Contribution of bacterial respiration to plankton respiration from 50 ˚N to 44 ˚S in the Atlantic Ocean.

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ABSTRACT

Marine bacteria play an important role in the global cycling of carbon and therefore in climate regulation. However, the paucity of direct measurements means that our understanding of the magnitude and variability of bacterial respiration in the ocean is poor. Estimations of respiration in the 0.2-0.8 µm size-fraction (considered as bacterial respiration), total plankton community respiration, and the contribution of bacterial respiration to total plankton community respiration were made along two latitudinal transects in the Atlantic Ocean (ca. 50 ºN – 44 ºS) during 2010 and 2011. Two different methodologies were used: determination of changes in dissolved O_2 concentration after standard 24 h dark bottle incubations, and measurements of in vivo reduction of 2-(ρ-iodophenyl)-3- (ρ-nitrophenyl)-5phenyl tetrazolium salt (INT). There was an overall significant correlation (r = 0.44, p <0.0001, n = 90) between the rates of community respiration estimated by both methods. Depth-integrated community respiration varied as much as three-fold between regions. Maximum rates occurred in waters of the western European shelf and Patagonian shelf, and minimum rates in the North and South oligotrophic gyres. Depth-integrated bacterial respiration followed the same pattern as community respiration. There was a significantly higher cell-specific bacterial respiration in the northern subtropical gyre than in the southern subtropical gyre which suggests that bacterial carbon turnover is faster in the northern gyre. The relationships between plankton respiration and physicochemical and biological variables were different in different years. In general, INT_T was correlated to both chlorophyll-*a* and bacterial abundance, while $INT_{0.2-0.8}$ was only correlated with bacterial abundance. However, in 2010 INT_T and $INT_{0.2-0.8}$ were also correlated with temperature and primary production while in 2011 they were correlated with nitrate + nitrite concentration. The bacterial contribution to depth integrated community respiration was highly variable within provinces

(4 - 77 %). Results from this study suggest that the proportion of total community respiration attributable to bacteria is similar between the 6 oceanographic regions studied.

Keywords: microbial plankton respiration, bacterial respiration, Atlantic Meridional Transect, oligotrophic, eutrophic regions.

1. Introduction

The variability of plankton community (i.e. auto- and heterotrophic pro- and eukaryotic micro-organisms) respiration in the ocean has been poorly characterized and there are even fewer studies focused on the spatial or temporal variability of bacterial respiration. Several studies of plankton community respiration across ocean basins have shown clear latitudinal trends related to the distribution of chlorophyll-*a* (Chl-*a*) and nutrients (Serret et al. 2001; Del Giorgio et al. 2011; Wilson et al. 2014; Serret et al. 2015). Information on bacterial respiration is limited and most of the studies focus on a specific area (Cottrell et al. 2008; Reinthaler et al. 2008; Teira et al. 2010; García-Martín et al. 2014; Martínez-García and Karl 2015) or along trophic gradients from coast to offshore or along estuaries (Biddanda et al. 1994; Del Giorgio et al. 2011).

Despite the importance of bacterial respiration for global carbon cycling and climate regulation (Azam 1998), there is a lack of consensus between estimates of the contribution of bacteria to the total microbial community respiration. Several studies based on in situ and laboratory experiments have reported bacterial contributions greater than 80 % in oligotrophic lakes (Biddanda et al. 2001), the North Atlantic oligotrophic gyre (Aranguren-Gassis et al. 2012) and across marine systems (Robinson 2008). Other authors applying carbon models have estimated that bacteria should contribute ≤50 % to the total respiration in

order to be consistent with estimations derived from bacterial production, growth efficiencies and dissolved organic carbon production models in the Subarctic North Pacific (Anderson and Ducklow 2001) and in the Subtropical North Atlantic (Marañón et al. 2007; Morán et al. 2007). Proportions of community respiration attributable to bacteria lower than 40 % have also been measured by means of dark incubations of filtered seawater in the Sargasso Sea (Obernosterer et al. 2003), western Arctic Ocean (Kirchman et al. 2009) and in the Arabian Sea (Robinson and Williams 1999), among others. Variability between regions and years is expected due to differences in physicochemical and biological characteristics, as well as differences due to the use of different methods (i.e. direct measurements versus model estimations).

Three main methods have been used to estimate bacterial respiration (BR): (i) direct measurements of the decrease in dissolved oxygen concentration after typically 24 h incubations in bottles enclosing seawater that has been pre-filtered through a standard pore size filter (1 or 0.8 µm are commonly used) to exclude most eukaryotic microbes (Biddanda et al. 1994; Biddanda et al. 2001; Cottrell et al. 2008); (ii) calculations derived from community respiration (CR) (Robinson 2008), bacterial production and bacterial growth efficiencies with or without considering temperature effects (Del Giorgio and Cole 1998; Rivkin and Legendre 2001; Morán et al. 2007) and (iii) measurements of the in vivo reduction of 2-(ρ-iodophenyl)-3- (ρ-nitrophenyl)-5phenyl tetrazolium salt (INT) inside the living cells without addition of substrate (Martínez-García et al. 2009; Aranguren-Gassis et al. 2012). None of these approaches is free of potential biases, and the major drawbacks to these techniques have been identified (Robinson 2008; Maldonado et al. 2012). The traditional in vitro oxygen consumption rates may incur greater potential biases when measuring bacterial respiration (i.e. separation of the bacteria from their predators during the size filtration and the potential increase of the concentration of dissolved organic material

(Pomeroy et al. 1994; Gasol and Morán 1999). Furthermore, the low sensitivity of the technique requires the collection of many replicates and incubations of up to 24h. As a result of these drawbacks, it is necessary to start using alternative approaches. The INT reduction method does not measure plankton respiration per se, but it is a good proxy to estimate plankton and bacterial respiration at short time scales (Martínez-García et al. 2009).

The scarcity of field data and the systematic undersampling of bacterial respiration, particularly in the open ocean, has led us to examine these rates within several biogeochemical provinces of the Atlantic Ocean. We tested the following hypothesis: 1) the percentage of bacterial respiration to total plankton community respiration is greater in oligotrophic regions than in temperate and equatorial regions and 2) bacterial contribution is higher in the north Atlantic gyre than the south Atlantic gyre as a result of the different metabolic behaviour of these two gyres (the north Atlantic gyre is predominantly heterotrophic while the south Atlantic gyre is balanced or autotrophic, Serret et al. 2015). In this study we have explored the latitudinal variability of respiration rates of both the total plankton community and the 0.2-0.8 µm size-fraction (considered as bacterial respiration), and the percentage of the bacterial respiration to total plankton community respiration using the in vivo INT reduction approach along two north-to-south transects in the Atlantic Ocean, covering a wide range of oligotrophic and eutrophic ecological conditions. Total plankton community respiration was also estimated by means of the classical 24 h dark incubation method in order to check for similarities in the results from each methodology.

2. Material and methods

2.1 Study site and sampling procedure

Water samples were collected at 67 stations on two Atlantic Meridional Transect (http://www.amt-uk.org/) cruises (AMT20 and AMT21) along north to south transects through the Atlantic Ocean, between 13 October and 21 November 2010 and 30 September and 08 November 2011. The transects run from 50.45 ˚N to 44.33 ˚S and encompassed six oceanographic provinces, as defined by Longhurst (1998): the North Atlantic Drift Province (NADR), North Atlantic Subtropical Gyral Province (NAST), North Atlantic Tropical Gyral Province (NATR), Western Tropical Atlantic Province (WTR), South Atlantic Gyral Province (SATL) and South Subtropical Convergence Province (SSTC) (Fig. 1).

Water samples (5-8 L) were collected from predawn SeaBird CTD casts at each station using 10 or 20 litre Niskin sampling bottles from 5 to 6 depths (for dissolved oxygen consumption, see below) and 3 depths (for in vivo INT reduction, see below) in the epipelagic zone, considered as the layer between the surface and the depth at which incident irradiance is 1% of surface irradiance (I_0). At the following three depths water was sampled for the in vivo INT and dissolved oxygen analyses in parallel: (i) the surface (2 m), (ii) the depth of 1 % I_0 and (iii) the depth of the deep chlorophyll maximum (DCM). When the DCM was coincident with the 1 % I_0 depth, a water sample was collected at an intermediate depth (33 % I_0). During the 2010 cruise, light depths were estimated on the basis that the 1 % I_0 corresponds to the depth of the DCM or, when the DCM was absent, to the base of the surface chlorophyll-rich layer (i.e. the mixed layer). However, during the 2011 cruise, the 1 % I_0 depths were estimated from the light profile obtained at the previous mid-day CTD cast. The water was then carefully transferred to 10 litre carboys, using a silicone tube, for subsequent subsampling and analyses of biological variables as outlined below. Due to the size of the sample bottles used in the dissolved oxygen and INT reduction techniques (100-200 mL), small microzooplankton are included in our samples, however the presence of larger organisms (>2 mm) were not observed in the samples and are considered unlikely to occur.

2.2 Physico-chemical and biological variables

Shipboard temperature, salinity and fluorescence measurements were undertaken using Sea-Bird Electronics SBE 911 and SBE 917 series CTD profilers fitted with a chlorophyll-*a* (Chl-*a*) fluorometer (Chelsea Technologies Group Aquatracka MKIII) and Chelsea Aqua 3 fluorometer, respectively. Temperature and salinity sensors were calibrated during the cruises. Micromolar nitrate+nitrite concentrations were determined using a Bran & Luebbe AAIII segmented flow, colourimetric, autoanalyser (Brewer and Riley 1965, Grasshoff 1976). Water samples were collected directly from the Niskin bottles at each station. All samples were analysed within 1-2 hours of sampling. At each station, 250 mL samples of seawater were collected at ≥5 depths and filtered through 0.2 µm polycarbonate filters for Chl-*a* analysis. Chlorophyll-*a* concentration was determined fluorometrically using a Turner Designs Trilogy fluorometer with a non-acidified chlorophyll module after extraction in 90 % acetone for 24 h at 4 ˚C following Welschmeyer (1994). Chl-*a* concentration is used in this study as an approximation of phytoplankton biomass. For analysis of primary production, water samples were collected into three 75 mL clear polycarbonate bottles and three black polycarbonate bottles from 6-8 depths, with 3-6 of these light depths matching those of the plankton community respiration sampling depths detailed above. Primary production rates were determined following Tilstone et al. 2009. Carbon-14 labelled sodium bicarbonate (5 - 15 µCi) was added to each bottle and then all bottles from each light depth were incubated in a on deck in a simulated in situ incubation system. Incubations were ended by sequential filtration through 25 mm 10, 2, and 0.2 µm polycarbonate filters. Filters were exposed to concentrated HCl fumes for 12 h immersed in scintillation cocktail. ^{14}C disintegration time per minute was measured on board using a Packard, Tricarb 2900 liquid scintillation counter.

Primary production was calculated as the sum of the primary production measured in the 10, 2 and 0.2 µm filters.

All environmental data, nitrate+nitrite, Chl-*a* and primary production are available through the British Oceanographic Data Centre (BODC).

2.3 Bacterial abundance

For the enumeration of bacterioplankton cells, a 1.2 mL aliquot from ≥7 depths (including the 3 depths at which INT reduction and dissolved oxygen consumption were measured) was fixed with paraformaldehyde (1%w/v final concentration) within half an hour of collection and stained with the nucleic acid dye SYBR Green I (Marie et al. 1997, Zubkov et al. 2000). To determine absolute bacterioplankton concentration a known volume of the custom-calibrated 0.5 µm multifluorescent bead standard (Zubkov and Burkill 2006) was added to stained samples before the samples were analysed using a FACSort instrument (Becton Dickinson, UK) within 24 h of sample collection. A scatter plot of light-scattering by particles (90 ° or side light scatter, SSC) versus particle Green fluorescence (FL1, 530 ± 30 nm) was used to discriminate bacterioplankton cells from other particles.

2.4 Plankton community and bacterial respiration by in vivo INT reduction assay

Reduction of the INT salt was used as a proxy for plankton respiration in two size-fractions: > 0.8 µm and 0.2-0.8 µm (referred to as $INT_{0.2\text{-}0.8}$). The total plankton community respiration is reported as the sum of the reduction of INT in each fraction (i.e. >0.8 µm and 0.2-0.8 µm, referred to as INT_T). Four 200 - 250 mL polypropylene plastic bottles were filled with seawater from each sampling depth. One replicate was immediately fixed by adding formaldehyde (2% w/v final concentration) and used as a killed control. Twenty minutes later all four replicates were inoculated with a sterile solution of 7.9 mM INT to give a final

concentration of 0.2 mM. The solution was freshly prepared for each experiment using Milli-Q water. Replicates were incubated for 1 - 4 h in deck incubators in complete darkness. Incubation temperatures were maintained with *in situ* water pumped from 4 - 6 m depth flowing through the incubation system for the surface samples, and by chilled water from a water bath maintained at *in situ* temperature ± 1 °C for the samples collected at the two deeper depths. After the incubation time, samples were fixed by adding formaldehyde, as for the killed control. Samples were sequentially filtered after 15 minutes through 0.8 µm and onto 0.2 µm pore size polycarbonate filters, air-dried, and stored frozen in 1.5 mL cryovials at –20 °C until further processing. The INT_T and $INT_{0.2\text{-}0.8}$ were determined from the absorbance at 485 nm of the reduced INT (formazan, INT_f) extracted with propanol and measured in quartz cuvettes using a Beckman model DU640 Spectrophotometer following Martínez-García et al. (2009). The magnitude of INT reduction in the killed control was substracted from the results of the incubated replicates, thus correcting for the potential reduction of INT caused by non-metabolic factors. The rate measured in the large size-fraction (>0.8 µm) will result mainly from the INT reduction by eukaryotes and particle attached prokaryotes. By contrast, the main respiring organisms in the small size-fraction ($INT_{0.2\text{-}0.8}$) would have been heterotrophic bacteria and *Prochlorococcus* cyanobacterial cells.

2.5 Dissolved oxygen consumption rate

During the 2011 cruise, total plankton community respiration (referred to hereafter as CR_{O2}) was also measured by monitoring changes in oxygen concentrations after 24 h dark bottle incubations. Dissolved oxygen concentration was measured by automated precision Winkler titration performed with a Metrohm 721 DMS Titrino titrator, using a potentiometric end point as described in Serret et al. (1999). Eight gravimetrically calibrated 125 mL opaque "dark" borosilicate glass bottles were carefully filled with water from 5-6 depths (97 %, 55 %, 33 %, 7 %, DCM and 1 % I_0). Water was allowed to overflow during the filling, and

special care was taken to prevent air bubble formation in the silicone tube. For each depth, four replicate dark bottles were fixed immediately with Winkler reagents (1 ml of 3 M $MnSO_4$ and 1 ml of (8 M KOH + 4 M KI) solutions) for the measurement of initial oxygen concentrations (t_0). The remaining four dark bottles were incubated in darkness for 24 h in the same deck incubators as the in vivo INT_T and $INT_{0.2\text{-}0.8}$ samples, and fixed for the measurement of final oxygen concentrations (t_{24}). CR_{O2} rates were estimated from the difference in oxygen concentration between the means of the initial (t_0) measurements and the replicate dark incubated (t_{24}) samples.

2.6 Calculations and statistical analysis

The INT reduced in the different size-fractions were converted to O_2 consumption rates by applying the conversion equation $\log CR_{O2} = 0.77 \log INT_T + 0.54$ derived from a comparison of 393 samples (including 95 from this study, AMT21) from eutrophic and oligotrophic marine studies with a $R^2 = 0.73$, $p < 0.0001$ (García-Martín et al. in prep). This new conversion equation has been adopted instead of the model regression with slope of 12.8 used in earlier studies (Martínez-García et al. 2009). Martínez-García's (2009) regression model was derived from respiration rates of cultured populations where the incubations for dissolved oxygen consumption were the same length of time as the incubations for INT reduction (i.e. 0-3 h) hours while the new model equation reported here compares daily oxygen consumption rates with short-term (1-4 h) INT reduction rates. A separate validation exercise comparing respiration rates calculated with the new model equation and with the previously reported regression slope (Martínez-García et al. 2009) showed that the 12.8 regression slope underestimated rates of respiration (when compared to measurements of oxygen consumption) by 20 %, underestimation not observed with the new model equation (Garcia-Martin et al. in prep).

Depth integrated INT_T, $INT_{0.2-0.8}$ and CR_{O2} rates were calculated by trapezoidal integration of the volumetric rates measured at the three to six depths within the epipelagic zone. These depth-integrated rates were then normalised by the depth of integration (weighted average respiration rate in the epipelagic zone) to compare rates between biogeochemical provinces. The standard errors (SE) of the integrated rates were calculated following the propagation procedure for independent measurements described by Miller and Miller (1988). The depth-integrated contribution of the 0.2-0.8 μm fraction to community respiration ($\%INT_{0.2-0.8}$) was calculated as the depth-integrated $INT_{0.2-0.8}$ divided by the depth-integrated INT_T and multiplied by 100.

Data were log-transformed when required to meet the normality and homocedasticity assumption required for the Analysis of Variance. A two-way ANOVA was used to determine the effects of year and region and any interaction effects between these two factors on INT_T, $INT_{0.2-0.8}$ and $\%INT_{0.2-0.8}$. Spearman non-parametric correlation tests were used to study the relation between volumetric INT_T, CR_{O2}, $INT_{0.2-0.8}$ and $\%INT_{0.2-0.8}$ and between each of these and physicochemical parameters (temperature, nitrate+nitrite concentration, Chl-*a* concentration). Statistical analyses were performed with SPSS software.

Plots shown in Figures 1, 2 and 4 were produced with Ocean Data View (ODV) software (Schlitzer 2015).

3. RESULTS

3.1 Hydrography

The distribution of temperature, salinity and Chl-*a* followed the same latitudinal pattern observed in previous cruises and described in Robinson et al. (2006). Cold, less saline water masses were observed at the European northern shelf and in Patagonian southern coastal-

influenced waters. In contrast, water in upper 200m of the North and South subtropical gyres were warmed and more saline (Fig. 2). The highest temperatures were measured at the surface near the Equator, coincident with lower salinity, which is characteristic of this province (Longhurst 1998). Temperature and salinity fronts marked the boundaries of the different provinces proposed by Longhurst (1998).

3.2 Chlorophyll-*a* and bacterial abundance

High Chl-*a* concentrations were measured in surface and sub-surface waters in the northern and southern temperate areas and at the 1 % I_0 in equatorial waters (Fig. 2). By contrast, the northern and southern subtropical gyres were characterized by low Chl-*a* concentrations throughout the epipelagic layer and with a DCM at depths ≥ 100m.

Bacterial abundances were 2- to 4-fold higher in temperate and equatorial regions than in the subtropical gyres (Fig. 2). Higher concentrations were found in surface and sub-surface waters than at the 1% I_0.

3.3 Latitudinal variability in plankton community respiration

Volumetric rates of total plankton community respiration followed a latitudinal pattern during both years (Fig. 3 A-B and Fig. 4), being more evident during 2010 (Fig 3A). Volumetric respiration rates were greater in the two temperate provinces (NADR and SSTC) and lower in the oligotrophic ones (NAST, NATR and SATL). INT_T rates varied up to six-fold between stations, with significant differences between provinces, years and the interaction between year and province (two-way ANOVA, $p<0.0001$ in all cases). These differences occurred mainly in the northern hemisphere as a result of high respiration rates measured in some

283 stations of the NAST and NATR provinces (Fig. 3B). The average of INT_T rates estimated in
284 2011 was significantly greater (mean [±SE], 3.16 [±0.28] µmol INT_f m^{-3} h^{-1}, equivalent to ca.
285 0.99 [±0.15] mmol O_2 m^{-3} d^{-1}) than the average of the 2010 rates (2.03 [±0.16] µmol INT_f m^{-3}
286 h^{-1}, equivalent to c.a. 0.70 [±0.1] mmol O_2 m^{-3} d^{-1}). The lack of CR_{O2} data from the 2010
287 cruise prevents a comparison of CR_{O2} between years. $INT_{0.2-0.8}$ represented on average 34 and
288 30 % (2010 and 2011, respectively) of INT_T and was significantly different between
289 provinces (p <0.0001) and there was a significant interaction between year and province (p =
290 0.015) (Fig. 3 A-B). In contrast to INT_T, $INT_{0.2-0.8}$ rates were not significantly different
291 between years (p = 0.22).
292 The latitudinal gradient in respiration rates was more evident for the epipelagic weighted
293 average INT_T and $INT_{0.2-0.8}$ rates (depth-integrated rates divided by the depth of integration)
294 (Fig. 5). Weighted average INT_T decreased from the European Atlantic shelf (NADR) to the
295 North Atlantic subtropical gyre (NAST and NATR), showing a slight increase in Equatorial
296 waters (WTRA). Rates decreased again in the South Atlantic gyre (SATL) and increased at
297 the Patagonian shelf (SSTC) reaching values comparable to the North Atlantic shelf during
298 2010 and 2011 (Fig. 5A-B). During 2011, INT_T in the northern subtropical provinces (NAST
299 and NATR) was highly variable with relatively high rates (> 5.0 µmol INT_{-f} m^{-3} h^{-1},
300 equivalent to c.a. >1.4 mmol O_2 m^{-3} d^{-1}) measured at two stations. INT_T rates in the northern
301 subtropical provinces (NAST and NATR) were higher than in the southern subtropical
302 province (SATL) during 2011. There were significant differences between provinces, years,
303 and the interaction of years and provinces (two-way ANOVA, p <0.001, p = 0.03, p = 0.012,
304 respectively). The weighted average INT_T rates were higher in temperate regions (NADR and
305 SSTC) than in the subtropical provinces (NAST, NATR and SATL). In general, the weighted
306 average CR_{O2} rates in 2011 followed a similar pattern to the INT_T rates (r = 0.678, p <0.0001,
307 n = 31) (Fig. 5C).

Weighted average $INT_{0.2\text{-}0.8}$ followed the same pattern as the INT_T rates in 2010 and 2011 (Fig. 5A-B) (r = 0.652, p <0.001, n = 28 and r = 0.858, p <0.001, n = 33, respectively). Significant differences were found between provinces (p = 0.001) and the interaction between years and provinces was significant (p = 0.032) but there was no difference between years (p = 0.157). Weighted average $INT_{0.2\text{-}0.8}$ was significantly higher in the SSTC than in the NATR and SATL (p = 0.008 and p = 0.001, respectively), and this increase in bacterial respiration was related to higher numbers of bacteria in the SSTC (r = 0.404, p <0.0001, n = 152). Despite the increase in bacterial numbers in temperate (NADR and SSTC) and equatorial regions (WTRA), the cell-specific $INT_{0.2\text{-}0.8}$ rates were from 1.4 to 2.8-fold higher in the temperate regions than in the subtropics (Fig. 5D). This difference in cell-specific respiration rates suggests that bacteria were more actively respiring the organic carbon in temperate waters than in oligotrophic regions. In addition, cell-specific $INT_{0.2\text{-}0.8}$ rates in the NAST and NATR were significantly higher than in the SATL (p = 0.02) during 2011.

3.4 Contribution of bacterial respiration to total plankton community respiration.

There was no significant difference between the $\%INT_{0.2\text{-}0.8}$ at the different depths sampled (p >0.05, t-paired test), suggesting an independency with depth. $\%INT_{0.2\text{-}0.8}$ values higher than 80 % (considered as unrealistic values, Aranguren-Gassis et al. 2012) were measured in 1 out of 92 samples during 2010 and 3 out of 96 samples in 2011. Three out of these four high percentages were found in the North and South Atlantic gyres.

There was no latitudinal trend in the bacterial contribution to depth integrated respiration during either 2010 or 2011 (Fig. 6). $\%INT_{0.2\text{-}0.8}$ ranged from 4 to 60 % during 2010 and from 8 to 77 % during 2011. No statistical differences were found between years, provinces or the interaction between year and province (two-way ANOVA, p >0.05 in all cases). During 2010,

the lowest contribution of bacterial respiration occurred in the NATR and through the SATL province (mean [±SE], 22 [±5] %), while the greatest contribution was found in the WTRA (43 [±11] %). During 2011, the lowest contribution occurred in the NADR and WTRA provinces (24 [±16] % and 24 [±2] %, respectively) and the highest in the NATR (36 [±11] %).

3.5 Microbial plankton respiration and the relationships with physicochemical and biological parameters

Correlations between volumetric INT_T, $INT_{0.2-0.8}$, $\%INT_{0.2-0.8}$, $CR_{O2,}$ key physicochemical parameters (temperature, nitrate+nitrite concentration, Chl-*a*), bacterial abundance and primary production (measured by ^{14}C and incubated from local dawn to dusk, 10-16 h, Tilstone et al. 2015) are presented in Table 1.

There were differences in the relationships between plankton respiration and to the physicochemical and biological parameters between the two years. During the 2010 cruise, INT_T and $INT_{0.2-0.8}$ were negatively correlated with temperature ($p \leq 0.003$) and positively correlated with bacterial abundance and primary production ($p < 0.0001$). INT_T was positively correlated with Chl-*a* ($p = 0.001$) (Table 1). During 2011 there was no significant relation between INT_T and temperature but there was a relation between INT_T and nitrate+nitrite concentration ($p \leq 0.038$) which did not occur in 2010. The $\%INT_{0.2-0.8}$ was not correlated with any of the environmental variables tested except for a negative correlation with Chl-*a* in 2011 ($p = 0.004$). During 2011 CR_{O2} was correlated with the same parameters as was INT_T (Table 1). In order to test any potential confounding bias between temperature and other factors that vary with depth we performed correlation analysis using only surface data. Surface INT_T and $INT_{0.2-0.8}$ were positively correlated with primary production ($p < 0.009$),

356 nitrate+nitrite (p <0.04) and Chl-*a* ($p \leq 0.001$). As in the statistical analysis with data from all
357 depths, there was not a relation between the respiratory rates and temperature, suggesting that
358 the respiratory rates were mainly controlled by environmental factors other than temperature.

359

360 **4 Discussion**

361 **4.1 Latitudinal trends in microbial plankton respiration**

362 Data from this study showed greater interannual variability in total plankton community
363 respiration compared to bacterial respiration. This interannual variability in respiration, as
364 well as seasonal and latitudinal variability, have been previously reported from similar
365 transects or specific provinces of the Atlantic Ocean (Robinson et al. 2002; Gist et al. 2009;
366 Serret et al. 2015) and the Pacific Ocean (Del Giorgio et al. 2011; Viviani et al. 2011; Wilson
367 et al. 2014). In general, the latitudinal pattern in total plankton community respiration (INT_T
368 and CR_{O2}) was similar to that of Chl-*a* and bacterial abundance, with higher respiration rates
369 in the temperate zones and lower in the oligotrophic gyres. Respiration rates increased near
370 the Equator alongside an increase in Chl-*a* and primary production. INT_T and CR_{O2} rates
371 reported here are within the range of previous results although different methodologies were
372 employed (in vivo INT reduction and dissolved oxygen incubations) (see references in Table
373 2). Comparisons of CR_{O2} in the two oligotrophic gyres during the 2011 showed a
374 geographical pattern similar to that reported in Serret et al. (2015), with higher rates in the
375 northern than in the southern gyre. This difference between the oligotrophic gyres was also
376 observed in the INT rates, with higher weighted average INT_T and cell-specific $INT_{0.2\text{-}0.8}$ in
377 the northern gyre than in the southern gyre. Results from this study suggest that bacteria, may
378 respire to a different extent in the two Atlantic subtropical gyres.

Respiration in the 0.2-0.8 µm size-fraction (or bacterial respiration) has not previously been measured in comparable North-to-South latitudinal transects, so we can only compare our $INT_{0.2\text{-}0.8}$ results with those from other studies in some of the sampled provinces but at different locations and seasons. The latitudinal pattern observed in this study is in agreement with the increase in bacterial respiration recorded from coastal waters to offshore waters in the Pacific Ocean (Del Giorgio et al. 2011). The range of $INT_{0.2\text{-}0.8}$ measured in this study (0.01 – 2.88 mmol O_2 m^{-3} d^{-1}, 5 – 137 mmol O_2 m^{-2} d^{-1} for volumetric and depth integrated data, respectively) are lower or in agreement with values reported for the NATR and NAST provinces in other studies (Morán et al. 2007; Alonso-Sáez et al. 2008; Reinthaler et al. 2008; Teira et al. 2010) (see references in Table 2) and in the NADR province (González et al. 2003 and Cottrell et al. 2008) (see references in Table 2), although the NADR locations were far from our sampling area.

In general, integrated total plankton community and bacterial respiration rates (expressed as weighted average rates in the epipelagic zone) and cell-specific $INT_{0.2\text{-}0.8}$ were between 1.5 and 3-fold higher in the high Chl-*a* temperate and upwelling regions (NADR, WTRA and SSTC provinces) compared with the low Chl-*a* oligotrophic gyres (NAST, NATR and SATL provinces) during 2010. Bacteria are usually considered to have a greater contribution to carbon fluxes in oligotrophic compared to eutrophic waters (Del Giorgio et al. 1997; Gasol and Duarte 2000; Zubkov 2014). The relative small size, and higher surface/volume ratio, increases the efficiency of nutrient acquisition and means these organisms are more competitive in low nutrient waters (Thingstad 2003). In this study the cell-specific bacterial respiration rates were lower in oligotrophic regions than in eutrophic regions which suggests, that even though they may be better competitors than phytoplankton for nutrients, they could have been limited or co-limited by nutrient resources (Hale et al. 2016, this issue). Other explanations to support the greater contribution of bacteria in oligotrophic conditions are

reduced bacterivory (Sanders et al. 1992) and lower bacterial mortality due to viral lysis (Weinbauer and Peduzzi 1995). This, together with the observation that heterotrophic bacteria support higher biomass in oligotrophic areas compared to autotrophic organisms (Ducklow and Carlson 1992; Gasol et al. 1997; Cotner et al. 2000) has led to the hypothesis that bacteria should have a higher contribution to total community respiration in these nutrient poor regions of the ocean. Experimental studies performed in oligotrophic lagoons and the open-ocean supported this hypothesis (Williams 1981; Cotner and Biddanda 2002). However, the bacterial contribution to total plankton community respiration determined in the present study does not agree with this hypothesis. The average of the depth-integrated bacterial contribution was 42 and 28 % in the northern subtropical gyre (2010 and 2011, respectively) and 22 and 34 % in the southern subtropical gyre (2010 and 2011, respectively), and was not significantly different to the bacterial contribution in the temperate and equatorial provinces. This lack of significant differences may be due to the high variability observed within the regions. Overall, our results do not support the conclusion that bacteria increase their relative contribution to plankton community respiration when the productivity decreases (Biddanda et al. 1994; Gasol and Duarte 2000; Roberts and Howarth 2006) and suggest that their contribution can not be determined only from the productivity of the system. In addition, the comparable rates of primary production (Serret et al. 2015) but higher INT_T and $INT_{0.2\text{-}0.8}$ rates recorded in the northern gyre than in the southern gyre corroborate this idea.

The 22 - 42 % contribution of $INT_{0.2\text{-}0.8}$ to INT_T observed in the oligotrophic provinces is much lower than the percentages previously reported for oligotrophic lakes (ca. 90 %, Cotner and Biddanda 2002) and marine systems (69 - 79 %, Biddanda et al. 1994; González et al. 2003; Del Giorgio et al. 2011). However, our results support the estimated bacterial contribution of 29 % from a study conducted in NASTE region (Teira et al. 2010), the 45 %

reported for the ALOHA station (Martínez-García and Karl 2015), the 33 % necessary to match community respiration with the sum of the contributions of the component microbial plankton classes in the North Atlantic Ocean (Morán et al. 2007), and the conclusions derived from meso- and oligotrophic studies that the bacterial contribution should be around 30 % independent of the ecosystem trophic status (Aranguren-Gassis et al. 2012).

4.2 Respiratory rates and their relation to environmental and biological factors

Results from this study show a significant, although weak, correlation between INT_T and Chl-*a* when all data are considered (r = 0.25 and 0.27 in the year 2010 and 2011, respectively). A more detailed analysis indicates that the significant relationship was driven by several data points with high (>1.2 mg m^{-3}) Chl-*a* concentration, and the relationship was not significant when these high Chl-*a* concentrations were removed. Lack of relationships between Chl-*a* and rates of respiration have been previously obtained (Williams 1981; Iriarte et al. 1991) and interpreted as a result of situations where the bacteria are the major respiring organisms at low levels of Chl-*a*. However, the $\%INT_{0.2\text{-}0.8}$ measured here during 2010 and 2011 at low Chl-*a* levels was very variable so our data do not corroborate this interpretation. It may be relevant however, that Chl-*a* concentrations during our study ranged between 0.03 and 1.83 mg m^{-3}, which is at the lowest extreme of the Chl-*a* concentrations measured during these previous studies (Williams 1981; Iriarte et al. 1991).

Temperature has been suggested to be a major factor that controls bacterial respiration either in natural populations (Rivkin and Legendre 2001) or in laboratory experiments (Kritzberg et al. 2010). Contrary to previous results, microbial plankton and bacterial respiration were inversely related to temperature in the 2010 survey. Although temperature is an environmental factor that controls metabolic rates, studying its effect on natural communities,

as in this study, is difficult. In addition the covariation of temperature with other potential controlling factors (nutrient concentration, primary productivity, bacterial abundance) complicates assessing the direct effect of temperature on respiration rates. Previous studies have proposed that other physicochemical factors apart from temperature may limit or co-limit the plankton activity. For example, Kirchman et al. (1995) suggested that bacterial production in the Equatorial Pacific was controlled primarily by the supply of dissolved organic matter and Hoppe et al. (2002) proved that although bacterial production and primary production were related to temperature, this relation was regulated by the trophic situation of the system. A more recent study performed in oligotrophic regions of the Atlantic Ocean showed that bacterial growth could also be limited by inorganic nutrients concentration (Hale et al. 2016, this issue) and suggested that previous data showing limitation by organic matter could be confounded by covariation between inorganic nutrients and organic matter availability. Moreover, the vertical distribution of physicochemical and biological factors could have counteracting influences on the proportion of bacteria to total plankton community respiration (del Giorgio et al. 2011). It is not clear what drives the increase or decrease in the contribution of bacterial to total microbial plankton respiration, but the interaction between multiple environmental and biological factors seems to play an important role and may be one explanation for the lack of relation with any single environmental or biological parameters tested in this study. If the respiratory activity of the total plankton community or the bacterial plankton is controlled by the interaction of several factors, predictions of the respiration of natural populations based on only temperature and cell size may not be possible. Moreover, bacterial respiration predicted from temperature, Chl-*a* or bacterial abundance relationships (López-Urrutia and Morán 2007) would not reveal the differences in respiration observed between the two gyres where temperature, Chl-*a* and productivity are similar.

In summary, microbial plankton and bacterial respiration showed latitudinal trends in the Atlantic Ocean related to chlorophyll-*a* and primary production trends, with higher rates in temperate provinces and lower rates in oligotrophic provinces. Bacteria in temperate and equatorial provinces had higher cell-specific bacterial respiration rates than bacteria in oligotrophic regions, and therefore had a faster turnover rate of organic carbon. The cell-specific bacterial respiration was higher in the northern gyre than in the southern gyre, which may explain the previously observed differences in plankton community respiration between the two gyres. The bacterial contribution to plankton community respiration was variable (4 - 77 %), without a clear latitudinal trend in contrast to our expectations, and could not be predicted from chlorophyll-*a*, temperature or nitrate+nitrite parameters. Further holistic studies including physicochemical and biological parameters (i.e. grazing, competition, bacterial production) should be undertaken to clarify the causes of variability in the proportion of total plankton community respiration attributable to bacteria.

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Table 1. Spearman correlation coefficients (*r*) between physicochemical variables (temperature, T; salinity, Sal; chlorophyll-*a*, Chl-*a*, nitrate+nitrite, NO_3+NO_2, bacterial abundance, BA; and primary production, PP) and respiration measured during the 2010 and 2011 cruises. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. nd denotes no data. The number of datapoints (*n*) are specified for each paramenter.

	2010					**2011**					
	T (n = 81)	**Chl-*a*** (n = 73)	**NO_3+NO_2** (n = 40)	**BA** (n = 68)	**PP** (n = 80)	**T** (n = 95)	**Chl-*a*** (n = 93)	**NO_3+NO_2** (n = 51)	**BA** (n = 84)	**PP** (n = 83)
INT_T	-0.40**	0.38**	0.3	0.52**	0.66**	-0.18	0.39**	.40**	.29**	0.18
$INT_{0.2\text{-}0.8}$	-0.32**	0.22	0.27	0.51**	0.68**	-0.09	0.1	.29*	.34**	0.2
$\%INT_{0.2\text{-}0.8}$	0.06	-0.23	0.09	0.147	0.13	0.14	-0.3**	0.02	0.04	0.02
CR_{O2}	nd	nd	nd	nd	nd	-0.23*	0.3**	.48**	.38**	.28*
BA	-0.13	0.19	-0.02		0.52**	-0.19	0.3**	.31*		.51**
PP	-.210	0.33**	0.03	0.52**		.35**	-0.17	-0.06	.51**	

693 Table 2. Reported rates of plankton (total plankton community, CR; and bacterial, BR)
694 respiration from similar Atlantic regions and the northern Pacific gyre, the period and year of
695 the studies and the methodology used for their estimations. Data reported in carbon units
696 have been converted to oxygen applying the respiratory quotient (RQ) reported in the article
697 or 1 in case of no mention.

			Volumetric		Integrated			
			CR	BR	CR	BR	Method	RQ
Author	Period and year	Province	mmol O_2 m^{-3} d^{-1}		mmol O_2 m^{-2} d^{-1}			
Serret et al. 2001	late spring 1998	NAST-NATR	0.5 - 1.5				DO	
González et al. 2003	summer 1999	NADR	1.9 - 2.5	1.4 - 2.5			DO	
Pérez et al. 2005	autumn 2000	WTRA			46 - 99		DO	
Marañón et al. 2007	spring - autumn 1992 -2001	NAST			54 - 181		DO	
Morán et al. 2007	autumn	NATR-NASTE	0.29 - 2.07		83.06	15.7 – 37.7	(BP/BGE) – BP	0.89
Alonso-Sáez et al. 2007	summer-autumn 2003	NAST-NATR		0.94 - 1.89			DO	0.88
Cottrell et al. 2008	summer 2005	NADR				8.4 – 41.8	DO	1
Reinthaler et al. 2008	autumn 2004	NASTE		1 - 30			DO	
Teira et al. 2010	late autumn 2006	NASTE	<0.01 - 0.74		1.4 -1.58	34 - 38	INT	1
del Giorgio et al. 2011	summer 2002	Northern Pacific Gyre		1 - 3.5			Leucine respiration	1
This study	autumn 2010 & 2011	NADR	0.38 - 2.8	0.05 - 0.78	63 - 222	5 - 61	INT	
		NAST	0.25 - 2.45	0.09 -0.87	89 - 670	13 - 137	INT	
		NATR	0.43 - 3.2	0.08 - 2.88	62 - 349	13 - 122	INT	
		WTRA	0.26 - 1.5	0.08 - 0.7	112 - 324	9 - 88	INT	
		SATL	0.22 - 1.27	0.01 - 0.84	47 - 371	6 - 72	INT	
		SSTC	0.76 - 2.02	0.3 - 1.5	89 - 250	18 - 108	INT	

DO: dissolved oxygen incubations
BP: bacterial production ; BGE: bacterial growth efficiencies
698 INT: in vivo INT reduction method

699

700 **Legends**

701 Figure 1. AMT20 and AMT21 cruise tracks. Black dots indicate the position of the sampling
702 stations during the 2010 cruise and white triangles the 2011 cruise. The approximate location
703 of the different regions is indicated.

704 Figure 2. Vertical and latitudinal sections of temperature, salinity, chlorophyll-*a*
705 concentration and bacterial abundance for the 2010 and 2011 cruises. Dashed line indicates
706 the epipelagic zone (corresponding to the depth of 1 % of incident irradiance). The
707 approximate boundaries between the different regions are indicated by dotted vertical lines.

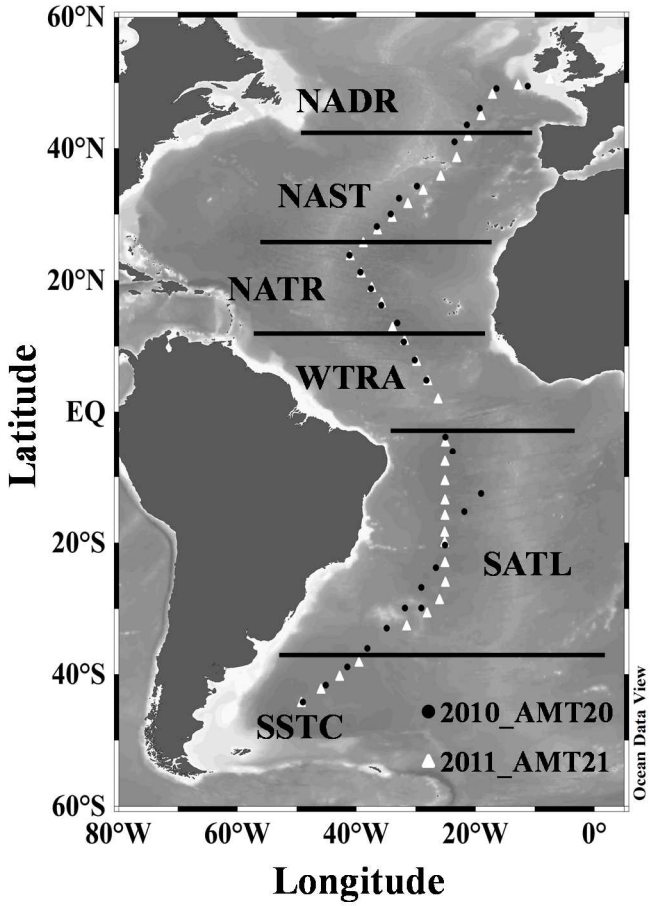
708 Figure 3. Total plankton community INT reduction (A and B) and INT reduction on the 0.2-
709 0.8 µm size-fraction (C and D) measured in surface (open triangles), 33% incident light (grey
710 circles) and 1% incident light (dark triangles) samples in the 2010 and 2011 surveys. The
711 approximate boundaries between the different regions are indicated by dotted vertical lines.

712 Figure 4. Latitudinal section of community respiration measured by means of dissolved
713 oxygen concentration during the 2011 survey. The approximate boundaries between the
714 different regions are indicated by dotted vertical lines.

715 Figure 5. Depth-integrated plankton community respiration (black dots) and respiration
716 measured in the 0.2-0.8 µm size-fraction (white dots) normalized by integrated depth
717 (weighted average rate in the epipelagic zone) measured with the INT reduction method (A,
718 B) and with the dissolved oxygen method (C); and cell-specific $INT_{0.2-0.8}$ rates (D) along the
719 north-south latitudinal transects. Only data from the 2011 is available for the CR_{O2}. Error bars
720 represent the standard error of the measurement. The approximate boundaries between the
721 different regions are indicated by dotted vertical lines.

722 Figure 6. Bacterial contribution to depth-integrated total plankton community respiration (%
723 $INT_{0.2\text{-}0.8}$) along the north-south latitudinal transect in the 2010 and 2011 surveys. Error bars
724 represent the standard error of the measurement (when error bars are not visible, they are
725 smaller than the symbol size). The approximate boundaries between the different regions are
726 indicated by dotted vertical lines.

727 Figure 1.



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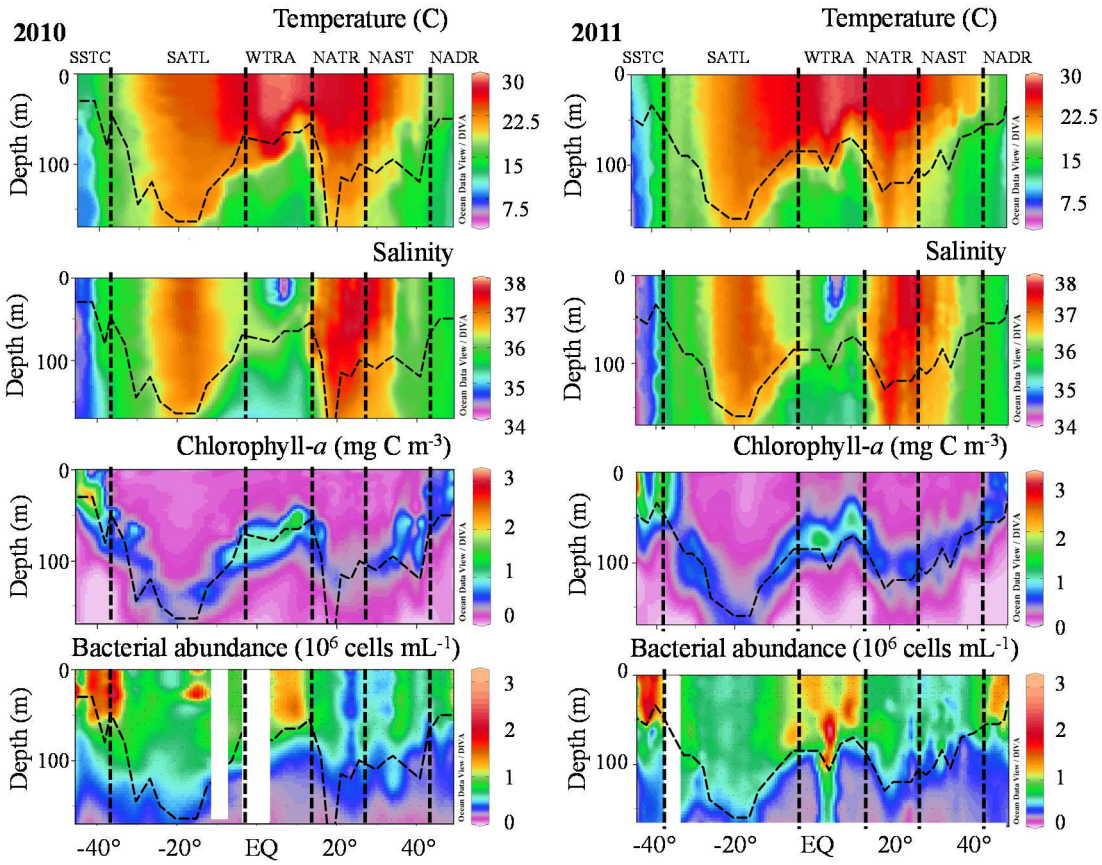
730 Figure 2.



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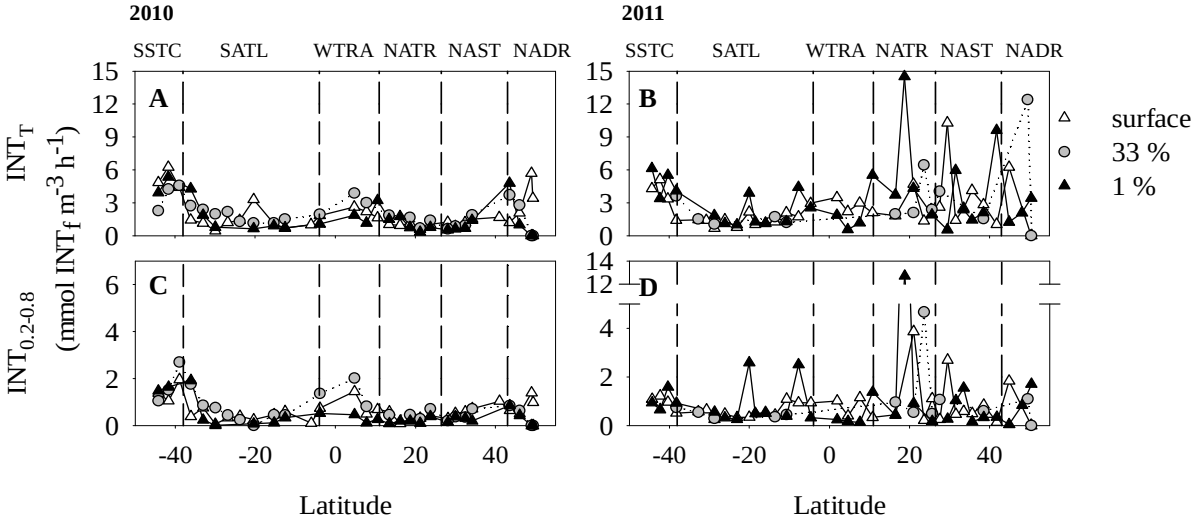
733 Figure 3.



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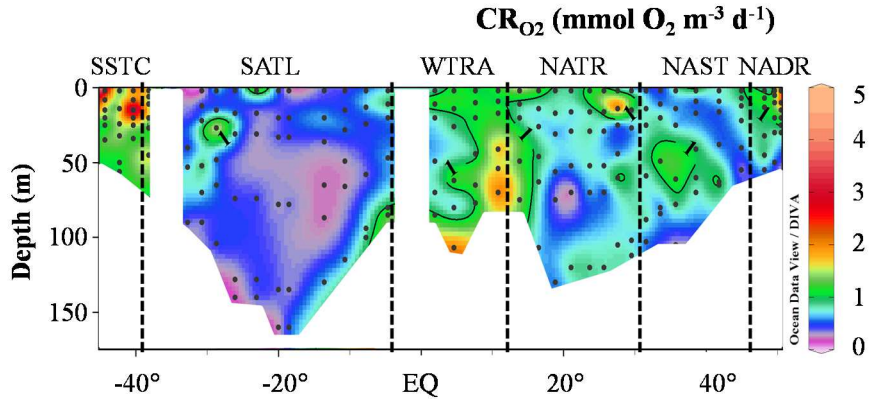
736 Figure 4.



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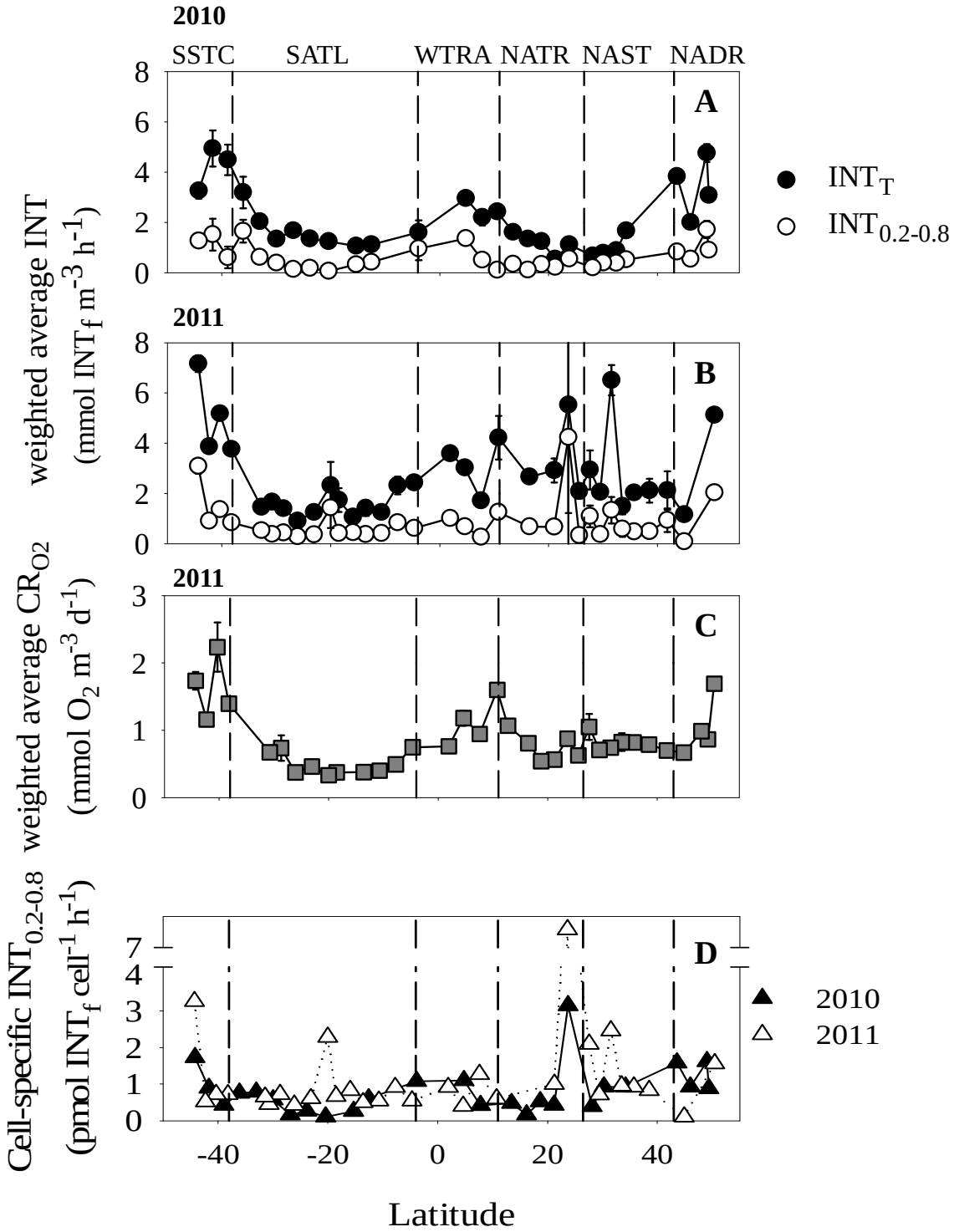
739 Figure 5.



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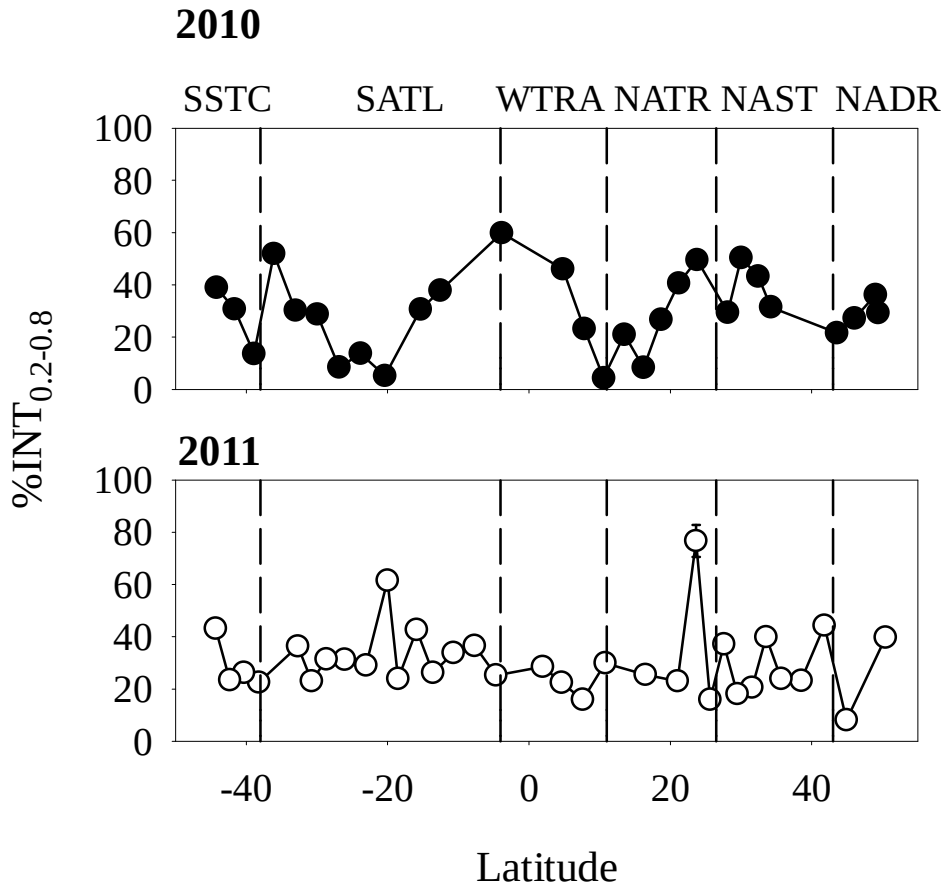
741

742 Figure 6.



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