Phytoplankton and heterotrophic respiration in the surface layer of the ocean

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Abstract We suggest a way to estimate phytoplankton respiration separately from heterotrophic respiration. The analysis is based on three previous observations: (1) that carbon loss in the plankton overnight is phytoplankton respiration; (2) that phytoplankton respiration occurs during the day at the same rate as at night; and (3) that uptake of ^{14}C estimates net primary production. Based on these three findings, it is proposed that phytoplankton respiration can be estimated as twice the overnight loss of organic carbon. Heterotrophic respiration can then be calculated as the difference between phytoplankton and total community respiration.

1. Introduction

A long-standing problem in biological oceanography is the measurement of the components of respiration (phytoplankton and heterotrophs) in plankton communities. Solution to the problem may even be considered something of a "Holy Grail" in biological oceanography. Respiration is critical to seasonal cycles [*Sverdrup, 1953*], net production rates, and growth dynamics. Respiration is the major sink for carbon in the ocean, yet we have only rudimentary understanding of its magnitude [*del Giorgio and Duarte, 2002*]. Net community production and net community respiration can be measured through changes in O_2 in samples incubated in daylight and in the dark, respectively [*e.g., Robinson et al., 2002*], and photosynthetic production can also be estimated through the use of ^{14}C or ^{18}O. But none of these methods, at first site, allows the estimation of respiration by the phytoplankton themselves, or respiration by heterotrophs: the bacteria, protozoa, and microzooplankton. Here we propose the hypothesis that phytoplankton and heterotrophic respiration can both be estimated in ocean samples as reliably as we can estimate primary production. The calculation is relatively simple, and we present tests of the method on data from two process studies from the Joint Global Ocean Flux Study, the North Atlantic Bloom Experiment (NABE) in 1989, and the Arabian Sea Expedition in 1995. Our hypothesis rests on three findings, as follows.

1. Over the last 15 years or so, we have conducted measurements of primary production in the ocean using the ^{14}C uptake method. The measurements were done on samples that were incubated *in situ*, suspended from a free-drifting buoy, from dawn until dusk. When the buoy was recovered at dusk, some replicates from each depth were kept in deck incubators overnight. Thus, each experiment had values for dawn-to-dusk and for 24 h incubations for carbon assimilation. When the nighttime loss is plotted against the daytime uptake, there is a positive relationship (Fig. 1). There are some outliers to the regression (from the tropics), but nighttime losses average about 20-25% of the previous 12 h uptake. There appears to be

little temperature dependence to the overnight loss of carbon; for these data temperatures range from about 10°C to 28°C. In none of the individual data sets is the regression intercept significantly different from zero, although there are some data for which there is no loss overnight. But *over most of the euphotic zone*, newly fixed carbon, represented by ^{14}C does not seem to be preferentially retained in the cells (or grazers), otherwise there would be a positive x-axis intercept. The simplest explanation is that ^{14}C and ^{12}C are equally likely to be respired, by the beginning of the dark period, and that the C loss is predominantly by phytoplankton.

2. The daytime uptake of ^{14}C over time is nearly always linear, evident both in natural populations (or before the setting sun reduces uptake) [*e.g., Marra et al., 1988*] and in laboratory culture [e.g., *Smith and Platt, 1984*]. To conform to a linear time course of carbon assimilation, models of isotope incorporation need to be constructed such that uptake depends only on the initial carbon in the cells, and also require some means of retaining newly-fixed carbon inside the cells. Retaining carbon can be done by having all the CO_2 respired during the day re-fixed in photosynthesis [*Dring and Jewson, 1982*], or by sequestering carbon in synthetic pathways [*Smith and Platt, 1984*]. Both models can explain the data, however, there is extensive evidence that re-fixation of respired CO_2 occurs in algal cells during photosynthesis [*Ryther, 1956, Raven, 1972*].

3. An analysis of the JGOFS data on primary productivity suggests that ^{14}C assimilation is, overall, closest to net O_2 production, albeit with some variability [*Marra, 2002*] (Fig. 2).

At first site, findings 2 and 3 above would seem to be at odds. Re-fixation means that all carbon is retained in the cells, and it can be concluded, therefore, that the ^{14}C technique measures gross production. But O_2 and CO_2 follow different metabolic pathways, and the data from the JGOFS cruises imply that the ^{14}C method measures net primary production *relative to oxygen fluxes*. The re-fixation of respired CO_2 means that, relative to O_2, photosynthesis is using proportionally more ambient H_2O (the source of evolved O_2) relative to CO_2. Stated another way, carbon uptake will always be less than the oxygen fluxes because there is a source for carbon within the cell from mitochondrial respiration. In this case, the ^{14}C method will be closest to net primary production relative to the evolution of oxygen by photosynthesis [*Marra, 2002*].

The foregoing three findings suggest a way to measure phytoplankton respiration in natural populations. Combined with measurements of community respiration, respiration by bacteria, protozoa, and microzooplankton contained in the same samples can also be estimated. The calculation is straightforward. The loss overnight (Fig. 1) implies that it is predominantly phytoplankton respiration. We then assume that the phytoplankton respire during the day at the same rate as at night. The evidence from laboratory culture [*Langdon, 1993*] on the day-night equivalence is ambiguous, however, natural populations show equal day and night rates of respiration, based on ^{18}O and O_2 fluxes [*Grande et al., 1989*]. Finally, from the linear time course of uptake, we assume that virtually all CO_2 respired during the day is re-fixed in photosynthesis. Our hypothesis, then, is twice the dark loss of

carbon equals the 24-h rate of phytoplankton respiration. We refer to this estimate of phytoplankton respiration as '2xDL.'

2. Methods

The data required to evaluate the method for calculating phytoplankton respiration include ^{14}C uptake, the measurement of gross primary production (^{18}O method), and the O_2 light-dark bottle method. All of these are standard, and the detailed techniques have been published previously [*Knudson et al., 1989; Bender et al., 1992; Dickson et al., 2001; Barber et al., 2001*]. We note that, unlike the data in Fig. 1, the data for this comparison extend only to the depth of 5% of surface irradiance instead of 1%. Cruises for which all these methods were used in a manner allowing the calculations are three JGOFS process cruises: Leg 4 of the North Atlantic Bloom Experiment (April-May 1989], and cruises TN043 and TN050 of the Arabian Sea Expedition. The data we use are available from the U.S. JGOFS website, http://www1.whoi.edu/jgofs.html.

3. Results and Discussion

^{14}C-based carbon uptake values, corrected by adding to them two times the dark loss (2xDL) should be equivalent to gross primary production (^{18}O method). For the NABE data (Fig 3a), the correlation between C uptake corrected for phytoplankton respiration and gross photosynthesis (measured independently) is nearly perfect ($r^2 = 0.96$), with a slope of 0.96 and an intercept not significantly different from zero. These data clearly support the hypothesis.

The analysis for the Arabian Sea is more complicated. For the stations nearest the coast of Oman (17-18°N/58°E; Fig. 3b), the slope of the linear regression is less than but not significantly different from 1 (0.89±0.06). For the offshore, more oligotrophic station (10°N/64°54'E; Fig. 3c) the data nearest the surface (highest values) show proportionately lower values for the corrected carbon assimilation compared to gross production, and which lowers the overall slope. Although we recognize these anomalies, we conclude that 2xDL is a reasonable estimate of phytoplankton respiration.

In the same data sets, community respiration can be calculated from net oxygen changes over 24 h (gross $^{18}O_2$ production minus net O_2 production) [*Bender et al., 1992; Dickson et al., 2001*]. Heterotrophic respiration (from bacteria, protozoa, and microzooplankton) is then the difference between total community and phytoplankton (autotrophic) respiration. Respiration by heterotrophs and autotrophs is plotted as a function of depth in Fig. 4. For the North Atlantic, heterotrophic and autotrophic components contribute equally to total respiration. For the Arabian Sea, however, the contribution of heterotrophic respiration is about twice that of the phytoplankton.

Heterotrophic respiration is more variable. There is evidence of anomalous water movements between 5/03 and 5/06, and by 5/08, a different water mass was probably

sampled [*McGillicuddy et al., 1995*]. Autotrophic respiration is light dependent, consistent with the idea that it is composed of respiratory energy used for maintenance and that associated with growth [*Laws and Caperon, 1976*]. As might be expected from Fig. 1, phytoplankton respiration is a positive function of daily C uptake (data not shown). As a function of depth, respiration by auto- and heterotrophs in the Arabian Sea behaves somewhat similarly, but there are anomalously low values of what we calculate as autotrophic respiration near the surface, and which may show up as underestimates in gross production in Fig. 3(b,c).

Deviations from the expected 1:1 relationship in Fig. 3 can be caused by processes such as the Mehler reaction, photorespiration, extra-cellular release of dissolved organic carbon (DOC), and grazing [see *Laws et al., 2000*]. The Mehler reaction would decrease the ability [by about 10%: *Laws et al., 2000*] of the ^{18}O technique to represent accurately gross photosynthesis. If important, it would decrease the values of gross photosynthesis in Figs 3b and c, and would improve the agreement with the corrected ^{14}C assimilation. Photorespiration seems to require a correction factor of about 10% but with an error of ±5%, under conditions of high light [*Falkowski and Raven, 1997*].

The extracellular release of dissolved organic carbon (DOC) is similarly difficult to evaluate. DOC may appear as particulate matter because of adsorption by filters [*Maske and Garcia-Marquez, 1994*]. Grazing by microheterotrophs, expected to be high in the Arabian Sea, would retain ^{14}C in particulate matter, effectively lowering respiration estimates [*Smith et al., 1984*]. On the other hand, it may be that bottle incubations are deleterious to microzooplankton [*Bender et al., 1997*]. The combined effects of grazing and extracellular release have been estimated to be about 25% [*Laws et al., 2000*]. Given the uncertainties surrounding these measurements, however, not only in magnitude, but in sign, we conclude that they are within the residuals of the correlation between gross photosynthesis measured by ^{18}O, and ^{14}C uptake corrected for phytoplankton respiration (Fig. 3).

Use of the ^{14}C method brings up issues regarding what it actually measures. The view taken here, and supported by a large number of observations of natural populations, is that the ^{14}C method measures net primary production [*Marra, 2002*]. One experiment, in 1982, with deck incubations of natural populations has suggested that ^{14}C uptake is closest to gross primary production [*Williams et al., 1983*]. This conclusion has been criticized in a model [*Smith et al., 1984*] showing how a slow-growing microheterotroph population could graze autotrophs and thereby retain ^{14}C. In this case, the ^{14}C technique would be measured as indistinguishable from gross production because of the grazing effect. Sampling in the same locale in 1985, however, showed the ^{14}C technique to agree with net production, (unpublished data available from JM) suggesting that the earlier results were an exception, perhaps because of the deck incubations [*Grande et al., 1989, Barber et al., 1997*].

A further problem with comparisons of oxygen and carbon fluxes is the photosynthetic quotient, or PQ. Following previous work we have used a PQ of 1.4 [*Laws, 1991, Bender et al., 1992; Dickson et al., 2001; Barber et al., 2001*]. PQ's can vary widely in laboratory culture, but depend upon the conditions and length of incubation and the quality of irradiance to which the cultures are exposed. Variations in the PQ represent a caveat, but the PQ is known well enough to allow us to make the comparison between carbon and oxygen fluxes.

Previous estimates of phytoplankton respiration in marine planktonic ecosystems have been inferred from abundance data and conversion efficiencies [*Langdon, 1993; Robinson and Williams, 1999*], have depended on data from laboratory culture [*Langdon, 1993*], or else they have not been verified with oxygen fluxes [*Eppley, Sharp, 1975, Laws et al., 1987*]. Wider application of the method presented here can address several problems in biological oceanography, such as the initiation of the spring phytoplankton bloom, compensation irradiances, and the role of heterotrophs in carbon cycling in planktonic communities [*del Georgio. et al., 1997; Williams, 1998*].

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Fig. 1. Comparison of C uptake for 12 h (dawn-to-dusk, x-axis) with the loss overnight (12h rate – 24h rate; y-axis). Units are mmols C m^{-3} $12h^{-1}$ or d^{-1}. Sources are data from programs in the North Atlantic (circles, crosses, stars) and Indonesia (squares, x's). Inset enlarges data near the origin.

Fig. 2. Comparison of 24-h net production (from O_2 fluxes) with carbon uptake via the ^{14}C technique using data from the JGOFS process cruises to the North Atlantic, Equatorial Pacific, and Arabian Sea. In all cases, nitrate was the dominant source of nitrogen. The line is for PQ =1.4 which is the appropriate conversion factor [*Laws, 1991*].

Fig. 3. ^{14}C uptake corrected for dark loss (day and night) compared to gross photosynthesis from the evolution of $^{18}O_2$. Units for both axes are μM O_2 d^{-1}, and where C uptake is adjusted for a PQ = 1.4 [*Laws, 1991*]. (a) North Atlantic spring bloom. The slope for the regression is 0.96±0.02 and the intercept is not significantly different from zero (r^2=0.96). Three points have been excluded from the original data as errors, where C uptake from ^{14}C assays was less than net production. (b) Arabian Sea at 17-18°N/58°E (near the coast of Oman). The slope of the linear regression (dotted line) is 0.89±0.06 and intercept is –0.31±0.51. Solid line has a slope of 1. (c) Central Arabian Sea (10°N/64°54'E). The regression slope is significantly less than 1, driven by the high values. For (b) and (c), diamond symbols are for the northeast monsoon, and squares are for the southwest monsoon.

Fig. 4. Autotrophic and heterotrophic respiration in the North Atlantic (a,b, respectively) and the Arabian Sea (c, d). The North Atlantic data are organized by day of experiment. Same-shaped symbols are from the same day. For the Arabian Sea (c,d), diamonds are from

the northeast monsoon and circles from the southwest monsoon. Respiration data are in units of μM O_2 d^{-1}.

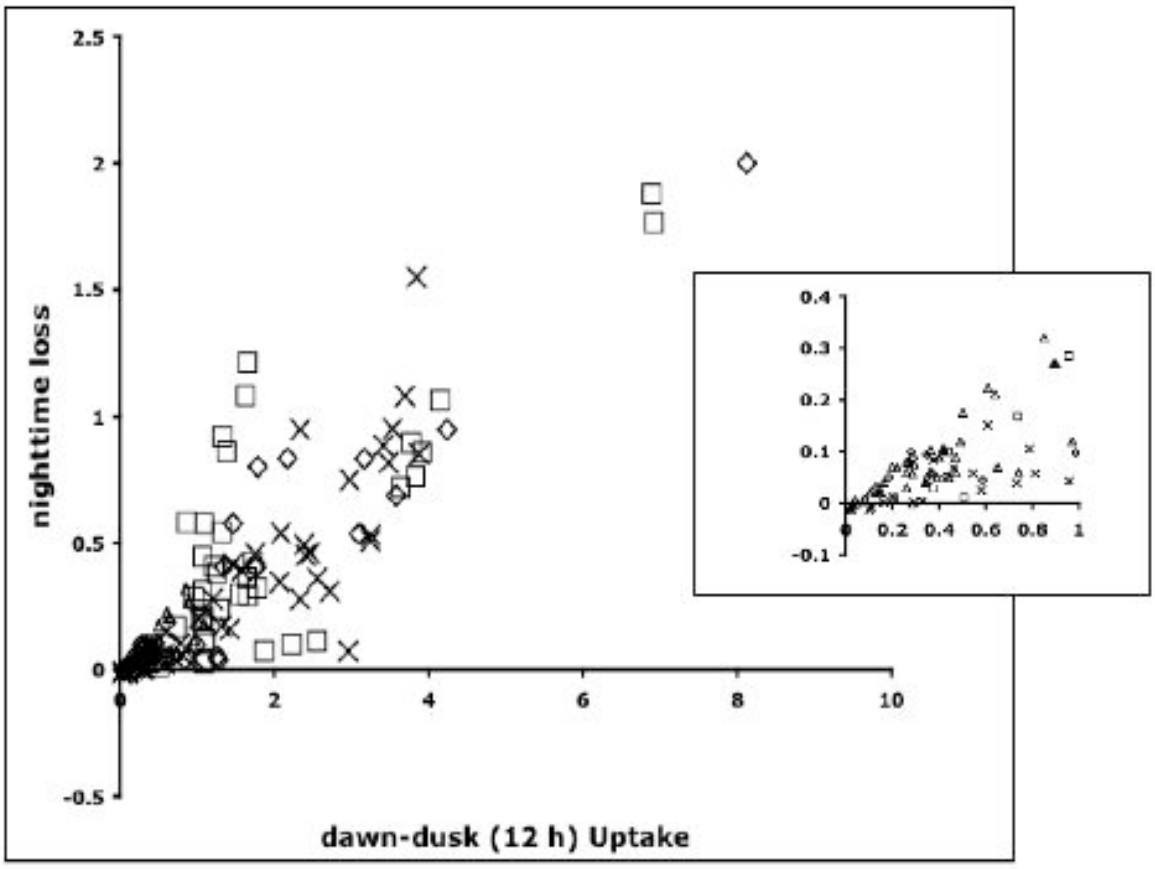


Fig. 1

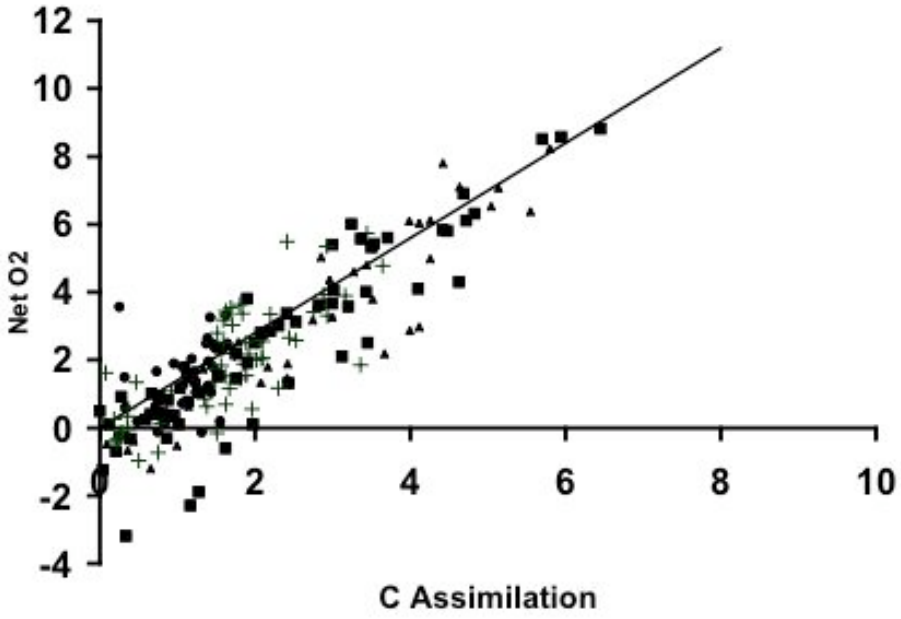
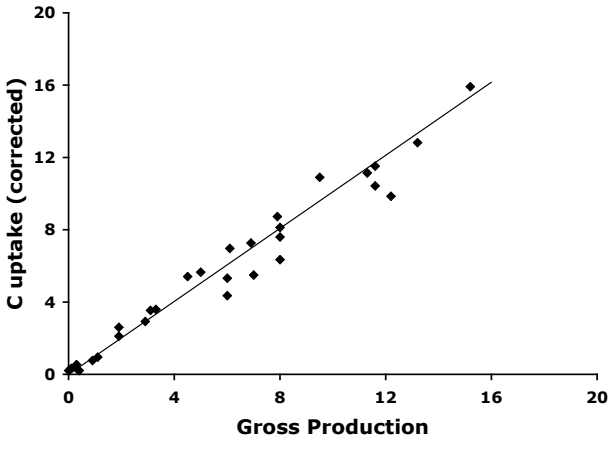


Fig. 2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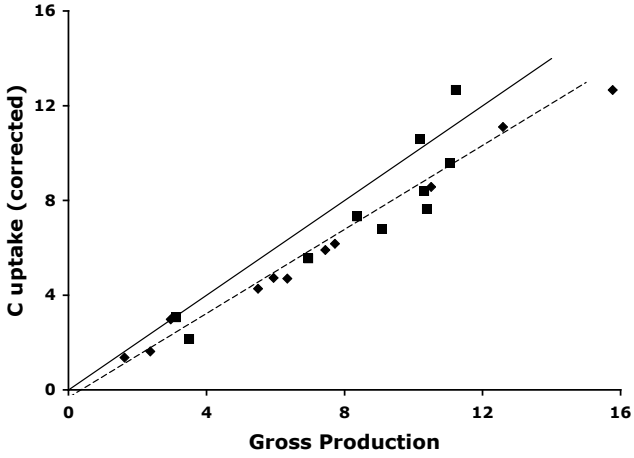
a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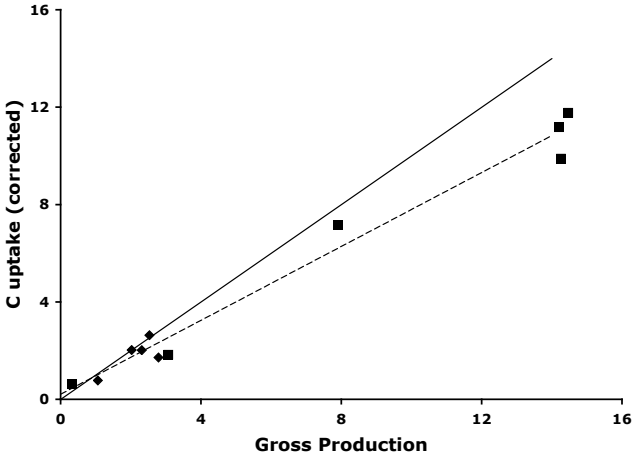
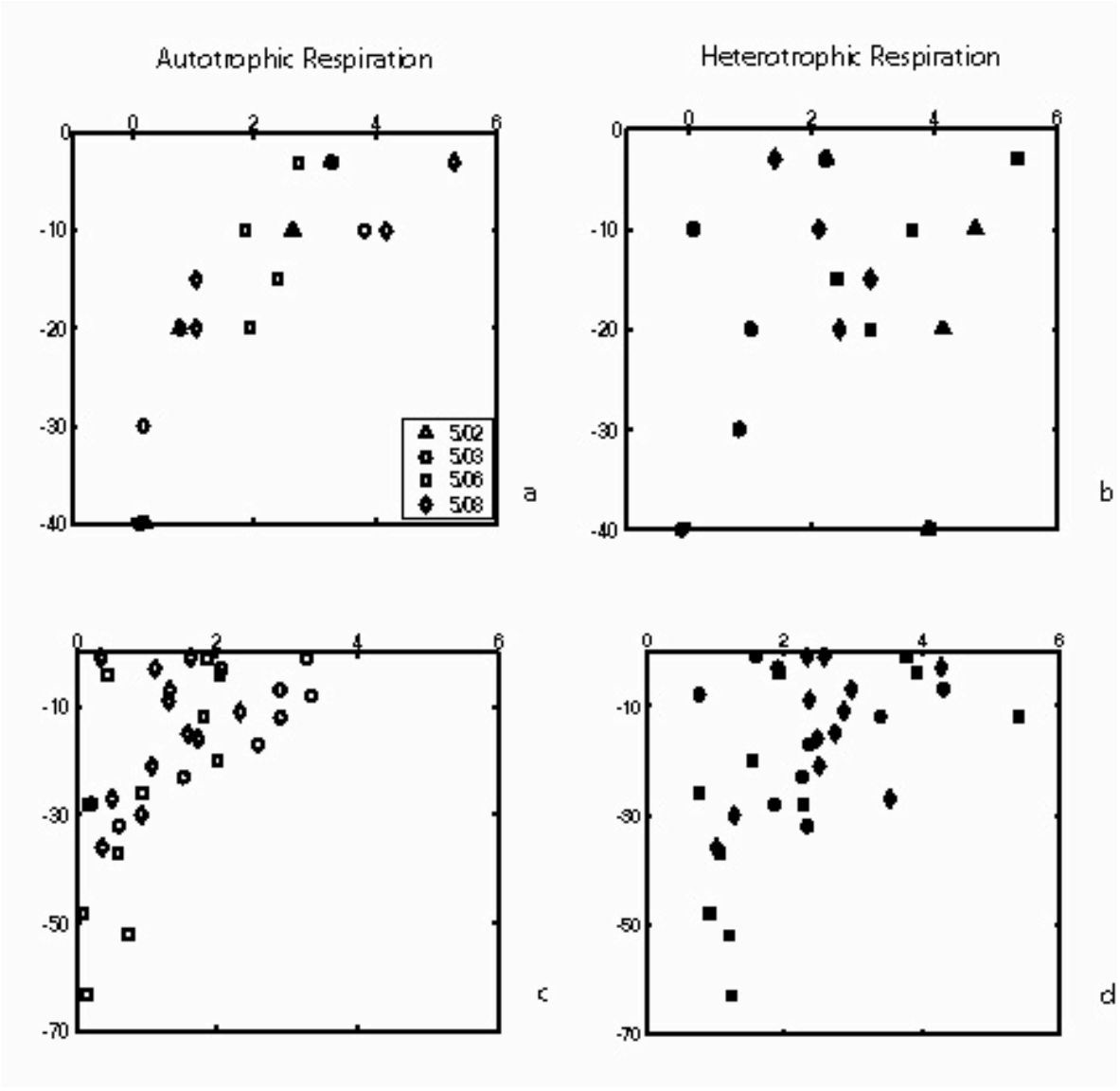


c

Figure 3, Marra and Barber


Autotrophic Respiration
Heterotrophic Respiration
502
503
506
508
a
b
c
d