Supplemental materials

Buoyant weight calculations

$$Wet\ weight\ of\ \ slide = dry\ weight\ \ slide - dry\ weight\ \ slide \times \frac{water\ \ density}{acrylic\ \ density}$$

$$Wet\ weight\ of\ \ cyanoacrylate = wet\ weight\ \ slide\ with\ coral - wet\ weight\ \ slide - coral\ \ wet\ weight$$

$$Dry\ weight\ cyanoacrylate = \frac{wet\ weight\ cyanoacrylate}{1 - \frac{water\ \ density}{cyanoacrylate\ \ density}}$$

$$Total\ \ plastic = dry\ weight\ \ slide + dry\ weight\ \ cyanoacrylate$$

$$Average\ \ plastic\ \ density = \frac{total\ \ plastic}{\frac{dry\ weight\ \ cyanoacrylate}{cyanoacrylate\ \ density} + \frac{dry\ \ slide\ \ weight}{acrylic\ \ density}}$$

$$Coral\ dry\ weight = \frac{wet\ weight\ \ slide\ with\ coral - (total\ \ plastic - \frac{total\ \ plastic \times water\ \ density}{average\ \ plastic\ \ density})}{1 - \frac{water\ \ density}{aragonite\ \ density}}$$

The following densities were used for calculations: acrylic density: 1.185 g cm^{-3}, cyanoacrylate density: 1.1 g cm^{-3}.

Time series

Figure S1 presents values for the various parameters measured (temperature, pCO_2, A_T, pH, S, nutrients, as well as some calculated values – saturation state and CO_2 concentration) over the course of the experiment versus time.

Total inorganic carbon measurement

C_T samples were taken in 20 ml glass (borosilicate) scintillation vials. Samples were poisoned immediately with 10-15 µl of a saturated $HgCl_2$ solution, capped, and stored refrigerated until sub-samples could be taken and sealed in glass ampoules (per McCorkle et al., 1985). C_T measurements were made using a manometer, per McCorkle et al. (1985).

Total alkalinity

As the protocols used here for A_T sampling and measurement depart from standard operating procedures (DOE 1994, Dickson et al., 2007), and in light of recent calls for protocols suitable for smaller samples (Hydes et al., 2010) we present here data concerning the accuracy, precision and limitations associated with the method used. Samples were taken in screw cap vials (20 ml) with foamed polyethylene liners, refrigerated, but not poisoned. Thus, there is a risk for evaporative loss of the sample to

affect the A_T, absorption of compounds (e.g. NH_3) during storage could similarly affect A_T, and biological activity post collection could change the A_T. In a previous experiment (Holcomb et al., 2010) evaporation affected A_T samples taken in Evergreen vials (Evergreen Scientific), leading to some samples being unusable. Several options were subsequently explored. Borosilicate and HDPE scintillation vials both proved suitable for storing A_T samples (once the vials were washed). Evaporation affected samples stored in both glass and plastic vials, however, with a few exceptions, evaporation rates were low enough that significant changes in A_T would not be observed over the time scale samples were stored. For samples in glass vials stored at room temperature all samples showed either no change in mass (storage periods of a few days) or a loss of mass during storage (periods from a few days to 520 d – reference weights were weighed regularly to verify that drift in the balance was not responsible for the change in weight). Average evaporation rates were 0.6±1 mg d^{-1}, n=763, occasional samples had much higher evaporation rates - <1% had evaporation rates greater than 5 mg d^{-1}, the median rate was 0.3 mg d^{-1}. For a ~20 g sample stored for 100 days in a glass vial, this corresponds to a loss of 0.3% on average, which if the sample initially had an A_T of 2000 μmol kg^{-1} would result in an A_T of 2006 μmol kg^{-1}, thus for samples stored less than one month, storage is unlikely to lead to a significant change in A_T due to evaporation. For plastic vials, average evaporation rates were 0.2±0.1 mg d^{-1}, n=362, the median rate was 0.2 mg d^{-1}, storage periods were up to 637 d.

Preliminary experiments suggested that evaporative loss could be further reduced by wrapping the lid with vinyl tape, however this step was deemed unnecessary for short term storage.

Biological activity occurring within the samples post collection could also affect the measured A_T value. To assess the potential for A_T values to change during storage, three comparisons were carried out:

1) Comparison of A_T values between A_T samples (not poisoned) and residual C_T samples (poisoned, see C_T, above).
2) Measurement of samples stored for varying lengths of time – in some instances, replicate samples were taken and one of the two stored for a longer period, in others, a single sample was measured, then stored and measured again.
3) Comparison of calculated (from A_T and pH_T) C_T versus measured C_T.

Comparison of poisoned versus non-poisoned samples (Fig. S2) shows poisoned samples tend to have lower A_T values than their non-poisoned counterparts, being on average 8 μmol kg^{-1} lower (standard deviation: 7 μmol kg^{-1}). The lower A_T values for poisoned samples can be explained in-part by sample dilution by the $HgCl_2$ solution used to poison samples (expected change < 2 μmol kg^{-1}). Whether the remaining difference reflects biological activity, differences in sample handling (since C_T vials were opened for C_T samples prior to A_T measurements), or day to day variations in A_T measurements is unknown, but, these data suggest biological activity in non-poisoned samples has a negligible effect on the A_T samples measured for this study.

Samples stored for varying lengths of time (Fig. S3) show a similar range of variation regardless of the length of storage (up to 172 d), though there may be a slight increase in A_T with time, consistent with evaporation.
Measured C_T values were in good agreement with calculated C_T values (Fig. S4), being on average 3±33 μmol kg^{-1} lower than calculated values. Thus the different carbonate system measurements are in good agreement, suggesting biological activity has not adversely affected A_T measurements.

Standards
With small volume measurements, a single bottle of certified reference material (CRM) provides far more material than needed to carry out calibrations for a given run, thus each bottle of CRM was subdivided into smaller aliquots and stored refrigerated in scintillation vials. In addition to CRM, an internal standard (poisoned Vineyard Sound seawater) was run regularly to assess system stability. Several measurements on multiple standards were made every day A_T measurements were made, and every few months a new bottle of CRM was opened and used to assess drift in the aliquots. Figure S5 shows the average values for every aliquot of CRM measured versus time – values are generally within 10 μmol/kg of the expected value.

Calculations
The A_T calculations used here (a normalized Gran function implemented by the Tiamo software which controls the titrator) depart from the recommended regression fit (DOE 1994; Dickson et al., 2007). To assess the magnitude of error introduced by using the Tiamo calculation routine, a subset of the titrations were manually copied out of the Tiamo database and recalculated following recommended protocols using a Matlab routine adapted from McDonnell and Dickson (unpublished). Alkalinity values were similar regardless of the calculation routine used (Fig S6), with an average difference of 1 μmol kg^{-1} between the two calculations, and in all instances, differences were less than 15 μmol kg^{-1}. Although the recommended calculations may be superior, the normalized Gran function gives similar values, adequate for monitoring treatment conditions.

Salinity
Salinity measurements made with the Hach conductivity probe (HQ40d meter with standard conductivity probe) were generally within 1 of values measured using a Guildline autosal salinometer (measurements performed by the WHOI CTD facility), however there is more variability in values measured using the Hach conductivity probe (Fig. S7).

Normalization
To compare physiological data, such as growth rates, data are commonly normalized in an effort to remove inherent differences between individuals (e.g. differences in initial size). Some of the more commonly used coral normalization parameters include: protein, ash free dry weight, polyp number, surface area, zooxanthellae counts, and initial mass (e.g. Kanwisher and Wainwright 1967; Edmunds and Gates 2002; Grottoli and Rodrigues

2004; Naumann et al., 2009; Ries et al., 2010). For normalizing buoyant weight based growth rate estimates, initial mass is by far the easiest approach as it requires no additional measurements. For similarly sized specimens with the same skeletal density, initial mass may well be an appropriate normalization approach. However, comparison of different studies which use initial mass as a normalization method is difficult. The mass of a coral specimen represents the combined contribution of pre-existing dead skeleton, recent still growing skeleton and tissue, with the bulk of the mass in the form of dead skeleton (in most cases). Thus studies which use differently sized specimens are likely to have inherent differences in mass normalized calcification rates due simply to the difference in the mass of dead skeleton. Any variation in sample size/shape will alter the ratio between the area of growing surface and pre-existing skeleton. Table S1 shows some example calculations of how the surface area to volume ratio changes for different shapes and sizes. Further complicating the use of initial mass are variations in skeletal density – coral skeletons are commonly host to boring organisms which can lead to reduced skeletal density and even in the absence of boring, skeletal density may change with age, season, environmental conditions, etc. Despite the limitations of initial mass normalizations, they are commonly used, thus data for both the current study and from a previous study (Holcomb et. al., 2010) are presented in Figure S8.

Tissue based normalization methods (e.g. protein, ash free dry weight, etc) estimate the amount of tissue covering the skeleton, and thus potentially capable of creating new skeleton. However, tissue based estimates tend to require that the specimen be sacrificed to make measurements of the normalizing parameter, which may be undesirable. Further, tissue based normalizations are generally based on the total tissue, yet the skeleton is formed by only a single cell layer – the calicoblastic epithelium. Tissue biomass has been found to vary substantially on seasonal cycles (Fitt et al., 2000), but biomass is not necessarily linked to changes in calcification (Rodrigues and Grottoli 2006, 2007), suggesting that tissue biomass does not necessarily represent the biomass or activity of the calicoblastic epithelium. Thus, for studies of the effects of a particular treatment on calcification, variation in tissue biomass could change the interpretation of the results.

The number of polyps may be a readily quantified parameter, and for specimens with similar polyp sizes and spacing, this may be a useful normalization parameter, however, *A. poculata* exhibits a range of polyp sizes and inconsistent spacing, thus limiting the utility of polyp counts.

Surface area represents the area over which new skeleton could potentially be deposited i.e. the surface covered by the calicoblastic epithelium, and thus should represent a useful normalization parameter, however, skeletal growth is not uniform, and there may be inherent differences in calcification rates among different parts of a colony (Elahi and Edmunds 2007).

Our current normalization methods all suffer from certain limitations, and which may be best for a given application is not necessarily obvious. Data from Holcomb et al. (2010) were used to assess potential normalization methods (Table S2). Prior growth rates appeared to be the best predictors of future growth rates, and thus normalization to

pre-treatment growth rates was chosen for the current study. The use of a pre-treatment phase allows any inherent differences in calcification rates between specimens to be measured (whether due to specimen size, age, etc.), and thus accounted for in the normalization of treatment data. There is an assumption that such differences are stable, to this end, corals were allowed to acclimate to aquarium conditions for almost two months prior to collecting several months of base-line growth rates for normalizing subsequent treatment rates.

Supplemental references

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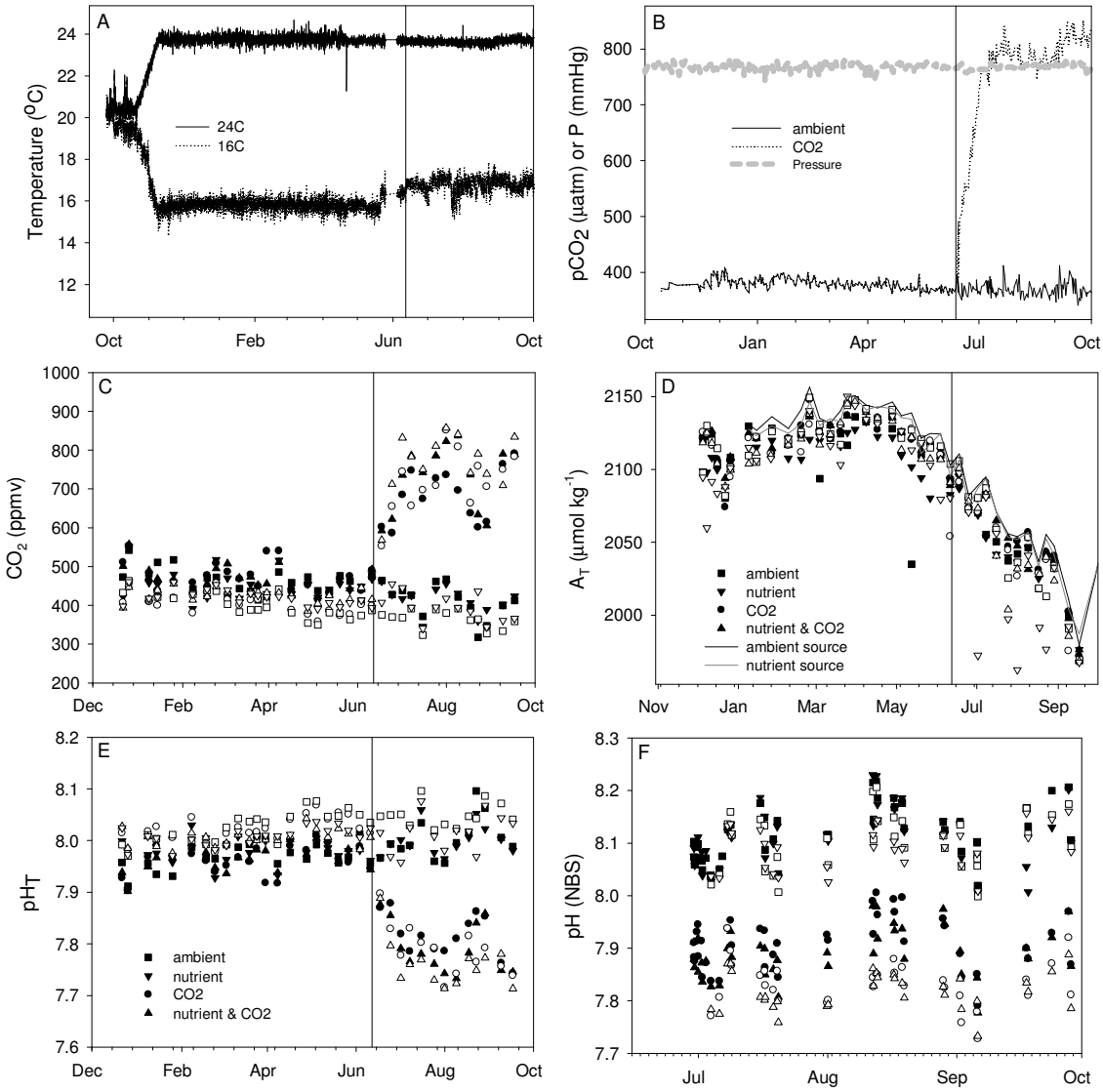
Rodrigues, L. J., and Grottoli, A. G.: Energy reserves and metabolism as indicators of coral recovery from bleaching, Limnol Oceanogr, 52, 1874-1882, DOI: 10.4319/lo.2007.52.5.1874, 2007.

Table S1. Surface area (SA) and volume (V) relationships for different geometric forms. Note that for a doubling of the volume (or ~equivalently, the dead skeletal mass) there is not a proportional increase in surface area, also note the change in the surface area/volume ratio as the form changes.

Form	Volume	Radius (r)	Height	SA	SA/V
Sphere	5	1.06	na	14.14	2.83
	10	1.34	na	22.45	2.24
Cylinder	5	0.93	2r	16.19	3.24
	10	1.17	2r	25.69	2.57
	5	0.74	4r	17.00	3.40
	10	0.93	4r	26.98	2.70
Cone	5	1.34	2r	18.16	3.63
	10	1.68	2r	28.83	2.88
	5	1.06	4r	18.11	3.62
	10	1.34	4r	28.75	2.87

Table S2. Correlations between growth rate and different normalization parameters. Significance of correlations and Pearson correlation coefficients for correlations of each normalization parameter with growth rate are listed for data from Holcomb et al. (2010) and the present study. Data are separated by temperature and symbiont status. Note that in Holcomb et al. (2010), a specific pre-treatment growth period was not performed as in the present study, initial growth rate values used include both pre-treatment rates and the transition to treatment conditions. Data are presented as the significance of correlation (if $p<0.05$, otherwise it is listed as non-significant, n.s.) with the correlation coefficient in parenthesis. Surface area was not determined in the present study (n.d.).

Growth rates from:	Initial mass	Surface area	Initial growth rate
Holcomb et al. 2010	n.s. (-0.213)	n.s. (0.184)	0.008 (0.529)
24 ^{o}C zooxanthellate	n.s. (0.33)	n.d.	0.000 (0.874)
24 ^{o}C azooxanthellate	0.001 (0.683)	n.d.	0.000 (0.952)
16 ^{o}C zooxanthellate	0.000 (0.85)	n.d.	0.000 (0.781)
16 ^{o}C azooxanthellate	0.001 (0.742)	n.d.	0.000 (0.875)


A
Temperature (°C)
24C
16C
Oct
Feb
Jun
Oct
B
pCO2 (µatm) or P (mmHg)
ambient
CO2
Pressure
Oct
Jan
Apr
Jul
Oct
C
CO2 (ppmv)
Dec
Feb
Apr
Jun
Aug
Oct
D
AT (µmol kg-1)
ambient
nutrient
CO2
nutrient & CO2
ambient source
nutrient source
Nov
Jan
Mar
May
Jul
Sep
E
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Jun
Aug
Oct
F
pH (NBS)
Jul
Aug
Sep
Oct

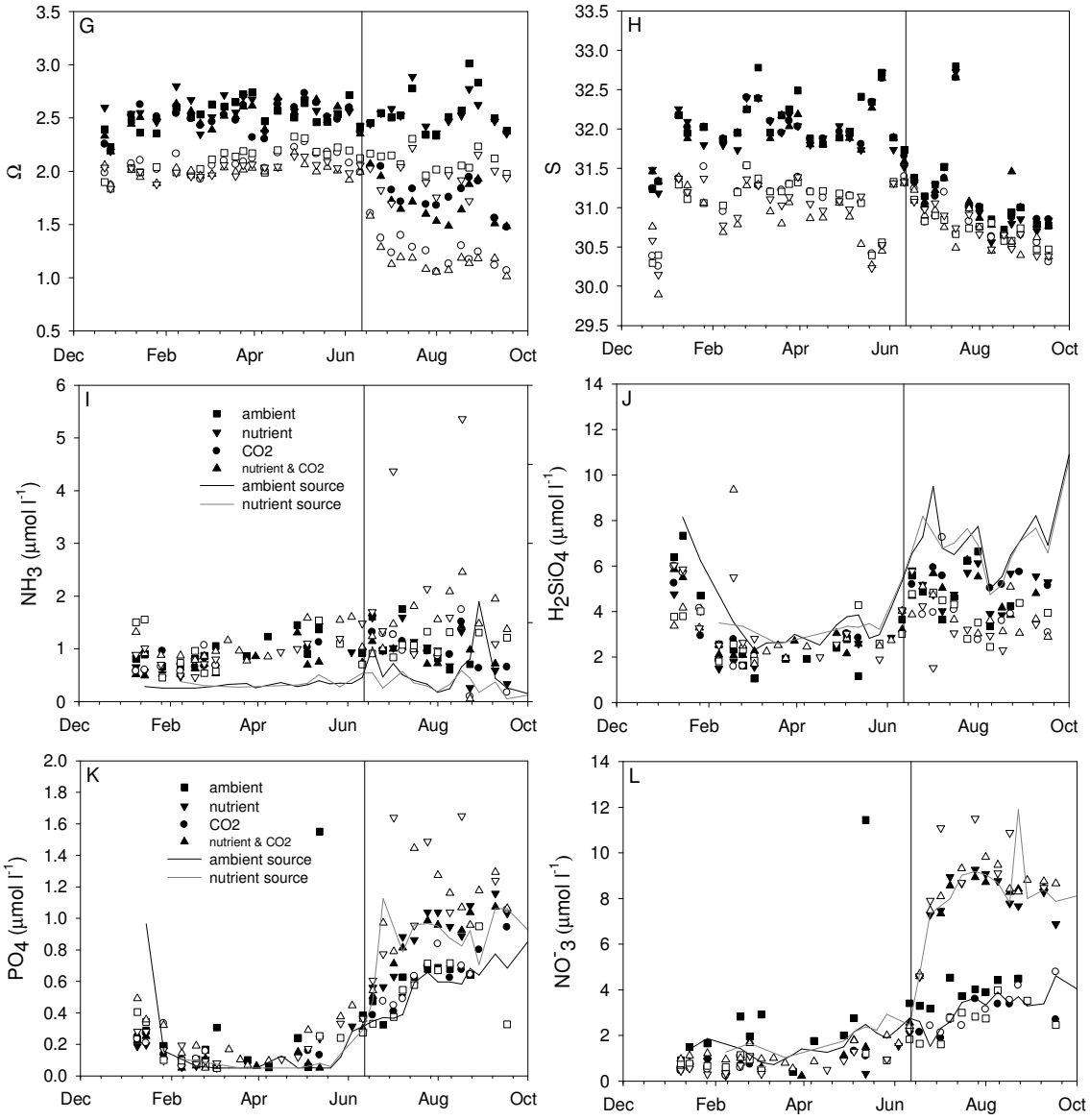


Figure S1. Aquarium conditions over time. A. A temperature logger recorded temperature every 12 min throughout the experiment starting before corals were added in September 2008. Plotted values are hourly averages for each water bath. Data were averaged to reduce artificial variability caused by electrical interference generated by fluorescent lighting. Artificial high frequency variability is still present in the plots. The rise in temperature during the last few months of the experiment for the 16 ^{o}C water bath was not detected by other thermometers and may reflect deterioration of the temperature sensor. B. Total atmospheric pressure (mmHg) and gas CO_2 partial pressures (µatm) measured on a daily basis on the supply lines (~dry air) for both the ambient and elevated pCO_2 treatments. C. Calculated CO_2 concentrations (gas phase) based on measured A_T and pH_T for each aquarium (symbols per 1E). D. A_T concentrations measured within

each aquarium and in the reservoirs. E. pH_T values measured spectrophotometrically within each aquarium. F. pH values measured with an electrode (calibrated with NBS buffers) on water from each aquarium on light/dark cycles over the course of the treatment phase (symbols per 1E). G. Saturation states calculated from A_T and pH for each aquarium (symbols per 1E). H. Salinity values measured within each aquarium. I. Ammonia concentrations measured both within each aquarium and in the reservoirs supplying the aquaria. J. Silicate concentrations measured within each aquarium and in the reservoirs. K. Phosphate concentrations measured within each aquarium and in the reservoirs. L. Nitrate/nitrite concentrations measured within each aquarium and in the reservoirs. Open symbols are used for 16 ^{o}C treatments, closed for 24 ^{o}C treatments. Data points are individual measurements or averages of replicates if replicate measurements on a given sample were made. The vertical line indicates the start of the transition to treatment conditions.

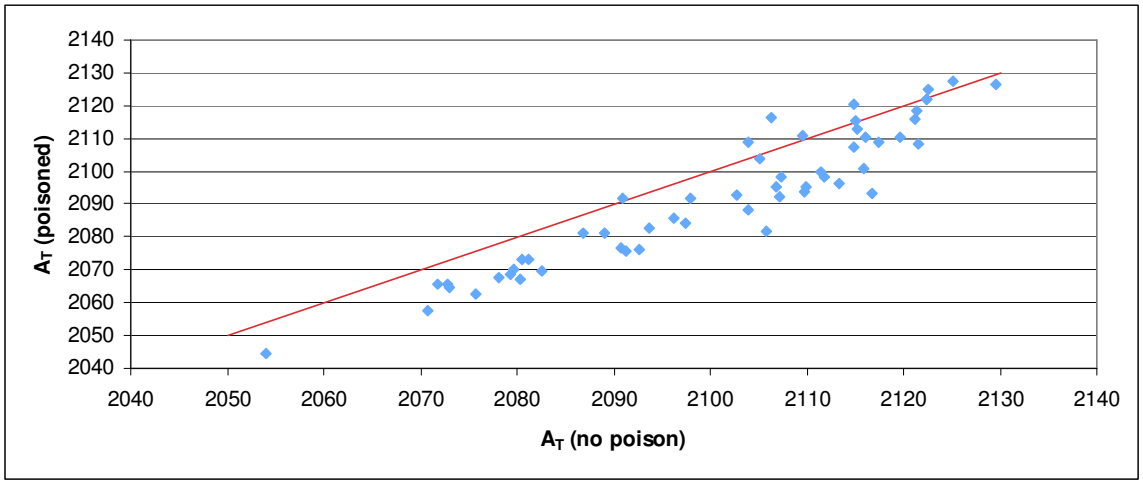


Figure S2. Comparison of poisoned and un-poisoned A_T samples. A_T (µmol kg^{-1}) was measured on replicate samples with one sample being poisoned with ~15 µl saturated $HgCl_2$, and the other not poisoned. Individual symbols (blue diamonds) are average A_T values measured for each sample, standard deviation of replicate measurements is in all cases less than 13 µmol kg^{-1}, and is generally better than 4 µmol kg^{-1}. The 1:1 line is plotted for reference (red line).

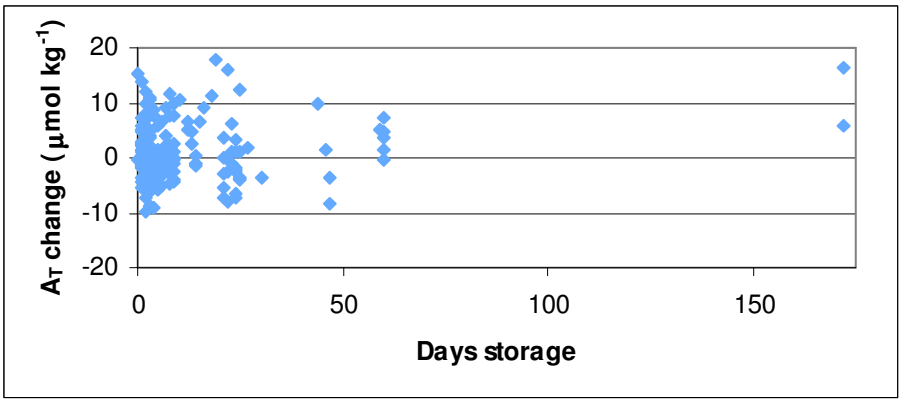


Figure S3. Effect of storage. Average A_T differences between non-poisoned samples measured on different days plotted versus the number of days between measurements. A_T differences show no significant correlation with storage.

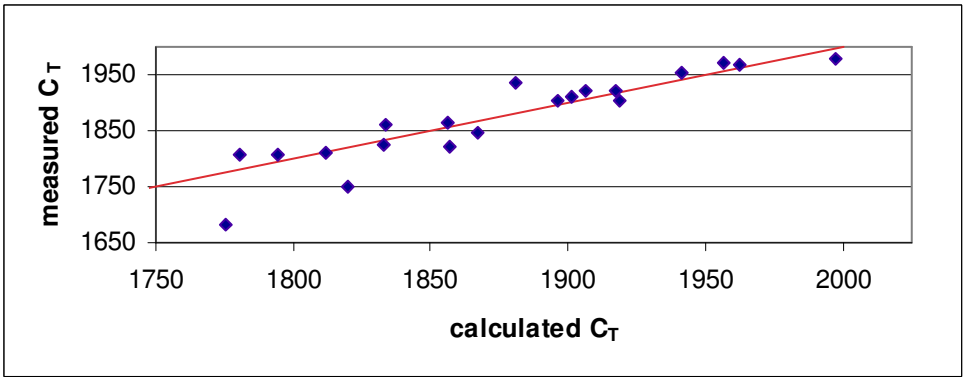
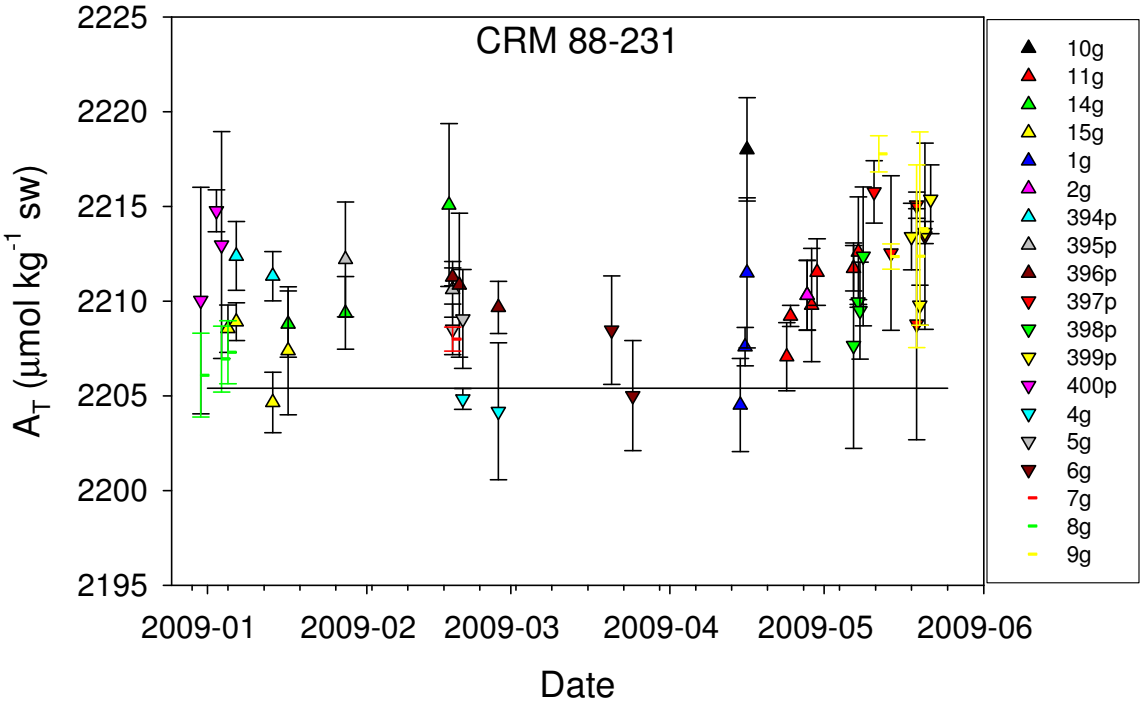
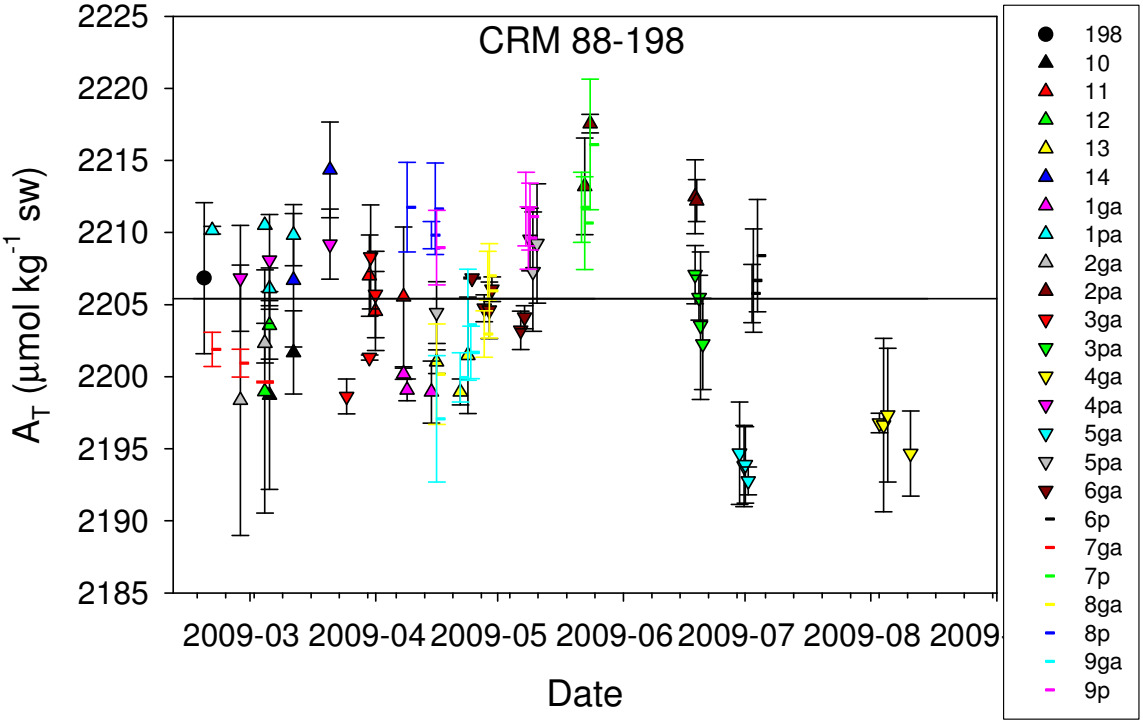


Figure S4. Agreement of measured and calculated C_T. Calculated (based on pH_T, A_T, T, S) C_T (µmol kg^{-1}) is plotted versus measured C_T. Symbols represent individual samples, the 1:1 line is shown for reference. Average difference is 3±33 µmol kg^{-1}.


CRM 88-231
A_T (µmol kg^{-1} sw)
2225
2220
2215
2210
2205
2200
2195
2009-01
2009-02
2009-03
2009-04
2009-05
2009-06
Date
10g
11g
14g
15g
1g
2g
394p
395p
396p
397p
398p
399p
400p
4g
5g
6g
7g
8g
9g


CRM 88-198
A_T (µmol kg^{-1} sw)
2225
2220
2215
2210
2205
2200
2195
2190
2185
2009-03
2009-04
2009-05
2009-06
2009-07
2009-08
2009
Date
198
10
11
12
13
14
1ga
1pa
2ga
2pa
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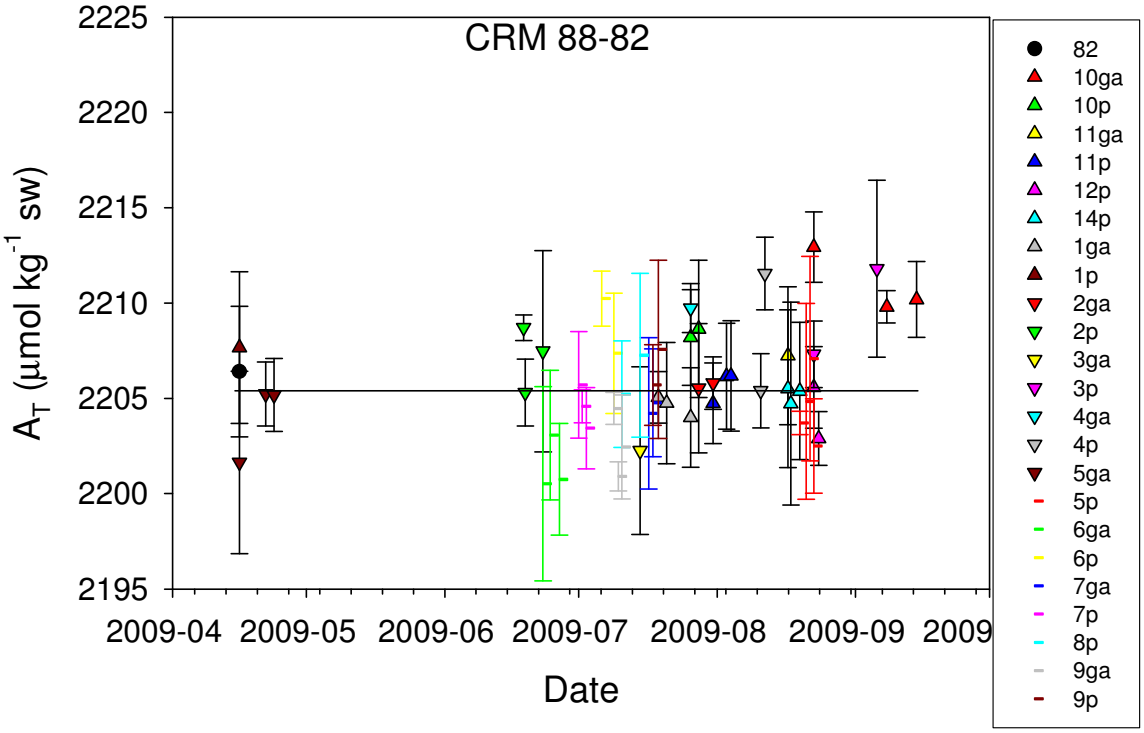
6p
7ga
7p
8ga
8p
9ga
9p


CRM 88-82
A_T (µmol kg^{-1} sw)
2225
2220
2215
2210
2205
2200
2195
2009-04
2009-05
2009-06
2009-07
2009-08
2009-09
2009
Date
82
10ga
10p
11ga
11p
12p
14p
1ga
1p
2ga
2p
3ga
3p
4ga
4p
5ga
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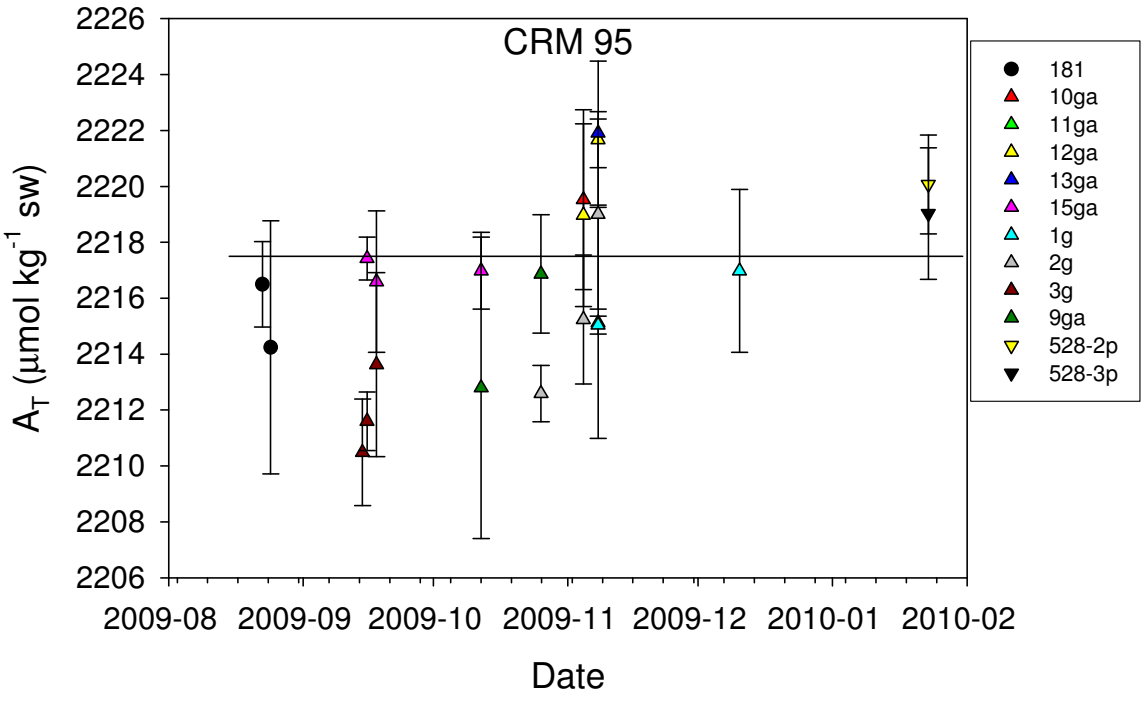
6ga
6p
7ga
7p
8p
9ga
9p


CRM 95
A_T (µmol kg^{-1} sw)
2226
2224
2222
2220
2218
2216
2214
2212
2210
2208
2206
2009-08
2009-09
2009-10
2009-11
2009-12
2010-01
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1g
2g
3g
9ga
528-2p
528-3p

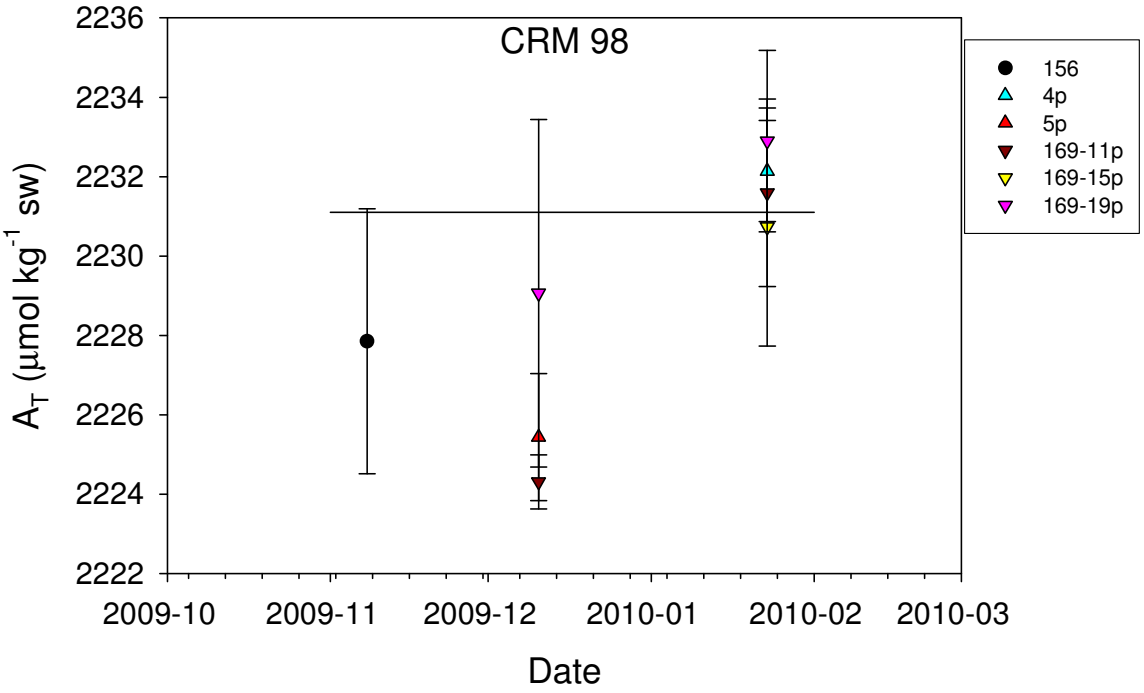

Figure S5. Stability of CRM aliquots. Average A_T values for each aliquot of CRM for each A_T run versus time. Each aliquot was measured on more than one day, thus there are several mean and standard deviation values for each aliquot – one for every A_T run in which the aliquot was measured. Different aliquots are indicated by different symbols, an aliquot for which a 'g' appears in the name (see figure legends) indicates the sample was stored in a glass vial, 'p' indicates plastic, an 'a' indicates the vial was acid washed (otherwise it was washed with distilled water only). A black circle is used for samples taken directly from a freshly opened CRM bottle. The graph title indicates the CRM batch number, and if all measurements were on aliquots from the same bottle, the bottle number as well. Symbols are averages, error bars are standard deviation. The horizontal black line in each plot represents the accepted A_T value. Note that CRM data are presented for a longer period than that covered by A_T measurements for the current study to allow data for additional CRM batches to be included in the plots.

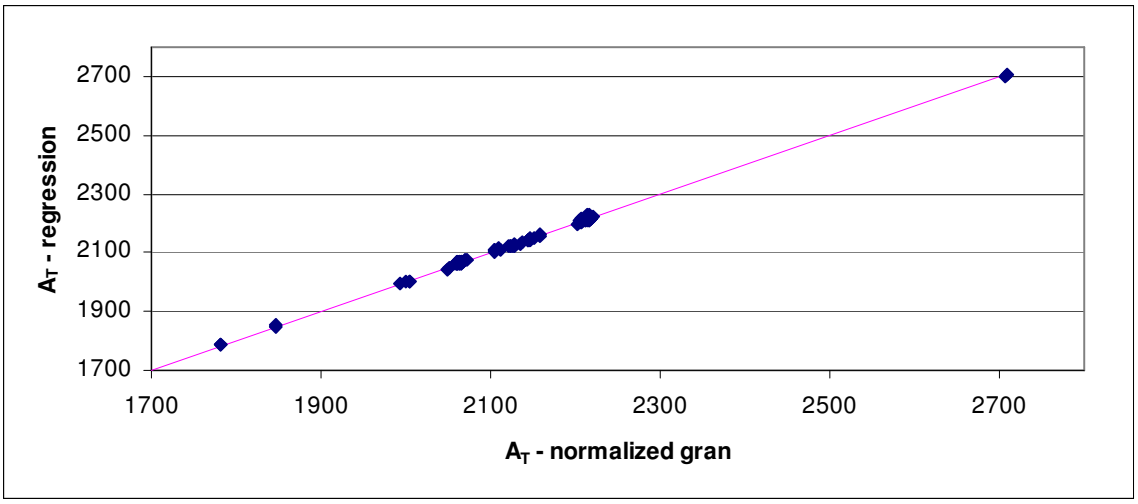

Figure S6. Comparison of A_T calculation routines – the recommended approach (regression) plotted versus the calculation carried out in the titration software (normalized gran). Points are values for individual titrations, the one to one line is shown for reference.

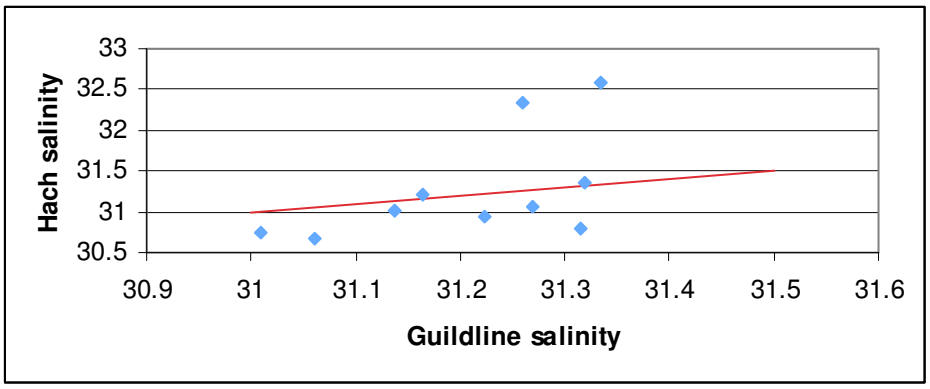

Figure S7. Agreement of different salinity measurements. Salinity values were obtained with a dip type conductivity probe (Hach) as well as measured by the WHOI CTD facility using a Guildline autosal. Points are values for an individual sample, the one to one line is shown for reference.

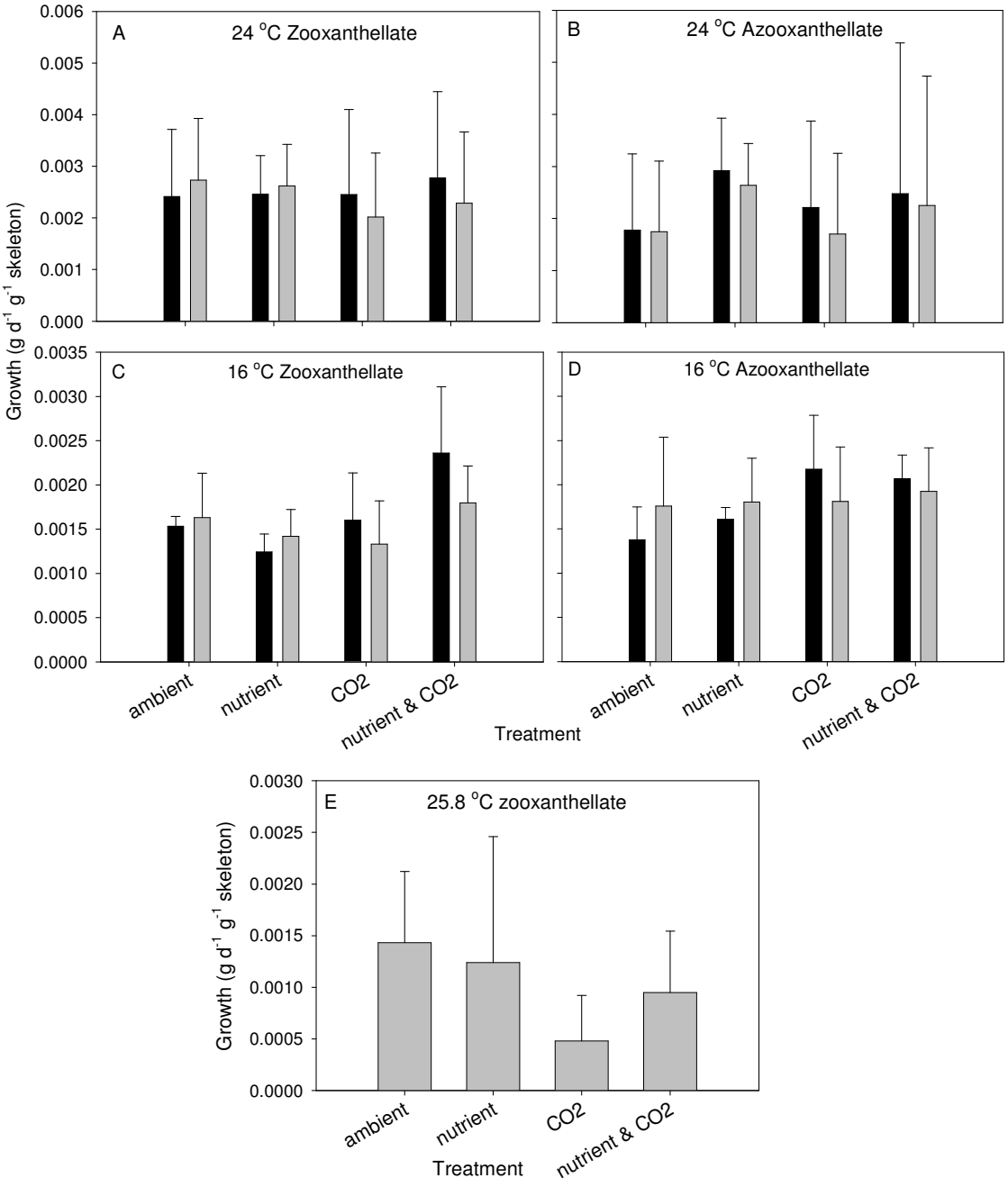

Figure S8. Mass normalized growth rates (g $CaCO_3$ d^{-1} g^{-1} dry skeleton). Both pre-treatment (black bars), and treatment (grey bars) rates are shown for data from the present study (A-D), treatment growth rates are shown for data replotted from Holcomb et al. (2010) (E). All normalizations are to initial skeletal dry mass (calculated) at the start of the given interval (pre-treatment or treatment). Bars represent means, error bars are 1 standard deviation.

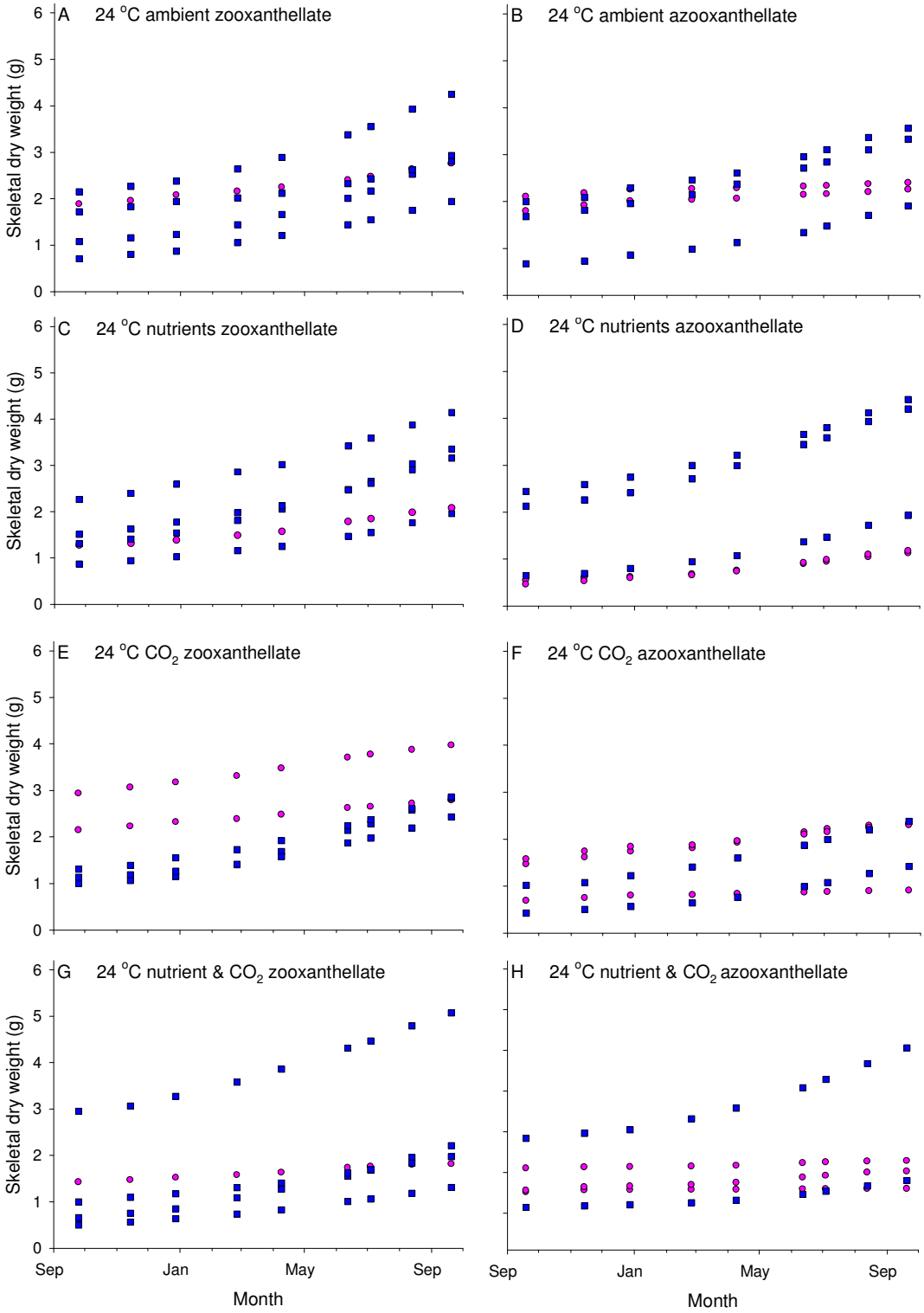
A 24 °C ambient zooxanthellate
B 24 °C ambient azooxanthellate
C 24 °C nutrients zooxanthellate
D 24 °C nutrients azooxanthellate
E 24 °C CO2 zooxanthellate
F 24 °C CO2 azooxanthellate
G 24 °C nutrient & CO2 zooxanthellate
H 24 °C nutrient & CO2 azooxanthellate
Skeletal dry weight (g)
0
1
2
3
4
5
6
Sep
Jan
May
Sep
Month

Figure S9. Calculated dry weight versus time for each coral fragment for each 24 ^{o}C treatment. Fragments from male colonies are indicated by blue squares, females by pink circles.

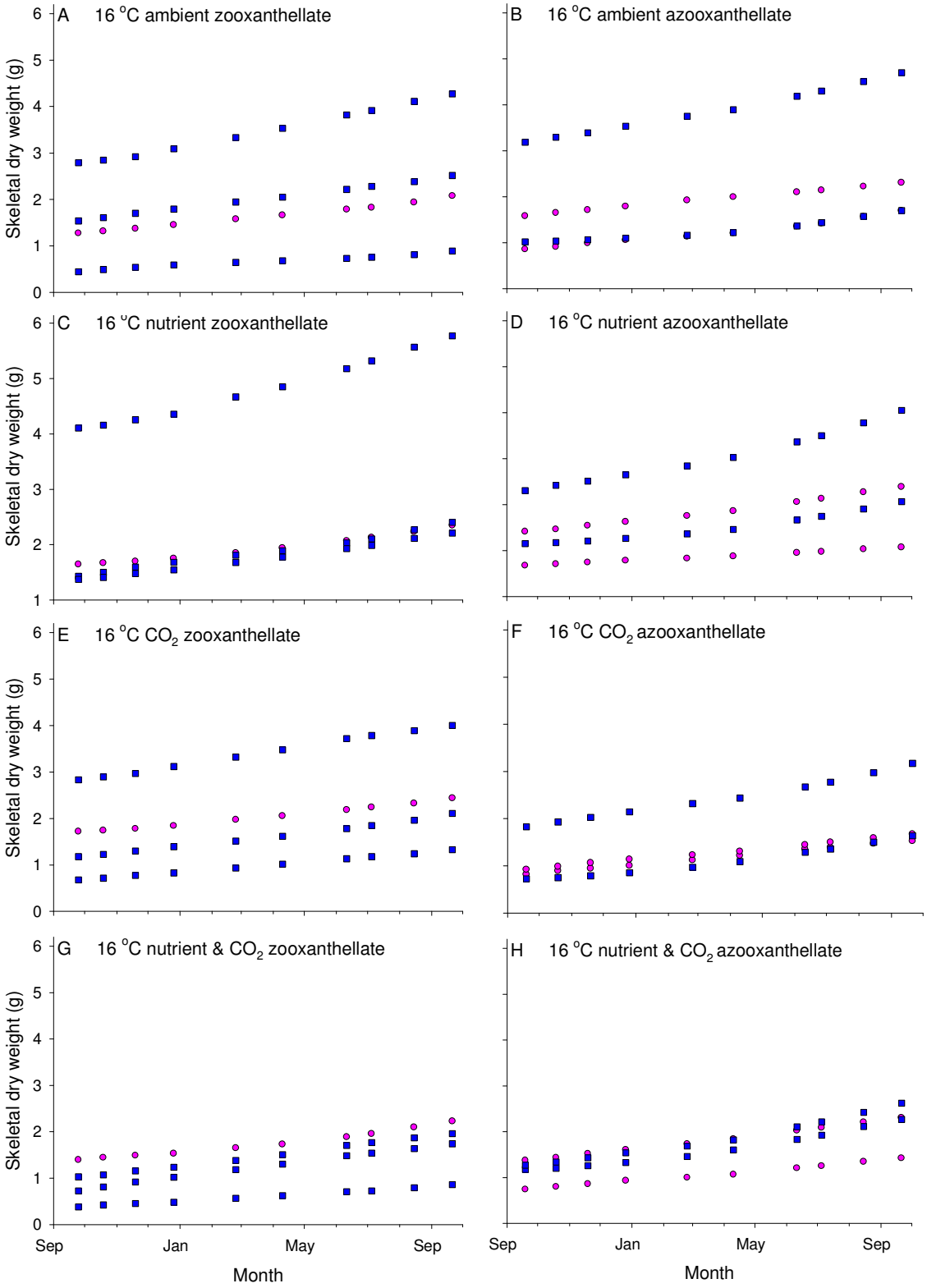
A 16 °C ambient zooxanthellate
B 16 °C ambient azooxanthellate
C 16 °C nutrient zooxanthellate
D 16 °C nutrient azooxanthellate
E 16 °C CO2 zooxanthellate
F 16 °C CO2 azooxanthellate
G 16 °C nutrient & CO2 zooxanthellate
H 16 °C nutrient & CO2 azooxanthellate
Skeletal dry weight (g)
0
1
2
3
4
5
6
Sep
Jan
May
Sep
Month

Figure S10. Calculated dry weight versus time for each coral fragment for each 16 ^{o}C treatment. Fragments from male colonies are indicated by blue squares, females by pink circles.

Supplemental appendix 1

Table SA1. Accompanying .csv file with water chemistry measured in each tank as well as in the source seawater for ambient and nutrient enriched treatments. Dates are dates of sample collection, units are μmol l^{-1} for nutrients, μmol kg^{-1} for A_T and C_T. A_T, pH_T, S and nutrient values were measured, the remaining values were calculated using CO2SYS. Empty cells indicate no data for the given parameters are available for that particular date.

Table SA2. Dry weights for each fragment (calculated from buoyant weight), date fragment was weighed, treatment, parent colony, gender of colony, and zooxanthellate status.