1 Bacterial carbon demand and growth efficiency in a coastal upwelling system

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3 Christian Lønborg [a, b, d*], Sandra Martínez-García[c], Eva Teira[c] & Xosé A. Álvarez-Salgado[a]

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5 [a] CSIC, Insituto de Investigacións Mariñas, Eduardo Cabello 6, 36208 Vigo, Spain

6 [b] Scottish Association for Marine Science, Oban, Argyll, PA37 1QA, United Kingdom

7 [c] Departamento de Ecoloxía e Bioloxía Animal, Universidade de Vigo,36200 Vigo, Spain

8 [d] Present address: Swansea University, College of Biosciences, Wallace Building, Singleton Park,

9 Swansea, Wales SA2 8PP, United Kingdom

10 [*]Corresponding author:

11 Swansea University, College of Biosciences,

12 Wallace Building, Singleton Park,

13 Swansea, Wales SA2 8PP

14 United Kingdom

15 Email: clonborg@gmail.com

16 Phone: 004528608841

**Abstract**

Eleven culture experiments were conducted in the coastal upwelling system of the Ría de Vigo (NW Iberian Peninsula) by inoculating GF/C filtrated (10%) in 0.2 μm filtrated (90%) surface seawater collected under contrasting hydrographic conditions. Short term (4 days) laboratory incubations were performed in the dark at 15ºC and dissolved organic carbon (DOC) concentration, bacterial biomass (BB) and production (BP), and ETS activity were monitored to: 1) study the course of bacterial carbon demand (BCD) and growth efficiency (BGE) during the incubation period; and 2) determine how BCD and BGE were linked with the changing environmental conditions. BP decreased by < 4 times (range from 3 to 11) and ETS activity increased by 6 times (range from 1 to 75) of the initial values after 4 days of incubation. As a result, the BCD increased by 5 times (range from 1 to 16) and the BGE decreased by 15 times (range 2 to 55) over the same time interval. The BGE integrated over the 4-day incubation period ranged between 7 ± 1 % and 41 ± 11% (average ± SD: 28 ± 11%); integrated BGE increased significantly ($R^2$ = 0.64, p< 0.003) with the initial concentration of inorganic N (DIN) and decreased significantly ($R^2$ = 0.55, p < 0.01) with the C:N ratio of the bioavailable dissolved organic matter (BDOM). A multiple linear regression with DIN and the C:N ratio of BDOM explained 89% of the observed variability in the integrated BGE, demonstrating the strong dependence of the growth efficiency on the nutrient conditions and the quality of the organic substrate feeding the community of this coastal embayment.

Keywords: Bacterial carbon demand, bacterial growth efficiency, inorganic nutrients, DOM stoichiometry, coastal upwelling

## Introduction

Dissolved organic carbon (DOC) constitutes the major carbon source for heterotrophic bacteria growth in marine pelagic systems (Hedges 2002). The bacterial use of DOC is therefore a central issue for understanding the carbon cycling through the microbial food web (Carlson 2002, Gasol et al 2008). After bacterial assimilation, organic carbon has two likely pathways: it is either transformed into new bacterial biomass (BB) or respired to $CO_2$ (BR). The ratio between production and the sum of bacterial production and respiration is termed the bacterial growth efficiency (BGE), typically ranging from 1 to 60% (del Giorgio & Cole 1998), meaning that between 40 to 99% of the assimilated carbon is used for respiration. The approach often used to determine the BGE are controlled laboratory incubations, where DOC concentration and microbial biomass are followed over time. This approach is associated with some methodological problems such as changes in bacterial community structure and physiology, and carbon and nutrient availability. This, together with the need for various conversion factors to convert bacterial abundance and uptake of radioactive substances into biomass adds uncertainties to the BGE estimates. In addition, the influence of short-term incubation (days) on the bacterial carbon demand (BCD) and growth efficiency is still unresolved and there are only a few seasonal studies describing how BGE change in relation to environmental conditions (Sherry et al. 1999, Reinthaler & Herndl 2005, Alonso-Sáez et al. 2008), which highly constrain our ability to model and predict the role of heterotrophic bacteria in the carbon cycle (Carlson et al. 1999).

The coastal ocean represents less than 10% of the ocean surface but contribute to 18-33% of the oceanic primary production and 27-50% of the new production (Walsh 1991, Wollast 1998, Gattuso et al. 1998). Coastal upwelling systems are particularly dynamic areas as they account for > 10% of global new production (Chavez & Toggweiler 1995). The Galician Rías Baixas are four coastal V-shaped embayments in the NW Iberian Peninsula, situated at the boundary between the temperate and

62 sub-polar regimes of the Eastern North Atlantic. The high productivity of the rías is driven by
63 upwelling and downwelling episodes leading to highly variable hydrographic and environmental
64 conditions (Álvarez-Salgado et al. 2010). Previous data on the functioning of the microbial system in
65 coastal upwelling areas suggest that most of the organic matter produced photosynthetically is
66 channelled through the microbial food web (Teira et al. 2003), although the links between bacterial
67 carbon cycling and changing environmental conditions were not examined in detail.
68 In this study we conducted eleven seawater cultures over an annual cycle in the highly dynamic
69 coastal upwelling system of the Ría de Vigo (NW Iberian Peninsula). This study is complementing the
70 work by Lønborg et al. (2010) where the degradation rates and C:N:P molar ratios of DOM in the Ría
71 de Vigo, was followed in the same incubations. The objectives of this study were (1) to determine the
72 time course of BCD and BGE during laboratory incubations; and (2) to find parametric relationships
73 between those variables and the availability of organic and inorganic substrates in the incubated water.
74
75 **Material and methods**
76 *Study area* – The Ría de Vigo is a coastal embayment on the western coast of the Iberian Peninsula. It
77 has a length of 33 Km, a surface area of 176 $Km^2$, a volume of 3.32 $Km^3$, and an average flushing time
78 of 7 days. The physical and chemical environment is influenced by periodic wind driven upwelling and
79 downwelling events (Álvarez-Salgado et al. 2010). The sampling site was located at 42º 14.5'N and 8º
80 45.8'W, in the middle sector of the embayment, a position appropriate for evaluating the main
81 processes occurring in the outer Ría de Vigo (Nogueira et al. 1997).
82 Water samples were collected during autumn (20 and 27 September, and 4 October 2007), winter
83 (31 January, and 7 and 14 February 2008), spring (17 and 24 April 2008) and summer (26 June, and 3
84 and 7 July 2008). Samples were taken at 5 m depth using a 25 l Niskin bottle. Salinity and temperature

were measured prior to the sampling with an SBE 9/11 CTD probe. Samples for chlorophyll a (Chl *a*) were collected by filtering seawater (100 to 200 ml) through a GF/F filter and analyzed after 90% acetone extraction with a Turner Designs 10000R fluorometer (Yentsch & Menzel 1963).

*Dilution culture setup* – The filtration of the seawater started within 10 min of collection; one part was filtered through pre-combusted (450°C for 4 h) GF/C filters to establish a microbial culture and the second part was gravity filtrated through a dual-stage (0.8 and 0.2 μm) filter cartridge (Pall-Acropak supor Membrane), which had been pre-washed with 10 l of Milli-Q. The filtrates were thereafter kept in the dark until arrival in the laboratory where sea water cultures were prepared by diluting the GF/C filtered in a 20 l carboy in a 1:9 ratio with the 0.2 μm filtrate.

The water was thereafter transferred into 500 mL glass bottles and incubated in the dark at a constant temperature of 15ºC, with four replicate bottles used for sub-sampling at incubation times 0, 1, 2, and 4 days. Unfiltered water from these bottles was used to follow changes in electron transport system (ETS) activity and bacterial abundance (BA). BA and ETS activity in experiments 1, 2, 3 and 11 was measured on incubation day 5 instead of day 4. For simplicity we refer to all samples as day 4 throughout the text. Samples for the analysis of dissolved inorganic nitrogen (DIN: $NH_4^+$ + $NO_2^-$ + $NO_3^-$) and phosphorus (DIP: $HPO_4^{2-}$), dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) were collected in four replicates at day 0 and 4. All samples for the dissolved phase were collected after filtration through 0.2 μm filters (Pall Supor membrane Disc). Glassware used was acid washed in 10 % HCl and rinsed with Milli-Q and sample water prior to use.

*Sample analysis* – Bacterial production (BP) was measured by [$^3$H] thymidine incorporation (Fuhrman & Azam 1980). Four replicate 9.9 ml samples and 2 trichloroacetic acid killed samples were spiked

107 with an aqueous stock solution of [$^{3}$H - methyl] thymidine (40 nmol final concentration). The samples
108 were incubated in the dark at 15ºC for 2 h, 10 ml of ice-cold Trichloracetat (TCA) was thereafter added
109 and samples were filtered onto 0.2 µm polycarbonate filters (presoaked in thymidine), washed with
110 95% ethanol and autoclaved Milli-Q water. The filters were hereafter dried at room temperature (24 h)
111 and mixed with 10 ml of scintillation fluid (Sigma-Flour). The radioactivity incorporated into cells was
112 counted using a spectral liquid scintillation counter. Thymidine incorporated into bacterial biomass was
113 converted to carbon production using the theoretical conversion factors 2 x $10^{18}$ cells $mol^{-1}$ thymidine
114 (Fuhrman & Azam 1980) and a carbon conversion factor of 30 fg C $cell^{-1}$, which is characteristic of
115 coastal bacterial assemblages (Fukuda et al. 1998).

116 Samples for determining bacterial abundance (BA) were fixed (1-2 h) with formol in the dark,
117 filtered onto 0.2 µm polycarbonate filters, and stored at -20°C until counted. The samples were stained
118 with a with a 4´,6-diamidino-2-phenylindole (DAPI)-mix before counted on a Leica DMBL
119 microscope equipped with a 100-W Hg-lamp and appropriate filters. More than 800 DAPI-stained cells
120 were counted per sample. Bacterial biomass was calculated from BA, using the same cell to carbon
121 conversion factor as for BP.

122 Samples for determining DIN and DIP were collected in 50 ml acid washed polyethylene bottles
123 and kept frozen (-20ºC) until analysis using standard colorimetric methods with an Alpkem segmented
124 flow analyser.

125 DOC and TDN were measured using a Shimadzu TOC analyser (Pt–catalyst) connected to an
126 Antek–TN measuring unit. Three to five replicate injections of 150 µl were performed per sample.
127 Concentrations were determined by subtracting a Milli-Q blank and dividing by the slope of a daily
128 standard curve of potassium hydrogen phthalate and glycine. Using the deep ocean reference (Sargasso
129 Sea deep water, 2600 m) we obtained a concentration of 46.0 ± 2.0 µM (average ± SD) for DOC and

130 22.0 ± 2.0 µM for TDN. Dissolved organic nitrogen (DON) concentrations were calculated as the
131 difference between TDN and DIN (DON = TDN – DIN). The concentration of TDP was determined by
132 wet-oxidizing in acid persulphate (120°C, 75 min) and measuring the liberated orthophosphate using
133 segment flow analysis calibrated with a daily calibration curve (Hansen & Koroleff, 1999). Dissolved
134 organic phosphorus (DOP) concentrations were calculated as the difference between TDP and DIP
135 (DOP = TDP – DIP).

136 The ETS activity was measured using the reduction of 2-(4-iodophenyl)-3-(4-nitro-phenyl)-5-
137 phenyl tetrazolium chloride (INT), as described by Martínez-García et al. (2009). This method has been
138 shown to provide real, non potential, respiration rates. The activity was measured using a final
139 concentration of 0.2 mM INT and 1 h incubations of 3 replicate samples (10 ml) and 1 formol-killed
140 control. The incubations were terminated by adding formol and filtering onto 0.2 µm polycarbonate
141 filters. The filters were hereafter stored frozen (-20°C) until further processing. The respiration rates
142 derived from ETS activity ($R_{ETS,}$ in µM $O_2$ $h^{-1}$) were obtained by multiplying the in vivo ETS activity
143 (in µM INTF) by the empirically derived conversion factor of 12.8 which has previously been shown to
144 be applicable in incubation studies (Martínez-García et al. 2009).

145 The microbial respiratory quotient (RQ), defined as the ratio of mol $CO_2$ production per mol $O_2$
146 used can fluctuate considerably, depending on the characteristics of the substrates being degraded. We
147 used the average RQ values (range 1.35-1.53) calculated by Lønborg et al. (2010) from the C:N:P
148 composition of bioavailable DOM (BDOM) for the same experiments.

149 The BCD at day 0 and 4 of the incubation was calculated as the sum of BP and ETS activity:

150 $$BCD = BP + ETS \quad (1)$$

151 The BGE at day 0 and 4 of the incubation was calculated as BP divided by the sum of BP and
152 ETS activity:

153 $$BGE = BP / (BP + ETS) \quad (2)$$

154 The integrated BCD over the 4 d incubation period was calculated as the DOC consumed
155 ($\Delta$DOC):

156 $$BCD = \Delta DOC / \Delta t \quad (3)$$

157 The integrated BGE over the 4 days was calculated as the net growth in bacterial biomass
158 between day 0 and 4 (BG) divided by $\Delta$DOC:

159 $$BGE = BG / \Delta DOC \quad (4)$$

160 Regression analyses were performed using the best–fit between the two variables X and Y
161 obtained by regression model II as described in Sokal & Rohlf (1995). In the cases were the intercept
162 was not significantly different from zero, it was set to zero and a new slope was calculated. Prior to the
163 regressions, normality was checked, the confidence level was set at 95% with all statistical analyses
164 conducted in Statistica 6.0.

165

166 **Results**

167 *Environmental conditions* – The environmental conditions during the sampling period in the Ría de
168 Vigo has previously been described in detail by Lønborg et al. (2010). Briefly, the autumn sampling
169 evolved from upwelling to moderate downwelling favourable winds resulting in a decreasing
170 temperature from > 16ºC to < 14ºC, increasing DIN concentration from 3 to 13 µM, DIP between 0.19
171 and 0.68 µM and Chl *a* was constant at around 3 mg $m^{-3}$ (Table 1). The winter sampling started with
172 relaxation evolving into strong downwelling conditions. Water temperature was relative constant at
173 around 13ºC, DIN and DIP concentrations were > 8 and > 0.42 µM respectively, and Chl *a* levels were
174 < 1.5 mg $m^{-3}$ (Table 1). The spring sampling were dominated by moderate downwelling winds, with
175 temperatures varying between 14 and 15ºC, DIN levels were > 5 µM, DIP was constant at 0.09 µM and

176 Chl *a* varied between 3.0 and 8.4 g $m^{-3}$. In the summer sampling, initial strong upwelling was followed
177 by moderate downwelling conditions. The water temperatures were > 17ºC, DIN levels were < 3 µM,
178 DIP varied between 0.02 and 0.38 µM and Chl *a* varied between 1.1 and 4.5 mg $m^{-3}$ (Table 1, see also
179 Figures 2 & 3 of Lønborg et al. 2010).

180 *Inorganic nutrients and DOC utilization* – The DIN and DIP concentration were in all cultures over the
181 detection limit (±0.1 µM and ±0.02 µM respectively) during the 4 days of incubation. Initial DOC
182 varied between 73 and 94 µM, DON from 4.5 to 7.2 µM and DOP from 0.12 to 0.32 µM (Table 1).
183 Bacterial DOM degradation over 4 days resulted in the removal of 11%, 28% and 44% of the initial
184 DOC, DON and DOP pools respectively, corresponding to 57%, 73% and 78% of the total bioavailable
185 DOC, DON and DOP (BDOC, BDON, BDOP) quantified by Lønborg et al. (2010) in long term
186 incubations (50-70 days) of the same sample water (Table 1).

187 *Bacterial abundance and production* –BA increased in all cultures (Figure 1) following the decreases
188 in DOM (Table 1). Initial BA varied from 1.3 to 6.1 × $10^5$ bacteria $ml^{-1}$, reaching 6.9 to 22.7 × $10^5$
189 bacteria $ml^{-1}$ after 4 days of incubation (Table 2), corresponding to a bacteria biomass growth (BG)
190 between 0.95 ± 0.14 and 5.10 ± 0.14 µM C (Table 2). The BA samples for days 2 and 3 of two summer
191 experiments were unfortunately lost (3 and 7 July 2008). As found by Teira et al. (2009) for the same
192 experiments, the bacterial growth rates varied between 0.5 to 0.8 $d^{-1}$, resulting in a average turnover
193 time of 29 h. There was no clear relationship between the obtained growth rates and the environmental
194 variables (Teira et al. 2009).

195 Initial bacterial production (BP) ranged from 0.02 ± 0.01 to 0.16 ± 0.02 µM C $d^{-1}$, with higher
196 values during autumn and summer (Table 2). The BP decreased in all experiments reaching values
197 between 0.01 ± 0.01 and 0.05 ± 0.01 µM C $d^{-1}$ after 4 days of incubation (Table 2). The specific growth

198 rate (μ) ($d^{-1}$) varied initially between 1.1 ± 0.2 to 7.8 ± 1.4 $d^{-1}$, declining to between 0.2 ± 0.1 and 0.9
199 ± 0.2 $d^{-1}$ at incubation day 4 (Table 2).

200 *ETS activity* – The respiratory ETS activity varied initially between 0.02 ± 0.01 and 0.95 ± 0.30 μM C
201 $day^{-1}$ increasing to between 0.30 ± 0.09 and 4.98 ± 0.26 μM C $d^{-1}$ at incubation day 4 (Figure 1).
202 Calculating the cell specific ETS activity a initial range from 0.11 ± 0.06 to 4.18 ± 1.53 fmol C $cell^{-1}$ $d^{-1}$
203 was found, reaching values between 0.83 ± 0.08 and 5.84 ± 0.62 fmol C $cell^{-1}$ $d^{-1}$ after 1 to 2 days of
204 incubation and values between 0.39 ± 0.13 and 5.34 ± 0.54 fmol C $cell^{-1}$ $d^{-1}$ at day 4 (Figure 1).
205
206 *Bacterial carbon demand and growth efficiency* – The BCD varied initially between 0.1 ± 0.1 and 1.0 ±
207 0.3 μM C $d^{-1}$ (average ± SD: 0.4 ± 0.3 μM C $d^{-1}$) increasing to between 0.3 ± 0.1 and 5.0 ± 0.3 μM C $d^{-1}$ (1.9 ± 1.7 μM C $d^{-1}$) after 4 days of incubation (Table 3a). Integrated BCD was highest in fall and
209 summer reaching values between 0.9 ± 0.3 and 6.6 ± 0.4 μM C $d^{-1}$ (4.5 ± 2.9 μM C $d^{-1}$) (Table 3a).
210 The instantaneous BGE had an initial average of 29 ± 15 % (range 6- 55%) declining to 3 ± 2 %
211 (range 1- 8%) after 4 days of incubation (Table 3b). The integrated BGE reached values between 10 ±
212 4 and 62 ± 19 % (28 ± 13%) (Table 3b). The availability of inorganic nutrients and the quality of the
213 bioavailable DOM pool played an important role in determining the integrated BGE as it was positively
214 correlated with the initial DIN ($R^2 = 0.67$, $p < 0.003$; Figure 2a) and negatively correlated with the C/N
215 mol ratio of BDOM ($R^2 = 0.55$, $p < 0.01$; Figure 2b). Furthermore, the multiple linear regression with
216 DIN and the C:N ratio of BDOM explained 89% of the observed variability in the integrated BGE ($R^2$
217 = 0.89, $p < 0.001$; Figure 2c).
218
219 **Discussion**

220 The variations in the environmental conditions of the Ría de Vigo have previously been shown to
221 affect the biogeochemical cycling and plankton community composition (e.g. Tilstone et al. 1999,
222 Álvarez-Salgado et al. 2001, Cermeño et al. 2006, Alonso-Gutiérrez et al. 2009). The microbial
223 parameters measured in this study showed variations that were related with the environmental
224 conditions such as the inorganic nutrient conditions and the quality of the bioavailable DOM (Figure
225 2).

226 *Bacterial metabolism changes during incubation* – During the course of the incubations BA,
227 BCD, and ETS activity increased between 1 and 75 times, while BP and BGE decreased between 1 and
228 55 times. Based on the BA, ETS activity and BP, we calculated the μ and cell-specific respiration rates.
229 In calculating these rates, we acknowledge the uncertainty associated with the presence of nonliving
230 cells, which could have caused an underestimation of the rates. However, Teira et al. (2009) observed
231 that after 24 h almost 100% of the DAPI-stained cells were detected by Fluorescence In Situ
232 Hybridization with eubacteria probes, which strongly suggests that the percent of non-living cells in the
233 dilution cultures was irrelevant. The respiration rates on a per-cell basis (Figure 1) increased during the
234 incubation, while μ declined (Table 2). This result suggests that respiration and BCD increases, while
235 BP and BGE decreases, as the labile DOM fraction gets depleted. A similar decrease in the bacterial
236 carbon conversion efficiency at the stationary phase of long-lasting (weeks) batch cultures was reported
237 by Turley & Lochte (1990). Therefore, as the incubations move towards more oligotrophic conditions
238 more carbon is used for energy production rather than being used for biomass production. Similar
239 results have been obtained in natural systems where BGE decreases relatively from eutrophic to
240 oligotrophic waters (del Giorgio & Cole 1998, Alonso-Sáez et al. 2007). The choice to use
241 instantaneous bacterial production and respiration estimates (short-term) or DOC degradation

experiments (long-term) to calculate the BGE are associated with some potential problems. Short-term methods are limited essentially by the very distinct duration of the incubations for estimating bacterial production (1-2 h) and respiration (usually 24 h) and the need for several carbon conversion factors. The in vivo ETS method allowed us to reduce incubation time for respiration, thus solving the first problem. On the other hand, long-term methods rely on a single carbon conversion factor (bacterial carbon content) but changes in DOM quality as well as in bacterial phylogenetic composition over time may severely affect BGE estimates. Changes in the phylogenetic composition of the bacteria were measured during our incubations (Teira et al. 2009) but a potential influence of these changes on the BGE was not evident. The decrease observed between the initial and the final instantaneous BGE estimates in our study indicate that long-term experiments could actually underestimate BGE. Nevertheless, the good agreement between our initial instantaneous (28 ± 13%) and integrated BGE (29 ± 15%) indicate that, in our study, the 4 days incubation did not severely underestimate BGE. Thus, the BGE estimated from changes in bacterial biomass and DOC concentration over few days seems to be a good approach as it relies on the use of less carbon conversion factors than the short-term approach based on simultaneous bacterial production and respiration estimates. We do not know whether and how the conversion factors may have changed seasonally and during our incubations, but as our BGE estimates are similar to previous values it suggests that our calculations are within the right range.

*Bacterial carbon cycling over an annual cycle* – The energy transfer from heterotrophic bacteria to higher trophic levels is ultimately dependent on the BGE. The range (8 ± 1 – 55 ± 11%) and average (28 ± 13%) of the integrated BGE found in this study was comparable with values reported for marine systems (~20%; Del Giorgio & Cole 1998) and previous estimates for the NW Iberian upwelling

265 system (12-20%, Barbosa et al. 2001 and 2-27%, Moran et al. 2002). The wide range of BGE showed
266 that a variable BGE can be obtained at this site over an annual cycle and the average indicates that the
267 bacterial community respired 72% of the assimilated carbon, suggesting a low bacterial carbon transfer
268 in this upwelling system.

269 Previous studies have indicated that BGE varies greatly depending on environmental factors such
270 as nutrient availability (Rivkin & Anderson 1997), DOM quality (Goldman et al. 1987, Lemée et al
271 2002, Reinthaler & Herndl 2005, Apple & Del Giorgio 2007) and temperature (Rivkin & Legendre,
272 2001). Our data suggested that the BGE in the Ría de Vigo was partly controlled by inorganic nutrients
273 availability and by the quality of the bioavailable DOM substrate (Figure 2). The relationship suggests
274 that the bacterial community requires energy (respiration) to take up DIN when the substrate has a high
275 C:N ratio resulting in lower BGE (Rivkin & Anderson 1997, Kirchman 2000). Considering the values
276 of the normalised regression slopes, $\beta_1 = 0.637$ (for DIN) and $\beta_2 = -0.491$ (for the C:N ratio of BDOM),
277 it results that BGE depends more on DIN than on the quality of BDOM ($\beta_2^2 / \beta_1^2 = 1.68$). The
278 importance of temperature in regulating BGE can not be determined in this study as all incubations
279 were conducted at the same constant temperature (15°C). Nevertheless, some recent studies suggest
280 that temperature only plays a minor role in controlling BGE (Vázquez-Dominguez et al 2007, Alonso-
281 Sáez et al 2008).

282 In summary, we have shown that the BCD and BGE changes over short-time scales in laboratory
283 incubations and that the BCD and BGE in the upwelling area of the Ría de Vigo is controlled by the
284 availability of carbon, inorganic nutrients and the composition of bioavailable DOM pool. The study
285 furthermore suggests that the BGE depends more on inorganic nitrogen concentration than on the
286 quality of DOM. Temporal and spatial differences in these variables may therefore have large influence
287 on the fate (new biomass vs. respiration) of organic carbon in the Ría de Vigo.

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389 Yentsch CS, Menzel DW (1963) A method for the determination of phytoplankton chlorophyll and
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391 Table 1. Initial ($DOC_0$, $DON_0$, $DOP_0$), 4 days change (ΔDOC) and total bioavailable (BDOC, BDON, BDOP) dissolved organic

392 carbon (DOC), nitrogen (DON) and Phosphorus (DOP) as found by Lønborg et al. (2010). The initial concentrations of dissolved

393 inorganic phosphate (DIP), Nitrate ($NO_3^-$), Nitrite ($NO_2^-$) and ammonium ($NH_4^+$). Values are averages of 4 replicates ± standard

394 deviation. n.d- not determined.

395

| Date | $DOC_0$ µM C | ΔDOC µM C | BDOC µM C | $DON_0$ µM N | BDON µM N | $DOP_0$ µM P | BDOP µM P | DIP µM P | $NO_3^-$ µM N | $NO_2^-$ µM N | $NH_4^+$ µM N |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 20-09-2007 | 94 ± 1 | 26 ± 2 | 29 ± 3 | 7.2 ± 0.7 | 3.6 ± 1.7 | 0.32 ± 0.02 | 0.25 ± 0.08 | 0.19 ± 0.02 | 0.14 ± 0.01 | 0.19 ± 0.01 | 3.67 ± 0.34 |
| 27-09-2007 | 79 ± 1 | 9 ± 3 | 15 ± 1 | 5.5 ± 0.4 | 2.2 ± 1.2 | 0.20 ± 0.03 | 0.11 ± 0.06 | 0.68 ± 0.01 | 5.28 ± 0.01 | 0.46 ± 0.01 | 5.23 ± 0.02 |
| 04-10-2007 | 75 ± 2 | 5 ± 2 | 12 ± 2 | 5.5 ± 0.3 | 2.0 ± 1.0 | 0.19 ± 0.04 | 0.11 ± 0.07 | 0.54 ± 0.03 | 9.09 ± 0.02 | 0.56 ± 0.01 | 2.81 ± 0.11 |
| 31-01-2008 | 75 ± 1 | 3 ± 2 | 9 ± 1 | 5.2 ± 0.3 | 1.7 ± 0.6 | 0.19 ± 0.01 | 0.11 ± 0.04 | 0.46 ± 0.01 | 6.78 ± 0.02 | 0.41 ± 0.05 | 2.11 ± 0.09 |
| 07-02-2008 | 77 ± 1 | 2 ± 1 | 7 ± 1 | 5.4 ± 0.6 | 1.7 ± 1.5 | 0.22 ± 0.02 | 0.13 ± 0.06 | 0.56 ± 0.02 | 7.00 ± 0.02 | 0.53 ± 0.01 | 3.18 ± 0.08 |
| 14-02-2008 | 73 ± 1 | 6 ± 2 | 10 ± 2 | 4.5 ± 0.4 | 1.3 ± 0.5 | 0.12 ± 0.02 | 0.09 ± 0.04 | 0.42 ± 0.01 | 5.77 ± 0.01 | 0.33 ± 0.01 | 2.62 ± 0.11 |
| 17-04-2008 | 81 ± 1 | 7 ± 2 | 13 ± 1 | 5.7 ± 0.5 | 2.2 ± 1.4 | 0.19 ± 0.04 | 0.14 ± 0.07 | 0.09 ± 0.02 | 0.00 ± 0.00 | 0.05 ± 0.03 | 1.87 ± 0.31 |
| 24-04-2008 | 85 ± 1 | 7 ± 1 | 11 ± 1 | 6.1 ± 0.2 | 2.5 ± 0.7 | n.d | n.d | 0.09 ± 0.01 | 3.94 ± 0.07 | 0.12 ± 0.01 | 2.43 ± 0.13 |
| 26-06-2008 | 88 ± 1 | 13 ± 2 | 17 ± 2 | 6.1 ± 0.5 | 2.5 ± 0.9 | 0.16 ± 0.03 | 0.10 ± 0.08 | 0.27 ± 0.01 | 0.22 ± 0.01 | 0.07 ± 0.01 | 0.65 ± 0.29 |
| 03-07-2008 | 82 ± 1 | 9 ± 1 | 14 ± 1 | 5.5 ± 0.4 | 2.0 ± 1.2 | 0.20 ± 0.03 | 0.11 ± 0.06 | 0.02 ± 0.01 | 0.05 ± 0.01 | 0.02 ± 0.01 | 1.23 ± 0.16 |
| 10-07-2008 | 89 ± 2 | 13 ± 1 | 20 ± 1 | 6.1 ± 0.4 | 2.9 ± 0.7 | 0.23 ± 0.03 | 0.17 ± 0.04 | 0.38 ± 0.01 | 0.70 ± 0.02 | 0.10 ± 0.01 | 2.87 ± 0.07 |
| Average ± SD | 82 ± 7 | 9 ± 7 | 14 ± 6 | 5.7 ± 0.7 | 2.2 ± 0.6 | 0.20 ± 0.05 | 0.13 ± 0.05 | 0.34 ± 0.22 | 3.54 ± 3.42 | 0.26 ± 0.21 | 2.61 ± 1.22 |

396 Table 2. Initial ($BP_0$, $BA_0$, $BB_0$) and final ($BP_4$, $BA_4$, $BB_4$) bacterial production (BP), abundance (BA) and biomass (BB). The
397 growth in bacterial biomass (BG) and the μ at day 0 ($BP_0$/ $BB_0$) and 4 ($BP_4$/ $BB_4$) of the incubations. Values are averages of 4
398 replicates ± standard deviation.
399

| Date | $BA_0$ | $BA_4$ | $BB_0$ | $BB_4$ | BG | $BP_0$ | $BP_4$ | BP/BB (0) | BP/BB (4) |
|---|---|---|---|---|---|---|---|---|---|
| | $\times 10^5$ ml$^{-1}$ | $\times 10^5$ ml$^{-1}$ | μM C | μM C | μM C | μM C d$^{-1}$ | μM C d$^{-1}$ | d$^{-1}$ | d$^{-1}$ |
| 20-09-2007 | 2.5 ± 0.2 | 11.4 ± 0.2 | 0.63 ± 0.05 | 2.84 ± 0.05 | 2.21 ± 0.10 | 0.16 ± 0.02 | 0.05 ± 0.01 | 7.8 ± 1.4 | 0.6 ± 0.1 |
| 27-09-2007 | 2.6 ± 0.1 | 11.3 ± 0.5 | 0.64 ± 0.02 | 2.81 ± 0.12 | 2.17 ± 0.13 | 0.06 ± 0.02 | 0.03 ± 0.01 | 3.0 ± 0.9 | 0.4 ± 0.1 |
| 04-10-2007 | 3.1 ± 0.1 | 11.4 ± 0.5 | 0.78 ± 0.02 | 2.84 ± 0.12 | 2.06 ± 0.13 | 0.06 ± 0.02 | 0.05 ± 0.01 | 2.3 ± 0.7 | 0.5 ± 0.1 |
| 31-01-2008 | 1.5 ± 0.1 | 6.9 ± 0.3 | 0.38 ± 0.01 | 1.71 ± 0.08 | 1.34 ± 0.09 | 0.08 ± 0.01 | 0.05 ± 0.01 | 6.2 ± 0.8 | 0.9 ± 0.2 |
| 07-02-2008 | 1.7 ± 0.1 | 8.9 ± 0.5 | 0.43 ± 0.02 | 2.23 ± 0.12 | 1.80 ± 0.14 | 0.02 ± 0.01 | 0.01 ± 0.01 | 1.5 ± 0.7 | 0.2 ± 0.1 |
| 14-02-2008 | 1.3 ± 0.1 | 7.6 ± 0.4 | 0.33 ± 0.01 | 1.89 ± 0.09 | 1.56 ± 0.11 | 0.06 ± 0.01 | 0.02 ± 0.01 | 5.6 ± 1.0 | 0.3 ± 0.1 |
| 17-04-2008 | 6.1 ± 0.3 | 9.9 ± 0.3 | 1.53 ± 0.07 | 2.48 ± 0.07 | 0.95 ± 0.14 | 0.05 ± 0.01 | 0.03 ± 0.01 | 1.1 ± 0.2 | 0.3 ± 0.1 |
| 24-04-2008 | 2.3 ± 0.1 | 9.3 ± 0.6 | 0.57 ± 0.03 | 2.32 ± 0.16 | 1.75 ± 0.19 | 0.06 ± 0.01 | 0.04 ± 0.01 | 3.3 ± 0.6 | 0.5 ± 0.1 |
| 26-06-2008 | 4.3 ± 0.4 | 10.9 ± 0.3 | 1.07 ± 0.09 | 2.72 ± 0.09 | 1.64 ± 0.18 | 0.08 ± 0.01 | 0.05 ± 0.01 | 2.3 ± 0.4 | 0.6 ± 0.1 |
| 03-07-2008 | 1.8 ± 0.8 | 9.3 ± 0.4 | 0.44 ± 0.03 | 2.33 ± 0.16 | 1.89 ± 0.19 | 0.09 ± 0.01 | 0.03 ± 0.01 | 6.3 ± 1.0 | 0.4 ± 0.1 |
| 10-07-2008 | 2.3 ± 0.6 | 22.7 ± 0.9 | 0.56 ± 0.02 | 5.67 ± 0.12 | 5.10 ± 0.14 | 0.11 ± 0.01 | 0.05 ± 0.01 | 5.7 ± 0.7 | 0.3 ± 0.1 |
| Average ± SD | 2.7 ± 1.4 | 10.9 ± 4.2 | 0.67 ± 0.35 | 2.71 ± 1.05 | 2.0 ± 1.1 | 0.08 ± 0.04 | 0.04 ± 0.01 | 4.1 ± 2.3 | 0.5 ± 0.2 |

400 Table 3. a) Bacterial carbon demand (BCD) and b) growth efficiency (BGE) at incubation day 0
401 ($BCD_0$,$BGE_0$) and 4 ($BCD_4$,$BGE_4$) and 4 days integrated values ($BCD_{Int}$, $BGE_{Int}$). Values are averages
402 of 4 replicates ± standard error.

a)

| Date | $BCD_0$ µM C $d^{-1}$ | $BCD_4$ µM C $d^{-1}$ | $BCD_{Int}$ µM C $d^{-1}$ |
|---|---|---|---|
| 20-09-2007 | 0.3 ± 0.1 | 3.2 ± 0.1 | 6.6 ± 0.4 |
| 27-09-2007 | 0.2 ± 0.1 | 1.3 ± 0.2 | 2.3 ± 0.6 |
| 04-10-2007 | 0.2 ± 0.1 | 0.6 ± 0.1 | 1.3 ± 0.5 |
| 31-01-2008 | 0.2 ± 0.1 | 2.2 ± 0.2 | 0.9 ± 0.3 |
| 07-02-2008 | 0.1 ± 0.1 | 1.4 ± 0.1 | 1.1 ± 0.2 |
| 14-02-2008 | 0.2 ± 0.1 | 0.3 ± 0.1 | 1.7 ± 0.4 |
| 17-04-2008 | 0.4 ± 0.1 | 0.6 ± 0.1 | 1.2 ± 0.6 |
| 24-04-2008 | 1.0 ± 0.3 | 1.0 ± 0.6 | 1.7 ± 0.4 |
| 26-06-2008 | 0.6 ± 0.1 | 2.7 ± 0.2 | 2.3 ± 0.5 |
| 03-07-2008 | 0.5 ± 0.2 | 5.0 ± 0.3 | 2.2 ± 0.2 |
| 10-07-2008 | 0.3 ± 0.2 | 2.5 ± 0.4 | 2.3 ± 0.4 |
| Average ± SD | 0.4 ± 0.3 | 1.9 ± 1.7 | 4.5 ± 2.9 |

b)

| Date | $BGE_0$ (%) | $BGE_4$ (%) | $BGE_{Int}$ (%) |
|---|---|---|---|
| 20-09-2007 | 48 ± 11 | 2 ± 1 | 8 ± 1 |
| 27-09-2007 | 38 ± 18 | 3 ± 1 | 23 ± 7 |
| 04-10-2007 | 28 ± 16 | 8 ± 2 | 40 ± 23 |
| 31-01-2008 | 32 ± 12 | 2 ± 1 | 36 ± 14 |
| 07-02-2008 | 55 ± 45 | 1 ± 1 | 41 ± 19 |
| 14-02-2008 | 27 ± 6 | 6 ± 4 | 23 ± 7 |
| 17-04-2008 | 14 ± 5 | 5 ± 2 | 19 ± 12 |
| 24-04-2008 | 6 ± 3 | 4 ± 3 | 26 ± 8 |
| 26-06-2008 | 14 ± 2 | 2 ± 1 | 18 ± 6 |
| 03-07-2008 | 20 ± 10 | 1 ± 1 | 7 ± 1 |
| 10-07-2008 | 32 ± 18 | 2 ± 1 | 55 ± 11 |
| Average ± SD | 29 ± 15 | 3 ± 2 | 27 ± 15 |

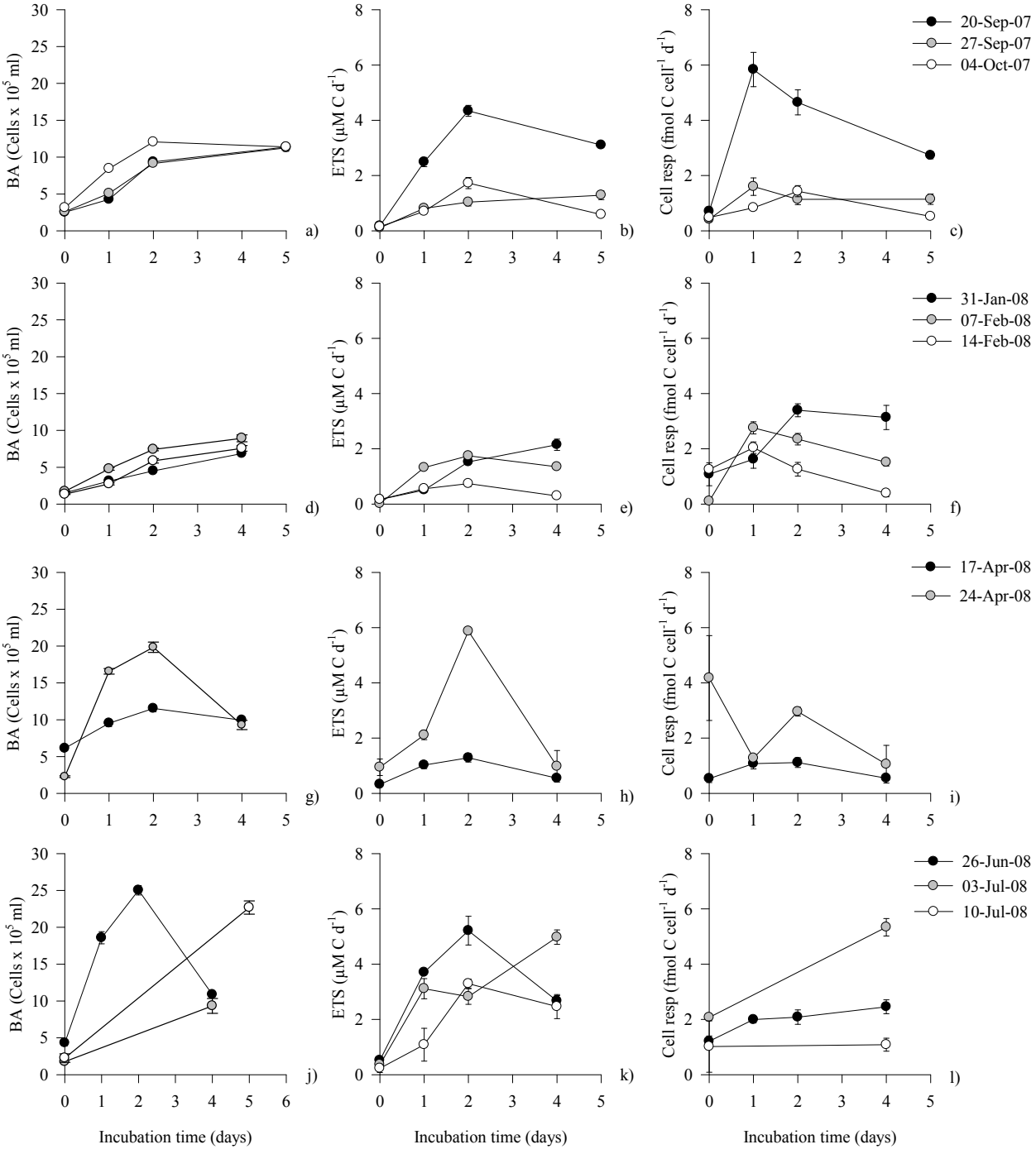
403

404 **Figure legends.**

405 Figure 1. Time evolution of a, d, g, j) bacterial abundance, b, e, h, k) ETS activity and c, f, i, l) cell
406 specific respiration during the incubations. Error bars represent standard deviations.

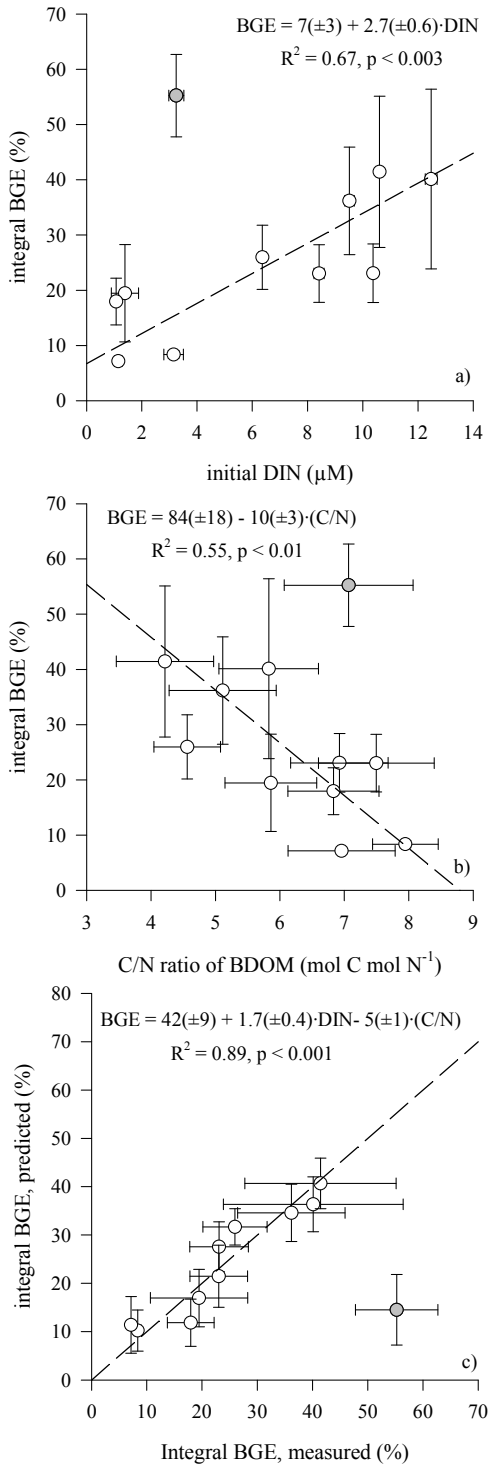
407

408 Figure 2. Relationships between a) the initial dissolved inorganic nitrogen concentration (DIN) and b)
409 the C:N ratio of the bioavailable dissolved organic matter (BDOM) with the 4 days integrated bacterial
410 growth efficiency (BGE); and c) relationship between the measured and the predicted BGE from the
411 multiple linear regression with DIN and C:N ratio of BDOM. The grey circles in the graphics indicate a
412 data point omitted from the linear regression (10-07-2008). Solid lines represent the corresponding
413 regression, $R^2$ = coefficient of determination, p = level of significance.




414

415 Figure 1.




416

417 Figure 2