in the seawater and the freshwater bacterial treatments, respectively (Fig. 3A). This was

216 in comparison to an *in situ* abundance of 7.4×10^6 and $3.1 \times 10^6 \text{ ml}^{-1}$ for the freshwater and
217 seawater stations, respectively (Table 1). Cell numbers continuously increased during the
218 experiment and reached values 2 to 3 times higher at the end of the incubation. This resulted in
219 maximum abundances that were always less than the *in situ* values by a factor of 2-3 (Table 1,
220 Fig. 3A). The only exception was in the seawater control treatment (SB-c), where bacterial
221 abundance declined to initial values at the endpoint.

222 Bacterial production increased throughout the incubation although controls and
223 transplanted treatments showed different patterns (Fig. 3B). While a lag period occurred in the
224 transplanted treatments, it greatly increased in the controls during the first 36 h. This trend
225 changed thereafter and bacterial production strongly increased and was up to 3-fold higher in the
226 transplanted treatments than in the controls. Thus, although bacterial production was low in the
227 transplantations in the short term (<36h), a two-fold stimulation was observed after 48 h in SB-t
228 and FB-t. At the end of the experiment the stimulation of bacterial production was nearly 3-fold
229 higher in SB-t than in FB-t.

230 During the first 91h of the incubation, bacterial respiration was significantly lower in the
231 transplanted treatments, relative to their respective controls (t-test, $p < 0.05$). During the second
232 part of the incubation (> 91h), respiration generally decreased as a function of time in all
233 treatments (Fig. 4A). Across the 7 day incubation period, respiration rates were statistically
234 significantly lower in FB-t than in FB-c (t-test, $p < 0.05$). This is in contrast to the seawater
235 bacterial transplantation (SB-t) and control (SB-c) where no significant difference (t-test,
236 $p > 0.05$) in respiration was observed over the 7-day incubation period.

237 Bacterial carbon demand, which is the sum of BR and BP was relatively stable
238 throughout the control incubations (ranging from 0.10 to $0.48 \mu\text{mol C L}^{-1} \text{ h}^{-1}$) and increased
239 slightly at the end of the experiment (data not shown). In contrast, in the transplanted treatments,
240 it was initially close to zero ($0.01 \mu\text{mol C L}^{-1} \text{ h}^{-1}$) but greatly increased after 2 d (up to $0.88 \mu\text{mol}$

241 C L⁻¹ h⁻¹) until the end of the experiment (data not shown). The stimulation of bacterial carbon
242 demand was stronger in SB-t (up to 239%) than in FB-t (up to 112%; Fig. 4B).

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

247 The apparent bacterial richness (i.e. the number of bands on DGGE gels) during the
248 incubation decreased in all treatments and this effect was detectable after 43 h or less (Fig. 5).
249 There were significant differences between the transplanted treatments and their respective
250 controls (t-test, $p < 0.05$). Both transplanted treatments showed a continuous decrease in apparent
251 richness whereas it stabilized and even slightly increased towards the end in both control
252 incubations. The largest loss in apparent richness occurred in SB-t, where a decrease of 16 bands
253 (41%) at the endpoint of the experiment was observed, compared to 13 in FB-t and only 6 in
254 both controls.

255 Transplantation effects, whether negative or positive, were always larger for SB-t than for
256 FB-t. The relative intensity of bands differed strongly during the confinement but varied also
257 between treatments (Fig. 6). For example, band no. 1 decreased from around 10% of the total at
258 the start of the experiment to less than 5% towards the end of the experiments in most
259 treatments. The relative abundance of bands 21, 23 and 27 was typically less than 5% at the start
260 of the experiments increased over the course of the experiment, with band 21 showing large
261 increases (up to almost 30%). Interestingly, in the FB-c the intensity of these three bands
262 remained comparatively stable throughout the experiment.

263

264 ***Viral dynamics.*** The initial viral abundance was higher in the freshwater-diluted
265 treatments (FB-c and SB-t) than in the seawater treatments (SB-c and FB-t) accounting for 10 x

266 10^7 and 1.5×10^7 particles ml^{-1} , respectively (Fig. 7A). This is in comparison to *in situ* values of
267 11.1 and 1.3×10^7 particles ml^{-1} for the freshwater and seawater station, respectively (Table 1).
268 Contrasting viral dynamics were observed between the treatments diluted with freshwater filtrate
269 (FB-c and SB-t), and the treatments diluted in seawater filtrate (SB-c and FB-t). The abundance
270 of freshwater viruses in FB-c and SB-t, varied more over time than the abundance of seawater
271 viruses in SB-c and FB-t, (Fig. 7A) and strongly decreased during the first day of incubation (by
272 33% and 42% in FB-c and SB-t, respectively). At the end of the experiment, the viral abundance
273 in FB-c and SB-t was slightly lower than at T_0 (15%). In FB-t viral abundance at the endpoint
274 was similar to T_0 , while in SB-c it was 60 % higher than the initial values.

275 Although viral production exhibited similar patterns during the control incubations, it was
276 43% to 235% higher in FB-c than in SB-c. Total viral production over the entire experiment was
277 lower than decay in FB-c, FB-t and SB-t and viral production significantly exceeded the decay
278 rate only in SB-c. By comparing the viral dynamics in FB-t and SB-t, relative to the seawater
279 and freshwater controls (i.e. FB-t compared to SB-c and SB-t compared to FB-c), respectively, it
280 is possible to evaluate the response of freshwater and marine viruses to the presence of a non-
281 indigenous bacterial community (Fig. 7B). Despite initially high viral production values, viral
282 production was repressed by up to 190% in the FB-t incubation, relative to the seawater control
283 (SB-c). In contrast, the transplantation of a seawater bacterial community (SB-t) into freshwater
284 viral community resulted in a strong stimulation of viral production (up to 840% after 91h).

285 The percentage of bacterial cells lysed per day can be estimated from the net increases in
286 viral abundance in the incubations and assuming a burst size of 50 viruses, which represents high
287 values from the North Sea (Winter et al. 2004a). Bacterial mortality due to viruses was higher in
288 FB-c than in SB-c (23% and 12% cells lysed per day, respectively) and while transplantation of
289 seawater bacteria increased virus-induced mortality to $52\% \text{ d}^{-1}$, the transplantation of freshwater
290 bacteria decreased mortality to $10\% \text{ d}^{-1}$.

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

297 **DISCUSSION**

298 *Metabolic and structural response of bacterial communities to transplantation.*

299 This study investigates how the metabolism and diversity of estuarine and riverine
300 bacteria responds to transplantation and to exposure to non-indigenous virus communities. The
301 results show that transplantation increased both bacterial production and carbon demand in
302 seawater as well as freshwater bacterial communities. This response is concomitant with a
303 decrease in apparent bacterial richness. It suggests that bacteria were able to grow under
304 allochthonous sources of dissolved organic matter supporting the conclusions of a previous study
305 carried out in a Danish estuary (Rochelle-Newall et al. 2004). The data also show that
306 transplantation stimulated total virus production and virus-induced mortality in SB-t but
307 repressed it in FB-t.

308 It is well established that incubations can significantly affect bacterial community
309 composition as well as activity parameters (Massana et al. 2001, Gattuso et al. 2002, Winter et
310 al. 2004b). However, incubations are often the only possible approach to investigate ecological
311 and biogeochemical issues. In the present study, the decrease in bacterial richness in the control
312 incubations reflects both a confinement effect and the impact of the initial dilution. The decline
313 of richness was similar in the two controls, where 6 bands were lost by the end of the
314 experiment. In contrast, band loss was more than twice as high in the transplanted communities
315 (13 and 16 bands for the FB-t and SB-t communities, respectively). This implies that the
316 negative effects of transplantation on bacterial richness were higher than those of confinement
317 alone. The lower apparent richness is probably related to the higher dominance of a few favored,
318 fast growing phylotypes in the transplanted treatments relative to the controls. Furthermore, the
319 loss of some of the phylotypes may well have helped to enhance the dominance of faster
320 growing ones, such as can be observed in the SB-t incubation with loss of band 15 and the
321 increase in band 23 intensity throughout the experiment.

322 These opportunistic species may have taken advantage of the nutrient amendment
323 resulting from the dilution thus out-competing other members of the assemblage (Massana et al.
324 2001, Gattuso et al. 2002, Winter et al. 2004b). Also, exposure of seawater bacteria to freshwater
325 dissolved organic matter (FW-DOM) could have activated specific ectoenzymes or have favored
326 certain members of the community capable of expressing them for hydrolyzing allochthonous
327 DOM (Kirchman et al. 2004). This could explain the decrease in richness and the changes in
328 community composition (Pinhassi et al. 1999).

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

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

342
343 ***Viral dynamics.*** The experimental setup (dilution of bacterial communities with $< 0.2 \mu\text{m}$
344 filtered water) artificially increased the initial virus to bacteria ratio. It was 5 to 9 times higher in
345 the controls than in the corresponding inoculum. It increased by a factor of 9 in SB-t but was 5

346 times lower in FB-t as compared to FB-c. The FB-t bacteria therefore experienced reduced viral
347 pressures relative to FB-c whereas SB-t bacteria experienced a stronger viral pressure than SB-c.

348 It has been shown that viral communities from different parts of an estuary exhibit
349 pronounced differences in the genotypic composition (Wommack et al. 1999). Thus, the viral
350 communities in the freshwater and seawater parts of the estuary should differ and this is
351 potentially supported by the difference in the proportions of viral groups, as measured by
352 fluorescence, between the two environments. There were differences between the FB-c and SB-c
353 treatments potentially suggesting the presence of different viral communities in seawater and
354 freshwater. These differences in relative proportion of low fluorescence viruses were also
355 evident in the FB-t and SB-t incubations, reflecting the seawater and freshwater viral sources,
356 respectively. The transplantation of seawater bacteria into a freshwater viral community resulted
357 in large changes in the relative proportion of the low fluorescence virus group which may
358 suggest that the seawater bacterial community produced viruses which differed from viruses
359 produced by the indigenous freshwater bacterial community. However, this remains to be
360 clarified in a more rigorous manner. Nevertheless, this hypothesis is also supported by the fact
361 that the SB-t transplantation resulted in an elevated viral production rate (Fig. 7B). Thus, it is
362 possible that the transplanted seawater bacteria were infected by viruses of the freshwater
363 community.

364 Similar observations have been made by Sano et al. (2004). Interestingly, this pattern did
365 not seem to hold for the seawater viruses in the FB-t incubation. Although viral production was
366 high in the first hours, viral production was much lower in the transplanted incubation than in the
367 control. This is suggestive of an increased survival of transplanted bacteria relative to that of the
368 control and points towards the hypothesis that the freshwater bacteria did not act as hosts for the
369 seawater viruses. Although the mechanisms for the lower viral production of the freshwater
370 bacteria are not clear, the salinity effects of transplanting freshwater bacteria into virus-

371 containing seawater may play a non-negligible role in altering the virus-bacterial encounter and
372 infection rates.

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

382 Viral production could be detected in the transplanted freshwater and the transplanted
383 seawater bacterial community (Fig. 7A). Several non-mutually exclusive mechanisms could
384 explain the production of viruses in transplanted bacterial communities. Firstly, the host range
385 could be broader than previously assumed, allowing viruses to infect hosts from different
386 environments. For example, Jensen et al. (1998) have argued that the concept derived from
387 isolated virus-hosts systems that viruses do not trespass the genus barrier (Ackerman & Dubow
388 1987) is an isolation artifact. This is also supported by Chiura (1997) who has shown that marine
389 viruses can infect *Escherichia coli*. Secondly, transplantation into a different environment (e.g.
390 with a large change in salinity) could cause induction of lysogenic cells (Jiang & Paul 1996).
391 The enhanced growth rates (Fig. 3 and 4) could have acted as inducing agent (Weinbauer 2004)
392 and caused the virus production observed. Finally, it is possible that cosmopolitan bacterial
393 phylotypes that can grow and produce viruses in freshwater and marine conditions exist. Indeed,
394 several identical bands were found in both environments and all treatments. Although there is no
395 definitive evidence to support the hypothesis that freshwater viruses can infect seawater bacteria

396 and vice versa, it is clear that transplanted bacteria were able to produce viruses in the new
397 environment, at least in the freshwater virus incubations. Moreover, as we found a strong
398 increase in virus production, it is clear that these viruses came from either the transplanted
399 community (by induction) or by new infection from the original viral community. Obviously,
400 our estimates of viral production and decay can only be considered as net changes and so must
401 be viewed as conservative estimates. Nevertheless, it is clear that these estimates were different
402 between the different incubations (e.g. strong stimulation in SB-t and strong repression in FB-t)
403 and so it is probable that there were real differences in viral production and decay between the
404 different treatments. To conclude, although the freshwater viral community seems to be able to
405 infect seawater hosts, the converse did not appear to occur.

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 557 **Table 1.** Physico-chemical and biological characteristics of surface water sampled at the
 558 freshwater (FW) and seawater (SW) ends of the Scheldt estuary. DOC: dissolved organic
 559 carbon \pm standard deviation. BP: bacterial production \pm standard deviation. DGGE:
 560 Denaturing Gradient Gel Electrophoresis.

561

	Stations	
	FW	SW
562 Temperature ($^{\circ}$ C)	11.04	7.73
563 Salinity	1.20	30.4
564 DOC (μ mol l $^{-1}$)	427 \pm 1.9	195.0 \pm 1.6
Virus (x 10 7 ml $^{-1}$)	11.1	1.3
Bacteria (x 10 6 ml $^{-1}$)	7.4	3.1
BP (μ mol C l $^{-1}$ h $^{-1}$)	0.255 \pm 0.006	0.070 \pm 0.002
Bacterial richness (# of DGGE bands)	40	37

565 **Figure legends**

566

567 **Figure 1.** Map of the estuary and sample sites

568

569 **Figure 2.** Experimental set-up. Seawater or Freshwater (60 L) was filtered sequentially through
 570 GF/F (Whatman) filters and 0.2 μ m Durapore (Millipore) cartridge filters. 18 L of each filtrate
 571 was dispatched into four 20 L polycarbonate carboys. 1 L of unfiltered freshwater or seawater
 572 was added to each incubation to provide the natural bacterial inoculum. Two transplants and two
 573 controls were used: SB-c: seawater + seawater bacteria; SB-t: freshwater + seawater bacteria;
 574 FB-c: freshwater + freshwater bacteria; FB-t: seawater + freshwater bacteria.

575

576 **Figure 3.** Bacterial abundance (BA; panel A) and production (BP; panel B) in the different
 577 treatments: SB-c: seawater + seawater bacteria; SB-t: seawater bacteria transplanted in
 578 freshwater; FB-c: freshwater + freshwater bacteria; FB-t: freshwater bacteria transplanted in
 579 seawater. In panel B values represent mean \pm standard deviation

580

581 **Figure 4.** Bacterial respiration (BR) in the four treatments during the different phases of the
 582 experiment (panel A). Transplantation effect on bacterial carbon demand (BCD) across the
 583 incubation period in transplanted treatments (SB-t and FB-t) relative to their corresponding
 584 controls (panel B).

585

586 **Figure 5.** DGGE gels from the incubations. Each lane is labeled with the incubation and the time
 587 point. The bands discussed in the text are also noted. Upper image: time points T0 to T43, lower
 588 image T91 to T157. SB-c: seawater + seawater bacteria; SB-t: seawater bacteria transplanted in
 589 freshwater; FB-c: freshwater + freshwater bacteria; FB-t: freshwater bacteria transplanted in
 590 seawater. SD: standard.

591

592 **Figure 6.** Bacterial richness in the four treatments.

593

594 **Figure 7.** Viral abundance (VA; panel A), relative virus production (panel B) and relative % of
 595 V1 (low fluorescence group) in the different treatments across the experiment. Relative virus
 596 production is expressed as a % of the respective viral control (SB-c for the FB-t and FB-c for
 597 SB-t). The relative percentage of V1 is expressed as the % of the total abundance. SB-c:

598 seawater + seawater bacteria; SB-t: transplanted seawater bacteria; FB-c: freshwater +
599 freshwater bacteria; FB-t: transplanted freshwater bacteria.

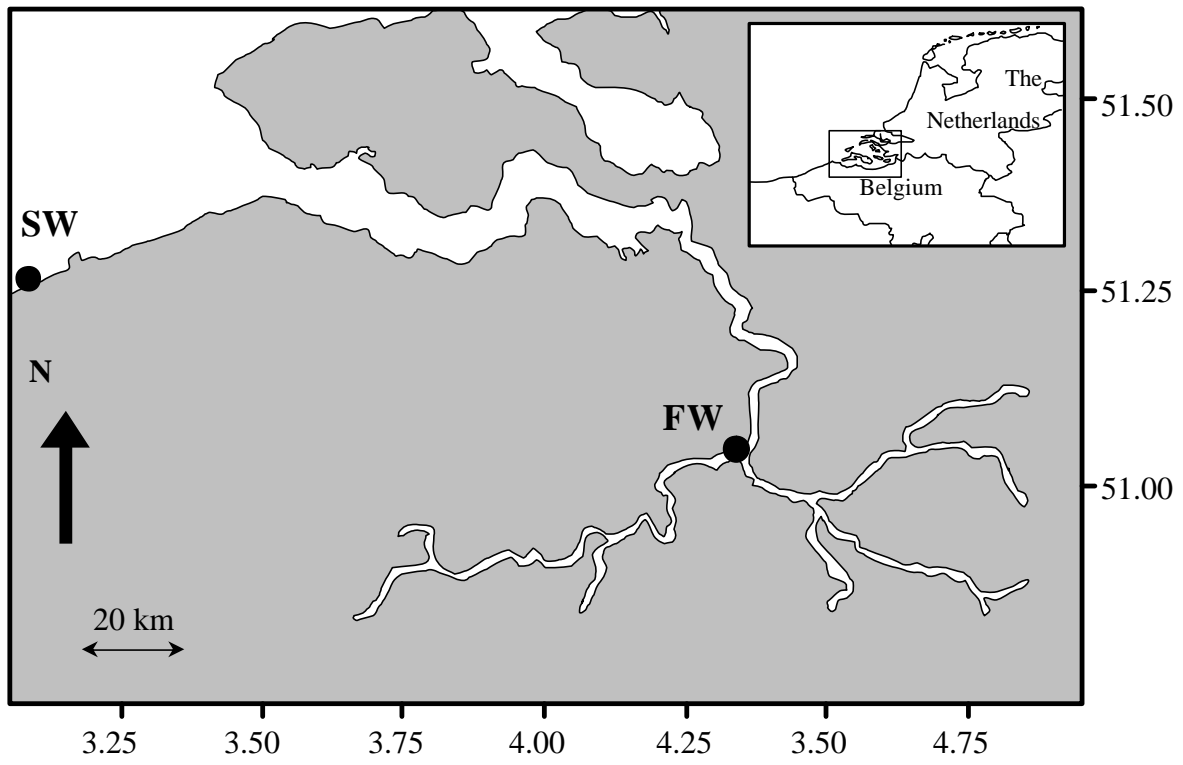


Fig. 1

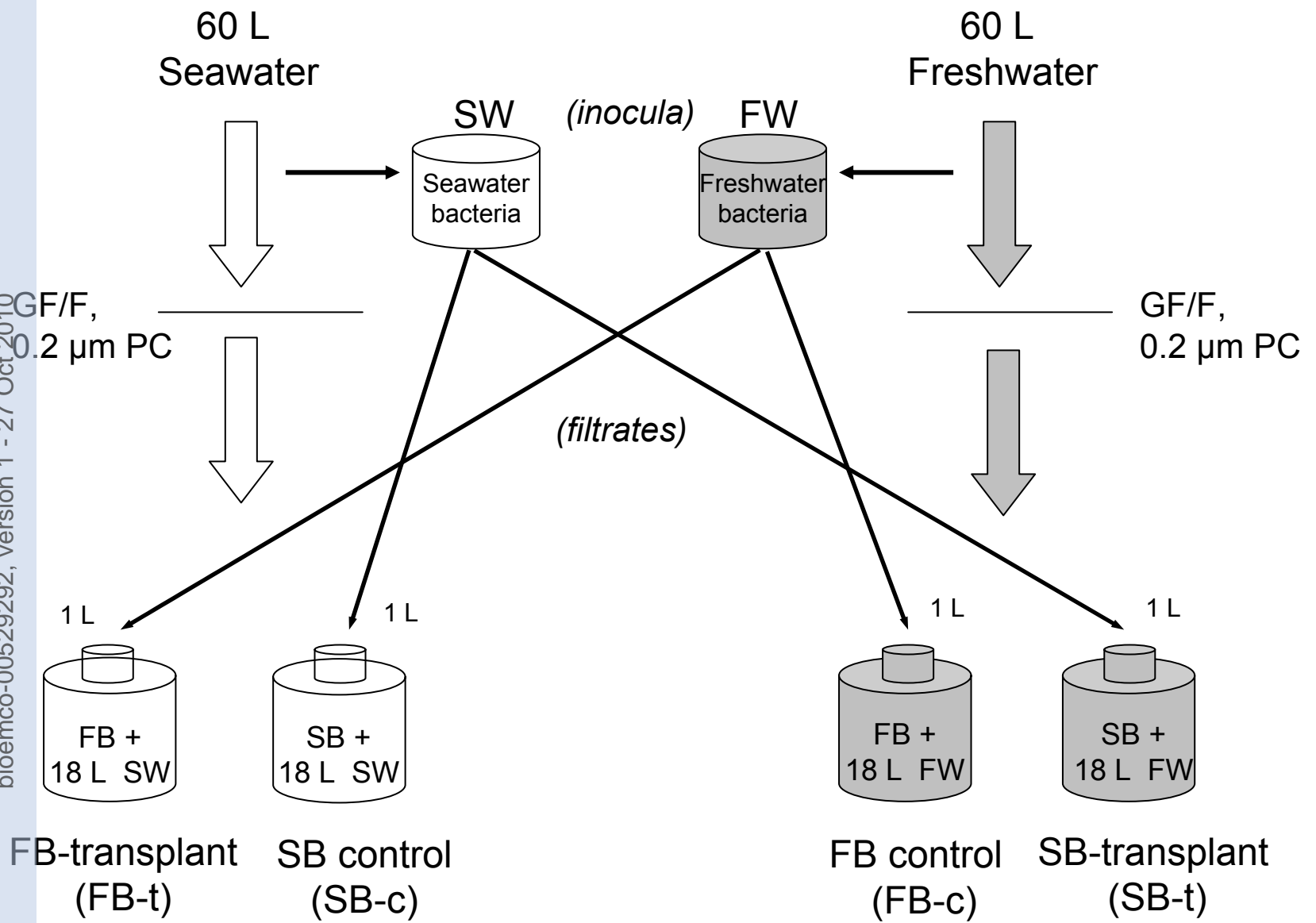


Fig. 2

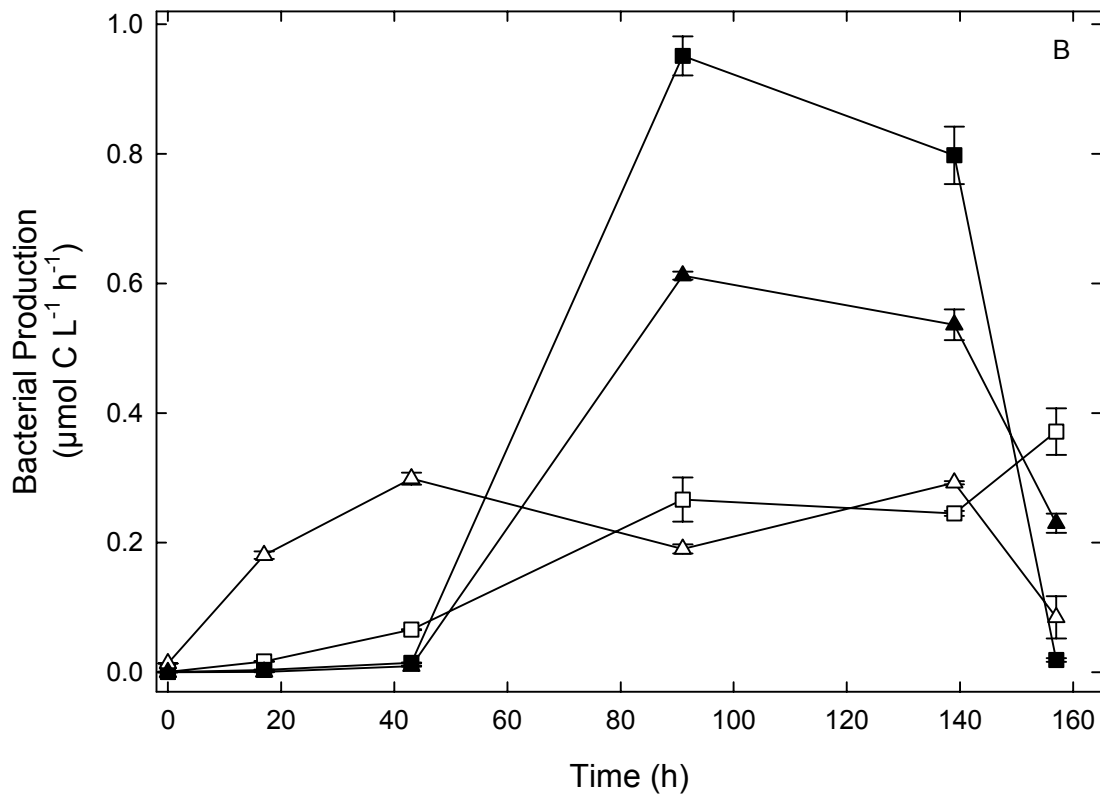
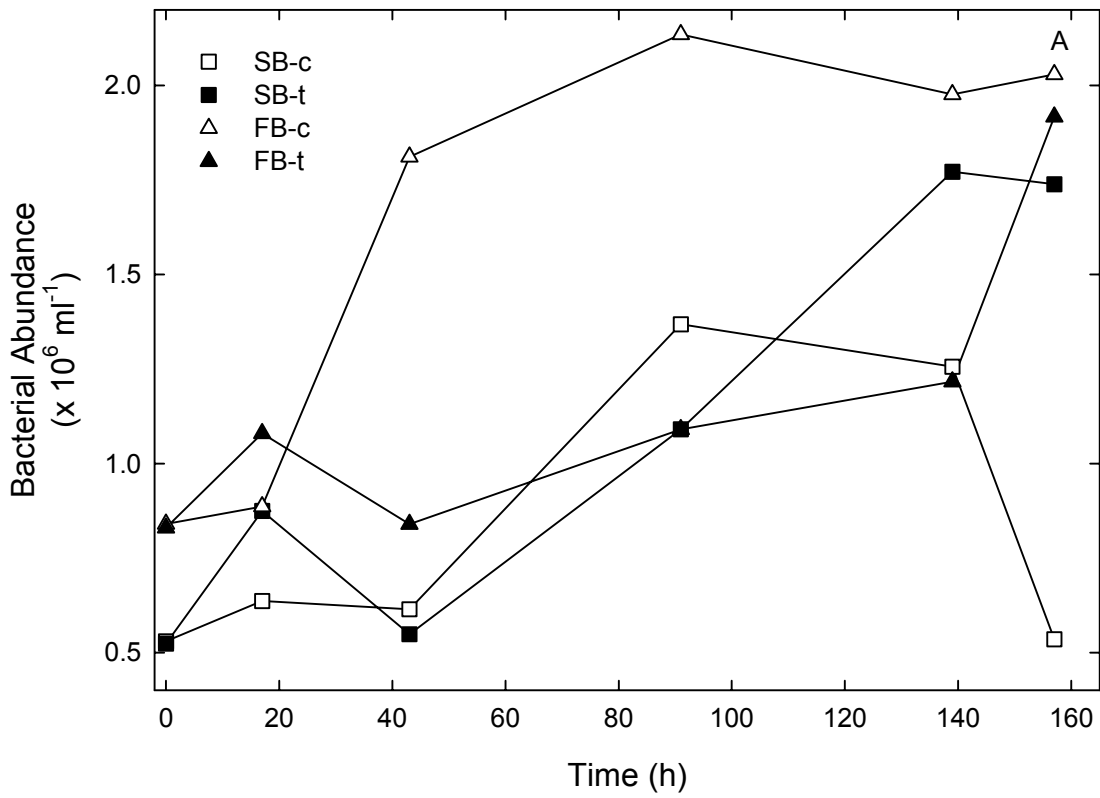


Fig. 3

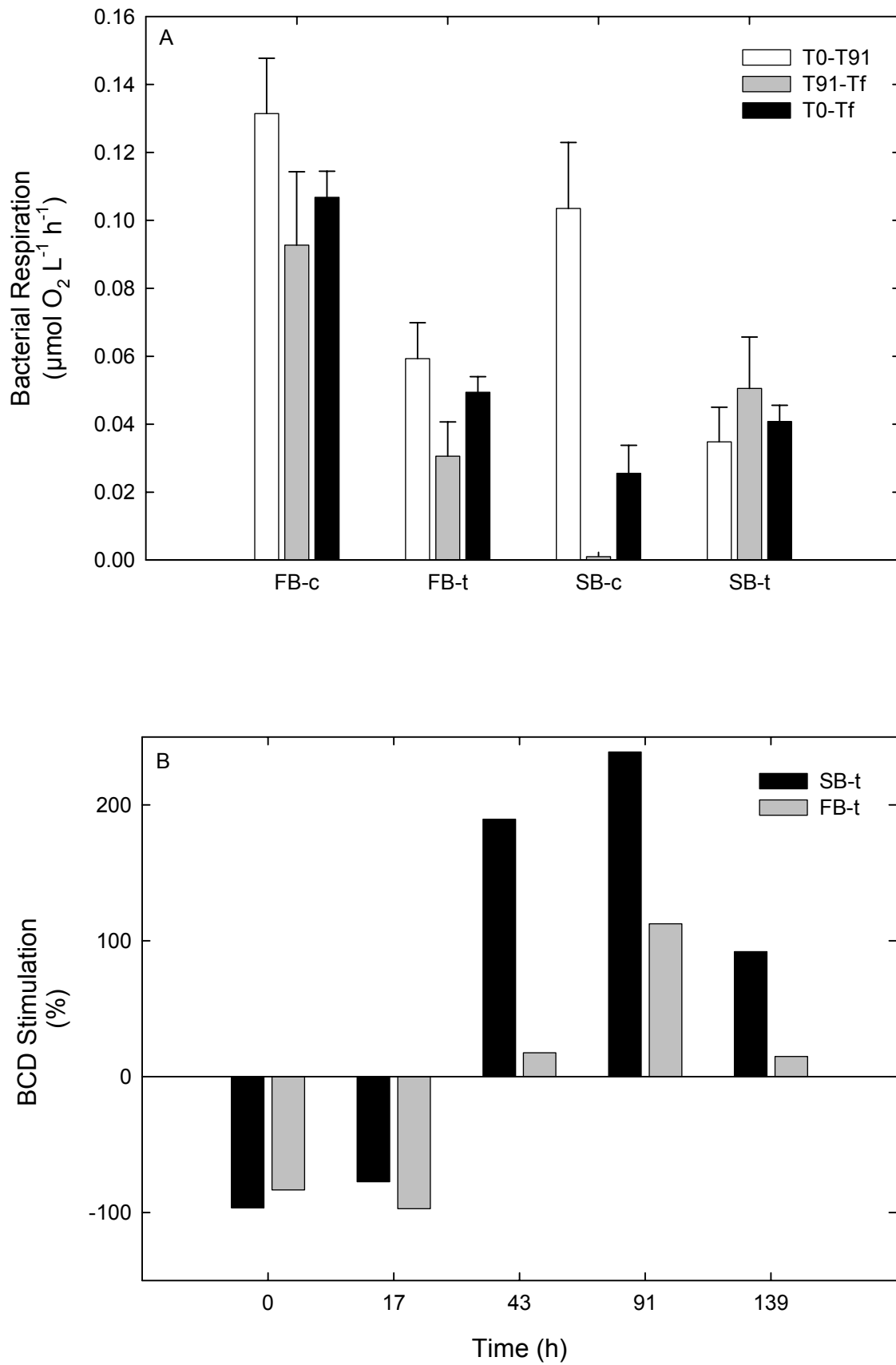


Fig. 4

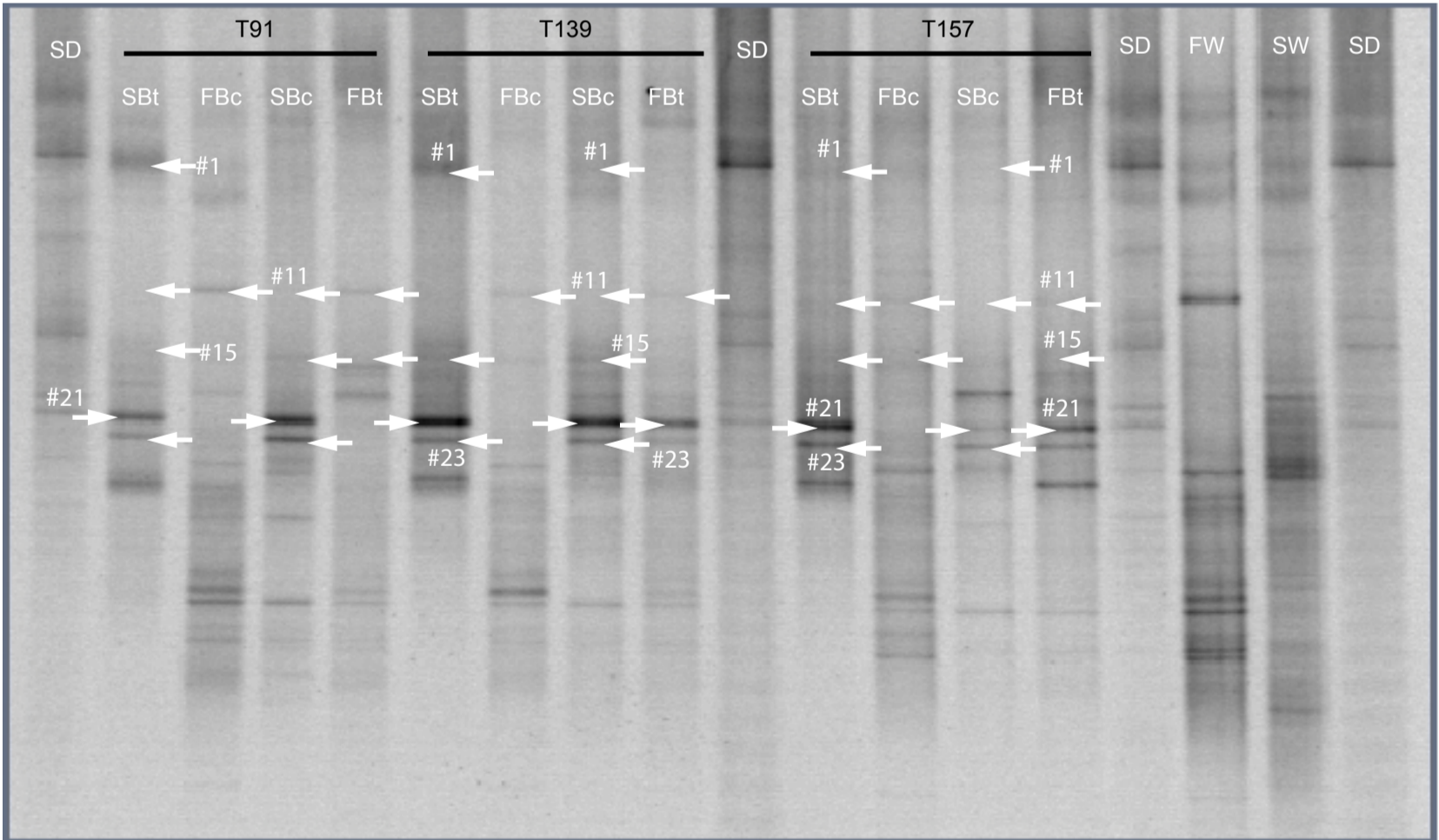
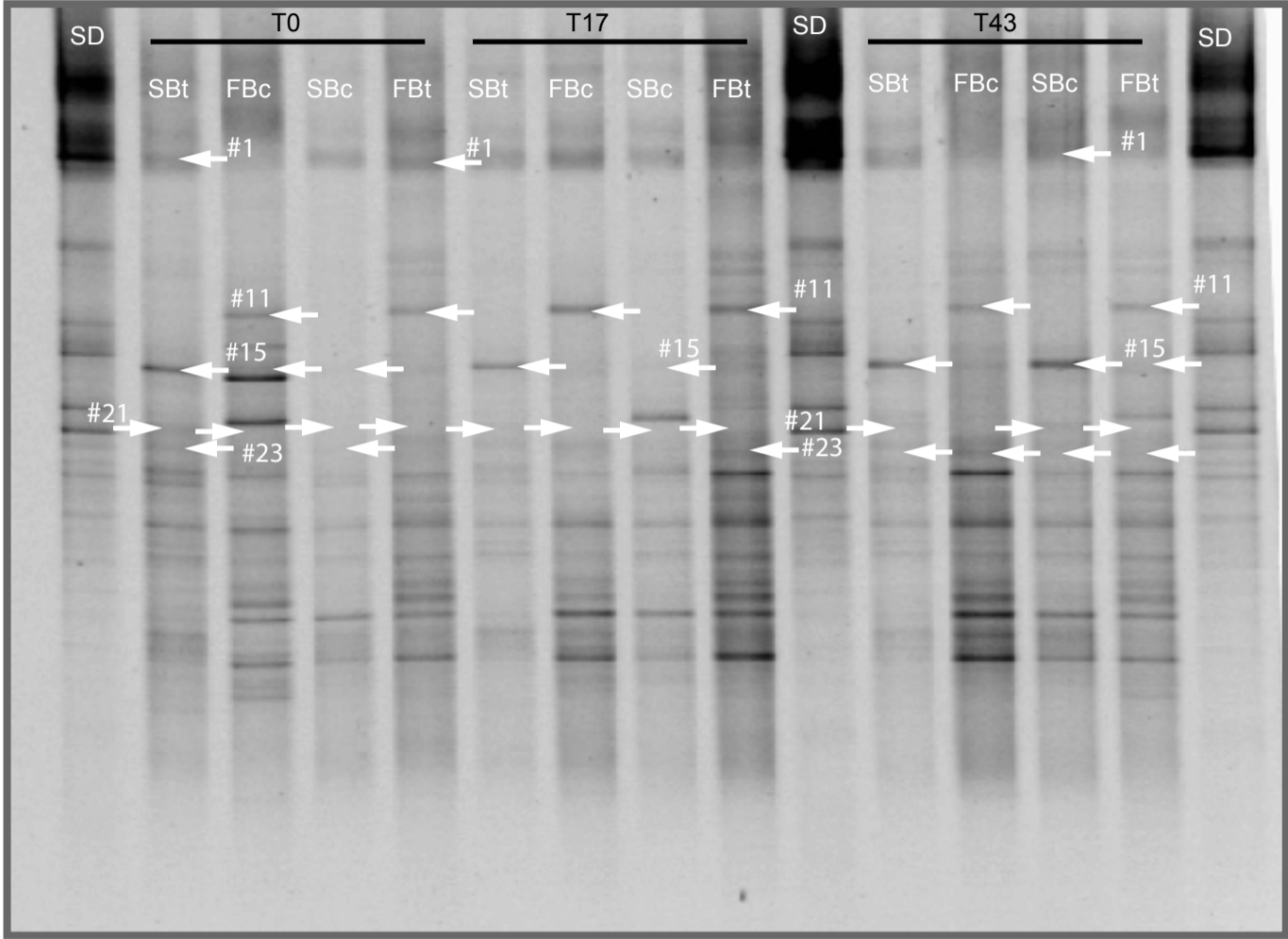


Fig. 5

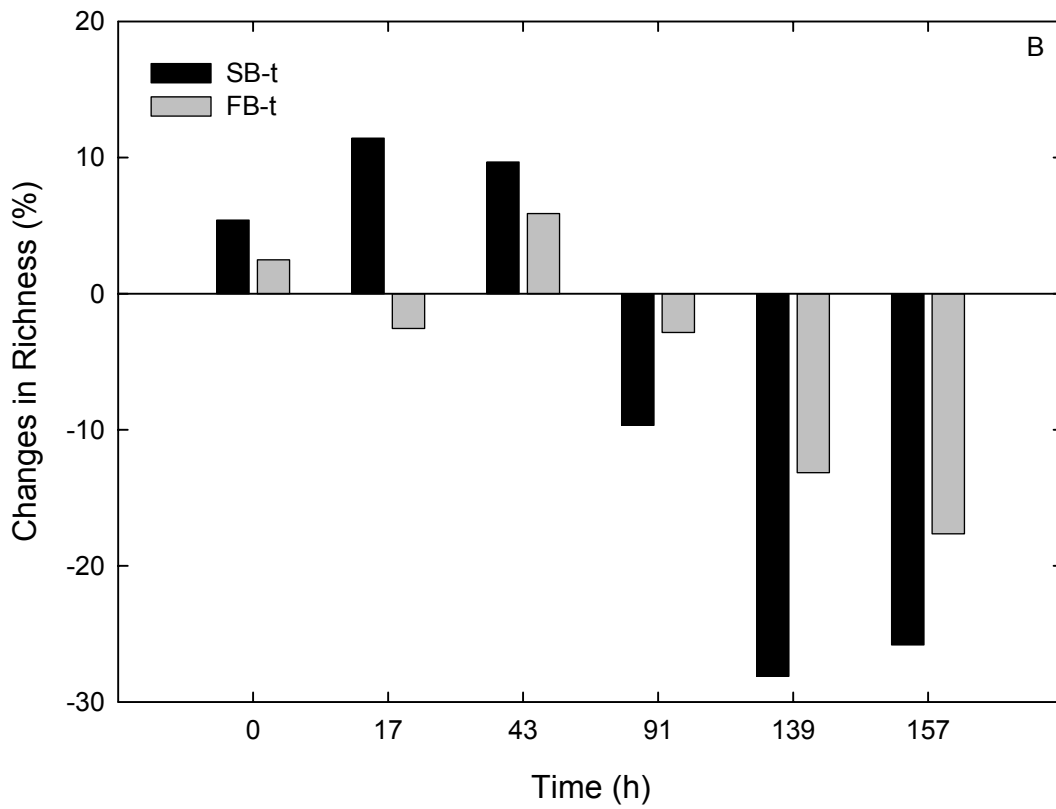
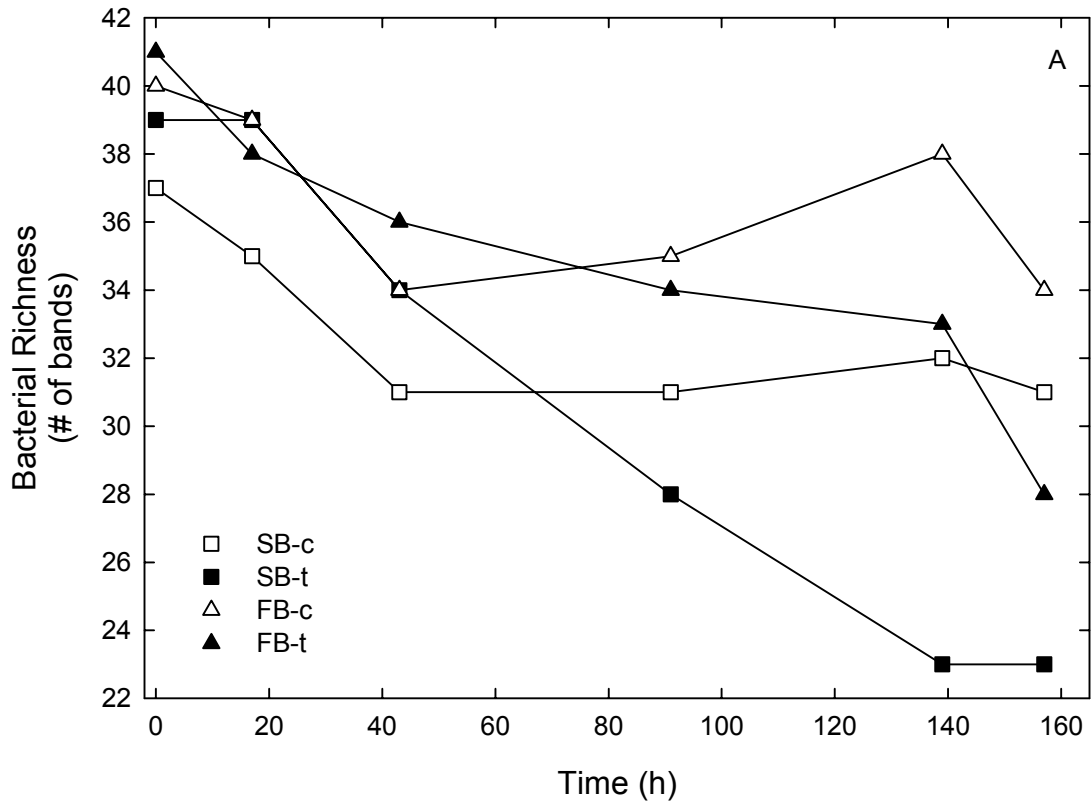


Fig. 6

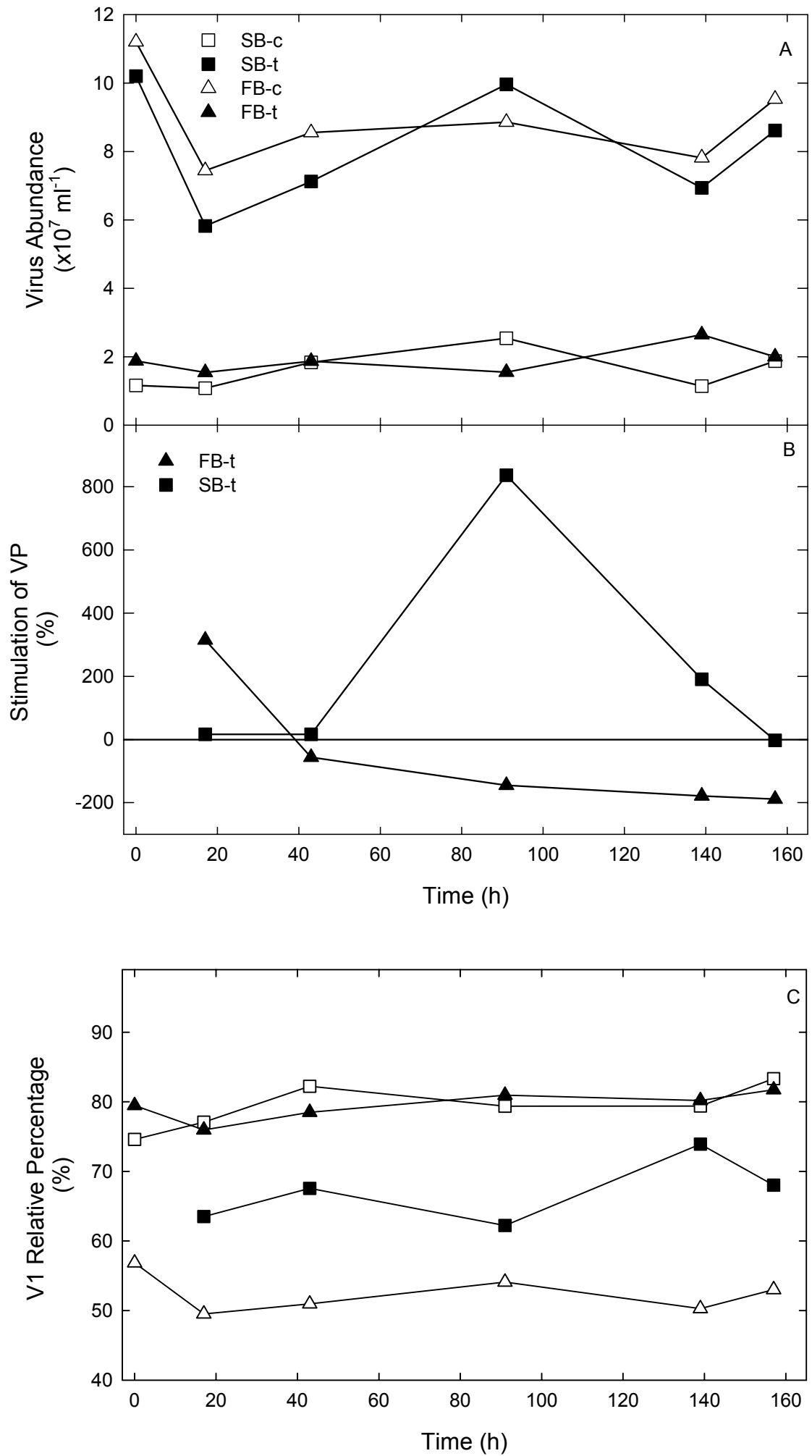


Fig. 7