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Pelagic food web patterns: do they modulate virus and nanoflagellate effects on picoplankton during the phytoplankton spring bloom?

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Running title:

Effects of viruses and nanoflagellates on picoplankton

Summary

As agents of mortality, viruses and nanoflagellates impact on picoplankton populations. We examined the differences in interactions between these compartments in two French Atlantic bays. Microbes, considered here as central actors of the planktonic food web, were first monitored seasonally in Arcachon (2005) and Marennes-Oléron (2006) bays. Their dynamics were evaluated to categorize trophic periods using the models of Legendre and Rassoulzadegan (1995) as a reference framework. Microbial interactions were then compared through 48h-batch-culture experiments performed during the phytoplankton spring bloom, identified as herbivorous in Marennes and multivorous in Arcachon. Marennes was spatially homogeneous compared to Arcachon. The former was potentially more productive, featuring a large number of heterotrophic pathways, while autotrophic mechanisms dominated in Arcachon. A link was found between viruses and phytoplankton in Marennes, suggesting a role of virus in the regulation of autotroph biomass. Moreover, the virus-bacteria relation was weaker in Marennes, with a bacterial lysis potential of 2.6% compared with 39% in Arcachon. The batch experiments (based on size-fractionation and viral enrichment) revealed different microbial interactions that corresponded to the spring-bloom trophic interactions in each bay. In Arcachon, where there is a multivorous web, flagellate predation and viral lysis acted in an opposite way on picophytoplankton. When together they both reduced viral production. Conversely, in Marennes (herbivorous web), flagellates and viruses together increased viral production. Differences in the composition of the bacterial community composition explained the combined flagellate-virus effects on viral production in the two bays.

Introduction

The long held paradigm that primary production is transferred through a herbivorous chain to small and large phytovores neglects the importance of most microorganisms. Yet bacteria, which are present at up to 10^6 cells.ml^{-1}, and viruses, whose concentration can reach 10^8 viral particles.ml^{-1},

dominate in the oceans (Borsheim et al., 1990; Hennes and Suttle, 1995; Li, 1998). Bacteria were primarily considered as remineralizers providing inorganic nutrients for primary producers. Later, the microbial loop concept (Azam et al., 1983) highlighted the role of bacteria as recyclers of DOM originating either from primary production or from losses at different trophic levels. Thus, the role of bacteria in the microbial loop evolved from that of a matter sink to a trophic link towards bacterivorous organisms. Moreover, bacterial community dynamics are not only controlled by resources i.e. bottom-up control, such as the availability of inorganic nutrients and the quality of DOM (Kirchman et al., 2000), but also by predation i.e. top-down control, examples being the mortality induced by protists such as nanoflagellates and ciliates and by viral lysis. (Sanders et al., 1992; Fuhrman, 1999). The selectivity of protist predation based on size, motility, shape and cell surface properties of prey (Gonzalez et al., 1990; Monger et al., 1999) can potentially impact on the morphological characteristics of bacteria and shape their community composition (BCC) (Hahn and Hofle, 2001; Posch et al., 2001). On an ecosystem level, protist predation may exert a stronger control in oligotrophic waters than in eutrophic waters (Gasol et al., 2002).

Since the 1990's, viruses have been considered as an additional factor in the mortality of bacteria (Fuhrman, 1999), other micro-organisms such as microalgae (Cottrell and Suttle, 1995) and unpigmented flagellates (Garza and Suttle, 1995; Massana et al., 2007). Virus-mediated bacterial mortality could account for 48-84% of bacterial production in oligotrophic areas and 59% in eutrophic areas (Boras et al., 2009). Regarding the biogeochemical aspects of the ecosystem, viral lysis shunts carbon flow (Fuhrman, 1999): 6 to 26% of photosynthetically fixed carbon flows back to DOM, becoming available for microbial uptake and consequently modifying the competitive balance of phytoplankton and bacteria for limiting nutrients (Wilhelm and Suttle, 1999). Moreover, viral infection could potentially maintain the species richness of bacteria, as described in the "kill the winner" concept (Thingstad and Lignell, 1997). The density dependence of viral infection could limit dominant and competitive bacteria and give an advantage to less competitive ones (Bouvier and Del Giorgio, 2007; Weinbauer et al., 2007; Auguet et al., 2009), although this idea is still

79 controversial (Winter et al., 2004). In addition, host specificity could concern strains instead of
80 species because of the phenotype plasticity of the viral permissivity-resistance characters
81 (Schwalbach et al., 2004; Fuhrman, 2009).
82 Even if virioplankton is mainly composed of bacteriophages (Fuhrman, 1999; Suttle, 2005), a link
83 with phytoplankton or chlorophyll *a* concentrations, suggesting phytophage pathogenicity
84 (Wommack and Colwell, 2000), has already been underlined in some long term surveys where there
85 were high densities of algal cells (Maranger et al., 1994) as well as in offshore stations (Boehme et
86 al., 1993; Guixa-Boixereu et al., 1999) or in coastal regions at the deep chlorophyll *a* maximum
87 (Baudoux et al., 2008). Organisms less than 3µm in size may be responsible of large part of primary
88 production, in particular in oligotrophic areas (Platt et al., 1983). Autotrophic as well as
89 heterotrophic pico- and nanoplankton are too small to be efficiently consumed by metazoans (Sherr
90 and Sherr, 1988); they are mainly consumed by flagellates and ciliates (Sherr and Sherr, 1994) and
91 can significantly contribute to their diet (Hirose et al., 2008). However, the grazing yield from
92 phytoplankton is lower than from bacteria. Furthermore, predation on protists indirectly affects
93 bacterial grazing, enhancing viral impact (Boras et al., 2009). Thus, as the preferential prey of
94 copepods (Nielsen and Kiorboe, 1994), ciliates can be considered as a trophic link between pico-
95 and nano-plankton and mesozooplankton (superior trophic levels) (Calbet and Saiz, 2005).
96 To assess the importance of microbiological plankton compartments in matter flow, Legendre and
97 Rassoulzadegan (1995) described 4 major trophic concepts each incorporating different dominant
98 carbon pathways: (1) The herbivorous or classical food web, which is based on large-phytoplankton
99 (>5µm) primary production leading to large zooplankton organisms. (2) The microbial food web, in
100 which primary production is mainly due to small phytoplankton and comprises pathways involving
101 heterotrophic bacteria and protozoa. (3) The microbial loop, a nearly closed system where carbon
102 flows are limited to heterotrophic bacteria and unpigmented protists; DOM released by the grazers
103 becomes substrate for bacteria. (4) Finally, the multivorous food web, a transitional trophic system

104 comprising diverse heterotrophic carbon pathways in which both large and small phytoplankters are
105 significant primary producers.
106 Accordingly, the question arises: do the interactions between microbial compartments (in particular
107 between viruses, picoplankton and nanoflagellates) vary depending on the trophic pathway? So far,
108 despite the clear importance of heterotrophic microorganisms in pelagic food webs, descriptions of
109 trophic models have mostly been based on primary producer size, water column stratification and
110 nutrients (Rivkin et al., 1996; Marquis et al., 2007). Thus, to answer this question, two sites along
111 the French Atlantic coast that are distinctive in terms of their river inputs and hydrology (shape,
112 mixing regime and water residence time) were chosen as study case areas to describe the temporal
113 succession of trophic pathways. The temporal dynamics of abiotic parameters and microbiological
114 compartments were assessed over 7 months in Arcachon Bay in 2005 and over one year in
115 Marennes Oléron Bay in 2006. Located North-Southward along the coast of the Bay of Biscay,
116 Arcachon and Marennes-Oléron Bays are both semi-enclosed and macrotidal lagoons, largely
117 composed of intertidal mudflats (Blanchet et al., 2008; Allard et al., 2009). Like many estuaries and
118 bays, the 2 basins are highly productive, featuring a large-phytoplankton spring bloom (Struski and
119 Bacher, 2006; Glé et al., 2008). In this context, does the large-phytoplankton spring bloom entail
120 similar trophic pathways in each bay and thus similar interactions between the above-mentioned
121 microbial compartments? To answer this question, we first defined the trophic succession for each
122 bay, then located the spring phytoplankton bloom within this succession, and finally *in vitro*
123 experiments were performed during this period to analyse the quantitative and qualitative impact of
124 nanoflagellate grazing and viral lysis on picoplankton.
125
126
127
128
129

Results

IN SITU SURVEYS

Environmental context

The evolution of the abiotic parameters in the 2 bays followed a typical temperate-coastal pattern featuring peak temperatures and salinities in the summer together with low nutrient levels during the period of low runoff. On average, Arcachon Bay (Arc) had fewer nutrients than Marennes (M-O) (Table S1 and S2). Arc waters were spatially heterogeneous, with significant differences of salinity, temperature and most nutrients between the 2 stations (ANOVA, H_0 no difference between stations is rejected, $p<0.05$). By contrast, M-O was homogeneous over 4 of the 5 stations (ANOVA $p>0.05$). The Charente station differed from the four others, with higher nutrient levels and lower salinity (ANOVA with Tukey multiple comparisons, $p>0.05$) (Table S1).

Zonation and seasonality of microbial distribution

The bacteria/virus relationship ($r^2 \geq 0.6$) in Arc was stronger than in M-O. However, the response of bacteria to increasing viruses was weaker in Arc (log/log regression slopes ≤ 0.6) than in M-O (slopes ≥ 0.84, Table 1). In Arc, bacterial (BA) and viral (VA) abundances in Comprian were significantly higher than in B13 (ANOVA, $p < 0.0002$, Table 1). In M-O, the BA/VA relationship was equal in 4 of the 5 stations (Slope comparison, ANCOVA, $p<0.05$, $n=96$), but the relationship was insignificant at Charente station ($p=0.09$) (Table 1). Nevertheless, no significant differences in the annual abundance of viruses and bacteria were found between the 5 stations (ANOVA, $p<0.05$). Consequently, the global monthly and annual means (BA: $4.11 \pm 0.34 \times 10^6$ cells.ml^{-1} and VA: $3.38 \pm 0.34 \times 10^7$ particles.ml^{-1}) are representative for the whole basin. The VBR (virus to bacteria ratio) was higher in M-O (annual mean= 11.16 ± 5.39) compared to Arc (4.06 ± 1.17 and 4.89 ± 1.96 at B13 and Comprian, respectively). In M-O, VBR tended to be higher from November to April (>10) and lower during summer (<10), while no trend was visible in Arc (data not shown).

155 The results of PCA and HAC were analysed to compare the spatial and temporal organization of the
156 2 bays (Fig. 1). Only samples from January to August were taken into account. Nutrients (23%) and
157 viruses (7.5%) contributed positively to axis 1 while Cryptophyceae (10%), salinity (5%), diatoms
158 (5%) and *Synechococcus* (5%) contributed negatively. Small phytoeukaryotes (16%), temperature
159 (14%), bacteria (12.5%) and ciliates (12.5%) contributed positively to axis 2. The 2 axes accounted
160 for 52.67% of the total variance among the samples. Along the first axis, Arc was distinguished
161 from M-O by low nutrients (Table S1), a high abundance of autotrophs [Diatoms (mean:
162 $3.52\pm4.17\text{x}10^5$cell.l^{-1} vs. $0.65\pm1.45\text{x}10^5$cell.l^{-1}), Cyanobacteria (mean: $1.77\pm2.83\text{x}10^4$ vs.
163 $3.9\pm2.9\text{x}10^3$cell.ml^{-1}), Cryptophyceae ($6.46\pm5.17\text{x}10^4$ vs. 13±28cell.l^{-1})], as well as low VA
164 ($5.71\pm5.23\text{x}10^6$ vs. $3.37\pm1.21\text{x}10^7$ particles.ml^{-1}). Seasonal clusters were found along the second
165 axis for both basins ("winter": January - April and "summer": May - August), principally due to
166 higher summer temperatures combined with a higher abundance of microorganism in Arc (bacteria,
167 ciliates and pico- nanophytoplankton). Furthermore, a spatial subdivision appeared in Arc along
168 axis 1, distinguishing the inner (Comprian) from the outer (B13) sampling station. By contrast, the
169 M-O stations were homogeneous, although the Charente station points were always shifted to the
170 right because of a lower salinity and a higher level of nutrients. Moreover, the Charente station was
171 also distinguished by large amounts of suspended matter (Table S1).
172 Based on the results of this multivariate assessment, the basin dynamics were further distinguished
173 using the inner station of Arc (Comprian) and averages of the five M-O stations (because of their
174 spatial homogeneity) using multiple regressions. VA and BA dynamics can be related to the more
175 complex relationships in M-O compared to Arc (Table 2). The opposite was observed for
176 heterotrophic nanoflagellates. There are several important details: (i) in winter, BA was positively
177 related to viruses in both bays and to HNF in M-O but negatively in Arc. In summer, only the link
178 with viruses remained along with supplementary links with nutrients (ammonium, nitrite and
179 phosphate); (ii) there was a negative relationship between viruses and picophytoplankton all year
180 long in both bays as well as with HNF in Arc during the summer; viruses were positively linked to

chla all year round in M-O but not in Arc. (iii) HNF were negatively related to ciliates during winter (Table 2).

Temporal succession of trophic pathways

Herbivorous food webs were identified for 20% of the sampling period in Arc (February and August) and for 27% of the period in M-O (May and July-August, Fig. 2A,B). Multivorous webs were present for 21% of the period in Arc (May and the second half of August) and for 25% of the period in M-O (3 weeks in February-March and 2 months between mid-October and mid-December). The microbial food web represented about half of the sampling period in both bays, yet they were not synchronized, except during March-April (Fig. 2). Finally, the microbial loop was absent in Arc, while it was present for 5% of the period in M-O (two weeks at the end of March and one in September). During these two periods, bacteria grew 2.0x and 2.4x, viruses 2.0x and 2.0x, nanoflagellates 6.7x and 1.5x and ciliates 0 and 20x, respectively (Fig. 2B, "Microbial loop").

BA and VA evolved in parallel during all the trophic periods in Arc. However, the synchronisation was less obvious in M-O, particularly during the herbivorous and multivorous phases when VA did not decrease as much (-1.6x) as BA (-2.1x).

In Arc, the small phytoplankton (annual mean = $3.4\pm1.5x10^4$ cells.ml^{-1}) were dominated by picoplankton, with 3 major growth periods (early April, early May accompanied by nanoplankton, and from mid-June to end of July, also accompanied by nanoplankton). Large-phytoplankton, with an annual mean of $4.73\pm4.16x10^5$ cells.l^{-1}, dominated at the beginning of the year, with mainly dinophyceae and cryptophyceae species; this was followed later by two diatom peaks (mid-May and late August). Three periods of growth of small phytoplankton were observed in M-O (annual mean = $1.98\pm1.71x10^4$ cell.ml^{-1}) that were mostly due to picoplankton (end of April, $4.9x10^4$ cells.ml^{-1}, early June, $6.8x10^4$ cells.ml^{-1} and early July, $4x10^4$ cells.ml^{-1}). Large-phytoplankton (annual mean=$7.05\pm14.6x10^4$ cells.l^{-1}) developed at the end of March (mainly diatoms), early May (mainly diatoms) and early July (diatoms and dinoflagellates). Moreover, picophytoplankton and large

phytoplankton accounted for the viral dynamics during the herbivorous and multivorous periods in M-O (simple regression, p=0.01 and p=0.006, respectively).

Nanoflagellate numbers increased systematically during the microbial phases in both Arc and M-O at the beginning of the herbivorous and multivorous periods, just before the large-phytoplankton blooms. Small cells dominated in the latter (annual mean: 88.2±6.9% <3µm cells, Fig. 2). In Arc, the pigmented/unpigmented ratio of nanoflagellates was higher (mean=11.6 vs. 3.9 in M-O).

Ciliate dynamics followed those of bacteria in M-O except in mid-February (Fig. 2B). Heterotrophic species dominated (annual mean of *Strombidium spp* plus *Strobilidium spp*: 55% and 48% of the total abundance, respectively). The only exception was during the spring phytoplankton bloom when autotrophic and potentially autotrophic taxa (*Myrionecta rubra*, *Tontonia spp*, *Laboea sp*) represented 60% and 80% of the total abundance. In Arc, ciliate dynamics followed those of nanoflagellates, increasing during the microbial food web periods.

Finally, beyond the fact that the changes in the trophic pathways in the two bays are not synchronized, the major difference was the contrasting organization at the beginning of the year, which was herbivorous in Arc and microbial in M-O. Moreover, during the large-phytoplankton spring blooms, M-O featured a herbivorous web pathway while Arc was in a multivorous phase.

IN VITRO **EXPERIMENTS**

Experiments were performed with water from Arcachon-Comprian (2006; T°*in situ*= 18.6°C; S= 32 PSU) and the Marennes-Station E (2007; T°*in situ*= 15°C; S= 32.5 PSU) collected at morning high tide. During incubation, the nighttime/daytime water temperature varied from 19.5°C to 23.3°C in Arc and from 18°C to 22°C in M-O, respectively.

Protists

In Arc, the abundance of picophytoeukaryote (picoeuk) was equivalent in all treatments at the beginning of the experiment (1.8×10^3cells.ml^{-1}). In the absence of predators, virus enrichment had a

233 positive effect from 18h to 38h, during which picoeuk abundance increased 18-fold (Fig. 3A). But
234 in the presence of flagellates, picoeuk abundance dropped 4.5-fold. In M-O water, the abundance of
235 picoeuk was not significantly different between treatments despite an initial difference due to the
236 filtration step.
237 Pigmented nanoflagellates were few and varied little in both sites, (means: $6.4x10^4$cells.l^{-1} in Arc
238 and $9.6x10^4$cells.l^{-1} in M-O), making up <1% and <10% of the total nanoflagellates, respectively.
239 The nanoflagellates started growing at 18h (Fig. 3E). Their growth rates were 2- to 4-fold higher in
240 Arc than in M-O (in 3µ-Tr and 20µ-Tr respectively). Moreover, in both bays, the nanoflagellates in
241 the latter were significantly higher than in 3µ-Tr (2way-ANOVA $p<0.01$) while the small cells
242 (<3µm) dominated and grew more rapidly than the large ones. During the 48h incubations, the
243 small/large flagellate ratio increased from 5 to 100 in Arc and from 1.5 to 20 in M-O.
244
245 *Bacterial abundance and growth rate*
246 During the different treatments, bacterial abundance after 48h increased 20-35 fold in Arc
247 (stationary phase) and 20-60 fold in M-O (logarithmic phase) (Fig. 3B). In Arc, the net bacterial
248 abundance increase (NBI) was always significantly greater in virus enriched treatments ($p=0.005$).
249 In M-O, the viral enrichment enhanced BA only in the 0.8µm treatments ($p<0.05$).
250 The maximum specific bacterial growth rate (µmax) showed significant differences between
251 treatments: µmax was significantly higher in both bays with virus-enriched treatments compared to
252 the corresponding controls (2way ANOVA, $p=0.02$) (cf Fig. 3B black versus clear symbols). The
253 growth stimulation was 9-14% in Arc and 12-33% in M-O. However, the presence of flagellates did
254 not significantly modify µmax in either of the bays (20µm and 3µm treatments versus 0.8µm
255 treatments; Fig. 3B)
256 In Arc, during the exponential phase, the growth rate of population 6 of HNA cells, which were the
257 most abundant, was stimulated in enriched-0.8µ-Tr (+44%) compared to the control, while
258 populations 3, 4 and 5 were inhibited. The presence of flagellates (3µ and 20µ treatments) masked

this particularity. In M-O, the growth rate of population 3 in the presence of flagellates was significantly higher than those of the other populations (data not shown).

Viral abundance and production

The initial viral abundance was multiplied 2-3-fold in enriched-treatments in both Arc and M-O (Fig. 3C). In Arc, some viruses were produced during the first 12 hours, except in 20µ-Tr. A second, stronger production phase was observed between 24 and 38h in all treatments, persisting till the end of the experiment in 3µ-Tr. The presence of flagellates significantly decreased the total viral production rate (-50% in 3µ-Tr and -75% in 20µ-Tr) from 0.04 h^{-1} in 0.8µ-Tr to 0.02 h^{-1} in 3µ-Tr and to 0.01 h^{-1} in 20µ-Tr (2way-ANOVA, p=0.0002). Moreover, viral enrichment decreased the viral production rate notably in presence of small predators (-47.5%, p=0.009) and in their absence, yet not significantly (-23%; p=0.1). In M-O, VA evolved differently with the various treatments: viruses were produced between 12 and 18h only in the presence of flagellates (3µ- and 20µ-treatments). There was a second production phase from 38h in all treatments except in the enriched-0.8µ-Tr and 20µ-Tr. The net viral production over 48h was negative (-31%, -72%, -5.3%, -70% and -65% in 0.8µ-Tr, enriched-0.8µ-Tr, 3µ-Tr, 20µ-Tr and enriched-20µ-Tr respectively) except in enriched-3µ-Tr (+5.8%). In contrast to Arc, a combination of the presence of flagellates and virus enrichment increased 2.5-fold the total viral production rate (from 0.01 h^{-1} in 0.8µ-Tr to 0.025 h^{-1} in enriched-3µ-Tr). The addition of viruses alone had the opposite effect (2way-ANOVA, interaction, p=0.006), inducing a significant 6.5-fold decrease (from 0.01 h^{-1} in 0.8µ-Tr to 0.0016 h^{-1} in enriched-0.8µ-Tr).

Virus mediated bacteriolysis

In Arc, we calculated that 70±15% of the bacterial stock was lysed per day (0.8µ-Tr). In the presence of flagellates, this impact decreased significantly to 33.7±1.9% (3µ-Tr) and 39±11% (20µ-Tr) (ANOVA: p=0.01). By contrast, virus mediated lysis was less severe in M-O (13.6±1.7% of

285 bacterial stock lysed per day without any predators). Small flagellates had no effect (9.4±4.6% in
286 3µ-Tr, $p>0.05$). However, in the presence of all the flagellates (20µ-Tr), the percentage of lysed
287 bacteria significantly decreased to 2.6±0.3% (ANOVA, $p=0.009$).
288
289 *Bacterial enzymatic activity and community composition*
290 In Arc, the change in specific maximum velocity of potential aminopeptidase activity (specific
291 Vmax) was similar in all treatments, with a peak at 24h. Specific activity dropped at 38h except in
292 3µ-Tr, where a strong activity was maintained until the end of the experiment (Fig. 3D). There was
293 a negative relationship between picoeuk abundance and bacterial proteolytic activity, which was
294 significant only in the presence of flagellates (Simple regression: $r^2=92\%$ $p<0.0001$ $n=15$ in 3µ-Tr,
295 $r^2=29\%$ $p=0.022$, $n=15$ in enriched-3µ-Tr). The specific Vmax peaked also at 24h in M-O, except in
296 the enriched-3µ-Tr at 12h. In both bays, peaks of activity occurred during the exponential growth
297 phase of bacteria (Fig. 3B, D). Moreover, a decline in bacterial activity coincided with an increase
298 in viral abundance in both bays: in M-O, there was a negative relationship between VA and specific
299 Vmax in the 0.8µ and 3µ treatments ($\log VA=4.11-0.63 \times \log Vmax$, $r^2=0.15$, $n=60$, $p=0.002$). This
300 relationship was still significant ($r^2=0.13$, $p=0.007$) when 20µ treatments were taken into account.
301
302 At the beginning of the incubations, the *Eubacteria* were less FISH-targeted in Arc (53% of
303 DAPI stained bacteria in 0.8µ-Tr and 35% in 3µ-Tr) than in M-O (62% in 0.8µ-Tr and 81% in 3µ-
304 Tr). In Arc, the dominance of *γ-proteobacteria* (Gamma) was further increased in the presence of
305 small nanoflagellates (15% versus 8%), while *α-* and *β-proteobacteria* (Alpha and Beta) were
306 present in relatively similar proportions (2-2.5%) (Fig. 4). In M-O, Gamma were more numerous in
307 the 0.8µ treatments (8.9%) than in the 3µ treatments (3%), while among the other groups, only Beta
308 in 0.8µ-Tr showed up significantly (2.6%).
309 Over 48h, the proportion of *Eubacteria* increased in both bays (78-85% in Arc, 83-90% of
310 total cells in M-O), with a dominance of Gamma. However, while the proportion of Gamma at 48h

311 was higher in Arc in the presence of flagellates (70±5.4%) than in their absence (58±3%),
312 independent of viral enrichment, Gamma dominated in M-O (≈80%) with no difference between
313 treatments (Fig. 4). Furthermore, at 48h in Arc, a positive effect of viruses was observed on Beta
314 and Alpha in the presence of flagellates (+38% in enriched-3µ-Tr compared to the control 0.8µ-Tr),
315 counterbalancing the negative impact of predators (-65% in 3µ-Tr). However, earlier on, at 12h,
316 Alpha cells were sensitive to virus inputs (-70% and -75% in enriched-0.8µ-Tr and enriched-3µ-Tr
317 respectively, compared to the corresponding control). In M-O, among the rare groups, Alpha
318 increased notably in virus-enriched treatments without predators at 48h (+50% in enriched-0.8µ-Tr).
319 By contrast, nanoflagellates restrained their growth (-49.6% in 3µ-Tr compared to the 0.8µ-Tr
320 control), and even more so after viral enrichment (-76.2% in enriched-3µ-Tr) (Fig. 4). Conversely,
321 the combined effect of nanoflagellates and viruses resulted in an increase of Beta cells at 12h
322 (enriched-3µ-Tr) while the presence of flagellates alone reduced Beta growth (3µ-Tr versus 0.8µ-
323 Tr). At 48h, this pattern was reversed: Beta cells increased in the presence of flagellates (+112%
324 compared to the control) while they decreased with additional viral enrichment (-50%).
325

326 ## Discussion

327 For each site, the survey concerned only one year without taking into account the potential inter-
328 annual variability of microbial dynamics in the pattern of trophic succession (Guarini et al., 2004;
329 Glé et al., 2007). Unfortunately, due to logistic restricitions, the comparison of the bays had to be
330 carried out for two adjacent years, and the bias of inter-annual variability cannot be assessed.
331 After *in situ* surveys of one year, 48h *in vitro* incubation experiments were performed with different
332 water categories treated by differential filtration, corresponding to a top-down manipulation of
333 microbial compartments and consequently the removal of predators. Because of the complexity of
334 the microbial food web, with parameters such as predation/competition links and the size overlap of
335 predators and competitors, the size-fractionation method may reduce or eliminate some elements of
336 normal trophic webs. Currently employed methods, including artificially composed trophic webs,

337 necessarily simplify natural systems. We consider that, of those currently employed, the filtration
338 method is one which involves the least disturbance of the webs (Chen et al., 2009).
339
340 *Temporal trophic succession*
341 Several previous studies have analysed seasonal production patterns in Arcachon Bay (Arc) (Robert
342 et al., 1987; Glé et al., 2008) and Marennes Bay (M-O) (Guarini et al., 1998; Struski and Bacher,
343 2006). In Arc, river runoff and high levels of tidal exchange create a gradient of nutrients along the
344 basin, except for phosphate (Glé et al., 2008). Seasonally, as in our study, Arc is early nutrient
345 limited with a depletion of nitrate from May to the autumn and of phosphate during spring. In M-O,
346 nutrient availability was higher and limitations were only encountered episodically during certain
347 years (Soletchnik et al., 1998; Struski, 2005). However, during our 2006 *in situ* survey, no
348 limitations were found. Based on our multivariate analysis, Arc is spatially heterogeneous while M-
349 O is spatially homogeneous, despite the influence of the Charente River. Moreover, as already
350 illustrated by Soletchnik et al. (2007), seasonal variations, characterized by changes in salinity,
351 temperature and chla are higher in M-O than in Arc.
352 Our trophic organization framework was based on microbiological and environmental dynamics, as
353 proposed by Legendre and Rassoulzadegan (1995). Mousseau et al. (2001) illustrated the
354 periodicity of trophic organization using ratios of biomasses, production and uptake rates. For the
355 present study, the entire spectrum of abundance from virioplankton to mesozooplankton were used
356 in a similar manner to Rodriguez et al. (2000). We used microbial abundance instead of biomass
357 since the contact rates between virus/host cells or prey/predator determine viral infections and
358 grazing rates. Our empirical trophic-period definitions were validated by a factorial discriminant
359 analysis (FDA) in which the discriminant variable was the defined trophic model (Fig. S1). The
360 clusters remained separated even in the absence of the abundance data used for characterizing the
361 trophic organization (Lambda Wilks test, $p<0.05$).

362 The microbial food web dominated in the two bays for 54% and 43% of the seven months of the
363 surveys in Arc and M-O, respectively; although this consisted of short periods of domination in M-
364 O and only 2 long ones in Arc. This domination was longer than what has been found in other
365 coastal sites: on the Canadian East coast and in Plymouth Channel, the microbial food web
366 dominated for less than 20% of the year and only during summer (Rodriguez et al., 2000; Mousseau
367 et al., 2001). A herbivorous food web dominated in Canada for 50% of the year while it represented
368 only 15% (2 short phytoplankton blooms) in Plymouth Channel. In the latter, there was a
369 domination of the transition model i.e. the multivorous food web. In our study sites, the herbivorous
370 web persisted over longer periods in M-O (43% of the first seven months) while the transitional
371 multivorous web dominated in Arc (22% over the same period). The trophic systems of Arc, a
372 largely closed lagoon, appear to be more stable and less subject to trophic variations than the more
373 open M-O (Fig. 2; see also Fig. 4 in Soletchnik et al., (2007). In the latter, there was a succession of
374 microbial and herbivorous models without a systematic passage through the transitional
375 multivorous model. Moreover, the microbial food web was easier to distinguish from the other webs
376 than in Arc (Fig. S1). Consequently, the dominance of the microbial food web attests the
377 importance of microbial compartments in both ecosystems.
378 Another major difference between the bays is the contrasting trophic organization during winter: a
379 herbivorous food web in Arc and a microbial food web in M-O. The presence of winter blooms has
380 already been described in the Bay of Biscay (Labry et al., 2001; Marquis et al., 2007) and suggested
381 for the two basins through analyses of long-term data series (Soletchnik et al., 2007). These
382 phytoplankton blooms are influenced neither by temperature nor by nitrogen or phosphorus
383 availability but are probably due to silicate input combined with a potential for salinity stratification
384 or light availability.
385 The spring phytoplankton bloom occurred at the same period in both bays (April-May) yet it was
386 greater in Arc, where it consisted of large phytoplankton forms such as diatoms and picoplankton.
387 However, estimations of pelagic primary production illustrate a higher annual productivity in M-O

388 of 185 $gC.m^{-2}.yr^{-1}$ compared with 103 $gC.m^{-2}.yr^{-1}$ in Arc (Struski and Bacher, 2006; Glé et al.,
389 2008).
390
391 *Grazing and viriolysis control*
392 Our results demonstrate that primary producers (small and large phytoplankton cells) and
393 autotrophy-based mechanisms (cf. PCA results) are important in Arcachon Bay (Arc). However,
394 Marennes Bay (M-O) is characterized by a higher potential for primary production, suggesting an
395 uncoupling between phytoplankton abundance and productivity. The difference in phytoplankton
396 abundance could be typically explained by (1) pelagic and benthic metazoan grazing, which is
397 relatively low in M-O (Sautour and Castel, 1998) compared to Arc; (2) flushing out, which is
398 relatively higher in M-O because it is quite an open system; (3) settling (Sautour and Castel, 1993);
399 (4) light limitation (Pomeroy and Deibel, 1986; Sautour and Castel, 1993) and trophic status (meso-
400 eutrophic in M-O vs. mesotrophic in Arc).
401 In addition, we show that in M-O, phytoplankton could also be controlled by viruses; this is
402 corroborated by the simultaneous virus-bacteria uncoupling (Fig. 2). Viruses have been shown to
403 modify the taxonomic diversity of their host communities, i.e. prokaryotes (Thingstad and Lignell,
404 1997), and protists (Suttle, 2007) restraining the rapidly growing and succeeding species. Moreover,
405 these successions of active phage-host systems would maintain a relatively constant baseline of host
406 standing stocks (Wommack and Colwell, 2000). In addition, in M-O, virus infection could maintain
407 the phytoplankton standing stock baseline at a low level. Thus, viral production may rely on
408 autotroph lysis as well as bacterial lysogeny events. Moreover, the *in vitro* experiments show that,
409 in the presence of protist predators, the potential for viral lysis of the bacteria standing stock was
410 lower in M-O (2.6%) than in Arc (39%) during the phytoplankton bloom. Indeed, viral control of
411 phytoplankton was less clear in Arc and the link between bacteria and viruses was strong. Without
412 nanoflagellates, the release of large numbers of free viruses was in line with an increase in bacterial
413 mortality of up to 70% of the bacterial standing stock, as already suggested by the contrasting

414 dynamics of nanoflagellates and viruses during the *in situ* survey (Fig. 2; Table 2). In contrast, the
415 dynamics of nanoflagellates and viruses were similar in M-O, and during the spring bloom, virus-
416 mediated bacterial mortality did not change significantly (9.4-13.6% with and without small
417 nanoflagellates, respectively).
418 Thus, the two trophic models that prevailed during the phytoplankton spring bloom are also
419 reflected in the interactions between the microbial compartments. The impact of nanoflagellates and
420 viruses on picoplankton differs in a multivorous web (Arc) compared to a herbivorous web (M-O).
421 The *in vitro* experiments carried out in Arc showed that the strong potential for viral bacteriolysis
422 (viral shunt: Wilhelm and Suttle, 1999) contributes to the development of picophytoeukaryotes
423 (picoeuk) due to an increased supply of organic and inorganic nutrients. The presence of
424 nanoflagellates, previously described as predators of picoeuk (Sherr and Sherr, 1994), appears to
425 counteract the indirect positive effect of viruses on the latter. Thus, the contrasting effect of viruses
426 and nanoflagellates on picoeuk can be defined as antagonist top-down control.
427 While the herbivorous web dominated in M-O, the antagonist impact could not be observed during
428 *in vitro* experiments because of the very low abundance of picoeuk in the sampling water. The
429 bacterioplankton were the only picoplankton compartment affected by viral enrichment in M-O:
430 through the viral shunt, the absence of picoeuk reduces the competition for nutrients and thus
431 increases the positive effect of viral enrichment on the growth of non-lysed bacteria.
432 In our study, nanoflagellate treatment in both bays did not affect bacterial abundance or growth, in
433 contrast to published data (Zhang et al., 2007). However, this does not necessarily mean that
434 predation by nanoflagellates had no effect. Indeed, flagellate grazing on picoeuk in Arc potentially
435 released particulate organic matter, provoking the observed increase of bacterial proteolysis.
436 Furthermore, with size-selective predation, the loss of bacteria through grazing is compensated by
437 the growth of predation-resistant cells, suggesting that nanoflagellates can affect the phenotypic
438 structure of bacterioplankton (Gonzalez et al., 1990; Monger and Landry, 1991; Simek et al., 1997;
439 Posch et al., 1999). Moreover, considering the BCC data, the morphological diversity of bacteria

and the mean biovolumes of bacterial sub-populations, there should be a preferential grazing of *α-proteobacteria* (small coccoid-shaped cells) by nanoflagellates compared to *β-proteobacteria* (large bacillus-shaped cells). Beta dominate in freshwater but can also be found in coastal waters, without a defined marine or riverine origin (Nold and Zwart, 1998). Indeed, they are present in both bays in proportions equivalent to more specifically marine groups like Alpha during spring phytoplankton blooms. More specifically, the hypothesis that Alpha are more vulnerable to grazing is confirmed in M-O. Moreover, Beta are not as opportunistic as Gamma but nevertheless are metabolically very reactive: the sensitivity of Alpha to grazing and to viral infection provides nutrients for Beta, which are resistant, and this is highlighted by an increase of bacterial proteolysis.

However, Beta were as vulnerable to grazing as Alpha in Arc. A different clonal composition of Beta could explain the variable response to predation pressure between the two bays (Brümmer et al., 2003; Schwalbach et al., 2004).

The variable responses of bacterial groups to viral lysis in the present study confirm previous results concerning BCC (Bouvier and Del Giorgio, 2007) and bacterial metabolism (Middelboe et al., 1996). Consequently, viral production can be modified according to the trophic period. During the multivorous phase of the phytoplankton spring bloom in Arc, viral production was synergistically depleted by the presence of flagellates and viral enrichment. The predation of picoeuk and bacteria decreases the number of host cells and could lead to a general drop of lytic or lysogenic viral production. Moreover, the acquisition of viral resistance, as observed with Alpha bacteria in virus-enriched incubation, would accentuate the negative impact on viral production. During the herbivorous phase of the phytoplankton spring bloom in M-O, the response in terms of viral production was the opposite. In the presence of flagellates, after virus enrichment, the development of predation-resistant and virus-sensitive Beta, at the expense of Alpha, led to a positive synergistic action of predators and viruses on viral production. This stimulation in the presence of flagellates has already been described (in contrast to the inhibition found in Arcachon) and explained by changes in BCC under grazing pressure (Simek et al., 2001; Weinbauer et al., 2003; Weinbauer et

al., 2007; Pradeep Ram and Sime-Ngando, 2008). Interestingly, our results also suggest that this stimulation occurs during the herbivorous period.

Conclusion

The temporal structure of trophic networks has been described in two bays on the French Atlantic coast, providing major new elements for future studies, in particular for comparing microbiological relationships during similar trophic periods.

Considering that trophic periods were defined in the same way for the two bays, the results of picoplankton and viral production can be extrapolated in relation to how multivorous and herbivorous trophic models work in general. Thus, during a multivorous period, viral lysis together with flagellate grazing may reduce viral production and act antagonistically toward picoeukaryotes. During a herbivorous period, flagellates and virus could both stimulate viral production. These contrasting responses sustain the debate about potential shifts in viral production due to grazing: positive or undetectable responses have already been found (Hornak et al., 2005; Weinbauer et al., 2007). For the first time, a negative effect of flagellate predation and viral lysis on viral production has been described and linked to a trophic model, the multivorous food web.

Experimental procedures

Study site and sampling strategy of in situ surveys

The study was carried out in two sites along the Bay of Biscay on the French Atlantic coast, Arcachon Bay and Marennes Oleron Bay, which are 150 km apart. Both are shallow, soft-bottom bays that are under strong tidal influence (Fig. S2). Arcachon Bay (44°40N, 1°10W) is a 180 km² triangular shaped coastal lagoon that communicates with the Atlantic through a broad channel to the south (Fig. S2). The Leyre River is the major source of freshwater for the lagoon (100 000 $m^3.y^{-1}$; Glé et al., 2007) which has a meso- to macrotidal regime with amplitudes from 1.1m to 4.9m. It is about 25 m deep at the entrance but the inside channels are shallow (5-10 m) , while about 70% of the bay is covered by intertidal flats (Blanchet et al., 2008). The water residence time is more than

492 3 weeks in the South East part of the bay. Marennes Oleron Bay to the North (46°00N, 1°10W) is
493 similar in size (175 km²) and depth (mean=8.6m; Stanisière et al., 2006), and intertidal mudflats
494 cover about 60% of its surface (Fig. S2). Freshwater arrives mainly from the Charente River, with
495 $3x10^9$ $m^3.y^{-1}$, or 90% of the total freshwater input (DDE-Charente Maritime/HYDRO-MEDD/DE).
496 The bay is characterised by a macrotidal system, with amplitudes from 2 to 6m. It communicates
497 with the ocean through two passes, the Pertuis de Maumusson to the South and the Pertuis
498 d'Antioche to the North, with a relatively short residence time of 11 days. Tidal exchanges are
499 greater through the northern pass, resulting in a north-south circulation of oceanic (Bay of Biscay)
500 water (Struski, 2005).
501 Sampling was carried out twice weekly, from January to August 2005, at 2 stations in Arcachon
502 Bay: B13, near the mouth of the Bay, which is under oceanic influence, and Comprian, in the inner
503 basin, which is affected by the continental runoff. Five stations were surveyed in Marennes Oleron
504 Bay on a bi-monthly basis (January to December 2006). The stations were spread out over the entire
505 basin: one was close to the southern pass (A= Auger), two were above mudflats close to the
506 continent (Br=Brouage) and island (O= Oleron) coasts, one was close to the northern pass
507 (B=Boyard) and one near the mouth of the Charente River (C=Charente).
508 Water samples were collected in both bays near the surface (<1m) using Niskin bottles. Subsamples
509 were fixed on board. The rest were kept in 8L polypropylene dark bottles for laboratory analyses
510 (transfer time <3h). Physical parameters (temperature, salinity, pH) were recorded on board with
511 multiparameter probes (Seabird SBE25 in Arcachon and YSI 6600EDS-M in Marennes).
512
513 *Batch culture design*
514 Experimental water was collected at high tide at the surface from the central Comprian station in
515 Arcachon Bay on 17 May 2006 and from the northern "E" station in Marennes Bay (Fig. S2) on 24
516 April 2007. The experimental protocols were based on a slightly modified version of the method of
517 Auguet et al. (2009). In the lab, three water categories were obtained by sequential filtration from

518 75L of seawater: (1) <20µm, filtered through nylon membranes, (2) <3µm, filtered through
519 Versapor acrylic polymer membranes (Gelman Sciences Inc.) and (3) <0.8µm, filtered through
520 cellulose acetate membranes (Sartorius). Viral particles were then concentrated from 40L of <0.2-
521 water, as a high molecular weight concentrate (HMWC) using a 30kDa polysulfone cartridge
522 (Sartorius). Viral-free water was kept for use as dilution water. Six different treatments were
523 prepared with the three water categories (Table 3), each diluted ten fold with the dilution water and
524 enriched (x2) or not with viruses (HMWC).
525 Each of the six treatments were triplicated in 2.4L Nalgene polycarbonate bottles filled with 2200
526 ml and incubated under *in situ* conditions of light and temperature in circulating seawater tanks.
527 Samples for bacterial, viral, phytoplankton and flagellate counts were collected at 0, 6, 12, 18, 24,
528 38 and 48h. Samples for bacterial activity and diversity were collected every 12h. Samples for
529 ciliate abundance were collected at the beginning and the end of the experiment (<20µm treatment).
530
531 *Physical and chemical parameters*
532 Seston was assessed by filtering 200 to 500 ml of water according to turbidity and processed
533 according to Auguet et al. (2005). Samples for dissolved inorganic nutrients (Silicate (Si),
534 Phosphate (PO_4), Urea, Ammonium (NH_4), Nitrite (NO_2) and Nitrate (NO_3)) were filtered (20ml,
535 Whatman GF/F) into glass flasks and stored at -20°C for analyses with an autoanalyser (Bran and
536 Luebbe, AA3 for Arcachon and Skalar for Marennes (Strickland and Parsons, 1972)).
537
538 *Biological parameters*
539 Chlorophyll *a* (chla) and pheopigment were analysed by filtering 200 ml of water through Whatman
540 GF/F filters (25 mm). Filters were stored for less than a month at -20°C before extraction (acetone
541 90% overnight at 4°C and shaken). Samples were analysed by the fluorimetry method (Turner, TD
542 700) according to Yentsch and Menzel (1963).

543 Subsamples (3ml) for viral and bacterial counts were fixed with 0.02-µm filtered formaldehyde (2%
544 final concentration) and stored for less than a week at 4°C. Samples were enumerated by
545 epifluorescence microscopy after staining for 30 min with Sybr Green I (Noble and Fuhrman, 1998).
546 Bacteria and viruses were counted in at least 15 fields chosen randomly under blue excitation (Zeiss
547 Axioskop 1000x). Using flow cytometry (FACSCalibur, Bekton Dickinson) and Sybr-GreenI
548 staining, bacteria cells were distinguished between High Nucleic Acid (HNA) and Low Nucleic
549 Acid (LNA) cells. Furthermore, for batch culture samples, HNA cells were clustered in 4 sub-
550 groups (populations 3, 4, 5 and 6) according to decreasing biovolume (2, 0.8, 0.3 and 0.15 μm^3,
551 respectively) and fluorescence. Small phytoplankton cells (<10µm; picoeukaryotes, nanoeukaryotes
552 and Synechococcus) were analysed using flow cytometry according to Joux et al. (2005).
553 Microphytoplankton (>10µm) and ciliate subsamples were fixed and stained in alkaline lugol (final
554 concentration, 1%). 10-20 ml subsamples were decanted in Utermöhl settling chambers before
555 counting and sizing by inverted microscopy (white light, Leica DMIRB, 400x – 630x). For ciliate
556 samples, the suspended matter of a 1L subsample was settled for 6h at *in situ* temperature before
557 siphoning off the top 850ml and fixing a 150ml aliquot of the siphoned water. Nanoflagellates
558 (60ml) were fixed with paraformaldehyde (final concentration 1%) and stored at 4°C for less than a
559 week. Cells filtered onto 0.8µm black polycarbonate membranes (Nucleopore) were stained with
560 DAPI (Porter and Feig, 1980) and counted under ultraviolet excitation (Zeiss Axioskop 1000x) with
561 a distinction between unpigmented (HNF) and pigmented (ANF) nanoflagellates based on the
562 absence of chlorophyll fluorescence under green excitation. At least 100 nanoflagellates were
563 counted for each slide.
564 Mesozooplankton were collected 1m below the surface in Arcachon Bay using a standard 200µm
565 WP-2 net towed slowly against the current. The catch was fixed in 5% seawater/formalin and
566 counted with a binocular loupe.
567
568

569 *Bacterial growth, viral production and bacterial mortality*

570 The maximum specific growth rate (h^{-1}) of bacteria was calculated from log transformed abundance
571 data during the exponential growth phase in each bottle. Total viral production rate (h^{-1}) was
572 calculated by adding the net increase of logarithmic viral abundance during each peak divided by
573 the incubation time. The bacterial mortality ($cell.ml^{-1}.h^{-1}$) induced by viral lysis was determined in
574 unenriched treatments from the viral production (sum of the net increase of viral abundance divided
575 by the duration of the experiment, $particles.ml^{-1}.h^{-1}$) divided by an assumed burst size of 50. The
576 percentage of bacterial cells lysed (d^{-1}) was equivalent to the bacterial mortality rate ($cell.ml^{-1}.d^{-1}$)
577 divided by the total bacterial abundance.

578

579 *Bacterial activity*

580 Leucine aminopeptidase activity was measured in duplicate at T0, 12, 24, 36 and T48h by using L-
581 Leucine-7-amino-4-methylcoumarin hydrochloride (Leu-MCA, Sigma) as a model protein substrate,
582 according to Hoppe (1993). The enzyme Vmax was determined by adding substrate to the sample,
583 giving a final saturating concentration of 1000µM. Incubations were performed in the dark and at *in*
584 *situ* temperature for around 5h, then stopped using Sodium-dodecyl sulfate (1% final concentration)
585 The release of 7-amino-4-methylcoumarin dye (MCA, Sigma) was measured with a
586 spectrofluorometer (Kontron, model SFM 25) at 380nm excitation and 440nm emission. Solutions
587 of MCA (20 to 2000nM) were used as a standard for calibration. The potential enzymatic activity
588 per cell was obtained by dividing Vmax by the corresponding bacterial abundance (specific Vmax,
589 $fM.cell^{-1}.h^{-1}$).

590

591 *Bacterial diversity*

592 Bacterial community composition was determined with fluorescent *in situ* hybridization using eight
593 Cy-3 labelled probes (MWG-Biotech): a mix of Eub 338 I,II and III for Eubacteria, Pla5a for
594 Planctomycetes, CF319a for Cytophaga-Flavobacterium cluster, Alf1b for *α-proteobacteria*, Bet43a

595 for *β-proteobacteria*, Gam42a for *γ-proteobacteria*, Arch915 for *archaea* and a non specific probe
596 as a control (Amann et al., 1995; Bouvier and Del Giorgio, 2007).
597 Samples were fixed in paraformaldehyde (2% final) and stored for at least 12h at 4°C before
598 freezing at -20°C. Bacteria were filtered onto 0.2µm polycarbonate membranes (Whatman), rinsed
599 with 0.2µm-filtered milli-Q water, dried and stored at -20°C. Pieces of membrane were hybridized
600 with 9µl of hybridization solution (Cy3-labelled probe, 2.5ng.µl^{-1}) for 4h30 at 46°C and washed at
601 48°C for 15 mn. The membrane was then rinsed for a few seconds successively in 0.2µm-filtered
602 Milli-Q water then 80% ethanol and finally air dried. Bacterial cells were counter-stained with
603 DAPI mounting solution (0.25µg.µl^{-1} in (Citifluor (Biovalley)/Vectashield (Ablys, SA, Paris): 4/1).
604 At least 10 fields were counted on an epifluorescence microscope (Axioskop, Zeiss) using Cy3
605 (blue) and DAPI (UV) filters.
606
607 *Identification of trophic dynamics*
608 Trophic periods were defined empirically using the 4 conceptual trophic flux models of Legendre
609 and Rassoulzadegan (1995), primarily from the abundances of phytoplankton and bacteria but also
610 taking into account the dynamics of viruses, HNF and ciliates (Table 4).
611 The herbivorous food web is characterized by blooms of large (>10µm) phytoplankton cells. During
612 this period there is in general an increase in the number of ciliates. The microbial food web is
613 defined by an increase of small autotrophs (phytoplankton <10µm) and heterotrophs (bacteria).
614 Their potential predators (HNF and ciliates) may increase in number. The microbial loop, a closed
615 system maintained by nutrient regeneration, is characterized by high abundance of exclusively
616 heterotrophic micro-organisms (bacteria, HNF and ciliates). Finally, the multivorous food web is a
617 transitional model where all trophic pathways are actively contributing to the dynamics of the
618 ecosystem.
619 The trophic organization identified for each bay was then formalized using multivariate factorial
620 discriminant analysis (FDA). FDA classifies the samples using predefined criteria (i.e. the trophic

621 model) to discriminate samples according to independent variables (all abiotic and biotic
622 parameters). Prior to the FDA analysis, data were normalized and tested for heteroskedasticity
623 (Levene test, Ho: equal variances between criteria). The purpose was to test whether samples are
624 classified as predicted by the empirical trophic model and to assess the relative importance of the
625 independent variables in the classification. The significance of clustering is tested by a Wilks
626 Lambda test (low Lambda values corresponds to significant ($p<0.05$) clustering).
627
628 *Statistical analysis*
629 To compare field data between the 2 bays, the Arcachon results (3-day intervals) were regrouped by
630 sliding means to obtain 15-day time steps, as in Marennes Bay. Statistical analyses were performed
631 with log transformed data using XlStat (Addinsoft; version 7.5.2: one way- or two way-ANOVA,
632 simple regression, Principal Component Analysis (PCA) combined with Hierarchical Ascendant
633 Classification (HAC), Factorial Discriminant Analysis (FDA)) and Minitab (version 15: stepwise
634 multiple regressions and Levene test for Heteroskedasticity).

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Table and figure legends:

Table 1: Annual means and simple linear regressions between bacterioplankton (BA) and virioplankton (VA) at the sampling stations of Marennes Oléron Bay (2006) and Arcachon Bay (2005). Linear regressions were performed with log transformed data.

	Stations	BA (cells.ml^{-1})	VA (particles.ml^{-1})	Equation	r^2	p	n
Marennes	Auger	$4.04x10^6$ (min-$8.70x10^5$/max-$1.01x10^7$)	$3.18x10^7$ (min-$5.24x10^6$/max-$5.97x10^7$)	log BA= 1.5logVA-4.7	0.60	<0.0001	20
	Boyard	$3.95x10^6$ (min-$1.01x10^6$/max-$1.30x10^6$)	$3.36x10^7$ (min-$1.82x10^7$/max-$5.59x10^7$)	log BA= 1.45logVA-4.39	0.46	0.001	20
	Charente	$4.60x10^6$ (min-$1.16x10^6$/max-$1.17x10^7$)	$3.96x10^7$ (min-$2.01x10^7$/max-$6.36x10^7$)	log BA= 0.84logVA+0.23	0.18	0.092	17
	Oléron	$3.70x10^6$ (min-$1.05x10^6$/max-$9.03x10^6$)	$3.07x10^7$ (min-$1.82x10^7$/max-$5.07x10^7$)	log BA= 1.74logVA-6.51	0.45	0.001	20
	Brouage	$4.28x10^6$ (min-$7.10x10^5$/max-$1.8x10^7$)	$3.35x10^7$ (min-$1.66x10^7$/max-$6.08x10^7$)	log BA= 1.6logVA-5.5	0.51	0.001	19
Arcachon	B13	$1.05x10^6$ (min-$3.10x10^5$/max-$1.58x10^6$)	$4.48x10^6$ (min-$7.50x10^5$/max-$8.11x10^6$)	log BA= 0.6logVA+2.06	0.83	<0.0001	31
	Comprian	$1.32x10^6$ (min-$5.00x10^5$/max-$2.38x10^6$)	$6.70x10^6$ (min-$1.44x10^6$/max-$1.01x10^7$)	log BA= 0.47logVA+2.93	0.60	<0.0001	31

Table 2. Stepwise linear regression of bacteria, virus, flagellate and ciliate abundance according to environmental parameters in Arcachon and Marennes bays for the entire year and according to the season. The seasons were defined based on the PCA results. Descriptors: BA: Bacterial abundance, VA: Viral abundance, HNF: Heterotrophic nanoflagellate abundance, ANF: Autotrophic nanoflagellate abundance, HNA: Abundance of high nucleic acid bacteria, Cil: Ciliate abundance, Pico: Picoplankton abundance, Nano: Nanoplankton abundance, Syn: *Synechococcus* abundance, Dino: Dinophyceae abundance, Diat: Diatom abundance, Cop: Copepods abundance T°w: Water temperature, S%: Salinity, Crypto: Cryptophyceae abundance, Seston Org: Organic seston concentration, NH_4: Ammonium, PO_4: Phosphate, NO_2: Nitrite, Chla: Chlorophyll *a* concentrations, Pheo: Pheopigment concentrations. Logarithmic transformations were applied to all data. The coefficient of determination (adjusted r^2) was corrected according to the number of variables used in the regression. Copepods were analysed only at Arcachon and taken into account in stepwise regression analysis, but their inclusion does not interfere with regression results except for HNF in winter. Bold parameters are discussed in the text.

		Multiple regression equations	r^2 adjusted	n
Marennes	Annual	log BA=2,72+0,02T°w***-0,48logSyn***+0,23logPico***+0,2logNano***+**0,39logVA*****+0,013S%**+0,09logHNF*	90.70	84
		log VA=6,18+0,42logBA***-0,007S%*-0,003coef***+0,31logChla***-0,14logCil*****-0,116logPico*****-0,22%HNA**	71.02	84
		log HNF=-5,23+0,86logpH***+0,125logDiat**	23.68	84
		log Cil=1,19+0,46logChla***+0,44logNano***+0,11logDiat**	60.15	84
	Winter	log BA= 2,02+0,02T°w*****+0,5logVA*****+0,002coef*****+0,09logHNF****	81.21	40
		log VA=4,35+0,68log BA***-0,004coef***-0,28logSyn**+0,14logPheo*	77.31	40
		log HNF= -8,16+1,23logpH***-0,3logSeston Org*+0,17logDiat**	47.01	40
		log Cil=7,45+1,27logChla***-0,58logVA**-0,41logPheo**	47.87	40
	Summer	log BA=2,94+0,32logNano*****+0,17logNH$_4$****-0,19logNO$_2$****-0,38logSyn***+0,2logPico*****+0,41logVA****+0,11logchla*	74.8	44
		log VA= 7,61-0,003coef**+0,2logchla**+0,11logNH$_4$*	27.21	44
		log HNF=…..		44
		log Cil=0,07+0,52logchla***+1,03logNano***-0,04T°w***+0,07logDino*	71.64	44
Arcachon	Annual	log BA= -2,16+0,55log VA***+0,52logPico***+0,24logSyn***	94.11	31
		log VA= 2,36+1,38log BA***-0,73logSyn*****-0,46logPico****-0,19logDino**-0,03T°w**+0,01S%*	92.83	31
		log HNF=18,19+0,22logCrypto***-0,9logVA***-1,04logCil***+1,94logSeston Org**-0,37logNH$_4$*	84.16	31
		log Cil= 1,36+1,29logSeston Org***+1,11%HNA**+0,74logPico***-0,4logPO$_4$***+0,13logDino**	97.38	31
	Winter	log BA= 0,97+**0,44logVA*****+0,99logANF*****-0,38logHNF****	91.04	14
		log VA= 8,48-0,52logDino***	64.67	14
		log HNF=15,47-2,1logNano*****-0,49logCil*****+0,28logCop**+0,3logPO$_4$*	94.96	14
		log Cil= -3,09+1,61logSyn***	82.23	14
	Summer	log BA= 2,47-**0,51logNH$_4$*****+0,57logVA*****+0,33logPO$_4$*****	95.86	17
		log VA= 2,93-**0,14logHNF****+0,75logChla***+0,76logBA***-0,22logSyn**	96.71	17
		log HNF= -14,53+0,4logCrypto***-0,55logDiat***+0,56S%**-0,61logPheo*	92.83	17
		log Cil= 15,49+1,58logSeston Org***-0,24S%***+0,2logVA**-0,53logNO$_2$*	98.16	17

***p<0.001 **p<0.01 *p<0.05

Table 3. Description of treatments used in batch culture experiments, with abbreviations employed in the text.

Water category	Organisms	Treatment	Abbreviation
<0.8µm	Bacteria Virus Picophytoeukaryotes	Control	0.8µ-Tr
		Virus enriched	Enriched-0.8µ-Tr
<3µm	+ Small nanoflagellates	Control	3µ-Tr
		Virus enriched	Enriched-3µ-Tr
<20µm	+ Large flagellates	Control	20µ-Tr
		Virus enriched	Enriched-20µ-Tr

Table 4. Definitions of the characteristics of the four trophic models defined by Legendre and Rassoulzadegan (1995) and according to Rodriguez et al. (2000) and Mousseau et al. (2001). Arrows describe the abundance dynamics of the biological compartments (increasing, decreasing or fluctuating). ** Major parameters taken into account for the definition of trophic periods; * secondary parameters. HNF: unpigmented nanoflagellates.

Trophic model	**Large phytoplankton (>10µm)****	**Small phytoplankton (<10µm)****	**Bacteria****	Virus*	HNF*	Ciliates*
Herbivorous food web	↗	↘	↘	→	→	↗
Multivorous food web	↗	↗	↗	↗	↗	↗
Microbial food web	↘	↗	↗	→	↗	↗
Microbial loop	↘	↘	↗	↗	↗	→

Table S1. Mean, minimum and maximum values of the abiotic and chlorophyll *a* parameters, with dates, at Marennes Bay (4 inner stations: A, B, O, Br and one riverine station: C). C was analysed separately because of the significant differences compared to the four other stations (H_0 rejected in a Tukey multiple comparison test). The seasons were defined based on the clustering of stations using PCA. Winter season: January to 5 April and November-December 2006. The remainder of the year was considered as the summer season. Runoff data were supplied by DDE-Charente Maritime/HYDRO-MEDD/DE for the Charente River.

Marennes	Winter			Summer		
	Mean	Min/*date*	Max/*date*	Mean	Min/*date*	Max/*date*
Water temperature (°C)						
A,B,O,Br	9.19	5.1/*1Mar.*	14.5/*28 Nov.*	18.74	13.3/*2 May*	24.7/*26 Jul.*
C	8.59	4.8/*7 Feb.*	12.9/*28 Nov.*	19.67	13.6/*2 May*	24.8/*26 Jul.*
Salinity (PSU)						
A,B,O,Br	31.96	28.3/*4 Apr.*	34.3/*7 Nov.*	33.86	31/*25 Apr.*	35.3/*24 Aug.*
C	20.61	10.4/*1 Mar.*	29.8/*24 Jan.*	28.2	12.7/*25 Apr.*	33.9/*1 Aug.*
Nitrate (µM)						
A,B,O,Br	42.40	12.09/*12 Dec.*	97.98/*28 Nov.*	15.12	0.53/*1 Aug.*	78.17/*25 Apr.*
C	90.76	46.95/*7 Nov.*	192.9/*12 Dec.*	47.46	6.65/*1 Aug.*	167.5/*25 Apr.*
Ammonium (µM)						
A,B,O,Br	7.16	0.01/*12 Dec.*	68.62/*28 Nov.*	2.43	0.35/*25 Apr.*	18.85/*24 Aug.*
C	13.07	1.15/*18 Jan.*	68.83/*12 Dec.*	2.66	0.41/*14 Jun.*	7.4/*25 Apr.*
Phosphate (µM)						
A,B,O,Br	0.97	0.5/*5 Apr.*	3.04/*1 Mar.*	0.48	0.01/*2 May*	1.22/*18 Sep.*
C	1.25	0.77/*12 Dec.*	1.61/*28 Nov.*	1.70	0.39/*4 Jul.*	3.8/*2 May*
Silicate (µM)						
A,B,O,Br	27.74	11.35/*18 Jan.*	94.28/12 *Dec.*	13.97	4.19/*2 May*	56.43/*26 Jul.*
C	39.14	27.03/*24 Jan.*	58.09/*28 Nov.*	31.97	18.3/*2 May*	60.45/*18 Sep.*
Chl *a* ($\mu g.l^{-1}$)						
A,B,O,Br	1.18	0.34/*7 Feb.*	8.31/*1 Mar.*	3.8	1.03/*18 Sep.*	16.78/*2 May*
C	3.58	0.43/*24 Jan.*	15.53/*7 Nov.*	4.34	1.35/*30 Oct.*	13.84/*2 May*
Total seston ($mg.l^{-1}$)						
A,B,O,Br	44.16	9.54/*24 Jan.*	163.34/*7 Nov.*	16.24	4.8/*5 Jun.*	134.6/*24 Aug.*
C	1637.9	18.31/*7 Feb.*	8820.5/*1 Mar.*	57.92	12.38/*5 Jun.*	263.5/*24 Aug.*
Charente runoff ($m^3.s^{-1}$)	104.19	24.50/*16 Nov.*	380/*10 Mar.*	29.46	9.83/*8 Sep.*	71.2/*25 Apr.*

Table S2. Mean, minimum and maximum values of the abiotic and chlorophyll *a* parameters with date occurrence at Arcachon Bay (B13 and Comprian stations). The seasons were defined based on the results of PCA clustering temporally the sampling stations; winter season from January to the end of April 2005. The rest of the year was considered as the summer season. Runoff data were supplied by DIREN-Aquitaine/HYDRO-MEDD/DE for Leyre River

Arcachon	Winter			Summer		
	Mean	Min/*date*	Max/*date*	Mean	Min/*date*	Max/*date*
Water temperature (°C)						
B13	10.30	7.4/*5Mar.*	14.8/*29 Apr.*	18.04	14.27/*14 May*	21.07/*23 Jul.*
Comprian	10.01	5.4/*5 Mar.*	16.42/*29 Apr.*	20.72	16.85/*14 May*	22.92/*16 Jul.*
Salinity (PSU)						
B13	34.26	24.02/*29 Apr.*	34.55/*9 Apr.*	34.49	32.3/*16 Jul.*	35.4/*2 Jul.*
Comprian	31.06	30.15/*10 Feb.*	32/*9 Apr.*	33.5	31.4/*6 May.*	35/*30 Aug.*
Nitrate (µM)						
B13	5	0.77/*29 Apr.*	11.21/*19 Feb.*	0.27	0.19/*23 Jul.*	0.48/*6 Ma.*
Comprian	8.19	2/*2 Apr.*	15.51/*31 Jan.*	0.93	0.26/*16 Jul.*	4.99/*6 May*
Ammonium (µM)						
B13	0.93	0.12/*29 Apr.*	4.71/*31 Jan.*	0.15	0.03/*14 May*	0.59/*30 Aug.*
Comprian	1.98	0.59/*12 Mar.*	6.46/*31 Jan.*	1.15	0.42/*16 Jul.*	2.38/*30 Aug.*
Phosphate (µM)						
B13	0.76	0.37/*29 Apr.*	2.34/*26 Mar.*	0.27	0.18/*30 Aug.*	0.36/*6 May*
Comprian	0.59	0.28/*10 Feb.*	1.8/*26 Mar.*	0.4	0.27/*9 Jul.*	0.82/*13 Aug.*
Silicate (µM)						
B13	4.65	0.82/*9 Apr.*	7.10/*23 Jan.*	3.6	1.31/*20 Aug.*	11.11/*25 Jun.*
Comprian	13.96	6.59/*2 Apr.*	21.93/*31 Jan.*	10.69	5.75/*30 Aug.*	17.78/*25 Jun.*
Chl *a* ($\mu g.l^{-1}$)						
B13	0.95	0.44/*12 Mar.*	1.61/*2 Apr.*	0.77	0/*30 Aug.*	1.55/*28 May*
Comprian	1.09	0.6/*5 Mar.*	1.9/*2 Apr.*	1.37	0.72/*18 Jun.*	2.32/*4 Jun.*
Total seston ($mg.l^{-1}$)						
B13	34.64	5.59/*2 Apr.*	216.2/*23 Jan.*	0.765	0.470/*6 May*	1.15/*18 Jun.*
Comprian	33.74	6.44/*19 Mar.*	208.7/*23 Jan.*	0.920	0.56/*30 Aug.*	1.36/*9 Jul.*
Leyre runoff ($m3.s^{-1}$)	13.46	9.69/*26 Mar.*	22.8/*27 Apr.*	6.44	4.02/*25 Aug.*	16.5/*1 May*

Figure 1

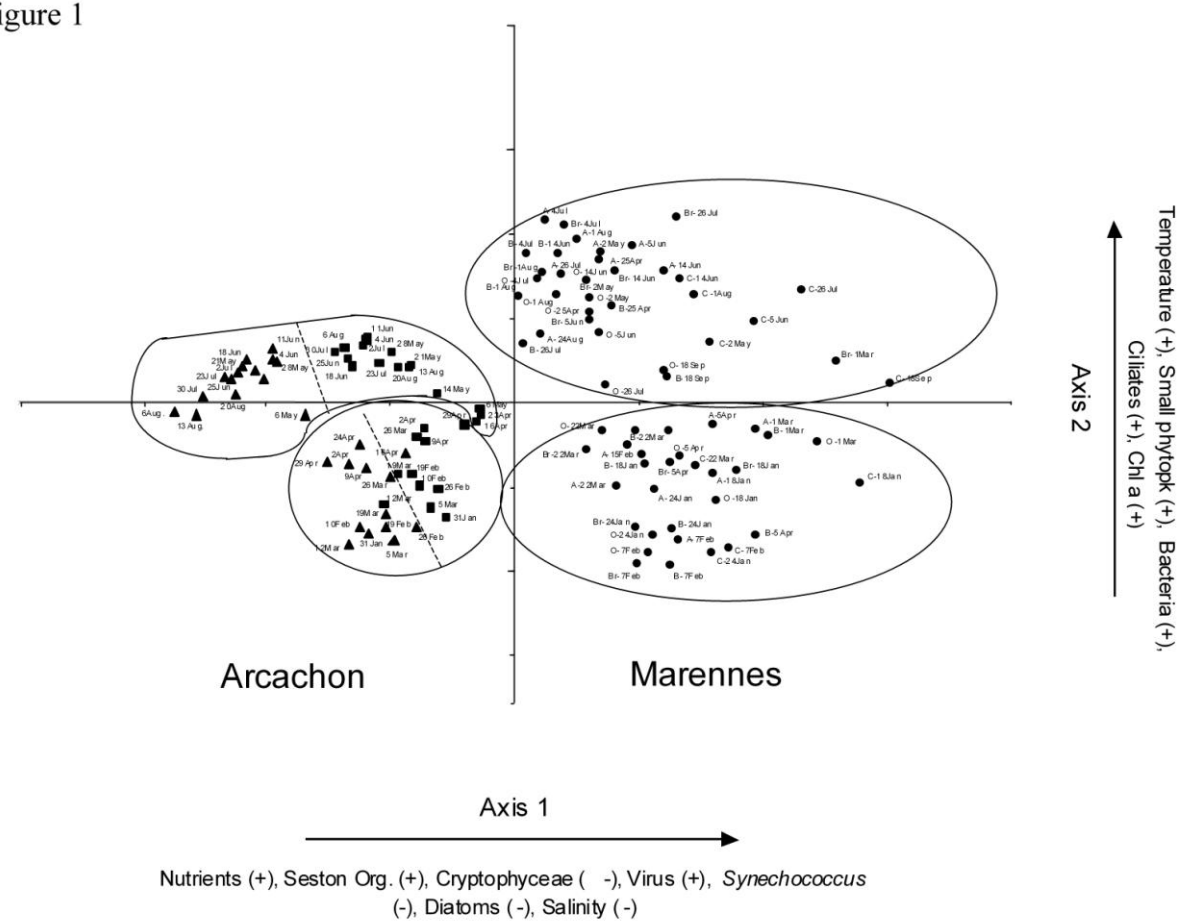

Fig. 1. Ordination by principal component analysis (PCA) of the basin stations obtained from microbiological, physical and chemical log transformed data in Arcachon (January to September 2005; ▲ B13, ■ Comprian, with sampling date) and Marennes-Oléron Bay (2006; ● with sampling stations and date). Arrows parallel to each axis: structuring variables. Ellipses: clusters based on hierarchical ascendant classification analysis (HAC) performed with coordinates of the station plots (straight and dotted lines: first and second discriminant in HAC, respectively).

Figure 2

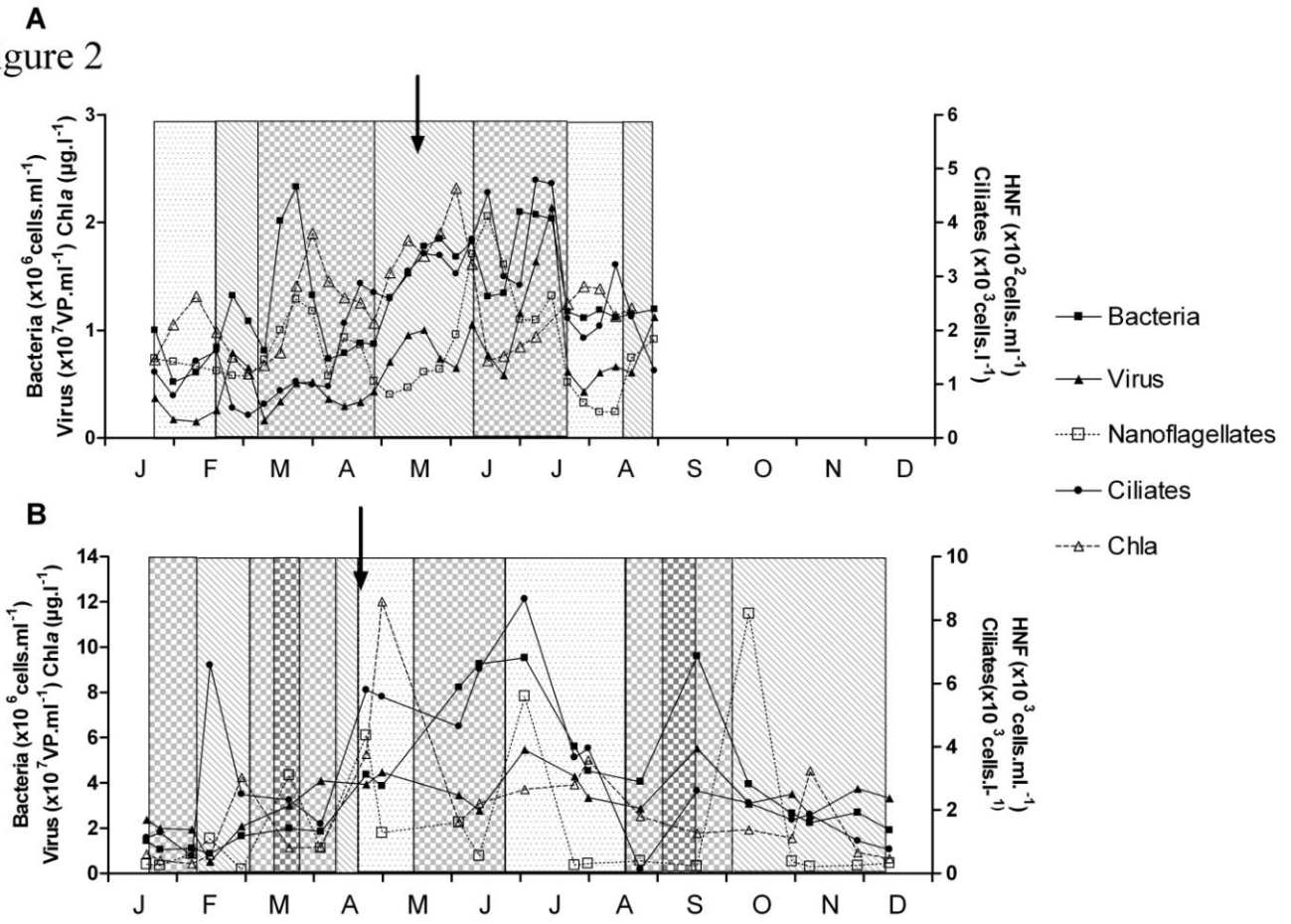

Fig. 2. Temporal dynamics of bacteria (▲), viruses (■), nanoflagellates (□), ciliates (x) abundance and chlorophyll *a* (Δ) concentration in the 2 study sites, Arcachon in 2005 (A: Comprian station) and Marennes in 2006 (B: Mean of the 5 stations). The trophic periods are represented by different background patterns: ⁘ Herbivorous food web; ▨ Multivorous food web; ▩ Microbial food web; ▩ Microbial loop. Arrows indicate the *in vitro* experiment periods.

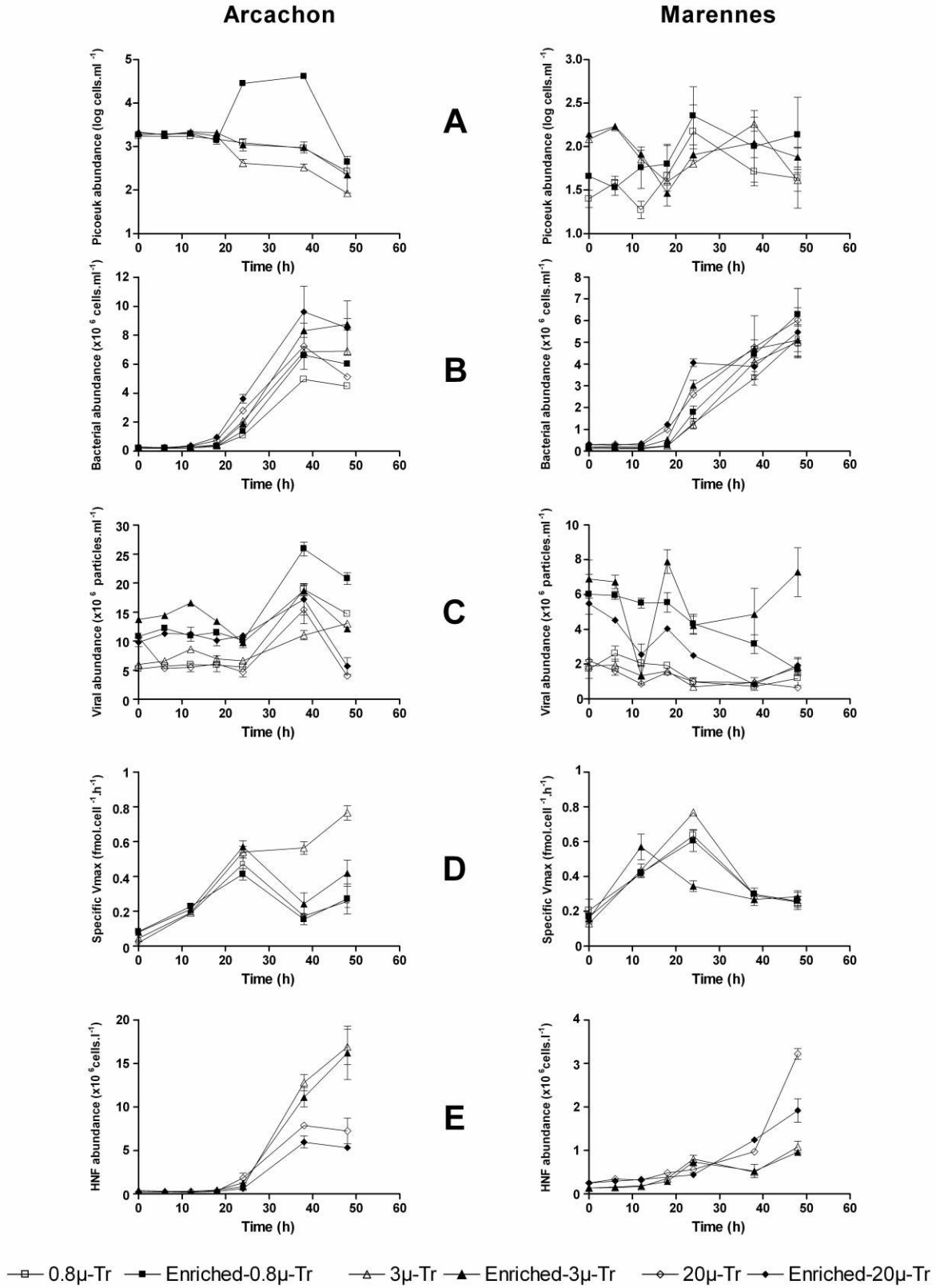

Fig. 3. Time course of the logarithmic abundance of picophytoeukaryotes (A), abundance of bacteria (B), viruses (C), heterotrophic nanoflagellates (HNF) (E) and specific aminopeptidase bacterial activity (D) in Arcachon and Marennes experiments. Means ± SD of triplicate incubations for each treatment.

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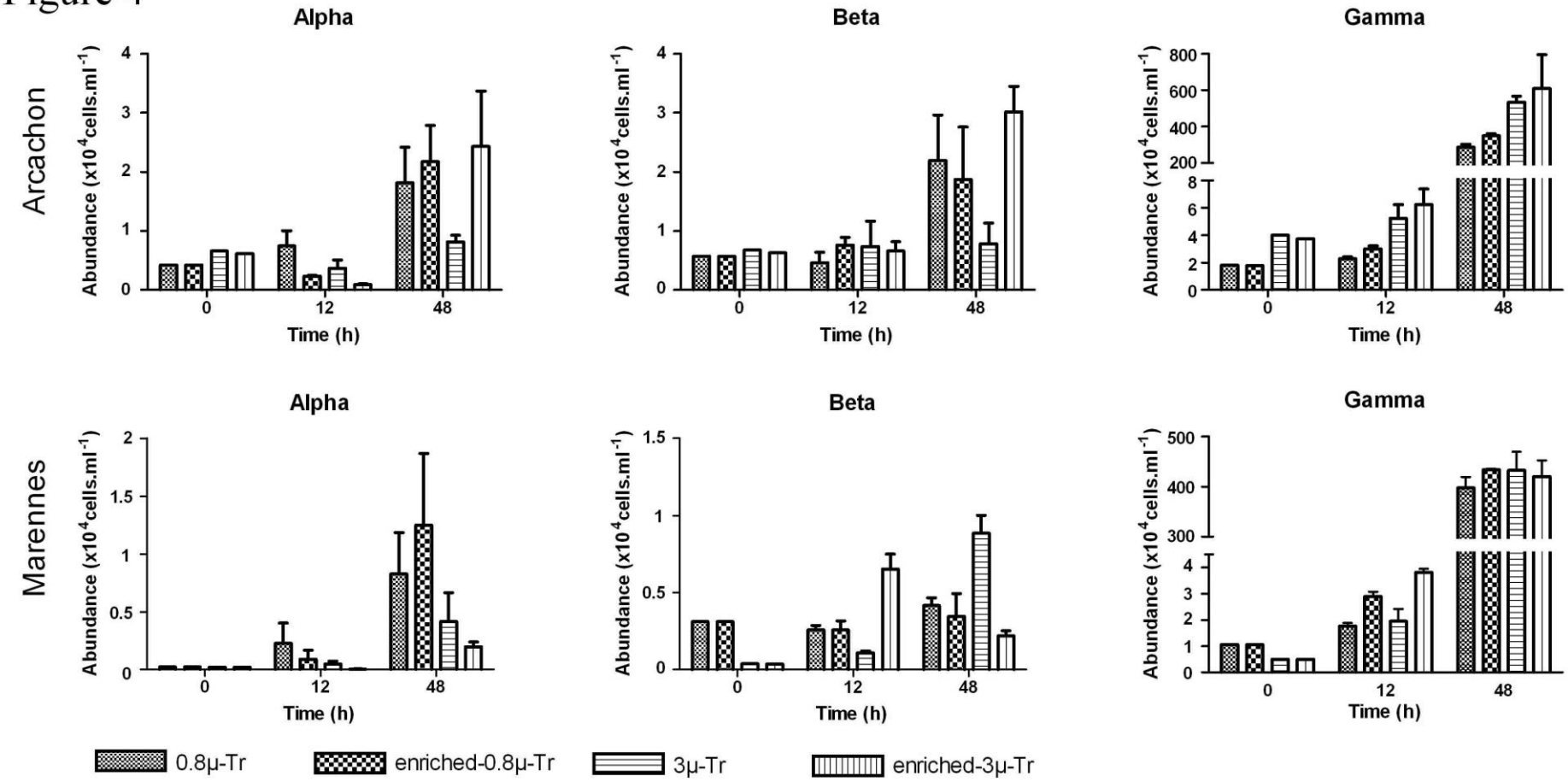
Figure 4



935
936
937 **Fig. 4.** Bacterial community composition based on FISH analysis: dynamics of Alpha-, Beta- and Gamma-proteobacteria in different treatments of
938 Arcarchon and Marennes experiments. Gamma abundance at T48 is read on the right axis of each graph. Means ± SE of triplicate incubations for each
939 treatment.
940

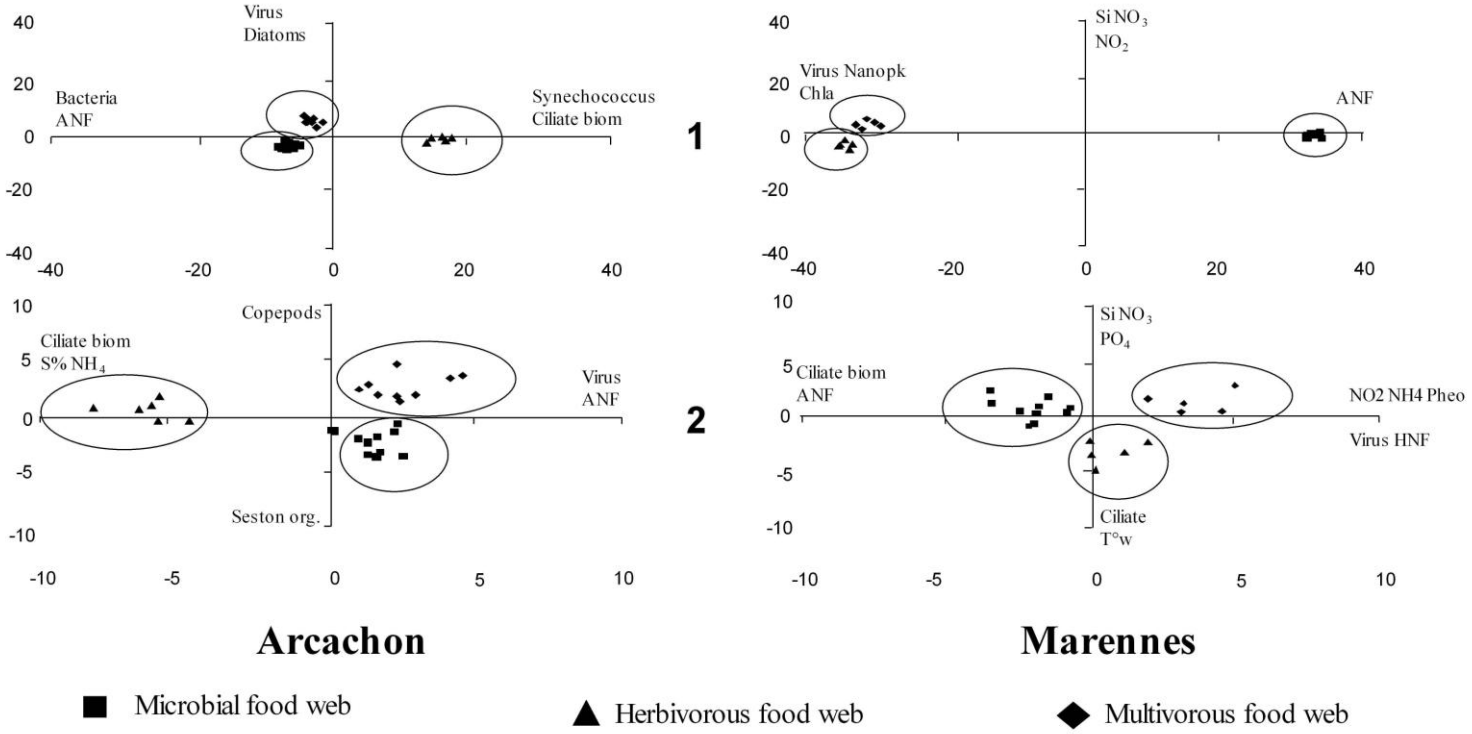


Fig. S1. Ordination by factorial discriminant analaysis (FDA) of samples from Arcachon (Comprian station) and Marennes (mean of the 5 stations). FDA generated from microbiological, physical and chemical data (log transformed) and using an empirical trophic model as a criterion variable (■, ▲, ♦): **(1)** with all the parameters and **(2)** excluding the principal factors used for the definition of trophic groups (large and small phytoplankton and bacteria abundance). Samples are clustered according to the trophic pattern. The microbial loop class was combined with the microbial food web class because few samples representing the former were found in Marennes and it was absent in Arcachon. Independent variables: salinity, water temperature, silicate, phosphate, ammonium, nitrate, nitrite, organic seston, pheopigment and chlorophyll *a* concentrations, abundance of viruses, bacteria, autotrophic nanoflagellates, heterotrophic nanoflagellates, ciliates, picoplankton, nanoplankton, Synechococcus, diatoms and Dinophyceae, biomass of ciliates and bacteria, copepods. Structuring variables are annotated along each axis: S%: salinity, ANF: Autotrophic nanoflagellates, Ciliate biom: Biomass of ciliates, Chla: Chlorophyll *a* concentration, Picopk: Picoplankton abundance, Nanopk: Nanoplankton abundance, Si: Silicate, NO_2: Nitrite, NO_3: Nitrate, NH_4: Ammonium, PO_4: Phosphate, Seston org.: Organic seston, Pheo: Pheopigment concentration, T°w: water temperature.

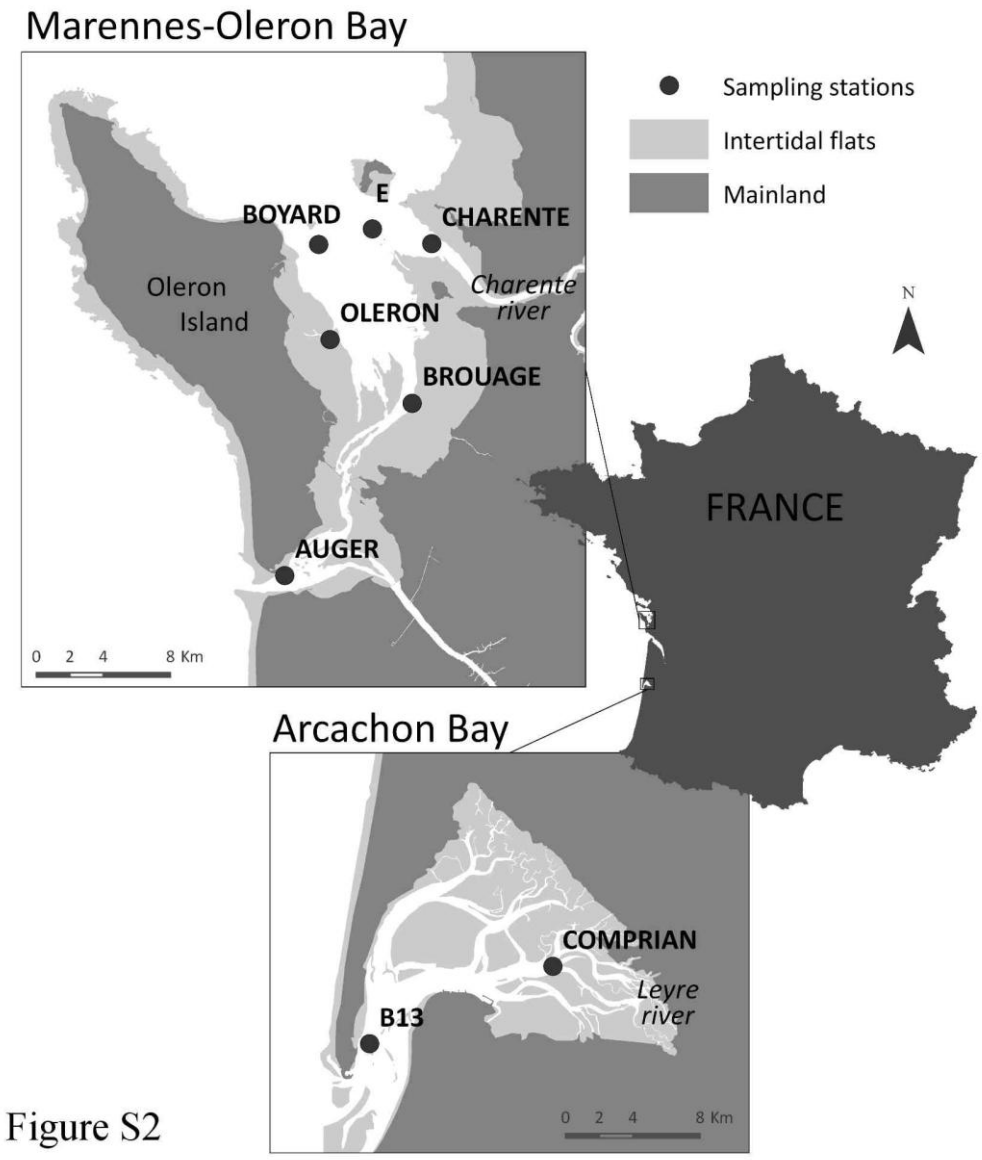


Fig. S2. Map of the 2 study sites, Marennes-Oléron Bay and Arcachon Bay, Atlantic coast, France, showing the locations of the *in situ* sampling stations: Marennes Bay, stations Boyard (45°58'43.73''N, 1°12'30.47''W), Charente (45°58'54.94''N, 1°07'17.28''W), Oléron (45°55'41.47''N, 1°11'47.1''W), Brouage (°53'45.58''N, 1°07'52.15''W) and Auger (45°48'02.3''N, 1°13'22.7''W). Arcachon Bay, stations B13 (44°36'54.98''N, 1°14'4.15''W) and Comprian (44°40'22.8''N, 1°04'38.1''W). Location of the sampling stations for *in vitro* experiments: in Marennes Bay, station E (45°59'18.7''N, 1°10'0.69''W), in Arcachon Bay, station Comprian.