

Carbon fluxes through estuarine bacteria reflect coupling with phytoplankton

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ABSTRACT: Microbial plankton carbon fluxes were comprehensively assessed at 4 contrasting sites in Waquoit Bay estuary (Massachusetts, USA) in 2008, focussing on the role of phytoplankton as dissolved organic matter suppliers for heterotrophic bacterioplankton. Strong seasonal patterns in environmental and microbial variables were observed, with concurrent summer maxima and minima in December for bacterial biomass ($17.7\text{--}164.7\ \mu\text{g C l}^{-1}$) and production ($4.8\text{--}121.5\ \mu\text{g C l}^{-1}\ \text{d}^{-1}$). Phytoplankton biomass ($0.7\text{--}32.6\ \mu\text{g chlorophyll l}^{-1}$) and primary production (PP, $164\text{--}3570\ \mu\text{g C l}^{-1}\ \text{d}^{-1}$) varied similarly to bacterial variables, with dissolved PP (DPP), i.e. released extracellularly, ranging from 3 to 36% of the total PP. The flux of DPP represented between 0.4 and 20% of bulk dissolved organic carbon (DOC, $92\text{--}346\ \mu\text{mol C l}^{-1}$) daily turnover. In addition to temperature, moderate bottom-up control of bacterial assemblages was suggested by biomass–production regressions. Bacteria apparently depended more on phytoplankton-derived DOC than on its total concentration. The difference became larger when the total flux of carbon or bacterial carbon demand (BCD, $19\text{--}468\ \mu\text{g C l}^{-1}\ \text{d}^{-1}$) was considered, with DPP explaining 53% of its variance compared with 37% from bulk DOC. On average, DPP could meet 50% of BCD, indicating a larger than expected trophic dependence of bacteria on phytoplankton in the whole estuarine ecosystem.

KEY WORDS: Bacteria · Phytoplankton · Carbon fluxes · Trophic coupling · Estuaries

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INTRODUCTION

The importance of pelagic bacteria in processing organic carbon in aquatic ecosystems is well established (Cotner & Biddanda 2002). The amount of dissolved organic carbon (DOC) consumed or bacterial carbon demand (BCD) is subsequently used for bacterial production (BP) and respiration (BR), with usually a greater fraction being returned as CO_2 . These variables are regulated by bacterial growth efficiency (BGE = BP/BCD), which has been reported to vary widely but usually shows higher values in nutrient-rich waters (del Giorgio & Cole 1998). In marine coastal and estuarine ecosystems, bacteria usually process more carbon than is produced *in situ*, result-

ing in net heterotrophy of the system (e.g. Hoch & Kirchman 1993, Thottathil et al. 2008). However, in the terrestrial–marine transition zone, recently produced DOC, either released by phytoplankton (here termed dissolved primary production, DPP) or by macrophytes is preferred to older, frequently terrestrially-derived sources (e.g. Norrman et al. 1995, Cherrier et al. 1999). Indeed, BGE is usually higher with high-quality DOC (del Giorgio & Cole 2000). The paradigm of the general trophic dependence of bacteria on phytoplankton has been recently challenged in a review comparing BP and particulate primary production (PPP) (Fouilland & Mostajir 2010). A drawback of the studies included in this large-scale meta-analysis is that they did not measure DPP and

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BCD, the most pertinent variables for assessing phytoplankton–bacterioplankton coupling (Morán et al. 2002a). Rather, these 2 variables were estimated from literature models largely differing in assumptions and results (Fouilland & Mostajir 2011, Morán & Alonso-Sáez 2011).

BP is by far the most common variable reported in studies of aquatic carbon fluxes mediated by heterotrophic prokaryotes due to the widespread use of leucine (Leu) and thymidine (TdR) incorporation methods (Kemp et al. 1993). However, the choice of theoretical or empirical substrate to C conversion factors (Ducklow 2000, Alonso-Sáez et al. 2010) may substantially alter the resulting BP values (Calvo-Díaz & Morán 2009). This uncertainty is further complicated with the need to obtain BCD for constraining the total amount of carbon flowing through bacteria (Gasol et al. 2008). When BR measurements are not available (which occurs frequently), BR or BGE values can be obtained from modelled relationships (del Giorgio & Cole 2000, Rivkin & Legendre 2001, López-Urrutia & Morán 2007, Robinson 2008). Similarly, only PPP is typically measured in most estimates of planktonic PP, despite the variable importance of DPP—the flux of photosynthate directly available for bacterial uptake—to total PP values in coastal waters (ca. 20% on average, but reported variations from <5% to >50%, Teira et al. 2001, Morán et al. 2002b, Marañón et al. 2004). As a consequence, claims that BP globally accounts for 10 to 20% of concurrent PPP in marine pelagic ecosystems (Ducklow 2000) may be substantially altered if BCD and DPP are the variables compared (Fouilland & Mostajir 2010, Morán & Alonso-Sáez 2011). Few studies have assessed these fluxes in estuarine waters.

Many factors interact to determine the amount and fate of the carbon processed by heterotrophic bacterioplankton. Among them, the quality of the available DOC and temperature are arguably the most important (White et al. 1991, Church 2008), although disentangling true temperature effects from covarying drivers is difficult (Pomeroy & Wiebe 2001, Lomas et al. 2002, Kirchman et al. 2009). The aims of this study were (1) to describe the temporal variability in carbon flow through heterotrophic bacteria—specifically addressing the conversion from Leu uptake to carbon biomass production—in an estuarine region characterized by different inorganic and organic nutrient loadings, and (2) to assess the importance of phytoplankton DPP in providing substrates for bacterial growth and metabolism. Experimental determinations were carried out in the estuarine ecosystem of Waquoit Bay (Massachusetts, USA) during

2008, covering a complete growing season of microbial plankton (Morán et al. 2011).

MATERIALS AND METHODS

Sampling site and environmental variables

The estuarine ecosystem of Waquoit Bay (41° 34' N, 70° 31' W, Fig. 1) belongs to the US network of National Estuarine Research Reserves (Apple et al. 2008). The shallow morphology and large amounts of nutrient loading received in the upper riverine reaches render it a meso- to eutrophic estuary dominated by macrophytes and benthic processes (Valiela et al. 1992). Waquoit Bay is made up of various sub-estuaries characterized by dissimilar nitrogen inputs caused by different land use. Relatively pristine areas coexist with heavily populated watersheds, therefore offering a wide range of environmental conditions (Valiela et al. 1997). The comprehensive assessment of carbon flow through heterotrophic bacterioplankton was conducted 6 times in 2008 from May to December in 3 sub-estuaries, Childs River, Quashnet River, Sage Lot Pond, as well as in Nantucket Sound coastal waters off the entrance canal. Sampling was conducted in ~4 hours by filling 3 acid-washed, rinsed Nalgene carboys over the side of a small boat at each site (Fig. 1). These 3 water samples were taken within an area roughly equivalent to a 10 m diameter circle. *In situ* measurements and samples for inorganic and organic nutrients, chlorophyll and PP were taken and processed as detailed in Morán et al. (2011).



Fig. 1. Waquoit Bay (Cape Cod, Massachusetts, USA, 41° 34' N, 70° 31' W) showing the 4 study sites (open circles). Image provided by Google Earth

Bacterial abundance, size and biomass

Bacterial abundance—the sum of low (LNA) and high (HNA) nucleic acid content cells—was determined by flow cytometry on a Becton-Dickinson FACSCalibur following the protocol of del Giorgio et al. (1996) as discussed in Gasol & del Giorgio (2000) but using Sybr-Green (Applied Biosystems) as stain. Cell size in biovolume (BB_v) was individually determined for LNA and HNA cells as described in Morán et al. (2011) and averaged for each site and sampling time. All samples were processed within 2 weeks after collection. Bacterial biomass was calculated from mean cell size using Norland's (1993) relationship.

Bacterial production and carbon demand

Bacterial activity was estimated in the 3 replicate samples per site as Leu incorporation rates as detailed elsewhere (Morán et al. 2011). The time scale and irradiance conditions were the same for bacteria and phytoplankton, making assessment of their trophic dependence more comparable. Activity was subsequently transformed into BP by means of empirically determined Leu to carbon conversion factors (Leu-to-C CFs) obtained with the cumulative method (Ducklow et al. 1992) in dilution re-growth experiments (Calvo-Díaz & Morán 2009). Experimental determinations of Leu-to-C CFs were performed with water from the 1st of the 3 replicate samples. The coefficient of variation of the 3 Leu incorporation replicates averaged 17%, so we applied the same CF to the other 2 replicates.

In order to constrain the total flux of carbon through heterotrophic bacteria, BCD was first obtained by adding estimates of BR using the literature models of López-Urrutia & Morán (2007) and Robinson (2008) to our measured BP values. These 2 models rely on different variables for estimating BR: temperature and bacterial abundance in the former, and BP in the latter. On 15 occasions (2 to 4 sub-estuaries per sampling time from May through October) BGE was also empirically determined in long-term DOC consumption experiments in which 50 ml unfiltered water samples were incubated in duplicates in the dark at *in situ* temperature ($\pm 1^\circ\text{C}$) for up to 25 d. At intervals (1 to 2 d at the beginning and then weekly), samples were filtered through pre-combusted GF/F filters to estimate DOC while aliquots were also taken to estimate bacterial biomass (BB) as described above. BGE was calculated as the increase of BB divided by the amount of DOC consumed during the exponential

phase, which averaged 4 d with a range of 1 to 21 d. Mean BGE values at each sub-estuary were 0.12 at Quashnet River, 0.10 at Nantucket Sound, 0.22 at Sage Lot Pond and 0.15 at Childs River.

Statistics

Except temperature, salinity and percentages, all variables were \log_{10} transformed in order to attain normality and homogeneity of variance for subsequent correlation and regression analyses. Pearson's product-moment correlations, ordinary least squares or Model I linear regressions, paired *t*-tests, and multiple linear regressions were performed with STATISTICA software, v. 7.1 (StaSoft). We used the Akaike information criterion (AIC) to select between the different multiple linear regression models. We first considered all models with $\Delta\text{AIC} \leq 2$ units from that with minimal AIC value, but finally we selected only those with AIC weights higher than 0.15. Differences between sub-estuaries were assessed by means of repeated measures analyses of variance (RM-ANOVA) and Bonferroni post-hoc tests at $p < 0.05$, with site as between-subject factor (within-subject factor, sampling time).

RESULTS

Environmental variability

Seasonal changes in temperature (Fig. 2A) were larger than those of salinity (Fig. 2B), which reflected the expected influence of rivers (Fig. 2A). Chlorophyll (Fig. 2C) attained significantly higher values at the 2 river-influenced sites, with maxima generally observed in July and minima in May or October. DOC concentrations (95 to $346 \mu\text{mol C l}^{-1}$) showed marked spatial differences, with higher values consistently observed at Sage Lot Pond, followed by similar values at both rivers and minima at Nantucket Sound (Fig. 2D). These geographical differences also held for DON concentrations (7.6 to $29.5 \mu\text{mol N l}^{-1}$, data not shown) but seasonality differed markedly, thereby affecting DOM C:N ratios. A maximum ratio of 12 from July through October with values around 6 at the beginning and end of the sampled period was found at the 2 riverine sites, compared with the rather constant (12 to 14) values at Sage Lot Pond and a steady increase from 10 to 15 at Nantucket Sound. Phosphate was generally above $0.1 \mu\text{mol l}^{-1}$ year-round (Morán et al. 2011) while nitrate (data not

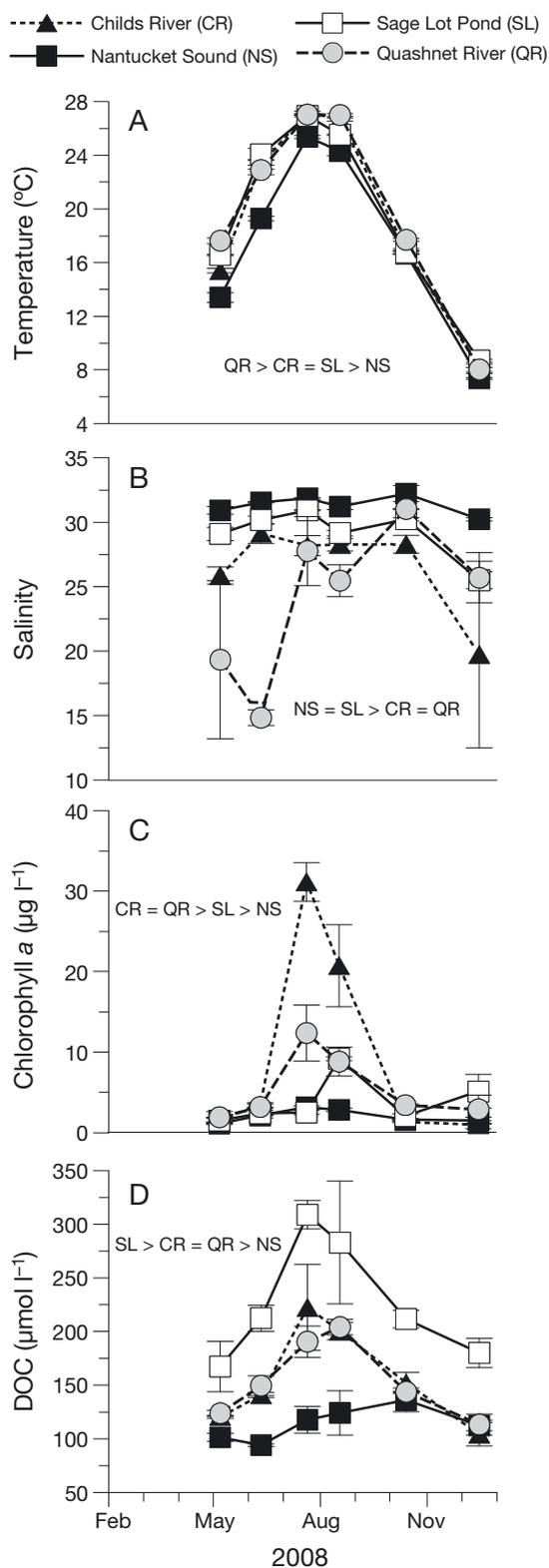


Fig. 2. (A) Mean temperature, (B) salinity, (C) chlorophyll and (D) dissolved organic carbon (DOC) concentrations at the 4 Waquoit Bay sites in 2008. Error bars represent standard deviations of 3 replicates per site. Significant differences between sites are indicated

shown), although generally covaried with phosphate, was at times below detection limits as previously reported (McClelland et al. 1997).

Phytoplanktonic PP

Daily PP rates closely followed changes in phytoplankton standing stocks (Fig. 2C). The sum of the particulate and dissolved fractions and parallel estimates of total PP (from unfiltered aliquots, see full details in Morán et al. 2011) were highly correlated ($r = 0.95$, $p \ll 0.001$, $n = 24$), although the latter gave slightly higher values (paired t -test, $p = 0.02$). PPP was usually highest at Childs River ($979 \pm 421 \mu\text{g C l}^{-1} \text{d}^{-1}$, mean \pm SE) and Quashnet River ($832 \pm 307 \mu\text{g C l}^{-1} \text{d}^{-1}$) and very similar except in August at Nantucket Sound and Sage Lot Pond (251 ± 50 and $429 \pm 157 \mu\text{g C l}^{-1} \text{d}^{-1}$, respectively, Fig. 3A). DPP showed higher temporal and spatial differences compared to PPP (Fig. 3B), yielding different patterns in percent extracellular release (PER) values. PER was usually $< 20\%$, with the conspicuous summer exception of Sage Lot Pond (Fig. 3C), which showed the largest range of variation (7 to 36%). PER was essentially constant at Nantucket Sound ($\sim 15\%$), while Childs River and Quashnet River showed no clear patterns. DPP and bulk DOC values covaried both within (except at Nantucket Sound, $r = 0.58\text{--}0.8$, $p < 0.05$, $n = 6$) and across sites (Table 1), suggesting that phytoplankton fluxes partially accounted for DOM variability in the estuary. DPP/DOC ranged between 0.004 and 0.20 d^{-1} and increased with temperature (Table 1).

BB and BP

Heterotrophic BB varied 6-fold, with maxima uniformly found in August (80 to $164 \mu\text{g C l}^{-1}$) and minima in December ($\sim 30 \mu\text{g C l}^{-1}$), and a clearly decreasing geographical gradient from Childs River to Nantucket Sound (Fig. 4A). The observed variability in BB was largely driven by changes in total abundance although geographical differences in cell size were also observed (RMANOVA $p = 0.003$), with values at Nantucket Sound ($0.0051 \pm 0.0001 \mu\text{m}^{-3}$, mean \pm SD) being significantly lower (Bonferroni post-hoc test) than at Quashnet River ($0.0063 \pm 0.0010 \mu\text{m}^{-3}$) and Sage Lot Pond ($0.0058 \pm 0.0002 \mu\text{m}^{-3}$) with intermediate values found at Childs River ($0.0055 \pm 0.0004 \mu\text{m}^{-3}$). The trend of larger bacteria at lower salinities could be caused by increasing DON concentrations with more freshwater present (Table 1).

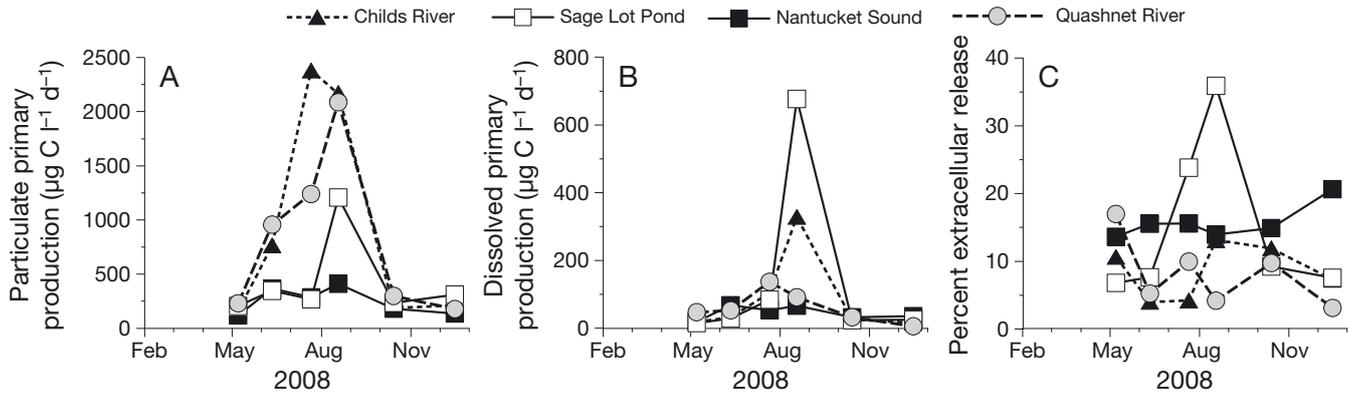


Fig. 3. Measured rates of (A) particulate, (B) dissolved primary production and (C) percent extracellular release (i.e. contribution of dissolved primary production to total primary production) in Waquoit Bay

Table 1. Pearson correlation coefficients between selected environmental variables, dissolved primary production and bacterial biomass and production in Waquoit Bay during 2008. Temp: temperature; Salt: salinity; Chl: chlorophyll; DPP: dissolved primary production, DPP/DOC: contribution of DPP to total DOC turnover; BBv: mean cell size; BB: bacterial biomass; LIR: leucine incorporation rates; LCF: Leu-to-C conversion factors, BP: bacterial production. Except for temperature, salinity and DPP/DOC, all variables were \log_{10} transformed. $n = 72$ except DPP, DPP/DOC and LCF, for which $n = 24$. Significant ($p < 0.05$) correlations are indicated in **bold** when the level of significance was $p < 0.00083$ after Bonferroni correction

	Temp	Salt	DOC	DON	Chl	DPP	DPP/DOC	BBv	BB	LIR	LCF
Salt	–										
DOC	0.57	–									
DON	–	-0.41	0.31								
Chl	0.63	–	0.56	–							
DPP	0.73	–	0.55	–	0.70						
DPP/DOC	0.47	–	–	–	0.57	0.87					
BBv	–	-0.29	–	0.36	–	–	–				
BB	0.87	–	0.64	–	0.75	0.75	0.55	–			
LIR	0.75	–	0.46	–	0.41	0.56	–	0.38	0.68		
LCF	–	0.61	–	–	–	–	–	–	–	-0.57	
BP	0.74	–	0.41	–	0.34	0.51	–	–	0.62	0.90	–

The overall high Leu incorporation rates (162 to $6562 \text{ pmol Leu l}^{-1} \text{ h}^{-1}$) showed distinct spatio-temporal patterns, with maxima in summer and a sharp decline towards December minima at all sites (Fig. 4B). Highest values at Quashnet River were only significantly higher than those at Nantucket Sound, which were also significantly lower than Sage Lot Pond values. Bacterial activity was significantly correlated with temperature, DPP, DOC and cell size (Table 1).

Leu-to-C CFs varied ~ 2.5 -fold within sites, with generally 2 relative maxima, in June and October or December and minima in May and July (Fig. 4C). Values increased with salinity but decreased with Leu incorporation rates (Table 1), resulting in lower variability of production (Fig. 4D) relative to activity (Fig. 4B) and not significantly different values between sub-estuaries (RMANOVA, $p = 0.10$). Overall, BP varied 2 orders of magnitude (4.8 to $121.5 \text{ } \mu\text{g C l}^{-1} \text{ d}^{-1}$), with maxima found in July and minima in December.

Modelled bacterial respiration varied over 2 orders of magnitude: 8.5 to $408 \text{ } \mu\text{g C l}^{-1} \text{ d}^{-1}$ according to López-Urrutia & Morán (2007) (BR_1) and 26 to $170 \text{ } \mu\text{g C l}^{-1} \text{ d}^{-1}$ according to Robinson (2008) (BR_2). Individual BGE values calculated in the long-term DOC consumption experiments (range 3 to 50%) compared reasonably well with BGEs estimated as $BP/(BP+BR_1)$ (4 to 70%), and $BP/(BP+BR_2)$ (16 to 42%), with a common mean value of 29%. Although the independent variables for estimating BR_1 and BR_2 differed, both BCD estimates were strongly correlated ($r = 0.80$, $p < 0.001$, $n = 72$) and were not significantly different (paired t -test, $p = 0.11$, $n = 72$). BCD calculated from the DOC consumption-based BGE values was also strongly correlated with the 2 aforementioned estimates ($r = 0.85$ and 0.92 , respectively, $p < 0.001$, $n = 24$). However, since the empirical BGEs were not available for all sites and sampling times, we decided to use BCD values of the 1st model, which ranged from to 19 to $468 \text{ } \mu\text{g C l}^{-1} \text{ d}^{-1}$, except when indicated otherwise.

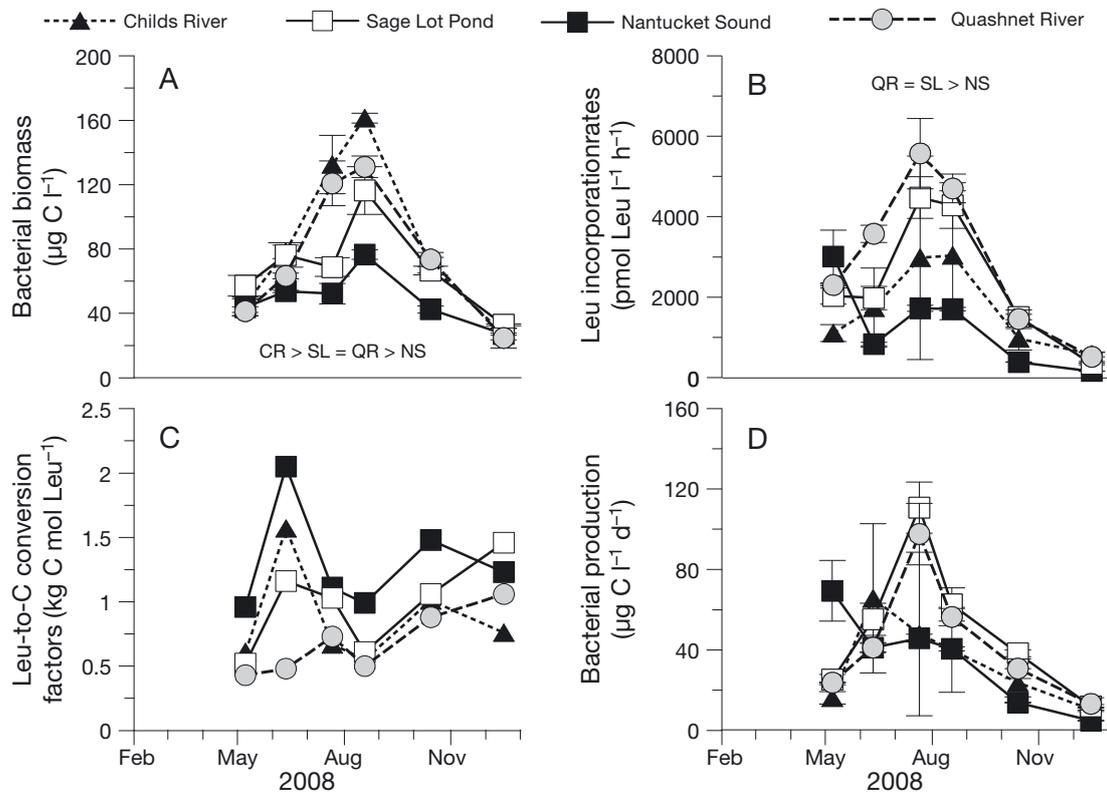


Fig. 4. (A) Mean bacterial biomass, (B) leucine incorporation rates, (C) Leu-to-C conversion factors and (D) bacterial production in Waquoit Bay. Error bars represent standard deviations of 3 replicates per site. In (C) only 1 replicate per site was carried out. Significant differences between sites are indicated in (A) and (B). In (D) the between-effect was not significant (RMANOVA, $p > 0.05$)

Controls of bacterial carbon flow

Good relationships were generally found in Waquoit Bay between bacterial biomass and Leu incorporation rates, a slight modification (Morán et al. 2010) of the assessment of bottom-up (resource) control suggested by Billen et al. (1990) and Ducklow (1992). With all data pooled, the slope of the log–log regression was 0.38, indicating close to moderate bottom-up control. The biomass-activity regressions varied across sites (Table 2), with absolute bottom-up control ranging from weak at Nantucket Sound to strong at Quashnet River.

Two candidates for exerting substrate control are DOC and the fraction of recent phytoplanktonic origin (DPP). Similar to LIR, BP was also significantly correlated with both of these variables (Table 1, Fig. 5B,C), with DPP explaining a higher percentage of total variance (26 vs. 17%). In an attempt to differentiate between bulk and phytoplanktonic DOC we performed multiple linear regressions with DOC, DON, DOC:DON and DPP as potential explanatory variables ($n = 24$). For bacterial biomass the best models were:

Table 2. Slopes of the log bacterial biomass vs. log leucine incorporation rates linear regressions with all data pooled ($n = 72$) and at each of the 4 Waquoit Bay sites ($n = 18$) with the indication of statistical significance and the strength of bottom-up control following Ducklow (1992)

	Slope	r^2	p	Bottom-up control
Waquoit Bay	0.38	0.68	<0.001	Weak
Childs River	0.36	0.22	0.048	Weak
Nantucket Sound	0.25	0.51	0.001	Weak
Sage Lot Pond	0.41	0.59	<0.001	Moderate
Quashnet River	0.85	0.86	<0.001	Strong

- (1) $\log BB = 0.09 + 0.56 \log DOC + 0.29 \log DPP$
AIC = -21.8, AIC weight = 0.39, $r^2 = 0.65$
- (2) $\log BB = -0.30 - 0.05 \log DOC:DON + 1.52 \log DPP$
AIC = -21.4, AIC weight = 0.31, $r^2 = 0.70$

The best models for bacterial production included the following variables:

- (1) $\log BP = -2.18 - 0.16 \log DOC:DON + 3.87 \log DPP$
AIC = 7.23, AIC weight = 0.36, $r^2 = 0.45$

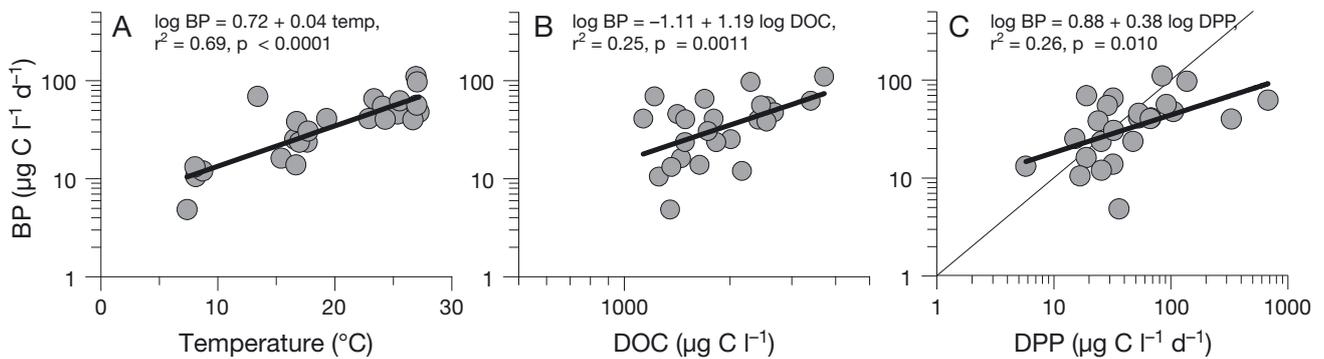


Fig. 5. Relationships between bacterial production (BP) and (A) temperature, (B) dissolved organic carbon (DOC) concentration and (C) dissolved primary production (DPP) rates for all data pooled. Continuous line in (C) represents the 1:1 relationship. All data except DPP are mean values of 3 replicates per site and sampling time. Fitted lines are ordinary least squares linear regressions. $n = 24$

$$(2) \quad \log BP = -1.67 - 0.15 \text{ DOC:DON} + 3.32 \log \text{ DOC} - 2.37 \log \text{ DON} + 0.23 \log \text{ DPP},$$

$$\text{AIC} = 7.56, \text{AIC weight} = 0.30, r^2 = 0.51$$

Both bulk DOC and DPP explained higher percentages of variance when the total flux of carbon (BP + BR) was considered. Moreover, DPP was a better predictor of BCD than bulk DOC regardless of the method used for estimating BR, the literature model (Fig. 6A) or the mean BGE values calculated for each site (Fig. 6B). In the latter case, only the linear regression with DPP was significant, providing stronger support to the role played by fresh photosynthate in fuelling bacterial metabolism.

This bacterial dependence on DPP differed between sub-estuaries (Fig. 7). Although BCD exceeded DPP on all except 2 occasions, released photosynthate could sporadically meet most bacterial requirements of carbon at the 4 sites. DPP/BCD ratios show that bacterial requirements for substrates could be provided mainly by phytoplankton (i.e. DPP/BCD > 0.5) on one third of the determinations, with no obvious temporal pattern (Fig. 7). Site-averaged values reflect that the contribution of DPP to BCD was higher in Sage Lot Pond and Nantucket Sound (64% and 60%, respectively) compared with the 2 riverine sites (39% at Childs River and 35% at Quashnet River). For the whole bay, DPP/BCD averaged 0.50 ± 0.11 .

DISCUSSION

Spatio-temporal variability of microbial carbon fluxes

Most physico-chemical and microbial plankton variables displayed strong between-site differences in Waquoit Bay (Figs. 2 & 3), largely dependent on

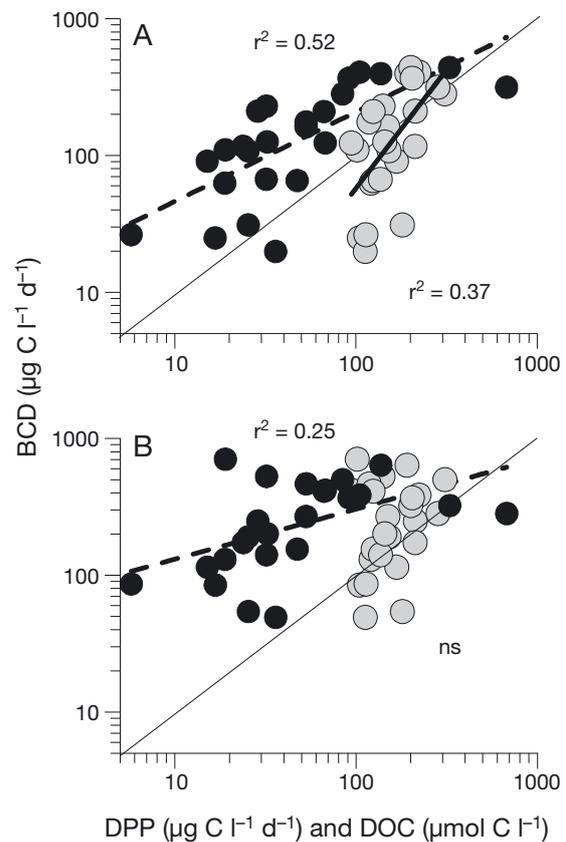


Fig. 6. Relationships between bacterial carbon demand (BCD) and dissolved primary production rates (DPP) and bulk dissolved organic carbon (DOC) concentrations for all Waquoit Bay data pooled using (A) the literature model of López-Urrutia & Morán (2007) for estimating bacterial respiration and (B) site-averaged empirical bacterial growth efficiencies obtained in long-term DOC consumption experiments. Note the different units for DPP and DOC on the x-axis. Fitted dashed and continuous lines represent significant ($p < 0.05$) BCD-DPP and BCD-DOC correlations, respectively. Coefficients of determination are also given. ns: non significant

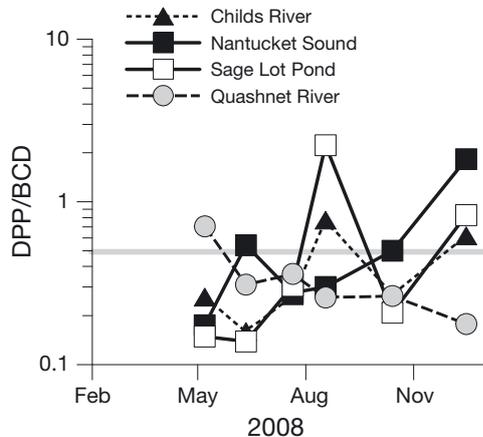


Fig. 7. Temporal evolution of the relative contribution of dissolved primary production to bacterial carbon demand (DPP/BCD). Grey line represents a DPP/BCD value of 0.50 (i.e. values above the line indicate that DPP would meet more than 50% of total BCD)

the varying influence of riverine nutrient inputs (Valiela et al. 1992, 1997, Martinetto et al. 2006) and associated changes in ecosystem functioning (McClelland & Valiela 1998, Olsen et al. 2011). Standing stocks and productivities tended to be higher at the freshwater-influenced sites Childs River and Quashnet River and usually the lowest values were found in coastal waters outside of the estuary mouth. Within-site differences in external forcings relevant for bacterial metabolism were more evident for bulk and phytoplankton-derived DOM (Fig. 2D and 3B) than for temperature (Fig. 2A).

PPP and DPP did not always covary, causing conspicuous differences in PER between sites (Fig. 3C). Although high PP is frequently associated with low PER (Nagata 2000), values >20%, perhaps caused by enhanced grazing activity (Teira et al. 2001) or transient inorganic nutrient limitation (Obernosterer & Herndl 1995), were found 3 times in Waquoit Bay. Strong nutrient and salinity gradients are likely behind the order of magnitude variation in PER, far from the hypothesized constancy at 20% in marine waters (Marañón et al. 2005). Interestingly, PER was more constant in Nantucket Sound coastal waters outside the estuary. Low PER variability was reported in stable environments compared to others experiencing high frequency physico-chemical forcing (Morán & Estrada 2001), like that expected upstream of the bay associated to changes in river discharge. A shift in the dominant phytoplankton species, with an increase in the *Synechococcus* to picoeukaryotes ratio in July and August (data not shown) could also be behind the substantial PER increase observed at Sage Lot Pond.

Spatio-temporal patterns in bacterial activity did not have a straightforward translation into production (cf. Fig. 4B,D) due to 4.8-fold variation in Leu-to-C CFs (Fig. 4C). Seasonal variations in tracer to carbon CFs had been previously documented for Leu (Alonso-Sáez et al. 2008, Calvo-Díaz & Morán 2009) and TdR (Kirchman et al. 1982), strongly cautioning against the use of fixed literature values. In spite of the overall nutrient-rich nature of Waquoit Bay waters, Leu-to-C CFs close to either 1.55 or 3.1 kg C mol Leu⁻¹, based on theoretical assumptions in nutrient replete bacteria (Simon & Azam 1989), were seldom achieved (Fig. 4C). The weak but inverse correlation with Leu incorporation rates, as in NE Atlantic continental shelf waters (Calvo-Díaz & Morán 2009), warns against the face value acceptance of activity as indicator of biomass production also in the freshwater-saltwater transition, where the lowest Leu-to-C CFs were surprisingly found in waters more affected by riverine discharge (Table 1).

Although the time needed to calculate BGE in the DOC consumption experiments was considerably larger than the <2 h needed for measuring Leu incorporation, the corresponding BGE estimates averaged for all sites available at each sampling time (data not shown) displayed a coherent seasonal cycle. Minima occurred in May and October and maxima in June or July, where they reached high values comparable to literature models (ca. 30%)—as expected for meso- to eutrophic sites, and specifically estuaries (del Giorgio & Cole 1998, 2000). Within-sites differences also reflected the expected increase in BGE with higher trophic status.

The large variability found across time and space in bacterial biomass and production in Waquoit Bay, likely reflecting the succession of distinct taxa, could be unexpectedly reduced to a few common variables explaining appreciable amounts of variance, as shown in Table 1, Fig. 6 and the multiple linear regressions.

Role of phytoplanktonic DOC in bottom-up control

Various lines of evidence point towards moderate bottom-up control of bacteria in Waquoit Bay as summarized in Table 2. Bacterial-specific growth rates were more controlled by substrates than by temperature, although temperature effects were also apparent (Morán et al. 2011). Both temperature and substrate supply affected bacterial biomass and production in this study, with the latter having a seemingly preponderant role (Fig. 5 and Table 1). There was no change in the slope of the BP-temperature

relationship at higher temperatures (Fig. 5A), considered elsewhere as evidence of summer shortage of bacterial substrates (Shiah & Ducklow 1994, Shiah et al. 1999, Morán et al. 2010). With substrates being available year-round, we aimed to determine to what extent bottom-up control was exerted by phytoplankton. Macrophytes (mostly seaweeds) are the dominant primary producers in Waquoit Bay proper (the 2 rivers and Sage Lot Pond), with phytoplankton contributions to gross PP ranging from 12 to 24% (D'Avanzo et al. 1996). Although this would leave a minor role of planktonic algae in sustaining bacterial growth, bacteria preferentially assimilate phytoplankton DOC relative to other sources (Norrman et al. 1995, del Giorgio & Cole 1998), and this preference appears to be widespread in coastal bacteria phylotypes (Sarmiento & Gasol 2012). DPP contribution to DOC turnover was significantly correlated with temperature but temporal changes in the relative degree of total versus phytoplanktonic bottom-up control of bacterial assemblages were not evident. Different associations with temperature in the southern Bay of Biscay were explained by strong limitations in phytoplanktonic DOM supply during summer stratification (Morán et al. 2010). In contrast to previous studies (Tomasky & Valiela 1995, York et al. 2007), the present study found that both phytoplankton biomass and production continued to increase, and also peaked, during the summer (Fig. 3), suggesting that our phytoplankton samples were not strongly limited by inorganic nutrients.

Organic substrates released by phytoplankton are usually a very small fraction of total DOC partly due to their rapid removal from the system by bacteria (Larsson & Hagström 1979). Interestingly, the correlations between DPP and bulk DOC concentration, indicative of sizeable phytoplanktonic contribution to total DOM fluxes, were significant at the 3 sites inside Waquoit Bay. That DOC concentrations at Childs River and Quashnet River, higher than at Nantucket Sound, also increased with increasing DPP gives further support to our view that phytoplankton products were important components of carbon flowing through bacteria in the whole estuary. Without considering temperature, the best multiple linear regression models for BB and BP always included DPP together with bulk DOM characteristics, explaining 55 to 60% and 45 to 50% of the respective variances. Further, DPP explained more variance in BCD than bulk DOC, regardless of the method used for estimating respiration (Fig. 6). Strictly speaking, bacterioplankton would not be tightly coupled to phytoplankton year-round in

Waquoit Bay according to our definition (Morán et al. 2002a) because BCD exceeded DPP in most of the samples. However, phytoplankton would be the dominant source of substrates for bacteria in 40% of all determinations (Fig. 7). The BCD–DPP covariation even held for the 2 riverine sites separately (data not shown), reinforcing the important role of dissolved photosynthate in fuelling bacterial metabolism in the entire system. Inside the bay, the highest correlation (0.92) was found at Quashnet River and the lowest at Sage Lot Pond (0.42), where benthic communities were dominated by macroalgae and eelgrass *Zostera marina*, respectively (Hauxwell et al. 2001, Fox et al. 2008). Interestingly, BGE differs between DOC of these 2 origins, with 3-fold higher values on DOC derived from vascular plants compared with seaweeds (del Giorgio & Cole 1998). These results suggest that the contribution of DPP to BCD may be also modulated by the quality of other DOC sources. Only a small fraction (<10%) of the bulk DOC pool would need to be turned over daily in order to satisfy bacterial needs, consistent with our estimated DPP/DOC values. The concurrent accumulations of bacteria and phytoplankton in eutrophic estuaries would thus not simply indicate common responses to other drivers, but suggest direct, trophic relationships—although the presence of large amounts of semi-labile to refractory DOC may have obscured this finding in the past.

Without considering here the role of sloppy feeding by meso- and micrograzers or cell lysis in supplying extra phytoplankton-derived DOC, DPP alone contributed on average 50% of bacterial carbon needs in the estuary and surrounding waters during an annual cycle (Fig. 7). When moving along the trophic gradient from freshwater to seawater DPP/BCD increased as predicted by Morán et al. (2002a), with a mean, but still noticeable 37% at the riverine sites and almost 2/3 BCD met by DPP at Nantucket Sound and Sage Lot Pond. Indications of phytoplankton–bacterioplankton coupling in estuaries are not completely new. Bacteria were entirely dependent on DOC of recent origin rather than old DOC in a subtropical mangrove-dominated estuary (Cherrier et al. 1999) while Fouilland & Mostajir (2010) show that the sum of PPP and DPP was able to meet BCD in highly productive waters ($>100 \mu\text{g C l}^{-1} \text{d}^{-1}$) such as those of Waquoit Bay (Fig. 3). After careful consideration of possible sources of error, we show that the strong seasonal changes were coherent with a major role of phytoplankton in supplying the substrates required for bacterial metabolism. We conclude that the frequently claimed need

of allochthonous DOM for supporting bacterial growth in estuarine ecosystems should not make us overlook the important function played by photosynthate freshly released by phytoplankton.

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