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#### DOCTOR OF PHILOSOPHY

The community photosynthetic quotient and the assimilation of nitrogen by oceanic plankton.

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### The Community Photosynthetic Quotient and the assimilation of Nitrogen by Oceanic Plankton.

Thesis submitted in accordance with the requirements of the University of Wales for the degree of Doctor of Philosophy.



School of Ocean Sciences, University College of North wales, Bangor, Gwynedd.

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# TEXT CUT OFF IN THE ORIGINAL

The Community Photosynthetic Quotient and the assimilation of Nitrogen by oceanic Plankton.

<u>Abstract</u> Emily Suzannah Wood

A high precision technique for measuring dissolved oxygen was used in conjunction with the coulometric back titration for total inorganic carbon in seawater, to make observations of the photosynthetic and respiratory quotients in a culture and an open ocean environment. The relationship between the PQ and the nitrogen source utilised was assessed by the concomitant measurement of nitrogen assimilation by the 15-N mass spectrometric technique, with the aim of resolving some of the uncertainty surrounding the calculated and observed PQ values.

For the most part the PQ values were found to lie within the constraints set by theory. A complete resolution of the exact relationship between the PQ and the nitrogen source was limited by the analysis of the ambient  $\rm NH_3$ concentrations.

Measurements of community production and respiration were made in the N.E.Atlantic as part of the BOFS programme. Concomitant 14-C rate measurements were made, enabling a comparison of the methodologies. The comparison of the oxygen and 14-C technique was found to be affected by the magnitude of the respiration rate, resulting in elevated PQ values.

The *in vitro* techniques employed in this study were compared to *in situ* techniques for the analysis of the fluxes of oxygen and carbon dioxide. It was confirmed that the *in vitro* techniques were not a serious or overriding cause for concern.

The productivity rate measurements from the oxygen technique were used to assess the critical depths which are consistent with the development of the phytoplankton bloom. A relationship was observed between the critical depth and the net community production rate.

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Table showing the scientific personnel from whom data has been used in this thesis. been used in this chesis. Cruise Measurement Disc182 CD46 CD47 \_\_\_\_\_ <sup>1</sup>E.Wood, <sup>1</sup>E.Wood <sup>4</sup>D.Purdie <sup>2</sup>C.Robinson, 0<sub>2</sub> metabolism <sup>3</sup>P.Williams <sup>1</sup>E.Wood <sup>1</sup>E.Wood TCO, metabolism <sup>1</sup>E.Wood <sup>5</sup>S.Bury <sup>1</sup>E.Wood 15-N uptake <sup>6</sup>G.Savidge, <sup>8</sup>A.Pomeroy <sup>7</sup>P.Boyd <sup>6</sup>G.Savidge, <sup>7</sup>P.Boyd 14-C uptake <sup>6</sup>G.Savidge, <sup>7</sup>P.Boyd <sup>6</sup>G.Savidge, <sup>8</sup>A.Pomeroy <sup>7</sup>P.Boyd Chlorophyll a <sup>6</sup>G.Savidge, <sup>7</sup>P.Boyd <sup>9</sup>P.McCardle <sup>10</sup>B.Head NH<sub>3</sub> and NO<sub>3</sub> concentration <sup>11</sup>H.Kennedy <sup>11</sup>H.Kennedy POC/PON determination -Culture work \_\_\_\_\_\_ <sup>12</sup>B.Saunders, <sup>1</sup>E.Wood Growth <sup>1</sup>E.Wood Chlorophyll a <sup>12</sup>B.Saunders Cell counts <sup>1</sup>E.Wood O<sub>2</sub> metabolism <sup>1</sup>E.Wood TCO<sub>2</sub> metabolism <sup>1</sup>E.Wood 15-N uptake <sup>13</sup>M.Woodward NH3 and NO3 concentration Emily Wood, School of Ocean Sciences, U.C.N.W. 1. Dr.Carol Robinson, School of Ocean Sciences, U.C.N.W. 2. Prof.P.J.LeB.Williams, School of Ocean Sciences, U.C.N.W. 3. 4. Dr. Duncan Purdie, Southampton University Dr.Sarah Bury, Plymouth Marine Laboratory
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#### <u>Chapter 1</u>

#### GENERAL INTRODUCTION AND GOALS OF STUDY.

#### Chapter 1.

#### General introduction and goals of study.

The physico-chemical environment of this planet is profoundly affected by the organisms which frequent it. The present day atmosphere evolved mainly as a consequence of the activity of ancestral stromatolite building bacteria (Rothschild and Mancinelli, 1990). Since life first began on the earth, the interactions of microorganisms with their environment is thought to have played a major role in controlling the planet's climate. Our own species, is now taking control.

The anthropogenic activities of man are interfering with the earths geochemical cycles, to such an extent, that it is now widely believed, we will force the earth to experience a global climate change over the next 50 to 100 years, greater than anything seen over the past 10,000 years (IPCC, 1990). A global mean temperature rise of 3°C is predicted by the end of the next century, associated with a mean sea level rise of 65cm over this period.

The greenhouse gas, carbon dioxide  $(CO_2)$ , accounts for about half of the atmospheric greenhouse effect

(Ramanathan et al., 1985) and is expected to be more dominant in the future. It is therefore the main focus of concern.

Atmospheric  $CO_2$  levels are under the control of the sea (Berger et al., 1990) with some fifty times more  $CO_2$  being stored in the ocean than in the atmosphere. Whilst it is known that human activity adds some 6 billion tonnes of carbon into the atmosphere each year, the annual increase in atmospheric  $CO_2$  is only 3 billion tonnes. It is believed that the remaining 3 billion tonnes is taken up by the oceans, but recent estimates leave 1 billion tones unaccounted for (SCOR, 1990).

In order to understand, predict or even forestall the climatic changes that await us, we must increase our understanding of the atmospheric - ocean  $CO_2$  balance.

In 1986, the Biogeochemical Ocean Flux Study (BOFS) was set up to improve our understanding of carbon flow through the marine system. BOFS became the U.K. component of the Joint Global Ocean Flux Study (JGOFS), the collaboration of national programmes being essential in understanding ocean processes on a world-wide scale.

The aims of the BOFS programme were:

- To improve our understanding of the biogeochemical processes influencing the dynamics of the cycling of the elements on the ocean, and related atmospheric exchanges, with particular reference to carbon;

- to develop, in collaboration with other national and international programmes, models capable of rationalising and eventually predicting the chemical and biological consequences of natural and man-induced changes to the atmosphere-ocean system.

The partitioning of carbon between the atmosphere and the oceans is controlled to a large extent by the "biological pump", the draw-down of  $CO_2$  from the atmosphere into the surface ocean, its assimilation into organic compounds by autotrophic activity, and its downward transport to be sequested at depth and deposited in the sediments (Berger et al., 1989).

Primary production is therefore the first step in the biological control of the oceanic regulation of atmospheric  $CO_2$  levels. Much of the  $CO_2$  taken up by photosynthesis will be recycled back into the atmosphere through respiration. In order to assess the draw-down of
CO<sub>2</sub> from the surface to the deep water, we need therefore, to measure the net export flux of carbon from the surface to the deep ocean.

Measurements of net community production can be made by measuring the metabolism of carbon or oxygen, as the two processes can be related by photosynthetic and respiratory quotients (P.Q. and R.Q.). Because nitrogen is believed to be assimilated in a semi-stoichiometric relationship to carbon (Redfield, 1934), it too can be used as a determinant for photosynthetic rate measurements.

The productivity of surface waters is dependent on the availability of nutrients, with nitrogen, regarded to be the limiting nutrient in most oceanic regions (Carpenter and Capone, 1983). If the net export flux from the surface ocean were zero, an unexploited ecosystem could run indefinitely on regenerated production. Losses from the euphotic zone however occur, due to sinking, mixing and vertical zooplankton migration. The available nitrogen in the euphotic zone, would rapidly become depleted without a replacement flux of nitrogen, this flux being the basis of new production. New production can be thought of as the maximum rate at which organic compounds can be extracted from the euphotic zone, and in the long

term therefore, can be equated with net community production (Platt et al., 1989). The relative rates of new and regenerated production are expressed in the f-ratio.

The fluxes of oxygen, carbon dioxide and nitrogen in the surface ocean can therefore be related by the P.Q. and its relationship to the f-ratio.

For a community, to increase, the gross production rate must exceed the community respiration rate. Physical processes will be of importance in determining whether this can occur. Sverdrup, (1953) outlined a 'critical depth' theory from which predictions could be made about the "vernal blooming of phytoplankton". Because the observed growth rate of a community is related to the net community production rate, measurements of the critical depth in relation to the depth of mixing, should enable predictions to be made about the potential for the export of carbon.

The goals of this study are, therefore;

- to make measurements of community production and respiration in the open ocean as part for the BOFS programme, and in so doing, to assess the respective

methodologies employed in measuring primary production rates.

- to assess the relationship between the P.Q and the assimilation of nitrogen, and in so doing, to pin down some of the uncertainty surrounding calculated and observed P.Q. values.

- to calculate the critical depth values from oxygen metabolism measurements, and relate them to the net available export flux of carbon.

# <u>Chapter 2</u>

# TERMINOLOGY AND DEFINATIONS

### Chapter 2

### Terminology and definitions.

It has now become common practice to make primary production rate measurements using two or more different techniques. No longer held back by the precision limits of the more typical of these techniques, (namely the oxygen and 14-C techniques) we are forced to question the accuracy with which we are able to make these measurements. The current dispute as to what each technique is measuring is not helped by the confusion in the literature over primary production terminology. Here, I will attempt to clarify the definitions used and outline precisely what each term is referring to.

I have included in this part of the thesis the section entitled, "Predicting the vernal blooming of phytoplankton". It is introduced at this point as it is necessary to define the terms which will be referred to throughout the thesis.

#### 2.1. Photosynthesis

'The conversion of radiant energy into the potential chemical energy of stable organic compounds, carried out by plants and autotrophic bacteria is the essence of photosynthesis'. Fogg, (1972).

Below is the all too familiar, over simplified equation of photosynthesis;

$$CO_2 + H_2O -> CH_2O + O_2$$

The reduction of carbon dioxide to organic carbon in the presence of energy from the sun with the subsequent in a mole to mole ratio: an evolution of free oxygen acceptable hypothesis until physiologists discovered that the photosynthetic oxygen liberated was derived not from the reduction of carbon dioxide but from water. Chosen to be ignored for simplicity (e.g. Ryther, 1956) the fact that the reduction of carbon dioxide and the evolution of oxygen are separate and quite unrelated processes is often overlooked. It follows that definitions of photosynthesis as the fixation of carbon, or the release of oxygen are problematic. As I shall outline below, the photochemical reactions of photosynthesis result in the formation of high energy adenosine tri-phosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH<sub>2</sub>), 'universal

biochemical currency', Fogg, (1972) used in a variety of other biochemical processes other than the fixation of carbon or the reduction of water to liberate oxygen.

### 2.1.1. 'Light' reactions of photosynthesis.

In the light reactions of photosynthesis there are two main photochemical processes, cyclic and non-cyclic photophosphorylation, which take place in the thylakoid membrane system.

# i. Cyclic photophosphorylation

In cyclic photophosphorylation, ADP (adenosine diphosphate), is converted into high energy ATP (adenosine tri-phosphate) from the absorption of light energy in the chloroplasts (Kirk, 1983). Arnon, (1966) proposed the scheme for cyclic photophosphorylation as shown in figure 2.1.(1) and can be summarised as;

> light ADP + Pi ----> ATP



### ii. Non-cyclic photophosphorylation

In non-cyclic photophosphorylation ATP formation is coupled with the reduction of NADP (nicotinamide adenine dinucleotide phosphate) to  $NADPH_2$ , thus a source of hydrogen ions is required, the hydrogen donor normally being water, with the subsequent release of oxygen. Non-cyclic photophosphorylation is shown schematically in figure 2.1.(2) and can be summarised as;

2NADP + 2H<sub>2</sub>O + 2ADP + 2Pi ----> 2NADPH<sub>2</sub> + O<sub>2</sub> + 2ATP

Non-cyclic photophosphorylation can be divided into two photochemical reactions.

Photosystem I: The absorption of long wave-length light, responsible for the reduction of NADP to NADPH<sub>2</sub>.

Photosystem II: The absorption of short-wavelengh light resulting in the production of oxygen.

Experiments show that response to light adaptation occurs in photosystem II, Megard et al., (1985), and because oxygen production is directly related to this system, (Arnon, 1966), the linkage between carbon fixation and oxygen production must vary with irradiance.



#### 2.1.2. 'Dark' reactions of photosynthesis.

The dark reactions of photosynthesis involve both the fixation and reduction of carbon dioxide. The fixation of carbon dioxide is termed carboxylation, and because reduction is not involved the energy requirement is small. Carboxylation occurs when the acceptor molecule RuDP (ribulose-1,5-diphosphate) reacts with carbon dioxide to give a highly unstable 6-carbon compound which splits to give two molecules of PGA (3-phosphoglyceric acid). This reaction proceeds spontaneously, and requires no energy or supply of hydrogen, and so is not directly related to the photochemical reactions (Fogg, 1972). It has been established that PGA is the first stable product of photosynthesis.

PGA then enters the Calvin cycle (figure 2.1.(3)) the most important reactions involved in the reduction of carbon. The regeneration of RuDP from PGA requires energy from ATP as does the reduction of carbon from PGA. For carbon dioxide to be reduced to a hexose (or 6 carbon) sugar, the hydrogen from two molecules of NADPH<sub>2</sub> and the energy from three ATP molecules is required.

The dark reactions of photosynthesis take place in the stroma of the chloroplast.



### 2.1.3. Photosynthetic processes other than carbon fixation

The high energy ATP and NADPH<sub>2</sub> produced by the photochemical reactions of photosynthesis are not only used in the carbon reduction cycle, but are 'universal biochemical currencies', used for a variety of other processes.

## i. Polyphosphate storage.

In certain circumstances when the production of ATP by photophosphorylation is in excess, algae will form polyphosphate stores, which act as a reserve of high energy phosphate (Craigie, 1974).

ii. Molecular synthesis.

ATP produced by cyclic photophosphorylation is also used for the synthesis of large molecules, for example the formation of starch.

iii. Ion uptake.

Another requirement for energy derived from ATP is in the active uptake of ions across the cell membrane.

iv. Assimilation of nitrogen.

Nitrogen, if taken up in the form of nitrate, must first be reduced to ammonia before it can be utilised by the cell. Photochemically generated reductive power is used in the reduction of nitrate. Nitrate reduction can occur in the dark, but the rates are accelerated in the light. It was first hypothesised that nitrate was reduced to ammonia at the expense of carbohydrate with the subsequent liberation of oxygen. It was found, however, that nitrate could be reduced, and oxygen liberated without any concomitant change in carbon dioxide. Thus the reduction of nitrate is not dependent on the subsequent reduction of carbohydrate. The overall equation for nitrate reduction then becomes:

$$HNO_3 + H_2O -> NH_3 + 2O_2$$

The process is, however, not as straight forward as the above equation would suggest. It was found that both nitrite and hydroxylamine were produced as intermediates in the reaction before the production of ammonia. It would appear that the linkage between photosynthesis and the reduction of nitrate, is such that photochemically produced hydrogen donors are necessary in the reduction of nitrate and that photochemically produced ATP may be

directly required in the uptake of nitrate into the cell.

## 2.1.4. The products of photosynthesis.

There is no substance that can be described as the product of photosynthesis (Fogg, 1972). One might consider the production of oxygen as an exception, but this is more commonly regarded as a by-product of photosynthesis. The first stable product of carbon fixation is PGA, in which the carbon has not yet been reduced. The PGA then either enters the Calvin cycle and is built up into carbohydrate, or only going some of the way round the cycle, ends up as sucrose, for instance. The PGA may not enter the cycle at all, its central position in the cellular metabolism meaning it may well be used for the synthesis of other molecules such as amino-acids, fats, and proteins.

An important early product of carbon fixation is glycollic acid, which has been reported as acting as a cellular overflow mechanism. Plankton have been shown to release as much as 95% of the carbon fixed in photosynthesis into their surrounding medium (Fogg, 1972). This high exudation rate being shown to occur at light intensities such that photosynthesis is inhibited. It would seem that carbon is

being fixed at a rate greater than can be dealt with by the synthetic mechanisms of the cell. Recent work by Zlotnik and Dudinsky, (1989) however, found that the percentage of excreted matter was never more than 10%. Even though the magnitude is debated, it is still an important physiological process which will influence measurements made of photosynthetic rates, and which will, of course, vary with species and environmental conditions.

# 2.2. Respiration

The biological functions of photosynthesis and respiration seem to be identical in that they both provide assimilatory power in the form of high energy ATP (adenosine triphosphate) and  $NADPH_2$  (nicotinamide adenine dinucleotide phosphate) and a pool of metabolic intermediates that can be used in the assimilation of more complex molecules.

There are several respiratory processes which seem to proceed in opposition to photosynthesis, the possibility of interactions between respiration and photosynthesis are numerous. However, the two processes occur in distinctly separate organelles implying that the end products of one need not necessarily be the starting material of the other. For example, the photosynthetic release of oxygen is a terminal process carried out by a distinct and

different enzyme system from that responsible for the respiratory uptake of oxygen. As has been said, photosynthesis occurs in the thylakoid system and chloroplasts, whereas respiration occurs in the mitochondria of the cell.

In studies of planktonic systems, respiration can not be thought of as an equal and opposite rate to photosynthesis. Though they may be regarded as essentially equal on an oceanic scale, planktonic respiration is encountered throughout the euphotic zone at illuminations below that possible for photosynthesis.

The ratio of respiration to photosynthesis will vary according to species, and environmental conditions, and can be used as an index of the trophic balance of the ecosystem (Iriarte et al., 1991).

# 2.3. Photorespiration.

It has been found that relatively weak illumination can suppress mitochondrial respiratory processes and stimulate another type of respiration, namely photorespiration. Photorespiration is apparently closely linked to photosynthesis, in that factors affecting the one affect the other, for example, the increased rate of the two

the two processes with increasing light intensity.

The susbstrate for photorespiration appears to be glycollate, an early product of photosynthesis. Like the release of extracellular glycollate, the only function of photorespiration seems to be an overflow mechanism for the disposal of excess photosynthetic products; as photosynthesis increases, the release of extracellular glycollate will increase, and subsequently the rate of photorespiration will increase.

Grande et al., (1989b) found in some algal species photorespiration rates were from between 20 percent to greater than 100 percent of mitochondrial respiration rates. High photorespiration rates were also found in species taken from experimental coastal ecosystems (Grande et al., 1989b).

It is of paramount importance to be able to determine photorespiration as all too often respiration is considered to be the same rate both in the light and in the dark.

## 2.4. The photosynthetic rate.

In order to assess the photosynthetic activity of a community it is necessary to determine the rate at which photosynthesis is proceeding rather than try and make deductions from a measure of the standing stock of carbon, for instance.

Photosynthesis will change according to the environmental conditions and the stage of development of the community. Because photosynthesis is by definition the conversion of radiant to chemical energy, the photosynthetic rate should be a measure of this energy flux. Traditionally, however, changes in the observed concentration of oxygen or carbon dioxide have been measured. We need first, then, to define what aspect of photosynthesis we are going to take as a measure of the photosynthetic process. Because we are measuring a rate process, we must then decide the time and space scales over which we are going to measure. With community rate measurements, the necessity arises to define exactly what the community is that we are measuring. It then becomes appropriate to subdivide the photosynthetic rate into operational definitions; namely, the gross production rate, the net primary production rate, and the net community production rate.

#### 2.4.1. Gross production rate.

'The gross rate of photosynthetic increase in plant crop before any corrections for respiration or excretion are made.' Strickland, (1960).

Gross production (Pg) is the total change due to autotrophic and heterotrophic production and respiration. It is "an intangible quantity" (Ryther, 1956), and it was recommended by an I.B.P committee that its use be dropped (Williams et al., 1979). Operationally it is measured from the total change in a chosen parameter in the light plus the total change in that parameter in the dark, acted upon by the whole community.

### 2.4.2. Net primary production rate.

'The net rate of autosynthesis of the organic constituents of plant material in water' (Strickland, 1960).

The term net primary production (Pn) refers only to the primary productivity associated with the autotrophic plankton.

If the respiration of the autotrophs (Ra) is deducted from the gross production (Pg) the resultant is Pn.

The term does not include losses due to death or predation.

$$Pn = Pg - Ra$$

Operationally it is a measure of the net change in a parameter in the light as a result only of autotrophic activity.

# 2.4.3. Net community production rate.

'This term is reserved to describe the rate of production (either positive or negative), of plant organisms under the influence of all environmental factors, and thus include losses by death and predation.' Strickland, (1960).

Net community production (Pc) is differentiated from net primary production by the inclusion of the heterotrophic community, thus if the respiration due to both the macroscopic and microscopic heterotrophs (Rh) is deducted from net primary production (Pn) the resultant is Pc; Platt et al., (1989). As can be seen from Strickland's definition of the term, we should also subtract those losses due to death and predation (D).

Operationally it is measured as the net change in a parameter in the light due to the activity of the entire community.

# 2.4.4. Total Production

"Total production is the nitrogen equivalent of Pg in the sense that it represents the sum total of the nitrogen assimilated by the photoautotrophs" (Platt et al., 1989).

Because nitrogen is believed to be the limiting nutrient in most oceanic regions (Carpenter and Capone, 1983), and that it is assimilated in a stoichiometric factor to carbon (Redfield *et al.*, 1963), it is used as a variable in rate measurements of photosynthesis. Because there is no evidence that phytoplankton remineralise nitrogen, Platt *et al.*, (1989) make the case that total production can be equated to net primary production.

Total production is subdivided into 'regenerated' production and 'new' production, depending on the nitrogen source

utilised.

#### 2.4.5. Regenerated production.

"Regenerated production is that production based on reduced forms of nitrogen" (Dugdale and Goering, 1967).

Because the principal source of regenerated nitrogen is ammonia, the assumption is made that regenerated production is that production based on the assimilation of ammonia. In an ideal situation, if there were no loses to the photic zone of the ocean, the ecosystem could run indefinitely on regenerated production. Because losses occur, however, due to sinking, mixing, predation, and the harvesting of fish, etc., the nitrogen pool would rapidly become depleted. The replacement flux of nitrogen is the basis of new production.

### 2.4.6. New production.

"New production", as defined by Dugdale and Goering, (1967), is "the primary production in the euphotic zone resulting from nutrient fluxes from outside the euphotic zone, such as upwelling, terrestrial runoff, or atmospheric

inputs".

The principal externally supplied nitrogen source is upwelled nitrate (Eppley et al., 1973). The assumption has therefore been made that new production is that production based on nitrate. New production, in the words of Platt et al., (1989), may be thought of as "the maximum rate at which organic compounds can be extracted from the photic zone without jeopardy to the long term integrity of the ecosystem contained therein"; therefore in the long term, it can be equated to net community production (Platt et al., 1984).

#### 2.5. The photosynthetic quotient

The photosynthetic quotient (P.Q.) is a term that expresses the relationship between photosynthetic oxygen and carbon metabolism. It is defined as the molar ratio of the rate of photosynthetic oxygen produced, divided by the rate of carbon dioxide consumed.

Until recently, the range of P.Q. values has been quoted as 1 - 1.6 (Strickland, 1960, 1965; Parsons et al., 1975), with the commonly adopted median value of 1.25 (Ryther and Vaccaro, 1954; Strickland and Parsons, 1972).

Because over an ecological time-scale, the cell is not able to harbour an excess or deficiency of photochemically produced reductant, the P.Q. has been assumed to be calculable from stoichiometric equations (Williams and Robertson, 1991). However, the exact stoichiometric relationship has, as yet, not been resolved. When making comparisons between the 14-C and oxygen techniques, the rate of carbon assimilated has often been found to be less than that expected in comparison with the oxygen evolved, e.g. Antia et al., (1963), McAllister et al., (1964), Ryther et al., (1971), Bryan, (1979).

The inter-comparison of techniques will be dealt with else where, (see section 3.8.); here the theoretical

relationship between oxygen production and carbon dioxide consumption will be discussed.

Based on the manometric work of Redfield, (1934), the P.Q. of marine plankton was first assumed to be unity. This value is derived from the early equation of photosynthesis, where carbon dioxide and water react together on a mole to mole basis, to produce the molar equivalent of oxygen and a molecule of 'sugar'. Rabinowitch, (1945) was the first to throw light on the quotient's disparities, by showing that the end products of photosynthesis are not always carbohydrates, but can be more reduced compounds such as lipids, or more oxidised compounds such as glycollic acid, which would increase and decrease the P.Q. accordingly. Myers, (1949) then introduced a second level of complication by putting forward the argument that nitrogen is actually competing with carbon for reductant generated during oxygenic photosynthesis thus affecting the P.Q.

Thomas et al., (1976) and Terry, (1982) have demonstrated that carbon assimilation will decrease if nitrate or nitrite is added to algal cultures. The argument is such that if the plankton are consuming organic material with a carbon to nitrogen to phosphorous ratio of 106:16:1 (Redfield, 1934), and nitrogen is being consumed in the

form of ammonia, then the following equation can be written;

 $106CO_2 + 16NH_3 + H_3PO_4 -> [(CH_2O)106(NH_3)16H_3PO_4] + 106O_2$ => PQ = 106/106 = 1

But, if the nitrogen source being utilised is nitrate, it must first be reduced to ammonia, the energy required coming from the photolysis of water, an oxygen producing process;

 $106HNO_3 + 16H_2O \rightarrow 16NH_3 + 32O_2$ => PQ = 106 + 32/106 = 1.3

The influence of the nitrogen source on the P.Q. has been demonstrated in culture experiments, e.g. Cramer and Myers, (1948), Myers and Cramer, (1948), Van Niel et al., (1953), Williams et al., (1979), and on natural algal populations, e.g. Williams et al., (1979), Raine, (1983), Davies and Williams, (1984), Irwin, (1991). Williams et al., (1979) made the quotient more workable by separating it into two components, a 'carbon' P.Q. controlled by the state of reduction of the end products of photosynthesis, and a 'nitrogen' P.Q. controlled by the state of reduction of the nitrogen source being utilised. The argument was extended to include sulphur (Williams and Robertson, 1991).

Thus;

 $PQ = PQ_{C} + PQ_{n} + PQ_{s}$ 

where;

 $PQ_C$  is the carbon P.Q. determined by the state of reduction of the end product of photosynthesis, and for a compound of the empirical formula -  $C(H)_Y(O)_X$  $PQ_C = (1+Y/4) - Z/2$ 

 $PQ_n$  is the state of oxidation of the nitrogen source, for nitrate,  $PQ_n = 2 X (NO_3/C)$ 

for nitrite,  $PQ_n = 1.5 \times (NO_2/C)$ 

and for ammonia and urea,  $PQ_n = 0$ 

 $PQ_S$  is the component of the P.Q. associated with the reduction of sulphur.  $PQ_S = 2 X (SO_4/C)$ .

Where  $NO_3/C$ ,  $NO_2/C$  and  $SO_4/C$  are the assimilation numbers of nitrate, nitrite and sulphate respectively.

The relative contribution of the carbon and nitrogen components to the overall P.Q. will be reflected in the carbon to nitrogen assimilation ratio of the cell, and for growth on nitrate as the nitrogen source, the C:N ratio will be the major factor influencing the P.Q. (Williams et al., 1979). The effect of  $PQ_n$  on the overall P.Q. is illustrated in table 2.5.(a) below, where the P.Q. has been calculated for various C:N ratios, and  $PQ_c$  is taken to be 1.1, from Laws, (1991).

#### Table 2.5.(a)

The relationship between the C:N assimilation ratio and the expected photosynthetic quotient for growth on nitrate with the 'carbon' P.Q. assumed to be 1.1

Atomic (	C:N	ratio	calculated	PQ
	3:1		1.77	
4	4:1		1.60	
	5:1		1.43	
8	8:1		1.35	
10	0:1		1.30	
15	5:1		1.23	

The P.Q. is constrained by biochemical limits pending the end products of photosynthesis, and the nitrogen source utilised. The theoretical range of P.Q. values are given in table 2.5.(b) below, taken from Williams and Robertson, (1991), showing the acceptable upper and lower boundary to the P.Q.

### Table 2.5.(b)

.Q. values calculated for 'conventional' metabolites						
Photosynthetic product	PQc	C:N	PQn	PQ		
lower boundary Glycollic acid (C <sub>2</sub> H <sub>3</sub> O <sub>3</sub> ) Glucose (C <sub>6</sub> H <sub>12</sub> O <sub>6</sub> )	0.63 1.0	-	0 0	0.63		
Upper boundary Saturated fatty acid (-CH <sub>2</sub> -) Nucleic acid ( $C_{38}H_{47}O_{28}N_{15}P_4$ ) Protein ( $C_{18}H_{24}O_6N_5$ ) 'Typical' algal cell (protein, 40%, carbohydrate, 40%, lipid, 15%, nucleic acid, 5%)	1.5 0.98 1.01 1.08	2.5 3.6 7.6	0 0.8 0.56 0.26	1.5 1.78 1.57 1.34		

from Williams and Robertson, (1991).

Proteins, polysacharides and lipids account for most of the carbon synthesised by actively growing microalgae. Proteins and nucleic acids constitute the principal end products of nitrogen metabolism, and the production of neither could give rise to P.Q. values greater than 1.78 with nitrate as the nitrogen source (Williams and Robertson, 1991).

The P.Q. associated with polysacharides is equal to 1 independent of the nitrogen source (Laws, 1991). For lipid synthesis, the highest P.Q. obtainable would aproach 2, (methane production), but because most lipids contain at least 12 carbon atoms, are unsaturated and contain cyclic components and oxygen (Suen *et al.*, 1987), lipid synthesis can be assumed to produce a P.Q. of 1.4 (Laws,

1991).

The lower limits of the P.Q., therefore, for conventional metabolites, are 1.0 for glucose production and 0.625 for glycollate production - independent of the nitrogen source being utilised; and the upper limits are; 1.78 for the production of nucleic acids with nitrate as the nitrogen source.

In order to calculate theoretical P.Q. values assumptions have to be made about the algal cellular composition, but Laws, (1991), has shown that the derived quotient is surprisingly insensitive to such assumptions, and is in fact only affected in the second decimal place. The cited 'expected' P.Q. for a 'typical' algal cell is 1.08 for ammonia based production and 1.34 for nitrate based production (Williams and Robertson, 1991). Laws, (1991) calculated an 'expected' P.Q. of 1.1 and 1.4 for ammonia and nitrate based production respectively.

Compounds such as dimethylsulphide and methylamine are known to be produced by algae. These compounds have low C:S amd C:N ratios and accordingly high P.Q.values. The formation of dimethylsulphide gives a P.Q. value of 2.75 (Sieburth, 1968; Iverson et al., 1989), and methylamine, a P.Q. of 3.5 (Williams and Robertson, 1991). The production of oxalic acid, the most oxidised plant product,

would produce a P.Q. of 0.25 (Williams and Robertson, 1991). These compounds have not been thought of as major products of photosynthesis, but should not be ignored, as the possibility of finding methane production, for example, being of greater relative importance, cannot be discounted.

Figure 2.5.(1) shows the full potential range of P.Q. values including these 'unconventional ' metabolites, taken from Williams and Robertson, (1991).

The story does not, however, end here. Work by Kessler, (1959) and Hatori, (1962) suggest that the enzymes responsible for the reduction of nitrate have a higher affinity for photochemically produced reductant than the enzymes for the reduction of carbon dioxide. This would imply that the P.Q. will also be a variable function of irradiance. Megard et al., (1985) demonstrate in lake Kinnerret that reduction of nitrate has priority over carbon dioxide reduction at low irradiances, where oxygenic photosynthesis is less than the threshold required for carbon assimilation. Lowered P.Q. values have been demonstrated by Anderson and Sand-Jensen, (1980), at low light intensities.

If the assimilation of carbon and generation of oxygen are decoupled, the build up of reduced intermediates in

the cell will not be of any great significance over the time scale of incubations as cells are unable to store any substantial amount of reductant power. However, the use of photochemically generated reductant for other processes than carbon assimilation will be of importance, for example the reduction of nitrate to nitrite, and nitrite to ammonia, as has been discussed.

If nitrate reduction is uncoupled from carbon dioxide assimilation, then constraints set by the C:N ratio on the  $PQ_n$  are released. To further complicate the issue, plankton may, in certain circumstances, be reducing more nitrate than they are able to assimilate.

An over assimilation of nitrate would lead to a build up of nitrite or ammonia in the water column. There is evidence of the accumulation of nitrite at the base of the euphotic zone (Kiefer *et al.*, 1976). Williams and Robertson, (1991), argue for the improbability of nitrate being over assimilated because of the effect of the subsequent build up of ammonia, which would then inhibit nitrate reduction, thus restoring the quotient. However, in line with the idea of Williams, (1990) that algae may be excreting organic material as a 'tax', a necessary payment for their survival in the community, so too may the algae be over assimilating nitrate as just such a tax.

The build up of ammmonia would be kept in check by the microheterotrophic community, and ambient concentrations sufficient to inhibit the reduction of nitrate would be prevented.

The microheterotrophic community will themselves affect the measured P.Q. in experiments where they are incubated concomitantly with the algae, (as is the case for oxygen Winkler measurements, for example). The most pertinent effect that the microheterotrophs will have on the quotient when sampling oligotrophic surface water, would be from nitrification. This process, however, is said to be inhibited in the light, Horrigan *et al.*, (1981), and so has been assumed to be of minor importance. The consequences of nitrification will be discussed further in chapter 3.

Another complication arises when considering photorespiration. If photorespiration is occurring the P.Q. will be reduced as at least 3 moles of oxygen are consumed for every mole of carbon released (Zelitch, 1971; Tolbert, 1974). The effect of photorespiration on measurements of P.Q. has been shown by Fock et al., (1968; 1969; 1971) and Bender et al., (1987). Because photorespiration is a variable function of light, the P.Q. will again be influenced by the light regime. Photorespiration is favoured at high oxygen concentrations, and Burris, (1981) showed that

as the oxygen concentration increased in cultures, the P.Q. subsequently decreased.

Finally the consumption of oxygen by non-biological processes must be considered as this will bias measurements of P.Q. values. Laane et al., (1985), demonstrated oxygen consumption can result from photo-oxidising proccesses in natural aquatic environments. Photoreactive dissolved and particulate matter is always present in marine water, and by absorbing light energy, a singlet oxygen is photochemically generated, which will then oxidise certain kinds of inorganic and organic molecules (Zafiriou et al., 1985). Laane et al., (1985) demonstrated a change in oxygen concentration of 5  $\mu$ mol L<sup>-1</sup> during a 6hr incubation of filter-sterilised North sea water, in a bottle.

Photochemical reactions are confined to the upper layer of the ocean surface, but in the open ocean this layer may extend as deep as 25m - the depth of penetration of ultra violet light (Smith and Baker, 1979). Laane et al., (1985) have shown that as much as 40 percent of the oxygen produced by photosynthesis in the oceans may be consumed by abiological processes. The relative contribution of oxygen consumption by photochemical reactions will, of course vary both spatially and temporally, but any significant abiologicol oxygen consumption will render

measurements of the P.Q. value artificial.

If significant oxygen consumption *is* occurring through photochemical reactions we cannot put any constraints on the observed P.Q. of illuminated surface waters, and the quotient then loses any physiological significance.


## 2.6.The respiration quotient.

The respiration quotient (R.Q.) is assumed to be the reciprocal of the photosynthetic quotient, *i.e* the biochemical stoichiometry of respiration is the reverse of photosynthesis. This assumption can not be valid as irradiance has a different effect on the carbon and oxygen assimilatory pathways. It must therefore have a different effect on the R.Q. values from the P.Q. values.

An important consideration is the effect of nitrate reduction on the quotient values. This effects the P.Q. values through the production of oxygen. The reverse of nitrate reduction, nitrification, is assumed to occur lower in the euphotic zone. Surface R.Q. values will not therefore reciprocate P.Q. values.

There is very little reported data of R.Q. values. Johnson et al., (1981) reported R.Q. values for the first time directly from oxygen and total carbon dioxide measurements. The R.Q. values were all lower than expected, (less than 1). Johnson et al., (1983) suggesteed that the R.Q. values were low due to bacterial activity occurring in anaerobic microzones within the water column. Robertson, (1989) reported R.Q. values from mesocosm experiments, in the range 0.52 to 2.87. The R.Q. values were reported as not being reciprocals of the P.Q. values concomitantly obtained.

## 2.6.1.Calculating the standard error on a quotient.

The photosynthetic quotient and respiration quotient are calculated from the division of two rate values. The rate values posses a standard error, therefore it is necessary to place an error bar on the quotient value obtained. The quotient (Z) is the result of the division of two values, X and Y which each possess a standard error,  $S.E._X$ and  $S.E._V$  respectively, where;

If the values for X and Y are values from two different normal distributions, and the standard deviation of each is much less than the mean, then the standard error of the quotient (Z) can be calculated from;

S.E.<sub>z</sub> =  $\frac{1}{\sqrt{2}}$  (Y<sup>2</sup> x S.E.<sub>x</sub><sup>2</sup>) + (X<sup>2</sup> x S.E.<sub>y</sub><sup>2</sup>)

(Baird, 1962; Barford, 1967).

## 2.7. The f-ratio

The classical view of the mechanism regulating primary production in the ocean is the supply of nitrogenous nutrients to the photic zone (Harrison, *et al.*, 1987). Dugdale and Goering, (1967) recognising the importance of the source of this nitrogenous nutrient supply, partitioned production between that based on allochonous nitrogen sources, (termed 'new' production), and that based on autochonous nitrogen sources, (termed 'regenerated' production). The concept of the f-ratio was then introduced (Eppley and Peterson, 1979), as a term used to define the proportion of the total nitrogen based production that is fuelled by new sources of nitrogen. The 'f-ratio' is defined as the ratio of new production

The 'f-ratio' is defined as the ratio of new production to total production;

 $f-ratio = P_{new}/P_t$ 

where;

Eppley, (1981) showed that the f-ratio varied asymptotically with total production, ranging from 0.05 in oligotrophic oceanic regions to greater than 0.5 in productive coastal upwelling regions. The f-ratio has also been shown to vary temporarily within regions (e.g. McCarthy et al., 1977; Eppley et al., 1979; Glibert et

al., 1982; Platt and Harrision, 1985), and with depth, (e.g. Olson, 1980; Harrison, 1983; Ronner et al., 1983; Lewis et al., 1986). The f-ratio has also been shown to vary with ambient ammonia concentrations (e.g. Olson, 1980; Garside, 1981; Cochlan, 1986), and with ambient nitrate concentration (Platt and Harrison, 1985; Harrison et al., 1987).

The absolute magnitude of the f-ratio is quite disputable, owing to the limitations in our understanding of the nitrogen story, and problems incurred with the methodology employed in measuring nitrogen assimilation rates, (namely the 15-N technique). The uptake of reduced forms of nitrogen other than ammonia, (urea, for example), will lead to an underestimate in the f-ratio. The main problem incurred when measuring f-ratios in oligotrophic regions is that the ambient levels of ammonia are often undetectable (McCarthy, 1980). The addition of tracer levels of ammonia will increase the ambient ammonia concentrations by an unknown proportion and subsequently give rise to an overestimate of the rates of regenerated production. As a consequence the f-ratio would be underestimated.

The importance of heterotrophic nitrogen uptake is not as yet clearly understood. Any heterotrophic assimilation of nitrate, for example, would lead to an overestimate of new

production, and consequently an overestimate of the fratio.

For the above reasons, measurements of the f-ratio cannot routinely be assumed to be either under or overestimates.

## 2.8. The C:N ratio

Based on oceanographic observations and calculations Redfield, (1934) concluded that the cellular composition of plankton should fall within a narrow range of values. The ratio of carbon to nitrogen to phophorous in the plankton was calculated as 106:16:1. Assimilation ratios of carbon to nitrogen should, over the life time of a cell, approximate to this ratio, (C:N = 6.6). Measurements on natural and cultured algae, revealed, however, that the ratio was variable (Ketchum, 1939; Mackereth, 1953).

Droop, (1968) hypothesized that the cell nutrient content controlled the plankton growth rate, and thus the C:N ratio could be related to the growth rate of the organism. Goldman and McCarthy, (1980) went further and stated that "the change in cellular chemical composition as a function of varying growth rate is a biochemical response to different degrees of nutrient limitation."

Near maximal growth rates have been reported at C:N ratios approximating to the ratios of Redfield, e.g. Goldman, (1980), but if some other factor such as light, is controlling the growth rate (Droop, 1974; Droop et al., 1982), then algae may have the same ratio at low growth rates. Tett et al., (1985), from observations of cultures and natural populations, stated that the relationship of C:N ratios to growth rate was "one-many", implying that their was no unique interpretation to be gleaned from a particular C:N ratio. Samples of natural plankton often contain substantial amounts of zooplanktonic, microbial, or detrital organic material with C:N ratios very different to that of algae.

Ranges of C:N ratios are quoted as 5.3-14 (Goldman, 1980) for oceanic particulate matter, 7.1 for nutrient sufficient phytoplankton (Goldman *et al.*, 1979), and 8.9 for oligotrophic open oceans (Perry, 1976).

The use of C:N ratios in interconverting between carbon and nitrogen fluxes in natural populations is subject to some difficulty, due to the inter-species variability as stated above. An emerging problem is due to the role of the picoplankton in nitrogen uptake. The importance of the picpolankton (<1  $\mu$ m), in ammonia uptake was measured by Probyn and Painting, (1985). They found that 50% of the

total uptake was due to the smaller fraction in Antarctic waters. Wheeler and Kirchman, (1986) differentiated this fraction into prokaryotes and eucaryotes by the use of protein synthesis inhibitors. They found that as much as 78% of the ammonia was taken up by the prokaryotes, a significant part of which were the heterotrophic bacteria. Contrary to the review of Billen, (1984) who concluded that amino acids were the most significant source of nitrogen to the bacteria, it would apear from new evidence the bacteria are consuming a considerable fraction that the of ammonia in the euphotic zone. Because the calculation of overall carbon assimilation includes a bacterial component and bacterial C:N assimilation ratios (3-4) are significantly lower than algae, the calculation of nitrogen flux from carbon flux by conventional C:N ratios of 6, will on occasion underestimate the nitrogen flux.

## 2.9. "Predicting the vernal blooming of phytoplankton."

Sverdrup wrote a paper in 1953 entitiled " predicting the vernal blooming of phytoplankton," in which he outlined a model whereby the phytoplankton bloom could be predicted from the calculations of depth of mixing, compensation depth and critical depth. These terms will be defined and the limitations imposed by their associated assumptions discussed. That a phytoplankton bloom exists *per se*, is open to debate. What is evident is that there is an annual ecological succession in the phytoplankton (Margelef, 1967), which can only proceed if excess production is invested into the system. For this reason the process of succession is linked to an increased stability in the water column. The terms "bloom" and "succession" are therefore interchangeable in this context.

Recently it has been envisaged that there exist two distinct forms of ecosystem structure in the sea (Cushing, 1989). One form is found in strongly stratified water (oligotrophic ocean), and the other in weakly stratified areas. Because this thesis is considering primarily open ocean oligotrophic systems, only the first form is of relevance, and thus the development of stratifaction is discussed in relation to this ecosystem structure.

#### 2.9.1. The compensation depth

The compensation depth is that depth where the energy intensity is such that gross photosynthesis balances destruction by respiration. The term 'destruction by respiration' is inclusive of both the autotrophic and heterotrophic components of respiration, and losses due to death and predation. Above the compensation point phytoplankton will end their daily cycle with a net increase in organic matter. Below this depth, "photosynthesis may still go on but there is a net loss of oxygen and life becomes impossible" (Marshall and Orr, 1928).

By the use of the oxygen technique we can define the compensation depth for the phytoplanktonic community as that depth where the net community production over 24hrs is zero. The compensation depth is shown schematically in figure 2.9.(2).

Theoretically photosynthesis is directly related to the photosynthetically available radiation (PAR), and so the compensation depth can also be derived from optical data. The expression is (from Tett, 1990);

 $Z_{\rm comp} = \ln (m_2 \times I_0'/I_c) \times (1/y(\min))$ 

#### where;

 $Z_{comp}$  = compensation depth  $M_2$  = correction factor for the additional attenuation of polychromatic light near the sea surface  $I_0'$  = 24hr mean PAR beneath the sea surface  $I_c$  = 24hr mean PAR at the compensation depth y(min) = minimum value of the diffusion attenuation coefficient

The value for  $I_C$  is derived from;

 $a \times I_c = r_b$ 

#### where;

a = the mean photosynthetic efficiency  $r_b$  = the mean respiration at the compensation depth.

The mean photosynthetic efficiency and mean respiration rate are assumed not to vary diurnally. When calculating the compensation depth from optical data, only autotrophic respiration is considered. The compensation depth therefore becomes that depth where net primary production is equal to zero and not the depth (as in the oxygen technique, which considers both autotrophic and heterotrophic

respiration) where net community production is equal to zero.

The value for the 24hr mean PAR at the compensation depth  $(I_C)$  was derived from oxygen measurements taken in the English channel (Jenkins, 1934). This value was later used by Sverdrup in his model of the critical depth (Sverdrup, 1953). The use of this value has resulted in the compensation depth often being assumed to be at the one percent light depth. It has been shown however that it may often extend below this.

### 2.9.2. The critical depth

"In order that the vernal blooming of phytoplankton shall begin it is necessary that in the surface layer the production of organic matter by phytoplankton exceeds the destruction by respiration" Sverdrup, (1953).

Because the photosynthetic rate is dependant on photosynthetically available irradiation but respiration is not, light must be a regulating factor in ascertaining this depth. Equally important, in such a concept, is the depth of mixing (Gran and Braarud, 1935). Heat input, convection and wind driven turbulence set up seasonal and diurnal

cycles of mixing and stratification in the ocean. The water column is structured into layers as shown in figure 2.9.(1). The time of mixing is a few hours in the surface wind mixed layer, a few days in the diurnal theromocline, and a substantial fraction of the year in the seasonal thermocline (Tett, 1990). Phytoplankton in the mixed layer will experience fluctuations not only due to the diurnal variation in PAR, but also from transport in eddies. If this mixed layer extends below the illuminated surface layer, in order for growth to occur, the plankton must spend more time above than below this layer. (In other words, spend more time above than below the compensation depth.)

"A depth must exist such that blooming can occur only if the depth of the mixed layer is less than this critical value" (Sverdrup, 1953). Such a depth is the critical depth, (figure 2.9.(2)).

For Sverdrup's model of the critical depth to work several assumptions have to be made. Photosynthesis is assumed to decrease logarithmically with light, to suffer no light inhibition at the surface, and to be controlled entirely by the light regime, and not be limited to any degree by nutrients. Respiration is assumed linear and constant with depth. The plankton are assumed homogeneous and

distributed evenly throughout the mixed layer.

Combining production measurements (and applying these assumptions) with optical data, an expression can be derived (from Tett, 1990) such that ;

$$Z_{crit} = (a/r^{D}) \times (m_2 \times I_0'/y(min))$$
  
= (m<sub>2</sub> × I<sub>0</sub>'/I<sub>c</sub>) × (1/y(min))  
(for definitions see above).

Smetaceck and Passou, (1990) challenged the current use of this model of critical depth, and suggest that it is put "to little if any practical use". Their reasoning for this is due to the assumptions that are inherent to the model. Their main criticism is that the assumption is made that 'respiration is constant with depth', and when applied, is assumed values such as 10% of gross production (e.g. Parsons et al., 1984; Tett and Edwards, 1984). The assumption that the mixed layer *is* well mixed may also not hold, as recent evidence (Lal and Lee, 1988) suggests that this layer is not well mixed over the time scale of 2-3 weeks.

It should be pointed out that Sverdrup was aware of the limitations of his model, and that criticism should be addressed at the misinterpreters. Sverdrup stated that phytoplankton may in fact spend more time above the com-

pensation depth if turbulence is weak, or may be unevenly distributed if they show a positive phototaxis. He also pointed out that any conclusions obtained from modelling the critical depths will be greatly modified if grazing rates are high, - production may be occurring but the population will remain small.





## Figure 2.9.(2)



The compensation depth and the critical depth

When Pg - R = 0; D = Zcomp When ZPg - ZR = 0; D = Zcrlt

where; R = respiration Pg = gross production ZPg = Integrated Pg

D = depthZcomp = compensation depth ZCrit = critical depth

ZR = Integrated R

<u>Chapter 3</u>

# TECHNIQUES FOR THE MEASUREMENT OF PRIMARY PRODUCTION

## Chapter 3.

### Techniques for the measurement of primary production.

When the first attempts were made to measure rates of primary production, the analytical precision of the technique employed was of foremost importance. With only one method being used, namely the oxygen Winkler titration (Gaarder and Gran, 1927), the accuracy of the technique was uncontestable. Today, faced with several techniques trying to measure the same process, and no-longer held back by precision limits, we are forced to question the accuracy of our measurements, and ask just what exactly it is that we are able to measure so precisely.

The terminology used in this section has been defined elsewhere, (chapter 2). Here the different techniques being used in this study will be discussed, compared, and a consensus of what we are able to measure by each method outlined. However, before a comparison of any of the techniques can be made the limitations of *in vitro* methodology itself must be addressed.

## 3.1. Problems associated with bottle confinement.

The main problem associated with measuring primary production in bottles (*in vitro*), is that the population in question is artificially confined to an enclosed water sample at a fixed depth. Bottle confinement subjects the population to possible bottle toxicity effects, a lack of internal turbulence, a lack of interaction with the external environment, and a fixed intensity of illumination.

When making concomitant measurements of oxygen production and carbon dioxide consumption, the problems associated with subjecting a population to a fixed illumination become of paramount importance.

The circulation time of a population in the surface mixed layer is about 40 minutes (Harris, 1984). A population enclosed in a bottle is confined to a certain irradiance for a much longer period, meaning that cells may become photoinhibited. The first response of cells to changes in irradiance is at the level of electron transport (Harris, 1980) causing subsequent physiological changes, such as the size and number of photosynthetic units (Falkowski, 1980). Irradiance has a direct affect on photosystem II, and thus will affect the production of oxygen directly,

and the linkage between carbon fixation and oxygen indirectly (Goldman et al., 1981).

Another problem associated with in vitro productivity measurements is the choice of length of duration of the incubation. Though not unique to in vitro measurements, the problem is more pertinent to a confined population than one in the open environment. The time of duration incubation leads to many assumptions chosen for the being made about the community and its response to the environment which are often not valid. A serious assumpmade is that populations are at a steady state tion during the length of their incubation. Evidence points to the fact that phytoplankton are actually not at steady state, and if fluctuations in environmental conditions are occurring on the same time scales as the physiological response time of the organism, there will be a lag between the environmental change and the observed response (Harris, 1984). On small, spatial, and temporal scales there is evidence of non steady state nutrient fluxes, their uptake and their storage. At non steady state, cell storage becomes much more important, as phytoplankton attempt to buffer their environment to achieve maximal growth rates. Harris and Piccinin, (1977) demonstrated that carbon fixation was most rapid in the first few minutes of an incubation, meaning the longer the incubation, the slower the apparent rate of

photosynthesis. When adding tracers to bottles such as the addition of 15-N, rapid initial uptake may occur, leading to a non linearity of uptake with time (Goldman *et al.*, 1981). Measured uptake may also not be directly attributable to photosynthesis, but may occur in order to replenish storage pools.

In bottle confined populations depletion of nutrients may also become a problem for incubations of any duration of time (Venrick et al., 1977).

Primary production is far from constant in space and time, and the way in which rate measurements are averaged will have a profound effect on the results (Platt and Harrison, 1985). Incubations of less than a day pose problems when extrapolated to daily rates, as assumptions of steady state, and linear incorporation rates are made. Likewise, daily rates integrated into weekly or yearly fluxes can only ever be at the best gross estimates.

With bottle confined estimates of production, losses due to mortality and the effects of immigration, and emigration, and grazing pressures cannot be estimated (Harris, 1986). There may even be changes in the taxonomy of the confined population (Venrick et al., 1977).

Gieskes et al., (1979) showed that the size of bottles in incubations was important and that mortality was more likely to occur in the smaller bottles. It is pertinent to note that in comparative studies between different techniques, the bottle sizes are invariably different.

Results from bottle confined incubations have been termed "manifestations of contemporaneous disequilibrium" (Hutchinson, 1961). Although a little erudite, this phrase captures the essence of the problem with in vitro incubations; - natural populations are not confined to a certain parcel of water at a certain depth, with no fluxes of nutrients or variability in the light field, and no grazing pressures upon the community.

## 3.2. In situ versus in vitro incubations

Despite the limitations of bottle confinements, in vitro sampling remains the most commonly used method for productivity studies. This is mainly due to the problems associated with in situ techniques. In situ techniques suffer from difficulties arising from the spatial heterogeneity of the oceans. Horizontal advection, turbulent mixing of water masses, in addition to transfer of gases across the air sea interface, make the interpretation of

in situ data as in situ rates difficult. In freewater in situ measurements, drogued marker buoys are employed to follow a sampled water mass. Details of the design of drogues and their ability to accurately track a water mass are described in Tijssen and Wetsteyn, (1984); McCormick et al., (1985); and Scavia and Fahnensteil, (1987).

Comparisons of in vitro and in situ rate measurements have been made. Some report agreement between the methods, (e.g. Williams et al., 1979; Davies and Williams, 1984; Fahnenstiel and Carrick, 1988; Williams and Purdie, 1991), and some report disagreement (e.g.Johnson et al., 1981; Daneri, 1990). The difficulties in interpolating between the two methods means that even that data which is reported as showing good agreement, has inherent problems associated with it. For example, Davies and Williams, (1984) compared in situ rates of oxygen production in a nominally 100m<sup>3</sup> body of water enclosed in a bag, with in vitro oxygen changes. The agreement was very good except for a small discrepancy in the night in both experiments. The reasons for this were not known. Williams and Purdie, (1990) found in situ and in vitro rates were not in serious discrepancy from work in the central north Pacific Gyre. The determinations were not made concurrently, however, and so discrepancies of a factor of two were unresolvable.

The differences between the *in vitro* and *in situ* methods are often quite large. Daneri, in press) for example, compared *in situ* changes in dissolved oxygen concentration with *in vitro* incubations in the southern North sea, and found that the *in vitro* rates accounted for only 50% of the observed *in situ* change.

Overall, the bulk of such comparisons between in situ and in vitro methods have been made using 24hr in vitro incubations and much longer time periods of in situ incubations (Schulenberger and Reid, 1981; Platt, 1984). The difficulties in reconciling in situ and in vitro rates become even more pronounced as different time scales are involved.

## 3.3. The O2 Winkler technique.

The methodology of the oxygen technique is discussed in chapter 4.3.1. In summary, the oxygen Winkler technique is used to measure rates of primary production by the change in oxygen concentration in vitro, after a definitive period in the light and in the dark. The total change in oxygen concentration in the light is termed net community production  $(P_C)$ ; the change in the dark, community respiration (R); and the sum of the two, gross production  $(P_g)$ . Because respiration is measured by the change in oxygen concentration in the dark, the assumption must be made that respiration is the same in both the light and the dark. This is probably the most important problem associated with the oxygen technique.

## 3.4. The 14-C technique

The methodology of the 14-C technique is discussed in chapter 3.4.5. The 14-C technique measures the rate of primary production by the incorporation of radio-labelled carbon into the cell *in vitro* in the light. Dissolved inorganic carbon is incorporated into the algae by photosynthesis, where it either remains as particulate organic

carbon, or is excreted into the water as dissolved organic carbon. By using a radio-tracer, the incorporation of carbon into the particulate fraction from the dissolved fraction can be measured quite easily. However the 14-C technique cannot routinely be used to measure respiration. During the incubation period, neither the extent that intracellular unlabelled carbon is being assimilated, nor the amount of radio-labelled carbon that is assimilated is being released back into the isotope pool, can readily be estimated. We are therefore unable to determine whether the rate measured is that of gross production or net primary production or some value between the two. This has been the cause of considerable debate in the literature, and is unsatisfactorily concluded as first one, then the other, and then settled as a value somewhere between the two, depending on environment, incubation times, light, Far from being solved by the passage of time, the etc. problem seems only to have increased as new data suggests that in fact the method may be measuring a rate less than net primary production. So what actually is the 14-C technique measuring?

3.4.1. Interpretation of the production rates from the 14-C technique.

The introduction of the 14-C technique offered a precise, and easy method to measure the apparent flux of carbon, directly. It soon became evident, however, that the interpretation of the results was not as easy as the measurement itself (Strickland, 1960; Harris, 1986).

The question of whether 14-C measures net or gross production was first addressed by Ryther, (1954) when he found that 10-20% of the carbon being assimilated in a 2hr incubation was subsequently lost over the next 4hrs of the incubation. Further experiments with fully labelled *Dunaliella* (Ryther, 1956) revealed that over a 24hr period in the light, no assimilated 14-C was being respired, but 20% was lost in the dark. Steeman Neilsen, (1955), however, found with cultured *Chlorella*, that 14-C was being respired in the light and that 60% was being reassimilated in photosynthesis. He calculated that if respiration is 10% of photosynthesis, the 14-C will return a result that is 94% of gross production.

The next obvious requirement was to develop a means of assessing the respiration term. Steeman Neilsen and Hansen, (1959) reported a method for estimating respiration

rates using 14-C. The method involved measuring carbon assimilation at various light intensities, and extrapolating the resultant curve back to zero to determine the respiration rate. The calculation, however, required too many assumptions to be made: that photosynthesis is linear at low light levels, that respiration is not affected by light intensity, and that excretion of dissolved organic carbon (DOC) does not differ at different light intensities. The method subsequently had to be abandoned.

In 1963, a review on the correction factors needed in calculating results from the 14-C technique was published (Thomas, 1963). Corrections were made for the isotope effect, excretion, dark uptake and respiration. When a number of workers corrected their 14-C data for the release of DOC they found that the rates they were measuring were less than net community production rates as measured by the oxygen technique (e.g. Eppley and Sloan, 1965; Antia et al., 1963; and McAllister et al., 1964). It soon became apparent that the early comparisons between the two techniques had been biased by the standardisation procedure for the 14-C stock solutions when a new protocol was published by Steeman Neilsen. However, even when using the new standardisation procedure and corrections for DOC release, Ryther et al., (1971) working in the Peru coastal current, went on to show that 14-C determined

rates were still a factor of 2 smaller than the net community production rates from oxygen.

Parsons et al., (1969) introduced particle size analysis to measure net primary production by the net change in particle volume. On comparison with the 14-C technique it shown that 14-C underestimated net primary was again production. Their conclusions, substantiated by work of Sheldon and Sutcliffe, (1978), who measured the change in adenosine triphosphate (ATP) in the small particle size fraction, was that 14-C labelled carbon was being recycled during the course of an incubation. The microplankton generation time was as low as 3hrs, which suggested that carbon was being recycled as many as four times in a 24hr incubation. Sorokin, (1971) found that in oligotrophic areas bacterial production could be several times greater than 14-C uptake. That 14-C estimates must be low was argued by Sorokin, (1971a) as the rates of production so obtained did not appear substantial enough to support the heterotrophic community.

A fundamental problem faced when making measurements with 'natural' plankton communities, is that the microheterotrophs are not discriminated. This problem is heightened in comparative studies between oxygen and 14-C methods as their interference with the tracer carbon flux is not the same as with the oxygen flux, and will therefore bias

comparisons between the two (Smith et al., 1984). There is as yet no established technique for measuring the heterotrophic consumption of primary production (Joint and Morris, 1982). Current estimates are very imprecise with a range in percentage error of 10 to 80 % (Joint and Morris, 1982; Bauerfeind, 1985). However there is evidence that the heterotrophic component may consume most of the primary production in some microplankton assemblages (Packard and Williams, 1981; Williams et al., 1983; Holligan et al., 1984; and Laanbroek et al., 1985). For example, Lochte and Turley, (1986), found heterotrophic bacterial production to be in excess by 20% of primary production in waters off the Isle of Man. Larsson and Hagstrom, (1979) showed that the assimilation of carbon by bacteria continued at the same rate over a 12hr incubation, assimilating and respiring the DOC as fast as it is released by the plankton, while the phytoplankton assimilation rates slowed markedly after the first few hours.

Smith et al., (1984), modelled 14-C and oxygen flux measurements and, considering the trophic interactions, showed that the active biomass of microheterotrophic organisms was large compared with that of the autotrophs, a situation which may be generally applicable to the pelagic zone of the open ocean. Their model attempts to show how the 14-C technique can measure something comparable to gross

production but which is in fact something very different. A large proportion of the 14-C accumulates in the heterotrophic biomass during the incubation, and will be measured concomitantly with the autotrophic pool. Coincidence of magnitude of 14-C uptake, and gross production as measured by the oxygen technique, is a consequence of a low autotrophic respiration rate, (i.e gross production approximates to net production), and the presence of a large heterotrophic population with a relatively slow turnover time.

The differential effect of light on oxygen and 14-C measurements was investigated by Harris and Lott (1973), Harris and Piccinin, (1977). They found that at low light 14-C compared well with gross production from the oxygen technique, but at high light intensities the agreement was poor. Photoinhibition was manifesting itself through; i), a rapid increase in respiration and a decrease in net production, which was characteristic of photorespiration; ii), a slower decline in gross production, which parallelled the decrease in chlorophyll.

To understand what exactly the 14-C technique is measuring it is necessary to understand the pathways by which it is taken up into, and released from, the cell. This approach is taken from a scheme used by Professor Williams. The assimilation and release of carbon into, and out of, the

cell is depicted in a four box diagram (figure 3.4.(1)). The cell is divided into photosynthetic (P), and respiratory (R) pools. These are then subdivided into inorganic (I) and organic (O) pools. The carbon can either be in the dissolved (D) or particulate (P) fraction. Figure 3.4.(1)b. shows the assimilation of 12-CO<sub>2</sub> from the dissolved inorganic pool to 12-CH<sub>2</sub>O in the photosynthetic particulate organic pool. The formation of CH<sub>2</sub>O is termed gross production. Respiration involves the reduction of CH<sub>2</sub>O in the respiratory partculate organic pool and its release as CO<sub>2</sub> into the dissolved inorganic pool. There are two more pathways of concern in the cell, shown in figure 3.4.(1)c. One is the transfer of organic material from the photosynthetic to respiratory pool (PGO -> PRO) and the other, the recycling of respiratory  $12-CO_2$  from the respiratory particulate inorganic pool to the photosynthetic particulate organic pool for subsequent assimilation.

Now consider the pathway of 14-C. The 14-C is added to the dissolved inorganic pool as sodium bicarbonate. It equilibriates with the photosynthetic particulate inorganic pool. Ideally 14-C follows the same pathway as 12-C (figure 3.4.(1)d., and is assimilated into the cell at the same rate as 12-C. A discrimination factor of 5% is employed in the calculations of 14-C assimilation rates to account for isotopic discrimination between 14-C and 12-C.



Figure 3.4.(1)d. shows the ideal situation where 14-C is measuring gross production. From knowing the initial 12-C concentration, the amount of 14-C added, and the final 14-C concentration in the particulate fraction, the gross production rate can be calculated. However, as has been shown, recycling may occur from the respiratory particulate organic to photosynthetic particulate organic pool. This will bias the estimate of gross production as the isotopic signature of the photosynthetic particulate inorganic pool will be diluted by recycled 12-C. The specific activity of the photosynthetic particulate inorganic pool can no longer be assumed to be the same as in the external dissolved inorganic pool. Only if there is no recycling can 14-C be measuring gross production. Further more, if any 14-C is transferred from the photosynthetic particulate organic pool into the respiratory particulate organic pool, and subsequently respired, the 14-C will be returned to the dissolved inorganic pool and thus the resultant production rate will tend towards a measure of net primary production depending on the extent of respi-This will depend to a large degree on ration of 14-C. the length of duration of the incubation. Initially only 12-C can be respired, but as more substrate becomes labelled so more 14-C can be respired. If 100 percent respiration of 14-C is occurring, the 14-C technique will measure net primary production. An important factor that must be considered is the possibility of a greater isotop-

ic discrimination between 14-C and 12-C occurring. That a factor of 5 percent is universally used may in fact be quite erroneous.

Consider again the ideal situation when 14-C is measuring gross production. As has already been shown, error will occur due to recycling from the respiratory particulate inorganic to photosynthetic particulate inorganic pools. If at any stage the cell is discriminating between 12-C and 14-C an additional error will occur. The ratio of 12-C to 14-C is assumed to be constant throughout the passage through the cell. If 12-C is being selected in preference to 14-C in the transfer from dissolved inorganic to photosynthetic particulate inorganic pools the final production When considering the situarate will be underestimated. tion where 14-C is measuring net primary production, if discrimination is occurring between the transfer of carbon from the photosynthetic particulate organic to the respiratory particulate organic pool, net production will be overestimated. The extent of isotopic discrimination may well be greater than 5 percent. It may quite possibly be species dependent, and may differ in the photosynthetic and respiratory pathways. It cannot be discounted that the discrimination factor of a cell may alter throughout its life history, for example a cell in conditions of low light intensity may not be able to afford the extra energy required in taking up the heavier isotope and so increase
the discrimination factor. At no stage is the cell obliged to assimilate 14-C, as 12-C is never in limited supply.

From these diagrams we can see the 14-C technique returns a rate of production equal to gross, or net, or somewhere between the two. But how can we explain 14-C returning a rate less than net primary production ?

Excretion involves the loss of carbon from the particulate organic pool to the dissolved organic pool. Chemical methods of measuring the change in 12-C are not affected by excretion as the change in total dissolved inorganic carbon is measured. However, if excretion is occurring, rate measurements made by the 14-C technique will be affected, as the assimilated 14-C in the particulate fraction is returned to the dissolved phase and so not accounted for as production. If the 14-C is subsequently taken up by bacteria into the particulate fraction, the 14-C will be accounted for, but if taken up by bacteria of a smaller size fraction than that retained on the filter, it will not be measured.

A final consideration is that carbon may be assimilated and incorporated into volatile molecules such as methane  $(CH_4)$  which will be lost from the filter. This again will not affect 12-C production measurements, as the net loss from the dissolved inorganic pool is still the same.

#### 3.5. The TCO2 technique

Comparisons between oxygen and 14-C rate measurements are limited by the fact that the two techniques do not share the same systematic errors (Williams and Robertson, 1991). A method that compares 'like' with 'like' was needed, thus for comparisons with the oxygen technique, a chemical method that did not involve tracer flow was sought. Johnson et al., (1983) compared oxygen measurements with TCO2 measurements from infrared photometry. The change in oxygen and TCO<sub>2</sub> was followed in free water masses over 24hrs, as an estimate of net community production. The expected correlation was not found between the two methods, with short term  $TCO_2$  and oxyggen variation showing significant departures from theoretical R.Q. and P.Q. values. The authors looked to the methanogens as possibly playing a more significant role in the carbon flux than originally assumed. This could be through the metabolism of the nonconservative gases, methane, carbon monoxide and hydrogen allowing for a greater flexibility in the range of P.Q. values. However, the estimates they made were 100 fold those reported in the literature for 14-C in the same area, and the measurements may very well have been due to an 'analytical artifact'.

In an earlier paper, using the same methodology, they report an 'enigmatic disparity' between the  $TCO_2$  and

oxygen measurements made in the North-Western Caribbean sea. Reported molar rates of net TCO, production were 2 -6 times greater than those of oxygen. Accompanying "precise" DOC data gave a good negative correlation with the  $TCO_2$  data, and suggested a time lag between the  $TCO_2$  and DOC release of 2.5hrs. They report an excretion rate of 9.5-62.3% of photosynthate into the open water. This is in accordance with that reported by Anderson and Zeutschel, (1970), in the same area, and the average value of 24% from the literature (Sieburth, 1968). Again they were aware at this stage of the possible sources of error in their calculations, (atmospheric exchange, carbonate dissolution, chemosynthesis, precipitation, or photorespiration, bacterial anoxyphotosynthesis in addition to analytical error). In none of the oxygen and TCO<sub>2</sub> comparisons was there a significant correlation. Bender et al., (1987), compared TCO2 by coulometry with oxygen and 18-0 measurements in experimental coastal ecosystems, (the Merl tanks). The molar rates they obtained from TCO, were higher than from oxygen, but only marginally, ( mean P.Q. = 0.98). The higher TCO<sub>2</sub> rates were attributed to a greater percentage of carbohydrate synthesis relative to protein synthesis.

Irwin (1991) made a comparison of *in vitro* rate measurements from  $TCO_2$  (by coulometry), oxygen and 14-C, in

natural assemblages of coastal phytoplankton in the Bedford basin. The agreement between  $TCO_2$  and oxygen was significant, with P.Q. values in the range 1.84 to 1.07. The 14-C method also agreed well with gross production from  $TCO_2$  over 24hr incubations, regression analysis revealing a 93% confidence limit on the comparison. It is implicit, however, in the methodology that the zero time samples were analysed together, and the light and dark samples analysed 24hrs later. Although the replication is quoted as having a typical coefficient of variation of 0.1% no account has been made for interbottle variability, nor for the day to day variability in the instrument, or the within day, instrumental drift. The precision of replication, I feel, is therefore biased. Williams and Robertson, (1991), compared oxygen and TCO2 rates both in situ and in vitro from an experimental mesocosm (Davies and Williams, 1984). In situ rates were not corrected for air sea exchange, or physical mixing. The in vitro rates have the 'highest precision' of the data sets, (not quoted), and fall within the expected P.Q. range of 1-1.36.

There is little data to be found comparing oxygen and  $TCO_2$  respiration rates. Johnson *et al.*, (1983) report low R.Q. values from oxygen and  $TCO_2$  which they attribute to bacterial activity occurring in anaerobic microzones within the water column. Robertson, (1989) reports R.Q. values in the

range 0.52 to 2.87 from work in mesocosms.

#### 3.6. The 18-0 technique

The  $H_2^{18}O$  technique offers an almost definitive measure of gross production (Bender et al., 1987). Water is enriched with the stable isotope  $H_2^{18}O$ . Photosynthetic oxygen evolution results in an increase in 18-O in the dissolved gas phase. Even though the 18-O technique is a tracer technique, it does not share the same limitations of the 14-C or 15-N techniques. This is because all the photosynthetically produced oxygen ends up in a single welldefined phase (dissolved oxygen), and the ambient pool of dissolved oxygen is comparatively very large ( 250  $\mu$ mol). The effect of respiration can be shown to be negligible. Calculations show that the only possible effect of respiration would be smaller by a factor than the expected precision (0.2 o) of the technique.

A possible error will incur with the technique if some photosynthetic oxygen is consumed within the cell immediately after its production. This will not affect the 18-0 concentration in the surrounding solution and, therefore, will not be counted as production. This intracellularily recycled oxygen can not be discriminated (Bender et al., 1987). Fractionation of the isotope can

also occur (Stevens et al., 1975; Kroopnik, 1975). However, with a large 18-0 enrichment of the water the effect of this fractionation has been shown to be negligible (Grande et al., 1988).

Comparison of 18-0 and oxygen production rates can be used to assess the difference between dark and light respiration rates (Grande et al., 1989). Dark respiration rates are calculated from the oxygen technique.

 $Pg(O_2) - Pc(O_2) = Rd$ where;  $PgO_2 = gross production from O_2$  $PcO_2 = net community production from O_2$ Rd = dark respiration

Light respiration rates are then calculated from the difference between gross production from 18-0 and community production from oxygen.

 $Pg(18-0) - Pc(0_2) = Rl$ where; Pg(18-0) = gross production from 18-0Rl = light respiration.

#### 3.7. The 15-N technique.

Measurements of total, new and regenerated production from 15-N tracer techniques. Problems inherent to the methodology and limitations imposed by our interpretation of the fluxes of nitrogen in the euphotic zone.

It is now generally accepted that nitrogenous nutrients play a central role in controlling primary production. The current paradigm is that ammonia, regenerated by the heterotrophic population, is cycled rapidly through the community in the euphotic zone of the oceans. At steady state, if no losses were incurred to the system, ammonia would be able to act as the sole sustaining nitrogen source - indefinitely. Because however losses incur, (sinking, mixing, death, predation, fish harvest, etc.), new nitrogen is introduced into the system, namely in the form of nitrate, produced at depth and mixed or upwelled into the euphotic zone. The assimilation and availability of nitrate is, therefore, assumed to be the controlling mechanism for net phytoplankton growth to occur.

For this scenario to work, it must be assumed that the heterotrophic consumption of phytoplankton and the recycling of nitrogen in the euphotic zone, results in the release of ammonia, that nitrate production in the

euphotic zone is negligible, and that any additional supplies of nitrate (from atmospheric or riverine inputs, for example), are negligible.

New and regenerated production rates are routinely measured from 15-N tracer techniques, in which a tracer addition is made to an incubated sample with an often undetectable ambient nitrogen concentration. Due to the inherent problems of making truly tracer additions, and the bottle confinement effects, the resultant measurements have been said to be "potentially artificial" (Ward et al., 1989). The correction factors employed for estimating the assimilation rates, are also not without problems, and are currently being debated in the literature, (e.g. Glibert et al., 1982a, 1985; Laws et. al., 1984, 1985; Harrison, 1983).

Recently, in the context of nitrogen based production, the importance of processes, other than nitrate or ammonia uptake, have been discussed. What, for example, would be the implications to new production measurements if nitrate is being *produced* in the euphotic zone? And where does the assimilation and production of nitrite fit into the current paradigm?

I will endeavour to address the following questions:
The problem of adding a truly tracer amount of sub-

strate.

2) The errors incurred when calculating 15-N rate measurements.

3) The importance of processes other than nitrate or ammonia uptake.

# 3.7.1. The problem of adding a truly tracer amount of substrate.

In 15-N tracer experiments it has become accepted practice (after Dugdale and Goering, 1967) to add labelled substrate at a concentration of 10% the ambient nitrogen levels to ensure an artificially enhanced nutrient regime is not created. The uptake of nitrate and ammonia can be explained by Michaelis-Menten kinetics (MacIsaac and Dugdale, 1969);

where V is the specific uptake rate, Vmax is the maximum specific uptake rate, S is the substrate concentration, and Ks is the half saturation constant.

The value of the half saturation constant sets the upper limit whereby , the response of the nutrient uptake to the

ambient nutrient concentration can be aproximated to first order kinetics, (i.e with a linear response curve). Thus, in situations where the ambient levels are below the half saturation constant, the addition of a 10% tracer will acount for a 10 % increase in uptake rate. It has become common practice not to correct for tracer additions of 10 to 15 % and to accept this as experimental error (McCarthy, 1980).

In oligotrophic surface water the ambient levels of nitrate and ammonia are often at, or below, the limits of detection by routine analytical methods necessitating the application of truly tracer additions. At such low nutrient concentrations, it has been shown (Harris, 1983), that even the smallest addition of a tracer can result in relatively large changes in the ambient substrate levels in an incubation vessel, and can significantly affect the uptake rates measured. When the ambient levels are undetectable, and a tracer addition is added qualitatively equivalent to the minimum limit of detection - it follows that the minimum enrichment must be 100%, and therefore calculated uptake rates will have a minimum error of 100 % (McCarthy, 1980).

To compensate for the impracticability (at the time) of making truly tracer additions, McIsaac and Dugdale, (1972)

devised a modification of the equations used in 15-N calculations. The uptake of substrate, after an addition of a larger amount of tracer, was determined and then back calculated with an independently obtained half-saturation constant to estimate the uptake rates at ambient concentrations. This was (and is not) an acceptable method, however, as it is impossible to relate calculations derived from a saturating pulse of substrate, to natural uptake rates. The ambient substrate levels will be greatly increased resulting in rapid response nutrient uptake (Glibert and Goldman, 1981), and in the case of large relative ammonia additions, possible inhibitory effects on nitrate assimilation (e.g. MacIsaac et al., 1977; Garside, 1981).

3.7.2. The errors incurred when calculating 15-N production rates.

Collos, (1987) stated that all the calculations used in the 15-N methodology make assumptions which have rarely been explicitly stated in the literature. There appear to be three major assumptions made, when making the calculations which are probably not realised in practice.

- That the 15-N isotope, in the incubated medium, is not diluted by the remineralization of organic matter produc-

ing unlabelled substrate.

- That there is no change in particulate nitrogen during the cause of the incubation.

- That there is no effect from the simultaneous uptake of nitrogen sources other than the labelled one.

Each of these points will be discussed in turn.

Harrison and Harris, (1986) measured 15-N ammonium uptake and remineralization rates off the coast of Hawaii and in the Sargasso sea. They found that isotope dilution from the *in vitro* production of unlabelled substrate was significant and, if unaccounted for would result in an underestimate of the uptake rates by 1.5-3 fold. Glibert *et al.*, (1982) derived isotope dilution models for the uptake and remineralization of ammonia, and showed that the ammonia assimilation rates were being underestimated by a factor of 2. It was evident that the equations used in the calculations of assimilation rates needed to be examined in more detail. Although the calculations pertinent to the assimilation of ammonia are discussed here, the same arguments can be extended to nitrate assimilation calculations.

The original equations used in the calculation of ammonia assimilation rates are shown below (Dugdale and Goering, 1967);

 $PNH_4 = PN \times \frac{(15-N \text{ atom } \$ \text{ excess})}{(15-N \text{ atom } \$ \text{ enrichment})}$ 

#### Where;

PNH<sub>4</sub> = assimilation rate of NH<sub>4</sub> ( $\mu$ mol 1<sup>-1</sup>day<sup>-1</sup>) 15-N atom percent excess = 15-N atom percent particulate the natural 15-N atom percent.) 15-N atom percent excess = 15-N atom percent enrichment the natural 15-N atom percent)

The equations make the assumptions that changes in atom percent enrichment are negligible throughout the incubation, and that the rate of assimilation of nitrogen is constant with time. It has, however, been shown that phytoplankton can utilise ammonia rapidly during the initial minutes of an incubation following even the most tracer additions of substrate (Glibert and Goldman, 1981). Because remineralization is occurring throughout the incubation, an assumption can be made that the ratio of 15-N to 14-N will be diluted in a linear fashion. However, the models of Caperon et al., (1979), and Blackburn, (1979), predicted an exponential decrease in 15-N atom percent, with time. Their models were derived independently, but predicted much the same results, and are now referred to as the Blackburn-Caperon model. The model makes the assumption that the remineralization and uptake rates are

constant with time, and that all the ammonia being remineralized is unenriched with 15-N. The isotopic discrimination is also assumed negligible, and the ambient ammonia concentrations are assumed to change linearly with time.

Glibert et al., (1982) showed that to calculate the uptake and remineralization rates, without making such gross assumptions, one must be able to measure the initial and final concentrations of the ambient ammonia concentration, as well as the initial and final concentrations of the 15-N atom percent enrichment. Thus, again, the accurate measurement of the ambient nitrogen source is shown to be of paramount importance.

Inherent to the calculations of 15-N assimilation rates is the assumption that the total particulate nitrogen does not change during the incubation. Even in oligotrophic water, MacIsaac and Dugdale, (1969) and Eplley et al., (1977) have shown a change in particulate nitrogen of up to 7% during an incubation. It would appear that high uptake rates may lead to significant changes in particulate nitrogen during an experiment and thus invalidate this assumption.

The uptake of nitrogen sources other than that carrying the label, is an inherent problem with stable isotope

tracer work. Because an absolute tracer amount is not being measured, (as with 14-C), but a ratio is being measured, any unlabelled compound being taken up concomitantly with the labelled compound will contribute a different 14-N to 15-N ratio to that of the labelled compound (Collos and Slawyk 1985). This may in fact be the cause of the observed inhibition of nitrate assimilation from an increased concentration of ammonia (Collos, 1987) as reported by e.g. McCarthy and Eplley, (1972); Conway, (1977); MacIsaac et al., (1977); Garside, (1981). The in nitrate assimilation decrease observed as the concentration of ammonia is increased, may reflect the increasing ammonia uptake relative to nitrate uptake, and the resulting dilution of 15-N atoms in the final particulate nitrogen fraction by the 14-N atoms from the unlabelled ammonia. Thus the process may, in fact, be due to a calculation artifact and not a physiological phenome-Likewise the observed inhibition of urea uptake by non. ammonia (Horrigan and McCarthy, 1982) may also be a calculation artifact. They observed that 15-N labelled urea was totally inhibited by the presence of ammonia, whereas the uptake of 14-C labelled urea was not, which suggests that inhibition was due to an artifact of the the stable isotope technique. Evidence for the inhibitory effects of ammonia on nitrate uptake in batch cultures is controversial (Collos, 1989), owing to the difficulty in separating effects due to growth, from the

effects due to ammonia, on the culture. For example, experiments which show an increase in nitrate uptake rate as the ammonia levels are depleted, may not be exhibiting the reported release of ammonia inhibitory effects. Initial nitrate uptake rates per unit volume may be low, but per unit biomass may be maximal, due to an initial low biomass. Over time the nitrate uptake rates will increase, not resulting from a decrease in ammonia, but because of an increase in biomass per unit volume.

Another problem inherent to tracer work is that the labelled tracer may be excreted and subsequently reincorporated, which would lead to unaccountable for underestimates of the assimilation rates.

3.7.3. The importance of processes other than nitrate or ammonia uptake.

#### i. The role of nitrite.

When measuring new production it is assumed that nitrate is the main source of new nitrogen. However, recent evidence suggests that nitrate may also be a source of regenerated production. A source of regenerated nitrate is that produced by nitrification.

Nitrification is the term given to the oxidation of ammonia by bacteria, with a subsequent release of energy, and nitrate, with nitrite as an intermediate. The reaction is summarized by the following equations;

$$2NH_4^+ + 2OH^- + 3O_2^- -> 2H^+ + 2NO_2^- + 4H_2O$$
  
 $2NO_2^- + O_2^- -> 2NO_3^-$ 

Nitrification has only been demonstrated relatively recently in seawater. Studies on the depth distribution of nitrification (Wada and Hattori, 1971; Olson, 1981; Ward et al., 1984; Ward, 1987) showed that nitrification occurred at the bottom of the euphotic zone. Ward et al., (1989) went on to unequivocally demonstrate, using time course experiments with isotope dilution and 15-N transformation techniques, the occurrence of nitrification in the euphotic zone, and, although the relative magnitude of nitrification and assimilation will change over the diel period, (due to the opposing effects of light on the two processes), in situ nitrification was found to be a significant source of nitrate to the phytoplankton at depths of 5-10% surface PAR.

The significance of this source of nitrate to new production measurements is greatest at the nitricline, where

nitrate assimilation rates are high due to the ambient nitrate concentrations, and the light regime is high enough for growth to occur, but low enough not to inhibit nitrification. That nitrification could be a significant source of nitrate at the nitricline had previously been contested on the grounds that nitrification is light inhibited, with the threshold appearing to be 0.2-2.0 % surface PAR (Hooper and Terry 1974; Olson 1981; Horrigan et al., 1982).

The existence of the primary nitrite maximum observed near the thermocline in stratified oceanic areas, has been explained by the nitrification of ammonia to nitrite, by the heterotrophic component of the community (Wada and Hattori, 1971; Olson, 1981). It has also been explained by the excretion of nitrite (Kiefer *et al.*, 1976) observed in many unicellular algae assimilating nitrate (Eppley and Coatsworth, 1968, Carlucci *et al.*, 1970). Miyasaki *et al.*, (1975) found that 50-63% of nitrate being assimilated by the phytoplankton in the North Western Pacific ocean was being excreted back as nitrite. Harrison and Davis, (1977) measured rates of nitrate assimilation and nitrite production, and calculated that as much as 90 % of the assimilated nitrate was being released back into the environment as nitrite.

The consideration of nitrite as a possible source of

regenerated production must therefore be realised.

It becomes clear that perhaps we are not measuring true rates of new and regenerated production. If nitrification is occurring to a significant extent, nitrate will be a source of regenerated production as well as new production. Thus by measuring nitrate uptake as new production only, we must be overestimating new production rates. It follows that the measured rates of regenerated production will be underestimated by the same amount. Regenerated production rates will also be underestimated by the assimilation of any nitrite or nitrate produced through nitrification. An overestimate of new production, and an underestimate of regenerated production will result in elevated f-ratios.

### ii. Non-biological processes affecting the nitrogen content of the surface ocean.

The surface ocean is a pool of inorganic and organic compounds produced and cycled biologically, which because of the warm, irradiated and oxygenated conditions are able to undergo photochemical and free radical reactions. It has been postulated that these observed photochemical reactions may compete with, and limit, primary production in the surface ocean (Zafiriou and True, 1979).

#### a. The photochemical oxidation of ammonia

The non-biological oxidation of ammonia has not been ascertained to any significant degree. That it occurs was demonstrated by Vaccaro and Ryther, (1954) with seawater kept in the dark for 48hrs. Hamilton, (1964) went on to show, using artificial and natural illuminations that the process was of very little significance in the marine nitrogen cycle.

#### b. The photochemical reduction of nitrate.

In the presence of sunlight nitrate is reduced photochemically. The process is in accordance with the following equation (Daniels et al., 1968);

 $NO_3$  + sunlight (y < 340nm) =  $NO_2$  + 1/20<sub>2</sub>

with a second photochemical process occurring concomitantly,

$$HOH + NO_3 + sunlight (y < 340nm) = NO_2 + OH + OH^2$$

Several investigators have demonstrated that the photolysis of nitrate can occur in the oceanic surface layer, but at unnaturally high concentrations of nitrate, e.g. Hamilton, 1964). By backward extrapolations, Zafiriou,

(1974) calculated from Hamilton's results that at a more acceptable nitrate concentration, (1µM), nitrate conversion rates of 10% per day could be expected. However, experimentally he was unable to measure any loss of nitrate in natural seawater samples held in guartz and borosilicate bottles, which he exposed to sunlight from 1 to 37 days. The results could not be held as conclusive evidence against nitrate photolysis occurring, because pernitrite, (ONOO<sup>-</sup>) may well have been analysed as part of the nitrate fraction. However, evidence was subsequently obtained indirectly. The accumulation of nitrite was observed in abiotic seawater, exposed to sunlight, which did not occur in control samples kept in the dark, nor in samples which contained very low nitrate concentrations. Nitrite itself is destroyed by sunlight, so in order to measure its production, the formation of nitrite needs to be in excess of its destruction by sunlight (Zafiriou and True, 1979).

The central equatorial Pacific is an area of high light and high surface nitrate concentrations where Zafiriou and True, (1979) and Zafiriou, (1980) measured nitrate photolysis rates of 0.02 to 0.5 percent of the nitrogen assimilated by primary production.

### 3.8. Incompatibility of techniques for measuring production rates.

Comparisons of the oxygen and 14-C techniques do not provide a powerful test of the accuracy of either of the techniques, both of which can be biased by the trophic interactions of the microplankton (Smith et al., 1984). A particular problem, when trying to relate fluxes from oxygen and 14-C, is that the activity of the microheterotrophs will interfere with the tracer flow of carbon in a different way to oxygen fluxes. The degree of bias will vary with the community structure, and, in particular, with the ratio of autotrophic to heterotrophic biomass.

Comparisons between the oxygen and total carbon dioxide techniques will offer a less biased rate due to the fact that both techniques share the same systematic errors (Williams and Robertson, 1991) and that the microheterotrophic community will affect both techniques to the same extent. This, of course, does not mean that either technique is giving an accurate measure of the production terms as they are defined. If, for example, bottle effects are biasing the biomass between the light and dark bottles, both techniques will underestimate production. However, a comparison of the two techniques is certainly the most accurate way of assessing the community photosynthetic and respiratory quotients. It may be argued

that errors shared by both techniques, of only being able to measure dark respiration rates, can be resolved by the simultaneous use of the 18-0 technique.

When making comparisons with the 15-N technique, again the difficulty arises that the 15-N technique is a tracer technique and does not share the same systematic errors as the oxygen or total carbon dioxide technique. Because а stable isotope is followed, and not a radiolabelled isotope, it cannot be *directly* compared to the 14-C method either, unless the basic differences between the stable and tracer techniques are recognised (Collos and Slawyk, 1985). Because a ratio of atom abundance is measured with the stable isotope technique, and not an absolute amount of isotope, as with the tracer technique, the former is liable to bias due to any unlabelled compound being taken up simultaneously to the labelled compound. Ideally 15-N measurements should be made concomitantly with 13-C measurements. Both techniques being stable isotope techniques, the same systematic errors are shared. The double labelling technique (Slawyk et al., 1977) allows the simultaneous measurement of carbon and nitrogen uptake during the same incubation. It is now possible to analyse both the particulate organic nitrogen and carbon and the 15-N and 13-C content of a single filter, simultaneously, by manual mass spectrometric methods (Slawyk et al., 1988). A fully

automated system (Preston and Owens, 1985) is not far off being fully developed. The simultaneous measurement of 15-N and 13-C uptake offers a method of measuring regenerated production without the errors implicit to ammonia assimilation measurements. Measurements of 15-N nitrate to 13-C assimilation ratios can be directly compared to the carbon to nitrogen composition ratios of the particulate matter (Collos and Slawyk, 1986). Although measurements of nitrate uptake is not problem free, when the ambient nitrate concentrations are higher than those for ammonia, problems of contamination and of uptake enhancement are less likely than with ammonia uptake measurements. Indirect methods of measuring regenerated production from the relative preference index, (the f-ratio divided by the ratio of the concentration of nitrate to sum of nitrate and ammonia), cannot be as precise as 6, rather than 2 error prone variables are involved.

## 3.9. A summary of primary production rate measurements made by $O_{21}$ TCO<sub>21</sub> and 18-0 techniques.

#### 3.9.1. Measuring gross production.

The oxygen and  $TCO_2$  techniques are used to measure gross production rates. Depending on the relative behaviour of the dark respiration to light respiration rates, the gross production rates may be in error.

If respiration rates are less in the dark bottle than in the light bottle, oxygen and  $TCO_2$  gross production measurements will be underestimates. Likewise, the oxygen and  $TCO_2$  techniques may be overestimating gross production, if the dark respiration rates are enhanced relative to the light respiration rates. The argument being;

 $P_{qC} = LB(O_2 \text{ or } TCO_2) + DB(O_2 \text{ or } TCO_2)$ 

Where;  $P_{gC}$  = calculated gross production rate LB = change in the light bottle DB = change in the dark bottle

therefore;

 $P_{qC} = (P_q - Rl - Rd) + (Ri + Rd)$ 

where; Rl = light stimulated respiration Rd = light insensitive respiration Ri = light inhibited respiration If Rl > Ri then; PgC < Pg If Ri > Rl then; PgC > Pg If Rd >> Rl or Ri then; PgC = Pg If Rl = Ri then; PgC = Pg

Underestimates of gross production can be brought about by an enhancement of respiration in the light, or a depression of respiration in the dark. Possible enhancement of light respiration may occur due to the increase of oxygen concentration surrounding algal cells *in vitro* (Gessner and Pannier, 1958). A depression in respiration in the dark may be attributable to population depletion (Edmondson and Edmondson, 1947). Smith *et al.*, (1984) state that the oxygen technique will always underestimate gross production rates due to unequal biomass accumulation in the light and dark bottles, (this must

also be applicable to the TCO<sub>2</sub> technique) causing respiration to be underestimated, - by as much as 10% - but fail to reference this claim.

When the 14-C technique was first used to measure production rates (Steeman Neilsen, 1952), the results were a factor of 10 times less than those obtained by the oxygen technique in the same area, (the Sargasso sea), by Riley, (1953). This discrepancy was attributed to enhanced bacterial respiration in the dark bottles, causing a consequential overestimate of gross production. Comparing gross production from  $H_2^{18}O$  and net community production from the oxygen technique, Grande *et al.*, (1989) found that respiration rates off the coast of Hawaii, were slightly greater in the dark than in the light.

The 14-C technique will return an estimate of gross production only in situations of high specific photosynthetic rates relative to the rates of autotrophic respiration (i.e. when gross production is comparable to net production), and during incubations of only a short duration.

The 14-C technique will underestimate gross production if any of the 14-C dissolved organic carbon or 14-C particulate organic carbon fraction is lost from the analysis,

(e.g. if volatiles are produced such as methane, or excretion is occurring). If  $14-CO_2$  is being respired and reassimilated, gross production will be underestimated. If recycling of  $14-CO_2$  is occurring within the cell, gross production will also be underestimated.

It is argued that the most accurate measurement of gross production is from the 18-0 technique (Bender et al., 1987), in which the effects of respiration are negligible. The 18-0 technique will underestimate gross production, if any oxygen is being intracellularily recycled.

#### 3.9.2. Measuring net primary production.

Net primary production cannot routinely be measured with the oxygen, or TCO<sub>2</sub> techniques, as the contribution to the respiration term must be apportioned between the autotrophic, and heterotrophic, component of the community. If it were possible to inhibit either the autotrophic or the heterotrophic component of the community, thereby being able to apportion the respiration term between the two, it would be possible to measure net primary production. Size fractionated experiments on respiration rates have been undertaken but this is generally an unsatisfactory approach as, more often than not, the respective components cannot be neatly subdivided into size groups.

If the situation arises where the respiration of the autotrophs is much greater than that of the heterotrophs then the net primary production rate will approximate to the community production rate, and thus the  $TCO_2$  and oxygen technique can return a rate comparable to net primary production.

The 14-C technique will measure a rate of net primary production, if all the respired carbon comes from the same carbon pool as the 14-C previously assimilated. If 14-C is being respired and reassimilated into the cell, then the net primary production rate will be underestimated.

#### 3.9.3. Measuring net community production.

The least disputable production measurement we can make (with the  $TCO_2$  and oxygen technique) is the net community production rate. Quite simply the total change in the oxygen or  $TCO_2$  concentration in the light bottle is measured, whether it is positive or negative, and the net community production rate is obtained. Apart from the bottle effects considered elsewhere, the only other inherent problem is the definition of the community. Larger plankton are excluded from bottle experiments, and grazing pressures are removed.

### 3.9.4. Measuring respiration.

The oxygen and TCO<sub>2</sub> techniques can be routinely used to measure dark community respiration rates. Comparisons with the 18-0 techniques allows the light respiration rates to be measured. The 14-C technique can not be routinely used to measure respiration. Chapter 4

METHODS

#### <u>Chapter 4</u>

#### <u>Methods</u>

#### 4.1. Sample collection and preparation.

Water was collected pre-dawn in acid cleaned 30L Go-Flo sampling bottles, attached to a Kevlar line. In the 1989 field programme water was siphoned into a 50L polycarbonate carboy, and then mixed thoroughly, with undue aeration being avoided. Samples were taken for the rate measurements in the following order:  $O_2$ ,  $18O_1$ , and then TCO<sub>2</sub>. Parallel <sup>14</sup>C (P.Boyd, G.Savidge, A.Pomroy) rate measurements were taken first, from the same water sample. Due to the volume of water required the 15N samples were taken from a separate Go-Flo bottle. In the 1990 field programme samples were taken directly from the Go-Flo sampling bottles. The sampling order was for oxygen, TCO<sub>2</sub>, 14-C and then 15-N. Supporting measurements were taken from all the bottles, for chlorophyll a and inorganic nutrients, ( nitrate, nitrite, ammonia, phosphate and silicate.)

#### 4.1.1. Oxygen sample collection and preperation.

Oxygen samples were collected in nominally 110cm<sup>3</sup> borosilicate glass bottles. For each depth, characteristically 6 time zero, 4 light and 4 dark replicates were taken. The bottles were carefully filled by silicon rubber syphon, in alternate sequence, and each bottle over flowed with three times its volume. The stoppers were then carefully placed to prevent bubbles. The bottles were kept cool and in the dark, if any delay was incurred before processing. The zero time bottles were then processed.

#### 4.1.2. 18-0 sample collection and preparation.

Samples were collected for  $H_2^{18}O$  studies in nominally  $110 \text{ cm}^3$  borosilicate glass bottles. Four replicates were taken at each depth, (two time zero and two experimental). The time zero samples were immediately siphoned into the pre-evacuated bottom flasks and the analysis procedure followed.

The samples were spiked with  $1 \text{cm}^3$  of 180 labelled  $H_20$  with a variable  $1 \text{cm}^3$  pipette and acid cleaned tips. Samples were spiked before placing the tops on the bottles so as not to cause air bubbles. The  $H_2^{18}0$  is 7% denser than water and so will not be lost when the top is inserted.

The <sup>18</sup>O labelled water used to spike the samples (95% <sup>18</sup>O, Monsanto Research Corp, Miamisburg, Ohio; courtesy M.Bender, U.R.I.), had been triple distilled in teflon bottles and tested for trace metal and nitrate contamination. Grande *et al.*, (1988) found Cu, Mn and Cd concentrations to be < 0.1 ppb and nitrate levels < 1.0  $\mu$ mol L<sup>-1</sup> causing [NO<sub>3</sub><sup>-</sup>] to increase by < 0.005  $\mu$ mol L<sup>-1</sup> in the incubated samples.

#### 4.1.3. TCO<sub>2</sub> sample collection and praperation.

Samples were collected in 250cm<sup>3</sup> glass bottles. Nine replicates were taken at each depth, (three time zero, three light and three dark). As with the oxygen technique, the bottles were filled in alternate sequence by silicon rubber syphon, and each bottle overflowed with three times its volume.

#### 4.1.4. 15-N sample collection and preparation.

Samples were collected in 2.3L polycarbonate bottles. Five replicates were taken at each depth, (one time zero, two for  $15-NO_3$  assimilation studies and two for  $15-NH_4$  assimilation studies.) Nitrate assimilation was measured by the

addition of  $Na^{15}NO_3$  (99.2 atom %), and ammonia assimilation by the addition of  $^{15}NH_4CL_2$  (99.7 atom %) to the samples at time zero. The time zero samples were filtered immediately through pre-ashed (450°C 24hr) Whatman GF/F 47mm filters, washed with filtered seawater, and then frozen for subsequent analysis at the laboratory. Time zero samples were taken for C:N analysis. Following the incubation period, the samples were filtered in the same way. The C:N ratio was measured from the same filters as the 15-N samples.

#### 4.2. Incubation procedure.

All rate measurements were either incubated in situ or on deck.

#### 4.2.1. In situ incubations.

The samples were taken pre-dawn and incubated for 12 and/or 24hrs. The light bottles were attached to the productivity rig, which was deployed from dawn to dusk for the 12hr samples, and dawn to dawn for the 24hr samples. Dark bottles were incubated on deck in the 1989 field programme, and incubated *in situ* in the 1990 field programme. The dark bottles were individually double wrapped in heavy gauge, black plastic bags.

The productivity rig was constructed by the workshop at UCNW according to the design shown in figure 4.2.(1). Six cages were suspended at the chosen depths, attached to one another by 9mm climbing rope, and supported by two surface marker buoys. The construction of the rigs was such to allow maximum protection with minimum shading. Each cage is able to rotate around its axis, ensuring unbiased illumination. The rig was tethered to a Dahn buoy which was attached to the ship in 1990. In 1989 the rig had been allowed to drift freely but is subsequently still drifting!

The oxygen, 18-0 and TCO<sub>2</sub> bottles were attached to polycarbonate holders which could then be transported to the cages, and attached easily and quickly with clips. The 15-N bottles were secured directly to the cage by a jubilee clip and elastic loops. The cages were then fastened to the rope, as it was released over the side, by screw gate caribeaners. The whole procedure for rig deployment need only take ten minutes. Retrieval was equally quick and easy. The deployed rig is shown in figure 4.2.(2).

The 14-C samples were incubated on a separate, but attached rig.




## 4.2.2. On-deck incubations.

Experiments were conducted using an on-deck incubator. In 1989 the incubator used was a white perspex box fitted with a lid, and kept at surface temperature by a continuous flow-through of surface water. The incubator used in 1990 was screened to 6 natural light levels and, again, kept at surface temperature by a flow-through of surface water.

## 4.3. Analytical techniques.

#### 4.3.1. The oxygen Winkler titrimetric technique.

## i. The principal of the oxygen technique.

The oxygen Winkler titrimetric technique is used to determine the change in oxygen concentration during an incubation giving a measure of net community production of oxygen, the gross production of oxygen and the respiration of oxygen.

Winkler first proposed the Winkler method for measuring the oxygen concentration of seawater in 1888. The procedure has not changed substantially since that time. The first use of the technique for making production measurements is credited to Gaarder and Gran, (1927) who used the Winkler titration to determine the change of dissolved oxygen in in vitro samples, however earlier work has been recorded (e.g. Putter, 1924) The first work carried outin oligotrophic water was by Riley, (1938). The sensitivity of the methods of that era was sufficient only for productive areas, and long incubation periods, i.e. several days were required. For more normal oceanic areas alternative techniques were sought; hence the introduction of the 14-C radio tracer technique (Steemann Nielsen, 1952). This was the start of a 40 year controversy over what exactly each technique was measuring, (see chapter 3.4).

The start of this long and 'lively' debate led a field of workers to re-examine the potential use of the oxygen technique for measuring productivity. Pomeroy and Johannes, (1966 and 1968) overcame the problem of long incubations by concentrating phytoplankton and were able measure the changes in oxygen concentration with to oxygen electrodes. The results were not satisfactory as losses of activity occurred during the concentration step (Williams, 1978). Increased sensitivity of the Winkler oxygen titration were sought, and instrumental methods of establishing the end point of the titration were devised ( Talling, 1973; Carritt and Carpenter, 1966; and Bryan et al., 1976). The whole process was then automated (Williams and Jenkinson, 1982) enabling oxygen samples to be titrated rapidly (less than 3 minutes), with a coefficient of variation of 0.03-0.1%. The development of a transportable and automated system allowed the rapid and immediate analysis of samples at sea.

## ii. The theory of the oxygen Winkler technique.

The principal of the Winkler titrimetric technique for measuring the change in oxygen concentration differs little from that first published by Winkler (1888). The nominal reactions involved in the Winkler method are out-

lined below:

1).  $2Mn^{2+} + 4OH^{-} -----> 2Mn(OH)_{2}$ 2).  $2Mn(OH)_{2} + O_{2} -----> 2[MnO(OH)_{2}]$ 3).  $2[MnO(OH)_{2}] + 4I^{-}+ 8H^{+} ----> 2I_{2} + 2Mn^{2+}$ 4).  $2I_{2} + 4S_{2}O_{3}^{2-} -----> 4I^{-} + 2S_{4}O_{6}^{2-}$ 

The overall reaction is:

 $2Mn^{2+} + 4OH^{-} + O_2 + 4I^{-} + 8H^{+} + 48_2O_3^{2-} - > 4I^{-} + 2S_4O_6^{2-}$ 

Manganous sulphate and sodium iodide are added to the sample of seawater, and a precipitate of manganous hydroxide results (1). This hydroxide is then converted to a tetravalent manganese compound by the oxygen present in the sample (2). Following the addition of acid the manganese compound oxidises iodide ions to iodine (3), the concentration of which is then determined by titration with sodium thiosulphate (4). It can be seen from the overall reaction that four moles of the titrated thiosulphate are equivalent to one mole of oxygen. It is, therefore, necessary to know the exact molarity of the thiosulphate in order to be able to accurately calculate the concentration of oxygen. Thus prior to the titration of oxygen the thiosulphate is standardisied.

iii. The automatic high precision Winkler titration system.

The precise automatic titration system of Williams and Jenkinson, (1982) is transportable and is able to be used at sea. The system used in the present study is now controlled by a Hewlett Packard series HP 85 microcomputer in place of the original RCA machine language based microprocessor. The automatic burette used was the Metrohm Dosimat E535, which gives accurate 0.2  $\mu$ l additions, 0.02 % of the total volume. This is coupled to a photometric end-point detector as described by Bryan et al., (1976), with the modifications outlined in Williams and Jenkinson, (1982). A voltage regulator drives the photomultiplier to cut out noise from the mains. (Problems of noise can occur during rough conditions at sea, the software can be modified to take some account of this, but with some loss to precision - it was found that the noise was further reduced by screening the lenses from changes in the ambient light field.)

It is a small matter to run the titrations manually by replacing the A/D digital convertor with a flat bed strip chart recorder. This enables fine judgement to be made on the end-point, and the precision is not impaired if care is taken while titrating.

Before titrating the burette must be flushed thoroughly to

ensure there are no air bubbles in the burette tubing, and that none of the thiosulphate has been standing for any length of time in the tubing where evaporation might have taken place.

#### iv. Fixation of samples.

At time zero and after the incubation, the samples were fixed in order that the oxygen concentration did not alter before being analysed. The stoppers were carefully removed and placed on the stopper rack. The temperature of the sample in each bottle was then taken with a fast response digital thermometer and recorded to 0.1°C. Manganous sulphate  $(1 \text{cm}^3)$  was then added to each bottle using а repetitive pipette (BCL 8000), followed by 1cm<sup>3</sup> of alkaline iodode solution. The precision of the chemical additions is important; if the pipetting error exceeds 3%, it becomes a significant source of error in the overall analysis. The stoppers were then carefully replaced and the contents of the bottles mixed twice to ensure the manganous hydroxide precipitate comes into contact with the whole sample. The bottles were then stored under water until titrated.

#### v. Titration of sample.

The samples were titrated in an alternate sequence, (zero, light, dark, zero, light, dark.. ) in order to eliminate instrumental bias in the results. Prior to the titration,  $1 \text{ cm}^3$  of 5M  $H_2SO_4$  was added to the sample bottles and gently swirled round. A magnetic stirrer, rinsed in distilled water, was lowered by the use of a magnetic rod carefully down the side of the bottle. The sample was then ready to be titrated.

## vi. Calculation of production rates.

Primary production rates are calculated from the change in oxygen concentration using the formulae below; for gross production, net community production, and respiration, respectively.

Gross production = Pg = (LB - DB) / tNet community production = Pc = (LB - ZB) / tRespiration = R = (ZB - DB) / t(expressed as  $\mu$ mol kg<sup>-1</sup> day<sup>-1</sup>)

+

Where;

 $LB = O_2$  concentration in the light bottle

 $DB = O_2$  concentration in the dark bottle  $ZB = O_2$  concentration at time zero t = incubation time

## vii. Performance of technique.

The automated oxygen Winkler titrimetric technique has a precision of 0.03-0.1% (Williams and Jenkinson, 1982), if used with due care and attention. Currently, if the correct procedures for bottle calibration, standardisation of the thiosulphate and preparation and dispension of the chemicals are employed, the limits of precision of the technique are set by the accuracy with which the automated Dosimat burette is able to dispense thiosulphate, the current model being able to dispense 0.2  $\mu$ l additions.

Pooled oxygen gross production rate data from cruise Charles Darwin 45/1990 has a standard deviation of 0.43  $\mu$ mol Kg<sup>-1</sup>.

# viii. Standardisation of thiosulphate.

The oxygen Winkler technique differs from other oxygen methods in that it does not use oxygen as a standard, although is technically possible. The accuracy of the

method has been extensively researched (e.g. Carpenter, 1965), and with advances in the technique, is now able to give a precision of 0.03% (Williams and Jenkinson, 1982). It is necessary that the molarity of the thiosulphate is known in order to be able to calculate the concentration of oxygen. Thiosulphate is not a primary analytical standard, and is, therefore, standardised prior to its use. In the standardisation procedure a known quantity of iodine is liberated on the addition of sulphuric acid to a standard potassium iodate solution. The 'liberated' iodine is then titrated against the thiosulphate producing a colour change which is photometrically determined. The thiosulphate molarity is then able to be calculated.

The chemical equation for the standardisation procedure is as outlined below:

 $10I^{-} + 2IO_{3}^{-} + 12H^{+} ----> 6I_{2} + 6H_{2}O$  $6I_{2} + 12S_{2}O_{3}^{2-} ----> 12I^{-} + 6S_{4}O_{6}^{2-}$ 

The procedure is as follows:

To a sea water sample,  $1 \text{cm}^3$  of sulphuric acid and  $1 \text{cm}^3$  of alkaline iodide are added. After thorough mixing  $10 \text{cm}^3$  of the potassium iodate standard are added (0.0100026N at  $20^{\circ}\text{C}$  (19/12/1988) P.J.leB.Williams, U.C.N.W.) using a custom made Volhardt precision pipette, (volume = 9.60731

 $\pm$  0.00022cm<sup>3</sup>). The solution is covered and left in the dark for ten minutes to allow the reaction to go to completion. The solution is then titrated with the thiosulphate and the molarity calculated from the following equation:

$$M_1 = ( \begin{array}{c} 6 \\ x \\ v_2 \\ x \\ v_1 \end{array})$$

where:

 $M_1$  = molarity of the thiosulphate  $V_1$  = volume of the thiosulphate added (cm<sup>3</sup>)  $V_2$  = volume of the iodate added (cm<sup>3</sup>)  $M_2$  = molarity of iodate standard = 0.0100026N.

## ix. Bottle calibration.

Nominally 110cm<sup>3</sup> borosilicate glass bottles are used for the oxygen technique with clear glass stoppers. Borosilicate offers better optical consistency than ordinary soda glass (Williams and Jenkinson, 1982), and are supposedly more robust. Each bottle and stopper are engraved with a number prior to calibration. Following the realisation that a main source of error in the oxygen technique was from the volatisation of iodine, occurring

during the transfer of the sample to the titration vessel (Carritt and Carpenter, 1966), the use of a whole bottle titration was introduced, initially suggested by Green and Carritt, (1966). In order to obtain the maximum precision from the oxygen Winkler method, the volume of sample titrated must be known to an accuracy of 0.03%. The oxygen bottles, therefore, need to be calibrated to this degree of accuracy: an initiation process into the world of oxygen measurements - the calibration of 200 oxygen bottles! A time consuming process which not only puts the intrinsic value of each bottle far above its actual cost, but also earns the technique the care and respect it deserves! The bottles are calibrated to an accuracy ten times that of 'A' grade volumetric glassware. The bottles are ideally calibrated at 25°C, but in lieu of a suitable constant temperature room, each bottle was corrected for the temperature of the contained water. The bottles and stoppers are cleaned and dried in a low temperature oven, and then weighed on a Sartorius balance, (model A7073), interfaced to an HP 85 microcomputer. They are then filled with distilled water and the stoppers inserted. Any excess water is removed and a fine tipped tube, connected to a pump, is used to remove any water from around the rim of the stopper. The bottles are then re-weighed, the temperature read to 0.1°C and the process then repeated. Software was written to convert the weighing with temperature correction, to bottle volume at 25°C. Since

each bottle was weighed empty and full, twice, an estimate of the random error could be made. It was no greater than  $0.01 \text{cm}^3$  i.e < 0.01%. The bottle volumes are calculated to a precision of  $0.01 \text{cm}^3$  or better.

## x. Bottle cleaning procedure.

The bottles are not cleaned between experiments but are stored with the Winkler reagents in the bottles. This is because iodine is generated photometrically, which keeps the bottles sterile and free from a bacterial film building on the walls (Williams and Jenkinson, 1982). Just prior to sampling, the bottles are rinsed vigorously with hot water to remove the iodine. On sampling, the bottles are rinsed once with the sample water.

#### 4.3.2. The 18-0 technique.

#### i. The principal of the 18-0 technique.

The measurement of gross production from the rate at which 18-0 labelled  $O_2$  is produced from 18-0 labelled  $H_2O$ .

The 18-O technique was introduced for the measurement of gross production of oxygen (Grande *et al.*, 1982) from a modified method of Kroopnik (1971).  $H_2O$  is labelled with 18-O, and the rate at which 18-O labelled  $O_2$  is produced is measured *in vitro* over 3-24 hours. The 18-O technique potentially offers a definitive method of measuring the gross production of oxygen without the limitations of other tracer techniques. 18-O is a stable isotope of oxygen with a natural abundance of 0.204 atom %. Because the dissolved  $O_2$  pool to which the labelled 18-O is added is much larger in comparison to that utilised in photosynthesis, respiration will affect the results to an insignificant degree (Grande *et al.*, 1989a).

## ii. The theory of the 18-0 technique.

Oxygen, in its natural form, is composed of three isotopes; 16-0 with an atomic abundance of 99.758 atom %, 17-0 with

an atomic abundance of 0.038 % and 18-0 with an atomic abundance of 0.204 atom %. The abundance of 18-0 is reported as  $\delta^{18}$ 0 where;

$$\delta(0/00) = [(^{18}0/^{16}\text{Osample}/^{18}0/^{16}\text{Ostandard}) - 1] \times 10^3$$

The standard mean ocean water (SMOW) is used as a reference for delta notation.

 $\delta^{18}$ O of O<sub>2</sub> in air = 23.5 %o wrt SMOW  $\delta^{18}$ O of O<sub>2</sub> in seawater = 24.2 %o (SMOW) + 0.7(AIR) at equilibrium.

During the incubation period 18-0 is fixed into  $O_2$  by the following reaction:

$$H_2^{18}O + C^{16}O_2 ---> {}^{18}O^{16}O + CH_2^{16}O.$$

The rate of oxygen production is calculated from the experimentally measured increase in the  $180^{16}$ O concentration. Fractionation has been found to occur (Guy et al., 1986; Fogel et al., 1986), but the effect is negligible due to the large 18-O enrichment of the sample.

The optimum addition of 18-0 for production measurements is that which will give a change in  $\delta^{18}$ 0 of 4 %0 (Bender, 1989 pers. comm.). To calculate the required addition a graph is drawn of the amount of tracer added against 18-0 production with the slope giving the change in;

 $d^{18}O(O_2) = 4$  o.

#### iii. Preparation for analysis.

A vacuum line is set up which is kept under vacuum throughout the cruise. Round bottomed flasks are heated at 60°C for 3 hrs to dry thoroughly. The round bottomed flasks are then greased with silicon grease and connected to the sample storage ampules and the manifold by a three way tap. The vacuated system is then checked for leaks. The required pressure for a 12 point outlet manifold is 120 microns (allowing a pressure of 10 microns per outlet). A vacuum gauge is connected to ensure the correct pressure is reached. It is essential that the system has no leaks and a thorough test, each time an experiment is run, is required.

## iv. Extraction of oxygen for analysis.

Gases were extracted from the seawater sample by siphoning  $50 \text{cm}^3$  from incubation bottles into flasks pre-evacuated to a pressure of  $<10^{-5}$  atm. Extensive degassing of the samples occur during the siphoning as the gas is introduced

into the vacuum via a small orifice. The sample is then allowed to equilibriate for an hour with the space in a second  $50 \text{cm}^3$  pre-evacuated sample flask. This is then sealed with an acetylene torch and the samples stored for analysis at Rhode Island University. In the laboratory the sealed ampule is broken, and the gas sample is treated cryogenically to remove water and carbon dioxide. The oxygen in the remaining gas is reacted with hot graphite to form  $CO_2$ . The 180160 ratio is then measured by mass spectrophotometry.

#### v. Calculation of production rates.

To calculate the change in 
$$d^{18}O$$
 of the sample the follow-  
ing equation is used,

$$d^{180}f = d^{180}i \times [0_2]i + d^{180}p \times [0_2]p$$
$$[0_2]i + [0_2]p$$

where:

 $d^{18}O_f = d^{18}O$  of the final sample  $d^{18}O_i = d^{18}O$  of the initial sample  $d^{18}O_p = d^{18}O$  of the photosynthetically produced oxygen  $[O_2]_p =$  the concentration of photosynthetically produced

#### oxygen

 $[0_2]_i$  = the concentration of the initial oxygen.

## vi. Performance of technique.

The accuracy and precision of the 18-0 technique was investigated by Bender and Grande, (1987). Seawater was fixed with  $HgCl_2$ , left stirring gently for 24hrs, then sampled in triplicate and analysed for  $d^{18}$ O of oxygen. The value obtained was  $\pm$  24.14 permil which was in agreement to  $\pm$  0.1 permil with the reported equilibrium value for seawater at the same temperature (Kroopnik and Craig, 1972). Bender and Grande, (1987) report a standard deviation from their data set from the P.R.P.O.O.S. cruise of  $\pm$  0.04 permil from 5 sets of duplicate samples. 4.3.3. Coulometric TCO<sub>2</sub> analysis.

## i. The principal of the TCO<sub>2</sub> technique.

The coulometric analysis of the change in total inorganic carbon in light and dark bottle incubations, gives a direct measurement of the gross production of carbon, the net production of carbon and the respiration of carbon, in vitro.

A coulometric method for the analysis of total carbon dioxide in seawater was introduced by Johnson *et al.*, (1985). Based on the coulometric titration of Szebelledy and Somogyi, (1938) the method offered a relative precision comparable to the oxygen technique (see chapter 3.5), and for the first time, the gross production, net production and respiration of carbon could be measured directly in open ocean systems.

The system was designed to analyse samples containing 2000  $\mu$ mol C L<sup>-1</sup> with an accuracy of < 0.1 %, (i.e. < 2  $\mu$ mol C).

ii. The theory of the coulometric technique.

The coulometric method for the analysis of TCO<sub>2</sub> measures the total inorganic carbon in seawater, i.e;

$$TCO_2 = [HCO_3^{-}] + [CO_3^{2-}] + [CO_2(ag)]$$

The coulometric technique measures the amount of electricity (coulombs), required to convert a chemical species to a different chemical state, the two being related by Faraday's constant. Based on Faraday's laws, the technique relies on the electron as the primary standard, thus, if an accurate coulometer can be built, the neccesity for difficult standardisation techniques is removed.

The principal of the carbon dioxide determination is that carbon dioxide is extracted from an acidified solution and absorbed on to an organic base (ethanolamine). The absorbed  $CO_2$  is then determined by the coulometric back titration.

The reaction procedure is outlined below (from Johnson, et al., 1985);

#### <u>Neutralisation</u>

1)...2CO<sub>2</sub> + 2NH<sub>2</sub>(CH<sub>2</sub>)2OH -> 2HO(CH<sub>2</sub>)2NHCOOH 2)...2HO(CH<sub>2</sub>)2NHCOOH + OH<sup>-</sup> -> 2HOH + 2HO(CH<sub>2</sub>)2NHCOO<sup>-</sup>

Oxidation-reduction

3)... $2Ag^{O}(s) \rightarrow 2Ag^{+} + 2e^{-}$  (anode) 4)... $2HOH + 2e^{-} \rightarrow H_{2} + 2OH^{-}$  (cathode)

## <u>Complexation</u>

5)... $2Ag^+ + 4I^-$ (saturated KI) ->  $2AgI_2^-$  (anode)

#### <u>Net reaction</u>

6)...Ag<sup>o</sup>(s) + 2I<sup>-</sup> + CO<sub>2</sub> + NH<sub>2</sub>(CH<sub>2</sub>)2OH -> AgI<sub>2</sub><sup>-</sup> +  $1/2H_2$  + HO(CH<sub>2</sub>)2NHCOO<sup>-</sup>

An extractor-stripper removes  $CO_2$  from the sample, this reacts with ethanolamine in the cell solution, to form the hydroxyethylcarbamic acid (1). Hydroxide ions are electrogenerated at the platinum cathode by the reduction of water (4). The weak acid (hydroxyethylcarbamic acid) is titrated by the hydroxide ions (2). The titration is thus quantified by the electrogeneration of these hydroxide

ions. The equivalence point of the titration is then detected photometrically with thymolphthalein (contained in the cell solution) as an indicator.

The procedure of the method can be subdivided into four main steps;

- i). The precise measurement of the sample volumeii). Acidification of the sampleiii). Extraction of TCO<sub>2</sub> in an organic base
- iv). The coulometric back titration of TCO<sub>2</sub>

#### iii. The coulometric back titration system.

The coulometric system used was a modified version (Robertson, 1989), of the UG-I model described by Johnson et al., (1987). The system is described in detail by Robertson, (1989) and only further modifications by Carol Robinson and myself need be outlined here.

The system is run automatically by a Hewlett Packard (HP86) computer and channel recorder controlling the action of solenoid and stainless steel valves. A sample is drawn into a calibrated pipette gravimetrically. The

pipette was calibrated twice (see chapter 4.3.3.viii.b). The pipette is cleaned with 10% HCl and then thoroughly rinsed with distilled water. Prior to the cruises, the pipette was washed with tri-sodium orthophosphate and again thoroughly rinsed with distilled water. 8% phosphoric acid is added to the extractor-stripper and sparged for 2 minutes with the carrier gas, analytical grade N<sub>2</sub> (BDH), to remove  $CO_2$ , ( the acid is cleaned by bubbling N<sub>2</sub> through for 1 hr each time it is replaced). The sample is pushed into the stripper and exits through a water cooler (Grant L.T.D. cooler), is passed through a magnesium perchlorate filled bulb to remove residual water, and then enters the coulometric cell. TCO<sub>2</sub> is detected by a Coulometric Inc. (Golden, CO, U.S.A.) coulometer, model 5010.

The cell solution is blue (due to thymolphthalein), at the equivalence point, (pH 10.5). As  $CO_2$  enters the cell, the pH is lowered, and the transmittance of the cell is raised. This initiates the above sequence, with a current being passed dependent on the percentage transmittance. As the acid is titrated, the current passed drops from high to low to zero, as the equivalence point is approached, which is sensed by an optical detector. The current is passed through a precision resistor, and the resultant voltage drop is converted into ' $\mu$ gC titrated' via a voltage-frequency convertor.

The pipette volume originally used was approximately  $31 \text{cm}^3$ in line with Johnson *et al.*, (1987). Without any detectable decrease in precision the pipette was replaced with one of nominally  $20 \text{cm}^3$  to reduce the sample manipulation time.

The pipette overflows into a level detector which, with the original stainless steel electrodes, often overflowed. The stainless steel electrodes were corroding, and subsequently reducing the reliability of the sensor. The detector was replaced with solid state optical liquid level switches (R.S. Components, Corby, Northants, stock no. 317-803.), and the problem was eradicated.

The cell reagents are supplied by Coulometrics Inc. The cell solution was changed if any discolouration of the electrodes occurred, or if the time of titration increased. This usually occurs after 25 milligrammes of Carbon has been titrated, which is only 25% of the recommended life of the chemicals (Coulometrics Inc.) This may in part, be due to the age of the chemical stock used. The cell is cleaned in distilled water, and then acetone is pumped through the frit with a hand pump followed by distilled water. If discolouration of the frit has occurred, anode solution is pumped through prior to cleaning. At sea the cell is routinely left soaking in 10 % potassium permanganate for 12 hrs and then sodium

sulphite + 15%Cl for a further 12 hrs. Prior to going to sea the cells are soaked for 24 hrs in aqua regia. The electrodes are rinsed in distilled water. Discolouration or 'furring' of the anode is removed with nitric acid. (The main reason for early high, and erratic blanks, was put down to the cell being insufficiently cleaned between analysis, which, by adopting a vigorous cleaning regime was contained). The main imprecision from the cell is from the retention of water droplets in the cell cap (D.Chipman, pers. comm.). This is removed by cleaning and thorough drying of the cap in an oven prior to use (Tefal mini-oven at 50°C for 3 hrs).

Problems with the solenoid values in the gas sampling unit 'sticking' were solved by regular cleaning and the replacement of their rectifiers with larger rectifiers which earthed separately.

The original PTFE tubing was replaced with 1/8'' stainless steel tubing where possible to minimize the contamination from atmospheric CO<sub>2</sub>. The remaining silicon tubing was replaced regularly.

iv. Fixation of samples.

The time zero bottles were fixed immediately by the addition of saturated mercuric chloride (250  $\mu$ l). The bottles were gently shaken to ensure thorough mixing of the sample. The light and dark bottles were fixed at the end of the experiment in the same way. The samples were stored under water, to minimise the temperature change, until analysed.

#### v. Titration of samples.

The samples were analysed in alternate sequence (zero, light, dark, zero, light , dark,) to minimise instrumental bias. Four replicates were taken from each sample bottle. On each analysis the pipette was rinsed with three times its volume of sample. Each replicate analysis takes 3-8 minutes on average.

## vi. Calculation of production rates.

The production rates are calculated for  $TCO_2$  in the same manner as that for oxygen;

Gross production = Pg = (DB - LB)/tNet community production = Pc = (ZB - LB)/t Respiration = R = (DB - ZB)/t(Expressed as  $\mu$ mol kg<sup>-1</sup> day<sup>-1</sup>)

where;

 $DB = TCO_2$  concentration in the dark bottle  $LB = TCO_2$  concentration in the light bottle  $ZB = TCO_2$  concentration at time zero

It is appropriate to mention here that an error was discovered in the original equations reported by Johnson et al., (1987), used to correct for the thermal expansion of water (Robinson and Williams, 1991), and subsequently amended. The corrected calculation is as follows:

```
V_{T} = V_{25}(1 + alpha) (T - 25)

TCO_{2} [\mu g L^{-1} at T^{O}C] = BCR \times 1000/V_{T}

TCO_{2} [\mu mol L^{-1} at 25^{O}C] = BCR \times 1000 \times d_{25}/(V_{T} \times 12.011 \times d_{T})

Dividing by d_{25} gives TCO_{2} in \mu mol kg^{-1} thus:
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```
TCO_2 [µmol kg<sup>-1</sup>] = BCR x 1000/(V<sub>T</sub> x 12.011 x d<sub>T</sub>)
```

where:

 $\begin{array}{l} v_{T} = \mbox{volume of pipette at sample temperature (cm^{3}),} \\ d_{T} = \mbox{density of sample at sample temperature,} \\ v_{25} = \mbox{volume of pipette at 25^{\circ}C} \ (cm^{3}), \\ d_{25} = \mbox{density of sample at 25^{\circ}C}, \\ BCR = \mbox{blank corrected reading from coulometer ($\mu gC$),} \\ alpha = \mbox{the volume coefficient of expansion for borosilicate glass, taken to be 1 x 10^{-5} \mbox{deg^{-1}} \ (Weast and Astle, 1983).} \end{array}$ 

The coulometric software automatically compensates for the instrumental 'blank' in calculating the amount of carbon titrated, according to the following equation:

Blank corrected reading = coulometer reading  $[\mu gC]$  - blank  $[\mu gC/minute] \times titration time [minutes].$ 

For the accurate determination of  $TCO_2$  a correction is made for the addition of  $HgCl_2$ .

#### vii. Performance of technique.

The precision of the technique is reported as 0.1% (Johnson et al., 1987) which permits the accurate analysis of production rate changes of > 0.2  $\mu$ mol kg<sup>-1</sup>. The pooled standard deviation of production measurements from the

standard deviation of production measurements from the field work in 1989 was 1.03  $\mu$ mol Kg<sup>-1</sup> and in 1990, 1.15  $\mu$ mol Kg<sup>-1</sup>, meaning that, realistically, this is the lower limit of rate change measurable with the coulometer used in this study. Already improvements to the precision have been made with the introduction of a new coulometer and adapted methodology, (See Robinson and Williams, 1991).

An important contributor to the overall precision of any analytical technique is the accurate determination of, and ensured stability of, the instrumental 'blank'. The blank in the TCO<sub>2</sub> technique is determined automatically and checked at the operators discretion, (before any analysis is undertaken, and then at hourly intervals.) The blank is determined by passing CO<sub>2</sub> free carrier gas through an aliquot of the acid, and into the coulometer cell. The coulometer is read at 20 second intervals, and the slope of the regression line with time is calculated (Robinson and Williams, 1991). The blank is considered acceptable if below 0.1  $\mu$ gC/minute. High blank values are associated with the cleaning of the cell. The cell solution itself has a finite life, and depending on the amount of carbon titrated, will start to deteriorate. The performance of the cell solution is the prime dictator of the techniques precision. No evidence has been found of any significant drift in the electronic accuracy of the coulometer itself (Robinson and Williams, 1991).

Imprecision of the technique no longer lies with the sample collection, due to the adoption of a more rigorous sampling protocol in line with the oxygen methodology, and analysis of variance revealed that the inter bottle variability (standard deviation = 0.3  $\mu$ mol Kg<sup>-1</sup>) is now much less than the inter bottle variability, (standard deviation = 0.9  $\mu$ mol kg<sup>-1</sup>) (Robertson and Williams, in press).

The analytical time of the system is 6 to 8 minutes per sample.

#### viii. Calibration Procedure.

#### a. Gas reference.

As a referencing procedure to ascertain the stability of the coulometer, a stainless steel loop is filled with pure  $CO_2$  at ambient temperature and pressure. The contents of this known volume of  $CO_2$  are analysed and the result obtained compared with the loop content calculated from the gas law (Johnson *et al.*, 1987). The calibration of the gas loop volume is a major problem, and although this procedure calibrates the  $CO_2$  absorption and subsequent titration by the cell, it has no check on the actual  $CO_2$ extraction procedure. The volume of  $CO_2$  extracted and

dispensed is therefore calibrated by using a 'known' volume of carbonate solution, and monitoring the pipette volume.

## b. Calibration of pipette volume.

The volume of the sample pipette is determined with a sodium carbonate solution after the methods of Johnson et al., (1987). The volume is calculated from the analysis of a known volume of NaCO<sub>3</sub>. Analytical grade Na<sub>2</sub>CO<sub>3</sub> is heated in a muffle furnace for 2 hrs at 270°C, cooled in a desiccator and weighed to 1  $\mu$ g accuracy. The carbonate is diluted with distilled deionised water as the diluent to 5L in a grade A volumteric flask. Corrections are applied for the thermal expansion of water and glassware, (Table F2 in Weast and Astle, 1983) and for the CO<sub>2</sub> content of the diluent. The carbonate solution is then analysed directly from the inverted volumetric flask.

The pipette was calibrated in both 1989 and 1990 prior to the BOFS cruises.

ix. Results of calibration of sample pipette.

The volume of the sample pipette is determined with analytical grade sodium carbonate solution. The diluent used must, therefore, have the minimal levels of carbon present. Analysis was undertaken of various sources of water in the laboratory to obtain the diluent with the lowest carbon content. The recommendations of Johnson et al., (1987) to add sodium hydroxide to the diluent to remove acid were also tried, but gave the worst results. lowest carbon content was found in The the distilled/deionised water sample, and so this was used as the diluent for all the ensuing calibrations. The results of the 1989 calibration are shown in table 4.3.(a) below:

Table 4.3.(a)

Calibration of pipette volume (1989).

Date	pipette	volume	(cm <sup>3</sup> )	8.E
20/02/89	30.	.2828 .2749	0	.0114
22/02/89	30.	3034	0	.0166
mean	30.	2870	0	.0282

The mean value obtained was  $30.2870 \text{ cm}^3$ ,  $0.0197 \text{ cm}^3$ smaller than that obtained by C. Robinson ( $30.3067 \pm 0.0166 \text{ cm}^3$ ).

In 1990 the calibrations were repeated on a new pipette

(nominally 20 cm<sup>3</sup>). Three calibrations were performed with unsatisfactory results. The calculated pipette volume increased over the analysis period by as much as 0.1 cm<sup>3</sup> and the standard error of the volume was a factor of 5 larger than expected. Statistics performed on a plot of calculated pipette volume against time, showed the variance was linear with an r squared value of 80.39 % and a correlation coefficient of 0.897. The relationship with temperature increase of the carbonate solution was statistically significant with an r squared value of 45.13 and a correlation coefficient of 0.672.

By running duplicate calibrations with the second coulometer it could be ensured that the carbonate standard was not at fault and analysis of the machine blank also discounted this as a possible source of error. The whole system was stripped and cleaned and the problem was traced to alkali being in the system which was slowly being released during the titration. This would have been due to an overflow in the extractor-stripper due to a faulty solenoid valve which was replaced. A repeat calibration was performed and the results shown in table 4.3.(b).

The problem of the drift in pipette volume was eradicated and the mean pipette volume drift over the calibration time was  $0.021 \pm 0.011$  cm<sup>3</sup>. Simple regression analysis of the change in pipette volume with time, as with tempera-

ture, were not statistically significant.

<b>Table 4.3.(b)</b> Calibration of pipette (1990).							
Date	pipe	tte	volume	(cm <sup>3</sup> )	8	. E	
04/05 05/04	/90 /90	21.	.4384 .5299	0 0	.00	0564	
19/04	/90 /90 	21	. 5097 	0 0	.00	0136	
mean		21	.4795	0	. 02	2363	

#### x. Bottle cleaning procedure.

In the 1989 field programme poor replication was found in the productivity measurements from  $TCO_2$  analysis. The protocol for fixing samples was to add 250  $\mu$ l of HgCl<sub>2</sub> to each bottle to ensure all organic activity is stopped. The problem of poor, between bottle, replication was put down to residual HgCl<sub>2</sub> remaining in the bottles after analysis and insufficient cleaning between experiments. Routinely, before going to sea the TCO<sub>2</sub> bottles are thoroughly cleaned by washing in 6N HNO<sub>3</sub> and HCl and leaving to soak for 12 hrs, but this is an undesirable procedure when at sea. A cleaning procedure was required which would ensure that all the HgCl<sub>2</sub> was removed from the glass surface, but was also practical for use at sea with

a large turn over of bottles.

# a. Analysis of the residual HgCl<sub>2</sub> in TCO<sub>2</sub> bottles: Determining a suitable cleaning procedure.

The effectiveness of different concentrations of Decon, HNO<sub>3</sub>, HCl, and APDC (8-hydroxy quinoline) were tested, and the duration of time needed to soak the bottles assessed. 0.05% APDC is used in the trace metal analysis for cleaning glassware. However, it is used hot following a rinse in 50% HNO<sub>3</sub>. It was envisaged that the complexing agent may be effective cold if the concentration were increased and if left to soak for 24 hrs.

## 1.Methodology.

A 50L carbuoy was filled with seawater, well mixed and then siphoned into  $TCO_2$  bottles. Three replicates were taken of each method with a control being run during each experiment. The seawater was analysed directly from the bottles into an atomic absorption spectrometer. The A.A was fitted with a VGA-76 with limits of determination of 0.5-10  $\mu$ g L<sup>-1</sup>; after the methods of Sturgeon *et al.*, (1979). The precision limits need be no smaller as ambient seawater contains an average of 0.1  $\mu$ g L<sup>-1</sup>.
#### 2. Results

A calibration curve was plotted on each day of analysis with standards made in 0.25% dichromate, 5% nitrate. Standards were run at concentrations of 5, 10, 25, 50 and 100  $\mu$ g l<sup>-1</sup>. The first experiment involved selecting bottles at random and analysing them for residual HgCl<sub>2</sub> released in the washing procedure. The results are shown in table 4.3.(c).

The results from the first experiment showed that the bottles had not been cleaned sufficiently and substantial quantities of  $HgCl_2$  were being released. The second experiment was run using cleaned bottles. Each bottle was soaked for 24hrs in 1:1 6N  $HNO_3$  and conc. HCl. The bottles were filled with seawater and spiked with 250  $\mu$ g  $HgCl_2$ . The effectiveness of Decon and acid cleaning and APDC were assessed, and the results shown in table 4.3.(d).

The results showed that it was quite critical how long the bottles were allowed to soak with the respective cleaning agents. The APDC was not satisfactory when used cold. Because it is also costly, the concentrations were not increased. The Decon wash actually contained less HGCl<sub>2</sub> than the acid wash, and was most effective after 24 hrs. It was envisaged that the acid wash removed most of the HgCl<sub>2</sub> in the first 6 hrs, which then vaporised out of the solution when left for the proceeding 18 hrs.

Table 4.3.(c) Results of the cleaning procedure for TCO<sub>2</sub> bottles

Exp no	). Clean	ing proce	adure	HgCl <sub>2</sub>
1	24 hrs	24 hrs	6 days	μg L-1
Ai ii	Blank			1.6 4.2
Bi ii iii	SW	TW	SW	1.9 0.8 1.2
Ci ii iii	SW	D	SW	1.0 1.1 1.1
Di ii iii	SW	APDC	SW	1.2 0.2 0.2
Blank SW = S D = 5% APDC =	= Seawat Seawater; Decon; 5 gL <sup>-1</sup>	er;		

Exp no.	Cleaning	proce	dure	Time	HgCL <sub>2</sub>
2				(hr)	μg L <sup>-1</sup>
Ei ii iii	Blank			(48)	0.0 0.0 0.0
Fi ii iii	SW(sp)	(24)	D	(24)	0.0 0.0 0.1
Gi ii iii	SW(sp) (	(24)	AW	(24)	0.0 0.2 0.0
Hi ii iii	SW(sp) (	(24)	D	(12)	0.1 0.2 0.2
II II II III	SW(sp) (	(24)	AW	(12)	0.2 0.4 0.4
Ji ii iii	SW(sp)	(24)	D	(6)	0.2 0.2 0.4
Ki ii iii	SW(sp)	(24)	AW	(6)	0.5 0.6 0.2
Li ii	SW(sp)	(24)	APD	C (24)	10.0 2.8 7.2

Table 4.3.(d) Results of the cleaning procedure for TCO<sub>2</sub> bottles 3.Conclusion.

From these experiments it was realised that the bottles were not being cleaned effectively when at sea. A cleaning protocol was established that was practical for use at sea with a large turn over of bottles. Bottles are acid cleaned in 10% HCL for 24 hrs and then after thorough rinsing soaked in 5% Decon for atleast a further 12hrs. The bottles are then rinsed thoroughly in seawater before use. It has been shown that the bottles will then contain no more than the ambient levels of HgCl<sub>2</sub>.

#### 4.3.4. The 15-N tracer technique.

Determination of new and regenerated production from the assimilation of 15-N labelled  $NH_4Cl_2$  and  $NaNO_3$  and the subsequent analysis by mass spectrometry of the 15-N isotopic ratio.

#### i. Theory.

15-N was first introduced into the studies of the nitrogen cycle by Dugdale *et al.*, (1961), and has since been used to determine the net community uptake of inorganic nitrogen *in vitro*. By the addition of a known quantity of 15-N labelled nitrogen compound, incubation, and subsequent analysis of the isotopic ratio of the particulate nitrogen, the assimilation of the nitrogen compound of interest can be calculated. The measurement of new production, taken to be that production based on nitrate (Dugdale and Goering, 1967), is measured by the assimilation of Na<sup>15</sup>NO<sub>3</sub>. The measurement of regenerated production, taken to be that production based on ammonia (Dugdale and Goering, 1967), is measured by the assimilation of  $^{15}NH_4Cl_2$ .

The analysis of the isotopic ratio of nitrogen is carried out by mass spectrometry. The original method for proc-

essing the nitrogen samples for mass spectrometric analysis was by Kjeldahl digestion (Kjeldahl, 1883) the ammonium produced then being oxidised to nitrogen gas by the Rittenburg technique (Sprinson and Rittenburg, 1949) and then isotopic analysis. This preparation has been replaced by the Dumas combustion technique (Steyermark, 1961). Its main advantage over the Kjeldahl technique is that it is a single step process.

The Dumas method involves the dry combustion of the total nitrogen containing compounds with copper oxide, or copper, in the presence of lime (Fielder and Proktsch, 1975). Ammonia is completely oxidised to nitrogen, and the nitrogen compounds reduced to nitrogen gas. Barsdate and Dugdale, (1965) modified a commercial nitrogen analyser for nitrogen isotope analysis. The technique was refined to increase the sample throughput and precision. Preston and Owens, (1983) then interfaced an automatic elemental analyser with an isotope ratio mass spectrometer. This enabled the rapid analysis of the total, and the isotopic ratios, of nitrogen to be obtained simultaneously. The system was then automated and adapted for shipboard use (Owens, 1988). The methodology employed in this thesis is after the methods of Owens, (1988).

#### ii. Sample preparation.

The frozen samples were dried in a moderate oven overnight. Sub-samples were removed from the filters by a 4-mm diameter punch. 6 or 8 discs were used for each analysis. The subsampling of the discs is taken randomly across the filter to minimise error due to unequal surface coverage of the filtrate. The advantage of subsampling is that replicate samples can be taken at the analysis stage. Two replicates for each sample were taken from each filter. The discs are carefully wrapped in tin caps, sealed, and then loaded into the autoanalyser.

#### iii.Analysis.

The 15-N analysis was carried out at Plymouth marine laboratory with the help of N.Owens and A.Rees. An elemental analyser interfaced with a triple collector isotope mass spectrometer (Europa Scientific Ltd, Crewe, England) was used. The protocol is according to the methods of Preston and Owens, (1983). The sample, in a pulse of high purity oxygen, is introduced automatically into a combustion tube in a furnace at 1020°C. The combustion is based on the Dumas technique. The products are then swept by the carrier gas flow of high purity helium, into a reduction tube, in a furnace at 550°C.

Copper in the reduction tube converts the nitrogen oxides produced into  $N_2$  gas. The gas is then passed through a carbosorb trap (BDH), to remove carbon dioxide, and a magnesium perchlorate trap (BDH), which removes water. The  $N_2$  is then passed through a chromatographic column to ensure complete separation of  $N_2$  from low molecular weight hydrocarbons and  $CO_2$ . The  $N_2$  is then admitted into the ion source of the mass spectrometer. The triple-collector is tuned to give a continuous output of the ion intensities of m/z 28, 29, and 30. The three ion intensities are integrated simultaneously over 1 second intervals for each sample. The complete analysis is microprocessor controlled.

Blank samples and standard samples are run throughout the analysis. The automated sample head allows up to 50 samples to be preprogrammed and run unmanned.

#### iv. Calculation of production rates.

The following equation is used for the analysis of nitrogen assimilation (Collos, 1987).

$$P = N (C_p - C_{po})$$
  
-----/t  
(C\_s - C\_{so})

where:

P = rate of nitrogen assimilation ( $\mu$ moles N L<sup>-1</sup> d<sup>-1</sup>) N = particulate nitrogen at the end of the incubation C<sub>p</sub> = atom % 15-N of the particulate fraction at the end of the incubation

 $C_{po}$  = atom % 15-N of the particulate fraction at the start of the incubation

 $C_s$  = atom % 15-N of the substrate at the start of the incubation, after the addition of the spike  $C_{so}$  = atom % 15-N of the substrate at the start of the incubation before the addition of the spike

N is equivalent to the total phytoplankton nitrogen, including the detrital fraction.

C<sub>po</sub> and C<sub>so</sub> are assumed to be equal with an atom % 15-N of 0.3663..oj on

The particulate nitrogen (N) at the end of the incubation is given by the mass spectrometer. This is machine blank corrected and then corrected for the paper blank, (the blank incurred from a tin cap containing the same number of filter subsamples.) This value is then converted to N L<sup>-1</sup> by multiplying the area of the subsample of the filter up to the area of the complete filter and then

correcting for the amount of sample actually filtered. The atom 3 of 15-N particulate at the end of the incubation (C<sub>p</sub>) is given directly by the mass spectrometer.

The 15-N atom % substrate is calculated from the following equation.

$$C_{s} = (N_{o} \times \text{atom } 15 - N_{o}) + (N_{add} \times \text{atom } 15 - N_{add})$$
$$(N_{o} + N_{add})$$

Where:

 $N_{o}$  = ambient concentration of natural nitrogen (nitrate or ammonia)

atom %  $15-N_0$  = atom % of natural nitrogen ( = 0.3663) N<sub>add</sub> = amount 15-N labelled nitrogen added in spike atom %  $15-N_{add}$  = atom % of added 15-N (  $NH_4Cl_2$  = 99.7,  $Na^{15}NO_3$  = 99.2).

#### v. Performance of technique.

The internal precision of the mass spectrometer is reported as + 0.00026 atom  $\frac{15}{N} \pm 0.74$  o/oo\* (Preston and Owens, 1983). The accuracy of the measurement of atom  $\frac{1}{3}$ is, however, currently impossible to establish as no internationally certified standard enriched in 15-N is available (Owens, 1988). The precision limits for the 15-N technique are clearly not determined by the mass spectrometry, but by the sampling strategy, and of paramount importance, by the determination of the ambient nutrient concentrations. Dugdale and Wilkerson, (1986) have reviewed the use of 15-N for determining rate measurements and it would not be out of place to summarise here their choice of factors which may create inaccuracies in the rates of nitrogen uptake.

1. Choice of equation to calculate nitrogen uptake

2. Accuracy of analytical procedure

i. DIN and DON analysis

ii. isotope ratio analysis

3. Presence of detrital nitrogen

4. Simultaneous uptake of unlabelled and labelled sources of nitrogen in incubations

5. Perturbation of uptake rates by addition of labelled compound

6. Changes of uptake rate during the incubation period

7. Effects from using glass incubation bottles

8. Discrepancies between 14-N disappearance and 15-N uptake

9. Ammonium production (ammonification) that creates dilution in isotope concentration during incubation.

A number of these points are not of paramount concern, for example, in answer to (1), the equation chosen for calculations are from Collos, (1987) and have been extensively used by Owens (Owens, 1986, 1988; Owens et al., 1987), and point (7), the use of glass bottles has been replaced with 2L polycarbonate bottles which, of all the techniques used in this study, are of the least concern due to their size.

The accuracy of the analytical technique (2), has already been demonstrated. However, the measurement of the ambient concentration of nitrate and ammonia is of paramount importance in the calculations and I would like to stress, is without a doubt the most critical determinant in the accuracy of the ammonia assimilation rates in this study. The method of nutrient analysis used in this study is by Auto-analyser. The reported precision of this technique for nitrate analysis has a standard deviation of 0.05  $\mu$ mol L<sup>-1</sup> and for ammonia, 0.06  $\mu$ mol L<sup>1-</sup> (Garside, pers.comm). However, the lower limit to the standard deviation for the ammonia analysis in the 1990 field programme was 0.5  $\mu$ mol L<sup>-1</sup>. More error will incur as the ambient nutrient concentration decreases, which is pertinent to the area studied in this thesis. By increasing the ammonia concentration by 0.5  $\mu$ mol L<sup>-1</sup> in my calculations, I effectively doubled the calculated ammonia uptake rate.

Another potentially important source of error, not mentioned previously, is from the subsampling from the filters. The uneven spread of filtrate often encountered caused me concern, however analysis of the data from 1990 gave a standard deviation of 0.009  $\mu$ mol L<sup>-1</sup> day<sup>-1</sup> from replicate subsamples, and a standard deviation of 0.024  $\mu$ mol L<sup>-1</sup> day<sup>-1</sup> from separate bottle replicates.

#### 4.3.5. The 14-C technique.

The 14-C rate measurements that are cited in this study were undertaken by Dr.G.Savidge, (Q.U.B.), Dr.P.Boyd, (Q.U.B.), and Alan Pomroy, (P.M.L.). The methodology employed is briefly outlined below.

The incubations for 14-C rate measurements were done in acid cleaned 60 cm<sup>3</sup>l polycarbonate bottles. The cleaning procedure entailed filling the bottles with 0.25 mol L<sup>-1</sup> Analar grade HCl, leaving to stand for three days, and then rinsing in Milli-Q grade water. The bottles were then left to stand for a further two days, filled with Milli-Q grade water. Stock 14-C solutions were prepared in serum bottles, cleaned in the same way as the incubation bottles, and sealed with teflon lined silicon caps.

The sampling procedure was as for the oxygen technique, with the water being taken from the same acid cleaned Go-Flo bottles. The samples were transferred to the  $60 \text{cm}^3$ polycarbonate incubation bottles. Each bottle was inoculated with 370 KBq (10 µCi) NaH<sup>14</sup>CO<sub>3</sub>, supplied by Amersham International. The specific activity of each stock solution was determined immediately after the inoculation of the experimental samples.

Three light replicates, and one dark bottle were attached

to an acrylic rack and suspended from a free floating incubation rig at the depths that the samples were taken. The samples were incubated for 24 hrs, in situ, and then filtered sequentially through 5  $\mu$ m, 1  $\mu$ m, and 0.2  $\mu$ m poresize Nucleopore polycarbonate filters in a cascade filtration apparatus (Joint and Pomroy, 1983). Samples were then placed in a desiccator for 2 minutes, with fuming HCl, and then dried. The samples were then counted on board ship in a liquid scintillation counter. The efficiency of counting was determined with an external standard, channels ratio method (Joint *et al.*, in press). <u>Chapter 5</u>

### AN INTERCOMPARISON OF THE OXYGEN AND THE TCO2 TECHNIQUE

#### Chapter 5

#### An intercomparison of the oxygen and TCO2 techniques.

During the BOFS 1990 field programme in the N.E.Atlantic, a rendevouz was made between the RRS Charles Darwin, the RRS Discovery, and the Dutch research ship, Tyro. An intercomparison of the oxygen and TCO<sub>2</sub> techniques was made between the three ships.

## 5.1. An intercomparison of the measurement of dissolved oxygen.

The measurement of dissolved oxygen was compared between the U.K. (Emily Wood; Charles Darwin 46), and the Dutch (Gijsbert Kraay; Tyro) on 16/05/1990 in the N.E.Atlantic. Samples were taken from the same water, collected from the CTD rosette sampler on board Tyro. The samples were fixed immediately on board Tyro, with the reagents personal to each researcher. Samples were taken from 24 depths, between the surface and 400m. Two replicate samples were taken from each depth. The U.K. samples, once fixed, were taken back to Darwin and analysed.

The results of the intercomparison are shown in table 5.1.(a). The pooled standard error of the oxygen replicates from CD46 are  $0.43\pm0.03 \ \mu mol \ Kg^{-1}$ . The depth distribution of dissolved oxygen is shown in figure 5.1.(1) from CD46 and Tyro. It can be seen that the measured oxygen concentration from the two research ships are in good agreement, however there is a mean offset of 2.9±0.09  $\mu$ mol Kg<sup>-1</sup>. The relationship between the data is better represented in figure 5.1.(2) where it can be seen that with the exception of only two points, all the data lies on the same line. The offset is mainly due to a difference in the calculation procedure. The Dutch values have been corrected for a seawater blank of 0.8  $\mu$ mol Kg<sup>-1</sup> and a reagent blank of 1.05  $\mu$ mol Kg<sup>-1</sup>. This gives a total correction of 1.85  $\mu$ mol Kg<sup>-1</sup>. If this is taken from the U.K. data the difference between the two rates becomes 1.05  $\mu$ mol Kg<sup>-1</sup>. This we have not as yet been able to account for.

Table 5.1.(a) Intercomparison of the measurement of dissolved oxygen from CD46 and Tyro, on 16/05/1990

Depth M	O <sub>2</sub> conc µmol/Kg	entn. ml/l	$O_2$ sol. $\mu$ mol/Kg	% satn	. insitu temp	insit sal	u Tyro µmol/Kg	
2	293.01	6.724	255.3	114.7	13.707	35.641	291.15	
2	294.16	6.751	255.3	115.2				
x	293.59	6.738		115.0				
s.e	+0.58	+.014		+0.3				
10	287.69	6.602	255.9	112.4	13.625	35.642	291.73	
20	291.16	6.682	256.0	113.7	13.174	35.649	288.67	
20	291.10	6.680	256.0	113.6				
X	291.13	0.081		113.7				
s.e	+0.0	3 + .00	)1	+0.1	10 330	25 652		
30	2/4.30	0.295	25/.2	106.6	13.772	35.653	2/0./9	
30	274.14	6.291	25/.2	106.5				
X	274.22	0.293		100.0				
s.e	+0.08	+.011	250 7	+0.1	10 100	25 (21	264 70	
40	268.21	0.100	209.7	103.2	12.109	32.031	204./9	
40	20/.2/	0.134	259.1	102.9				
X	20/./4	D.143		103.1				
5,e	TU.4/	T.UII	260 9	+0.2	11 970	25 642	256 20	
50	200.04	5.733	200.0	<b>33.1</b>	11.0/9	33.043	200.20	
50 •	200.00	5.727	200.0	99.0				
<b>A</b>	±0 15	J. J.J.Z		<b>77.1</b>				
3.C 40	250 47	5 954	262 A	09 9	11 777	35 622	256 71	
60	259.47	5 9/9	262.4	98.8	11.///	55.022	250.71	
¥	259.21	5.947	20214	98.8				
<b>a</b> . C A	+0.13	+.002		+0.1				
3.C 75	257.58	5.911	265.4	97.0	11.613	35.610	254.93	
75 75	257.54	5.910	265.4	97.0	11,013	JJ.010	234133	
¥	257.56	5.911		97.0				
- S.A	+0.02	+.000		+0.0				
100	261.30	5.996	266.2	98.1	11.471	35,596	258.90	
100	261.36	5.998	266.2	98.1		••••		1
X	261.33	5.997		98.1				
s.e	+0.03	+.001		+0.0				
150	260.04	5.976	266.9	97.4	11.353	35.583	258.45	
150	260.54	5.979	266.9	97.5				
X	260.29	5.978		97.5				
s.e	+0.25	+.002		+0.1				
200	259.82	5.962	267.3	97.1	11.263	35.565	257.41	
200	260.04	5.967	267.3	97.2				
ĸ	259.93	5.965		97.2				
s.e	+0.11	+.003		+0.1				
300	257.90	5.918	269.0	95.8	10.941	35.513	255.09	
300	257.10	5.900	269.0	95.8				
K	257.50	5.909		95.8				
	+0.40	+.009		+0.0				

Table 5.1.(a) (cont.)

Depth M	O <sub>2</sub> conce µmol/Kg	entn. ml/l	O <sub>2</sub> sol. µmol/Kg	<pre>%satn</pre>	Insitu temp	Insitu sal	Tyro µmol/Kg
400	258.41	5.795	336.3	76.8	10.530	35.446	249.64
500	239.57	5.497	273.8	87.4	10.183	35.407	236.48
500	240.10	5.509	273.8	87.6			
x	239.76	5.503		87.5			
s.e	+0.35	+.006		+0.1			
600	241.81	5.548	276.6	87.3	9.843	35.383	239.37
600	242.33	5.560	276.6	87.6			
x	242.07	5.554		87.5			
s.e	+0.26	+.006		+0.1			
700	214.36	4.918	279.9	76.5	9.323	35.346	211.18
700	215.77	4.951	279.9	77.0			
X	215.07	4.933		10.8			
S.e	+0.71	T.UI/	292 5	TU.J	8 703	25 250	198 02
1000	200.00	4.004	202.5	71.0	0./33	39.339	190.02
	201.31	4.612	202.3	71.1			
	+0.32	+.008		+0.1			
1000	207.52	4.761	290.5	71.4	7.578	35.344	204.25
1000	207.60	4.763	290.5	71.4			
x	207.56	4.762		71.4			1
s.e	+0.04	+.001		+0.0			1
1200	229.11	5.256	301.5	75.9	6.047	35.189	226.59
1200	229.37	5.262	301.5	76.0			
x	229.24	5.259		76.0			
s.e	+0.13	+.003		+0.1			
1500	261.74	6.004	313.6	83.4	4.268	34.969	259.12
1500	262.73	6.026	313.6	83.7			
x	262.24	6.015		83.6			
s.e	+0.13	+.003	<b>-</b>	+0.2			
2000	272.72	6.255	319.5	85.3	3.607	34.931	270.43
2000	273.26	6.268	319.5	85.5			
	2/2.99	0.202		03.9 +0 1			
5.e	TU.2/	T.00/	222 0	TU.I	3 222	24 050	262 52
2500	205.04	6 096	322.0	02.2 93 7	J. 222	34.333	202.55
2500	203.32	6.083	522.0	82.1			
	+0 14	+ 004		+0.1			
3000	251.12	5.760	325.1	77.2	2.837	34.949	249,11
3000	253.73	5.820	325.1	78.0	<i></i>	¥31838	
	252.43	5.790		77.6			
s.e	+1.31	+.030		+0.4			
5000	249.46	5.722	327.6	76.1	2.277	34.908	239.46
5000	242.44	5.561	327.6	73.9			
x	245.95	5.642		75.0			
s.e	+3.51	+.081		+1.1			

All oxygen concentrations corrected to 25°C.



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## 5.2.An intercomparison of the measurement of the metabolism of oxygen.

Samples were collected on board Darwin from the same water sample, taken from the 50L Go-Flo sample bottles. The oxygen bottles were filled after the methods in chapter 4. The bottles were kept in the dark while the Dutch filled their bottles and returned to Tyro. The samples were then incubated on each incubation rig. The samples were incubated from 10:50 until 19:45. The time zero bottles were fixed at 10:40. The U.K samples were fixed as outlined in chapter 4. The dark bottles were incubated on board ship.

The results of the productivity intercomparison are shown in table 5.2.(a). The difference between the rate measurements and the standard error on the difference is also shown. The rates agree to within ten percent of each other. The discreet values are however around 10  $\mu$ mol Kg<sup>-1</sup> different, the Dutch values this time being higher. (No corrections have been made to either data sets). This difference in discreet values would effect the eventual calculated rates by about 3 %, which is about the same order as the random error due to the titration. The mean standard error of the U.K. results was 0.25  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> 1, the Tyro mean standard error was three times as large as this being 0.72  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup>.

At the surface the gross oxygen production rates measured by the Dutch are 7.4 % higher than the U.K. rates, however the respiration rates are 11 % lower. If we are to expect any marked difference between the rates it is most probably likely to manifest itself in the surface samples owing to the different incubation rigs used. At 10M the rates are in close agreement, with the U.K. rates of gross production being only 4 % higher than the Dutch, and the U.K. respiration rates, some 8 % higher. At 20M the agreement is not so good, however the production rates are very close to the analytical precision of the technique. At 35M the rates are undetectable.

Although there are differences in the productivity rates, for the most part they are not significant at the 95 **%** level, (less than twice the value of the standard error).

#### Table 5.2.(a)

Table showing the intercompariosn of oxygen metabolism with depth between the U.K. (Emily Wood; Darwin 46) and the Dutch, (Gijsbert Kraay; Tyro) on 16/05/1990 between 10:50 - 19:45.

	Darwin 46	Tyro	D-T			
Depth = 2M						
Z	285.52+0.25	295.78+0.11				
<u></u> ተ	295.64+0.23	306.92 <u>+</u> 0.51				
D	283.44 <u>+</u> 0.15	293.90 <u>+</u> 0.61				
L						
Ea	$12.12 \pm 0.27$	13.02 <u>+</u> 0.80	0.90 <u>+</u> 0.84			
Rc LC	$10.04 \pm 0.34$	11.00 <u>+</u> 0.52	0.90 <u>+</u> 0.62			
K	2.08 <u>+</u> 0.29	1.88 <u>+</u> U.62	-U.2U <u>+</u> U.68			
Depth = 10M						
Z	285.33 <u>+</u> 0.17	293.27 <u>+</u> 0.27				
L	288.48 <u>+</u> 0.15	296.39 <u>+</u> 0.87				
D	282.57 <u>+</u> 0.28	290.72 <u>+</u> 0.50				
<b>D</b>		E (711 AA	_0 24+1 22			
rg	5.91 <u>+</u> 0.32 2 15±0 22	$5.0/\pm 1.00$ 2.12±0.01	-0.241.22			
	3.15 <u>+</u> U.23 3.76±0.23	3.12 <u>1</u> 0.91 2 5510 57	$-0.03 \pm 0.94$			
л 	2./0 <u>t</u> u.jj	2.33 <u>7</u> 0.37	U.24 <u>T</u> U.UO			
Depth = 20M						
2	265.89 <u>+</u> 0.23	274.27 <u>+</u> 0.46				
L	266.19 <u>+</u> 0.14	274.22 <u>+</u> 0.58				
D	265.35 <u>+</u> 0.03	274.54 <u>+</u> 0.46				
Pa	0.84+0.14	-0.32+0.74	-1,16+0.75			
PC	0.30+0.27	-0.05+0.74	-0.35+0.79			
R	0.54+0.23	-0.27+0.65	-0.81+0.69			
Depth = 35M		0/0 00 0				
	261.11 <u>+</u> 0.17	269.89 <u>+</u> 0.58				
<u>г</u>	261.40 <u>+</u> 0.08	209.55 <u>+</u> 0.33				
ע	201.29 <u>+</u> 0.12	209.60 <u>+</u> 0.54				
Pa	$0.11 \pm 0.14$	-0.05+0.63	-0.16±0.65			
Pc	0.29 <u>+</u> 0.18	$-0.34 \pm 0.67$	-0.63 <u>+</u> 0.69			
R	-0.18 <u>+</u> 0.21	0.29 <u>+</u> 0.79	0.47 <u>+</u> 0.82			
Where; 2 = zero time oxyge: L = light bottle oxy D = dark bottle oxyge: Pg = gross productions	There;					
Pc = net community   R = respiration (um	production (µmo	1 Kg <sup>-1</sup> 9hr <sup>-1</sup> )				

D-T = difference in production rate from Darwin and Tyro.

#### 5.3.An intercompariosn of the measurement of TCO2.

An intercomparison of the measurement of  $TCO_2$  was made between R.R.S.Charles Darwin 47, and R.R.S.Discovery 191. The intercomparison offered an opportunity to compare the two coulometric systems used at UCNW, the HP5010 system the HP86 computer controlled coulometer, model 5010 -(this study), and the IBM5011 system - the IBM PC computer controlled coulometer, model 5011 (C. Robinson, UCNW).

On 29/05/1990 samples were collected for  $TCO_2$  from a CTD cast on each ship from the surface to 150m. Samples were collected on CD47 at 07:05 hours at 48.36°N 17.41°W. The samples were fixed and analysed on the HP5010 system on board ship over the next 48hrs. Samples were collected on Discovery 191 at 06:56 hours at 48.41°N 17.45°W. The samples were fixed and returned to the laboratory to be analysed on the IBM5011 system.

The results of the intercomparison are shown in table 5.3.(a). The difference between the two measurements is also shown. A very precise intercomparison is not possible as the bottles were fired at different depths on the two ships. The depth distribution of  $TCO_2$  is shown in figure 5.3.(1). The mean difference in concentration is 7.6  $\mu$ mol Kg<sup>-1</sup> assuming that the depths are the same to within

6M. This is a maximum difference in the measured TCO<sub>2</sub> concentration of 0.4 %.

#### Table 5.3.(a)

Table showing the TCO<sub>2</sub> in situ data obtained on Discovery 191 (D191) and Darwin 47 (CD47) on 29/05/1989 where Discovery 191 data analysed on IBM (C.Robinson).

Depth M	<b>TCO2 (D191)</b> μmol/Kg	Depth M	<b>TCO2 (CD47</b> μmol/Kg	) D-C µmol/Kg
1.6	2037.1	0.5	2041.88	4.78
8.9	2050.6	3.4	2041.36	-9.24
19.8	2055.7	13.4	2054.25	-1.45
28.4	2085.8	23.3	2071.64	-14.15
-	-	33.2	2110.07	-
48.7	2119.8	42.7	2107.81	-11.19
-	-	53.5	2116.99	-
-	-	67.2	2116.99	-
73.9	2123.6	-	-	-
98.7	2125.6	93.3	2118.81	-6.79
148.3	2126.4	142.8	2121.53	-4.87

Where; D-C = difference between  $TCO_2$  concentration from Discovery 191 and CD47.

A second, less stringent parallel set of measurements was made between the two coulometric systems. Because the samples were taken on consecutive days the exercise could not be treated as an intercomparison. Samples were taken from the CTD cast from the surface to 150m on each ship. Samples were collected on Discovery 191 on 01/06/1990 at 06:33 hours at 48.28°N 17.06°W and stored for later analysis on the IBM5011 system at the laboratory. Samples



were collected on CD47 on 02/06/1990 at 08:00 hours at 48.07°N 16.85°W and analysed on board ship on the HP5010 system.

The results of the intercomparison are shown in table 5.3.(b). The depth distribution of TCO<sub>2</sub> is shown in figure 5.3.(2).

The mean difference in concentration is 10.5  $\mu$ mol Kg<sup>-1</sup>. The depths sampled are within 7m of each other, and the samples were taken on Darwin 24 hrs after the samples were taken on Discovery 191. The difference in TCO<sub>2</sub> concentration between the two systems is 0.5 percent.

#### Table 5.3.(b)

(CD47) on 02/06/1990 where analysis on D191 on the IBM analyser (C.Robinson).							
Depth M	<b>TCO2 (D191)</b> μmol/Kg	Depth M	<b>TCO2(CD47)</b> μmol/Kg	<b>C-D</b> µmol/Kg			
1.4	2040.56	0.5	2039.15	-1.41			
	2033.30	5.5					

Table showing TCO<sub>2</sub> in situ data from a Damiin 47

М	μmol/Kg	Deptn M	μmol/Kg	<b>C-D</b> μmol/Kg	
1.4	2040.56	0.5	2039.15	-1.41	
8.9	2039.90	3.5	2039.78	-0.12	
19.4	2100.31	13.2	2041.89	-58.42	
29.1	2115.42	22.5	2100.64	-14.78	
38.5	2117.72	34.3	2118.00	0.28	
48.7	2119.19	42.8	2118.26	-0.93	
-	-	53.7	2119.40	-	
-	-	67.6	2117.91	-	1
73.2	2118.40	-	-	-	ļ
99.3	2115.72	92.5	2121.07	5.35	
148.7	2117.19	142.8	2120.03	2.84	ļ

where;

C-D = difference between  $TCO_2$  concentration from Discovery 191 and CD47.



#### <u>Chapter 6</u>

EXPERIMENTAL STUDY OF THE RELATIONSHIP BETWEEN THE PHOTOSYNTHETIC QUOTIENT AND THE F-RATIO WITH A CULTURE OF THE DIATOM THALASSIOSIRA WEISSFLOGGI

#### Chapter 6.

# Experimental study of the relationship between the photosynthetic quotient and the f-ratio with a culture of the diatom Thalassiosira weissfloggi.

#### 6.1.Objectives.

The principal objective of this aspect of the study was to examine the relationship between the photosynthetic quotients derived from the oxygen and  $TCO_2$  technique  $(PQ[O_2/TCO_2])$  with the f-ratio (Pnew+Preg/Pnew), obtained from observations on the uptake of 15-NO<sub>3</sub> and 15-NH<sub>4</sub>, on a batch culture of the diatom Thalassiosira weissfloggi.

The strategy behind the culture experiments was to try and address questions concerning the relationship between the P.Q. and f-ratio in a controlled environment. In the field, observations too often have been made with insufficient knowledge of the ecological and biological circumstances to enable a rigorous analysis to be made. The most important aspect of this section of the study is that the supply of nutrients and their history can be known and monitored.

An axenic culture of the diatom Thalassiosira weissfloggi

was chosen because of its abundance in the area of the 1989 BOFS field programme, and because of the amount of literature that is available on culturing and maintaining this species.

#### 6.2. Experimental.

A batch culture of the diatom Thalassiosira Weissfloggi was grown in a light and temperature controlled room. The growth curve was monitored by measuring daily chlorophyll a concentrations and counting the cell numbers. The experiments were undertaken on the cultures during the exponential phase of their growth. When the stationary phase of growth had been reached a new batch of culture was subcultured and the experiments repeated.

For the nitrate assimilation experiments the culture was grown on an ammonium free medium with nitrate as the nitrogen source. Ammonia was then added to the culture in a quantity in excess of the concentration known to inhibit nitrate uptake.

The ammonia assimilation experiments were conducted 24hrs after the initial addition of ammonia.

#### 6.2.1.Culture preperation and growth.

The batch culture was prepared in flasks containing 2L of chemically cleaned and  $0.2\mu$ m filtered seawater and grown on f/2 Guillard medium. The culture was grown in a temperature controlled room (at 17°C) with a 12hr constant light and dark cycle. All handling of the culture was done

in a laminar flow unit.

6.2.2. Experimental protocol.

i) The growth phase of the culture was determined by chlorophyll a and cell counts.

ii) The required volume of culture was decanted and diluted to give the same number of cells per unit volume for every incubation.

iii.a) The diluted culture was then siphoned into 14,  $100 \text{cm}^3$  calibrated glass bottles for oxygen light/dark bottle incubations, and 9,  $250 \text{cm}^3$  glass bottles for TCO<sub>2</sub> light/dark bottle incubations.

b) Samples were collected in acid cleaned 10cm<sup>3</sup> vials for measurement of the ambient nutrient concentration. The samples were then frozen for subsequent analysis.

c) For the measurement of nitrate and ammonia assimilation the diluted culture was siphoned into 2, 2.4L polycarbonate bottles and inoculated with a 10 percent addition of  $15-NH_4Cl$  and  $15-NaNO_3$  respectively.

iv) The light oxygen and  $TCO_2$  bottles, and the 15-N bottles, were stored in a fixed temperature incubator for

a 12hr light and 12hr dark period. The dark oxygen and  $TCO_2$  bottles were stored for 24hrs in a dark incubator at the corresponding temperature.

v) The zero time bottles were fixed according to the protocol in chapter 4.

vi) After 24hrs the light oxygen and TCO<sub>2</sub> bottles were fixed. The 15-N samples were filtered on to ashed 47mm GF/F filters and frozen. Oxygen concentrations were measured using a manual Winkler titration system, (chapter 3.3). Carbon dioxide concentrations were measured using the automated coulometric system of Robinson and Williams, (1991), (chapter 3.4). The 15-N samples were analysed on the mass spectrometer by the methods of Owens, (1988), (chapter 3.5).

#### 6.2.3.Cell counts.

A 500  $\mu$ l volume of the culture was removed and the cell number counted on a coulter multisizer. The number of cells was taken as the mean of three counts.

#### 6.2.4.Chlorophyll a extraction.

The concentration of chlorophyll a was measured from
acetone extraction and flurometric analysis. The procedure was to filter  $10 \text{ cm}^3$  of the sample on to an ashed GF/F filter, which was then frozen, (three replicate samples were taken). Prior to analysis,  $10 \text{ cm}^3$  of acetone was added, the sample was homogenized, and then separated in a centrifuge. A subsample was decanted into a vial and placed in a flurometer. The flurometric signal was then measured before and after the addition of  $1 \text{ cm}^3$  of hydrochloric acid, (the mean of three readings per filter was taken). The chlorophyll *a* concentration was then measured from;

A x (Rb-Ra) x F =  $\mu$ g Chla ml<sup>-1</sup> where; A = calibration setting Rb= mean fluorescence before acid addition Ra= mean fluorescence after acid addition F = dilution factor = <u>volume extract</u> sample volume

# 6.2.5.Culture dilution.

The culture dilution was chosen such that the concentration of cellular carbon per unit volume was twice as high as that recorded in the 1989 BOFS field programme. This was to ensure that the measured rates of production would be in excess of 10 times the analytical error of analysis. The carbon concentration for each experiment was 200  $\mu$ g C L<sup>-1</sup>. From reference, the carbon

equivalent for Thalassiosira weissfloggi is  $100\mu$ g C per 716 cells.

The required dilution for  $200\mu g$  C L<sup>-1</sup> is therefore:

 $2 \times 716 \times \frac{\text{Total volume of culture}}{\text{number of cells (cm}^3)}$ 

## 6.3.Results.

6.3.1.Growth curves of culture from cell counts and chlorophyll a.

A total of six batches of culture were grown, with three experiments being undertaken on each batch. Figure 6.3.(1) shows the growth curves of the cultures derived from the chlorophyll a concentrations and from the cell counts. The time of sampling and the time of the addition of ammonia is shown. The daily chlorophyll a concentrations and cell counts for each culture are listed in table 6.3.(a). The change in chlorophyll a in  $\mu$ g L<sup>-1</sup> day<sup>-1</sup> and as a total percentage change over the 24hr experimental incubation, are shown in table 6.3.(e).

# Table 6.3.(a)

Table showing the cell counts and Chlorophyll a concentration for each day of the culture experiments.

Culture batch	Date	Experiment	Cell count (m1 <sup>-1</sup> )	chlor a ( $\mu g$ ml <sup>-1</sup> )
A	30/10	Ali	36,500	166
λ	31/10	Alii	53,800	219
λ	01/11	A2iii	70,900	258
λ	02/11	A2iV	82,600	331
λ	03/11	A2V	-	274
λ	06/11	A2Viii	-	216
В	03/11	Bli	-	3
В	04/11	B111	-	/
В	05/11	B1111	700	9
В	06/11	B11V	20,700	80
В	07/11	B2V	30,000	169
В	08/11	B3V1	44,300	251
В	09/11	B3V11	-	300
В	10/11	B3V111	-	000
В	11/11	BJIX	-	/31 631
D	12/11	DJA	-	692
	14/11	DJAL	-	627
D	14/11	DOXII	-	720
D B	15/11	DJAIII	_	794
D	17/11	DJAIV	_	615
B C	1//11	D3AV C11	-	17
C	10/11		_	43
C	11/11		-	54
C	12/11		-	85
C	13/11		-	132
Ċ	$\frac{13}{11}$	C2Vi	-	203
Ċ	15/11	C3Vii	-	264
č	16/11	C3Viii	-	315
Ċ	17/11	C3iX	-	273
c	18/11	C3X	-	293
c	19/11	C3Xi	-	363
D	17/11	Dli	-	67
D	18/11	Dlii	-	105
D	19/11	Dliii	30,300	172
D	20/11	£2iV	-	342
D	21/11	D3V	-	318
Е	03/12	Eli	-	14
E	04/12	Elii	-	20
E	05/12	Eliii	10,800	67
E	06/12	EliV	39,000	126
E	07/12	ElV	-	241
E	08/12	E2Vi	-	267
E	09/12	E3Vii	-	315
F	08/12	Fli	-	15
F	09/12	Flii	-	36
F	11/12	FliV	6,900	86
F	12/12	F2V	28,100	169
F	13/12	F3Vi	-	210



## 6.3.2. Primary production rate measurements.

Primary production rate measurements were made using two chemical techniques, the oxygen Winkler titrimetric technique and the  $TCO_2$  coulometric technique. The photosynthetic quotient (P.Q.) was calculated from PgO<sub>2</sub> and PgTCO<sub>2</sub>, and the respiratory quotient (R.Q.) was calculated from RTCO<sub>2</sub> and RO<sub>2</sub>.

The measured rates of gross production (Pg), net community production (Pc), and respiration (R), from oxygen and  $TCO_2$  measurements are shown in table 6.3.(b). The calculated P.Q. and R.Q. values are shown in table 6.3.(c).

## Table 6.3.(b)

Table showing the Gross production, (Pg), Net community production, (Pc), and respiration (R) from  $O_2$  and  $TCO_2$ ,  $(\mu molkg^{-1}day^{-1} \pm S.E)$ .

Expt Pg O <sub>2</sub>	Pc O2	R O2	Pg TCO <sub>2</sub>	Po TCO2	R TCO2
A2 7.75 $\pm$ 0.45 B1 8.22 $\pm$ 0.58 B2 13.77 $\pm$ 0.35 B3 17.65 $\pm$ 0.73 1 C1 13.14 $\pm$ 0.86 C2 6.28 $\pm$ 1.39 C3 9.65 $\pm$ 0.73 D1 8.95 $\pm$ 0.92 D2 12.51 $\pm$ 0.42 E1 17.57 $\pm$ 0.99 1 E2 14.12 $\pm$ 0.35 E3 16.58 $\pm$ 0.79 1 F1 11.42 $\pm$ 1.53 1 F2 16.62 $\pm$ 1.76 1	$\begin{array}{c}$	$\begin{array}{c}$	$\begin{array}{c} 6.05 \pm 1.00\\ 6.73 \pm 1.18\\ 6.12 \pm 1.52\\ 11.36 \pm 0.51\\ 10.32 \pm 0.87\\ 4.93 \pm 0.92\\ 6.67 \pm 1.59\\ 7.36 \pm 0.69\\ 9.54 \pm 1.35\\ 12.80 \pm 1.62\\ 9.88 \pm 0.57\\ 12.93 \pm 1.51\\ 9.98 \pm 0.79\\ 14.72 \pm 0.42\\ 12.16 \pm 1.74\\ \end{array}$	$\begin{array}{c} 0.11\pm1.45\\ 0.59\pm1.29\\ 0.76\pm2.01\\ 4.68\pm1.39\\ 3.86\pm1.36\\ 1.57\pm1.24\\ 0.98\pm2.21\\ 3.12\pm1.66\\ 5.07\pm1.78\\ 8.40\pm2.60\\ 6.04\pm0.89\\ 5.96\pm2.18\\ 9.72\pm0.89\\ 13.29\pm1.69\\ 8.48\pm2.39 \end{array}$	5.93 $\pm$ 1.09 6.14 $\pm$ 0.54 5.36 $\pm$ 1.33 6.68 $\pm$ 1.29 6.46 $\pm$ 1.05 3.36 $\pm$ 0.82 5.69 $\pm$ 1.54 4.24 $\pm$ 1.51 4.47 $\pm$ 1.16 4.40 $\pm$ 2.03 3.84 $\pm$ 0.68 6.97 $\pm$ 1.57 0.26 $\pm$ 0.40 1.43 $\pm$ 0.93 3.68 $\pm$ 1.64

# Table 6.3.(c)

Table showing the photosynthetic quotient  $PQ[O_2/TCO_2] \pm S.E$ and respiration quotient  $RQ[TCO_2/O_2] \pm S.E$ . Where PQ(T) = theoretically caluclated PQ from C:N ratio and f-ratio.

Nitrogen sourc	e Expt	PQ <u>+</u> 8.E	PQ(T)	RQ ± 8.5	RQ(T)
Nitrate	A2	1.28 <u>+</u> 0.22	-	1.46 <u>+</u> 0.28	-
	B1	$1.22 \pm 0.23$	-	$1.44 \pm 0.22$	-
	B2	$1.25 \pm 0.56$		1.14 <u>+</u> 0.29	-
	B3	1.55 <u>+</u> 0.09	-	1.24 <u>+</u> 0.26	-
	C1	1.27 <u>+</u> 0.12	1.14	1.51 <u>+</u> 0.35	0.88
	C2	1.27 <u>+</u> 0.37	1.17	1.04 <u>+</u> 0.63	0.85
	C3	1.45 <u>+</u> 0.36	1.13	1.18 <u>+</u> 0.35	0.88
	D1	1.22 <u>+</u> 0.17	1.16	0.59 <u>+</u> 0.22	0.86
	E1	1.37 <u>+</u> 0.19	1.13	0.96 <u>+</u> 0.50	0.88
	F1	1.14 <u>+</u> 0.18	1.12	0.32 <u>+</u> 0.75	0.89
Mean quotient		1.30 <u>+</u> 0.41		1.09 <u>+</u> 0.34	
Ammonia	D2	1.31 <u>+</u> 0.19	1.17	0.64 <u>+</u> 0.18	0.85
	E2	1.43 <u>+</u> 0.09	1.15	0.62 <u>+</u> 0.12	0.87
	E3	$1.28\pm0.16$	1.12	$1.18 \pm 0.36$	0.89
	F2	$1.13 \pm 0.18$	1.12	$1.01 \pm 0.25$	0.89
	F3	1.31 <u>+</u> 0.18	1.12	1.22 <u>+</u> 0.15	0.89
Mean quotient		1.29 <u>+</u> 0.05	0.93	<u>+</u> 0.13	
Mean quotient	(total)	1.30 <u>+</u> 0.03	1.04	<u>+</u> 0.09	

The mean P.Q. for the cultures grown with nitrate as the main nitrogen source, is  $1.30\pm0.04$  with a mean standard error of 0.25. The mean P.Q. for the culture fed on ammonia is  $1.29\pm0.07$  with a mean standard error of 0.22. The overall mean P.Q. obtained is  $1.30\pm0.03$  with a mean standard error of 0.12. The range of P.Q. values is 1.14 to 1.68.

Figure 6.3.(2) shows the frequency distribution of P.Q. values in relation to the nitrogen source utilised.



The mean R.Q. values for the culture grown on nitrate is  $1.09\pm0.12$  with a mean standard error of 0.75. The mean R.Q. of the culture grown on ammonia is  $0.93\pm0.14$  with a mean standard error of 0.21. The total mean R.Q. is  $1.04\pm0.1$  with a mean standard error of 0.33. The range of R.Q. values is 0.32 to 1.51.

Figure 6.3.(3) shows the frequency distribution of R.Q. values in relation to the nitrogen source utilised.

## 6.3.3.Nutrient analysis and 15-N assimilation rates..

For the calculation of  $15-NO_3$  and  $15-NH_4$  assimilation rates the ambient nitrogen concentration must be determined. The nitrogen concentrations ( $NO_3$ ,  $NO_2$  and  $NH_4$ ) were analysed on the auto-analyser. The timing of the experiments did not always permit the immediate analysis of the nutrients and storing samples is not satisfactory. As a consequence the nutrient concentrations were, therefore, only measured for two of the experiments, prior to and after the addition of ammonia.

The initial concentration of ammonia added to the culture was known and so subsequent ammonia concentrations were able to be calculated from the initial concentration minus that taken up over the ensuing 24hrs. The  $15-NH_4$  spikes



added to the incubations were all acceptable tracer additions (7.8±1.4 percent of the ambient concentration).

Because the initial NO<sub>3</sub> concentration was not known until after the experiments had been conducted, the  $15-NO_3$ spikes were often too high, (26.8±4.8 percent of the ambient nitrate concentrations). The calculated rates of nitrate assimilation (P<sub>new</sub>), ammonia assimilation (P<sub>reg</sub>), and total nitrogen assimilation (P<sub>t</sub>) are shown in table 6.3.(d) along with the calculated f-ratio (P<sub>new</sub>/P<sub>t</sub>).

## Table 6.3.(d)

Table showing the assimilation rates of 15-NO<sub>3</sub> ( $P_{new}$ ); 15-NH<sub>4</sub> ( $P_{reg}$ ); 15-NO<sub>3</sub> + 15-NH<sub>4</sub> ( $P_t$ ); and f-ratio ( $\mu$ moll<sup>-1</sup>day<sup>-1</sup> ± S.E)

Nitrogen	source	Expt	Pnew ±	8.E	Preg	± 8.5	Pt	f-ratio
Nitrate		D1	0.206 <u>+</u> (	0.008				1.00
		E1	0.160 <u>+</u>	0.020	-	-	-	1.00
		F1	0.122±	0.011	-	-	-	1.00
Ammonia		D2	0.313 <u>+</u>	0.002	1.039	<u>+</u> 0.018	1.35	2 0.23
		E2	0.235 <u>+</u>	0.001	1.333	<u>+</u> 0.189	1.56	8 0.15
		E3	0.290 <u>+</u> (	0.009	1.314	<u>+</u> 0.191	1.60	4 0.18
		F2	0.110	-	1.416	<u>+</u> 0.022	1.42	6 0.08
		F3	0.086 <u>+</u> (	0.004	0.968	±0.004	1.05	4 0.08

## 6.3.4.C:N assimilation ratios.

The ratio of carbon to nitrogen assimilated over the 24hr incubation is calculated for the indices of gross and net production. Gross production C:N assimilation ratios  $(C:N_{(g)})$  are calculated from  $P_g(TCO_2)$  and  $P_t(15-N)$ . Net production C:N assimilation ratios  $(C:N_{(n)})$  are calculated from  $P_c(TCO_2)$  and  $P_{new}(15-NO_3)$ .  $C:N_{(g)}$  and  $C:N_{(n)}$  ratios are shown in table 6.3.(e).

# Table 6.3.(e)

Table showing the C:N assimillation ratios (C:N(g) from  $Pg(TCO_2:Pt)$  and  $C:N_{(n)}$  from  $Pc(TCO_2:Pnew)$  and the change in chlorophyll a concentration ( $\mu g L^{-1} day^{-1}$  and as a percentage increase.)

Nitrogen	source	Expt	C:N(g)	C:N(n)	Chl a	\$
Nitrate		A2	-	-	73.58	28
		B1	-	-	88.62	110
		B2	-',	-	81.79	48
		B3	- <sup>v</sup>	-	115.55	46
		C1	-	-	70.51	53
		C2	-	-	61.02	30
		C3	-	-	51.36	19
		D1 :	35.73	15.15	-24.31	-7
		E1 8	B0.00	52.50	25.68	11
		F1 8	81.80	79.67	86.33	96
Ammonia		D2	7.06	16.20	-24.31	-7
		E2	6.30	25.70	47.81	18
		E3	8.06	20.55	-	-
		F2 3	10.32	120.82	48.78	29
		F3	9.25	86.98	-	

# 6.4.Discussion

The distribution of P.Q. values observed from these experiments are within the expected range of P.Q. values for conventional metabolites of photosynthesis. The values are in the range of those reported by Williams and Robertson (1991) who have used the same chemical techniques in their experiments.

However it is evident that certain aspects of the data are difficult to account for. The distribution of P.Q. values in relation to the nitrogen source utilised is in contradiction to the expected distribution (Williams et al., 1979; Laws, 1991; Williams and Robertson, 1991). The mean P.Q. values obtained from these experiments are 1.29 for the culture grown on ammonia and 1.30 for the culture grown on nitrate. We would expect there to be a difference in these two values, a lower P.Q. of 1.1 being attributable to ammonia assimilation and a higher P.Q. of 1.4 being attributable to nitrate assimilation. It would appear that during all the experiments the cultures were being grown in a predominantly ammonia driven environment. The following reasoning is put forward to support this case.

The ambient ammonia concentrations at the start of the incubations were undetectable. However, the culture may

have been excreting ammonia during their growth. On one account prior to the addition of ammonia,  $0.15\mu$ mol L<sup>-1</sup> of ammonia was detected. The possibility that this is due to analytical error can not be discounted, but that it is has been excreted by the plankton is also possible. That plankton can assimilate ammonia rapidly in response to very low ambient levels, has been demonstrated by a number of workers, (e.g. McCarthy and Goldman, 1979; Glibert and Goldman, 1981; and Goldman et al., 1981). It has also been shown that remineralized ammonia is sufficient to supply most of the nitrogenous demand of the phytoplankton (Glibert, 1982). The phytoplankton may therefore have been growing predominantly on remineralized ammonia.

An asymptotic relationship has been demonstrated between the assimilation of nitrate and the ambient nitrate concentrations, (Harrison et al., 1987). It has also been shown however, that the presence of ammonia will inhibit nitrate uptake (McCarthy et al., 1977;). A relationship has been demonstrated between the ambient ammonia concentration and the maximum attainable f-ratio for any increase in nitrate concentration (Harrison et al., 1987). For example, with an ambient ammonia concentration of 0.1  $\mu$ mol L<sup>-1</sup>, the maximum attainable f-ratio is 0.7, and at ambient levels of 0.5  $\mu$ mol L<sup>-1</sup>, the maximum f-ratio is 0.4. The range of f-ratios seen in these culture experiments is between 0.08 and 0.23 even though the ambient

nitrate concentration reaches levels of 7  $\mu$ mol L<sup>-1</sup> while the ambient ammonia concentration is only 0.5  $\mu$ mol 1<sup>-1</sup>. This is evidence that ammonia must be inhibiting nitrate assimilation as it is the preferred nitrogen source throughout the experiments (the mean f-ratio being 0.14). The notion that nitrate inhibition from the presence of ammonia is an experimental artifact (Collos, 1987) must be discounted.

The C:N(q) ratios for the nitrate grown culture, are all far higher than expected, (mean ratio = 65.9), which is further evidence that nitrate is not the total source of nitrogen, and that one (or several) other nitrogen sources must be being utilised. Once the addition of ammonia was made to the incubations, the assimilation rate of nitrate actually increased in experiments D (0.206-0.313  $\mu$ mol L<sup>-1</sup>) and E (0.160-0.235  $\mu$ mol L<sup>-1</sup>), and only decreased very slightly in experiment F (0.122 - 0.110  $\mu$ mol L<sup>-1</sup>). If the addition of ammonia was inhibiting nitrate uptake, and nitrate had been the predominant nitrogen source, we would expect to see a reduction in nitrate assimilation rates. This further supports the notion that the culture was at no time ammonia limited and that the addition of excess ammonia did not change their source of nitrogen substantially.

The C:N<sub>(g)</sub> ratio from the ammonia based culture are within the expected range, (the mean value being 8.2). This would suggest that nitrate and ammonia are the only substantial sources of nitrogen to the culture, discounting the necessity to look for any other source of nitrogen. That the C:N<sub>(g)</sub> ratios are within the accepted range is supportive of the fact that the gross primary production rate, and the total production rate are equatable parameters of primary production.

Though it is not possible to draw any general conclusions about the relationship between the growth rate and C:N ratio, that C:N ratios can approximate to Redfield ratios at low growth rates (Tett *et al.*, 1985), is supported by experiment D2, where the ratio of carbon assimilated to nitrogen is 7.06 even though there is a net decrease in chlorophyll a by 7 percent.

The R.Q. is considered to be the reciprocal of the P.Q. A P.Q. range of 1.1-1.4 (Laws, 1991) would therefore give a R.Q. range of 0.91 - 0.71. The mean R.Q. obtained from these experiments of  $0.93\pm0.1$  is satisfactory, however the range of R.Q. values is larger than one would expect (0.32-1.51) and does not reciprocate the P.Q. values. That the R.Q. does not reciprocate the P.Q. is supported by such workers as Robertson, (1989) who derived P.Q. and R.Q. data from the same two chemical techniques.

The low R.Q. values may possibly be due to temperature affects, differentially affecting the release of carbon to the utilisation of oxygen. The temperature of the oxygen samples is taken prior to fixing which allows one to look at any change of temperature during the incubations. In several experiments the temperature of the dark bottles was elevated in relation to the light bottles.

The  $Q_{10}$  is defined as the ratio of a rate process at one temperature to the rate at a temperature  $10^{\circ}$ C higher. The calculations of  $Q_{10}$  are based on a single enzyme theory and for respiration are cited as 2. Williams and Robinson, (*in prep*) have found that the  $Q_{10}$  for respiration can in fact vary between 1 and 10. There is no evidence I am aware of that carbon and oxygen will be affected differently, but because the consumption of oxygen is directly related to the energy flux and carbon dioxide production is not, one may expect the former to be more tightly controlled by temperature than the latter.

In these experiments the increase in temperature is concomitant with an increase in respiration rates from oxygen, relative to respiration rates from  $TCO_2$ , (experiments D2, E2, F1,F2 and F3 all show temperature differences of up to 3<sup>O</sup>C, and exhibit low R.Q. values). This

effect may be due in part to the oxygen bottles being warmed to a greater extent than the larger TCO<sub>2</sub> bottles, but because both bottles were incubated in the same dark incubator for the same period of time this seems an unsatisfactory explanation.

The theoretically calculated P.Q. and R.Q. values are shown in table 6.3.(c). The P.Q. value is calculated from the C:N assimilation ratios and f-ratios obtained from the TCO<sub>2</sub> and 15-N measurments. The R.Q. is taken to be the reciprocal of the theoretical P.Q. The range of deviations in P.Q. values from the theoretically derived values is 0.01 to 0.28 with a mean deviation of 0.14. It is apparent from the theoretical P.Q. values that the culture was predominantly assimilating ammonia. The deviation from the theoretical values is at the limit of the obtainable precision of the combined analytical techniques.

In conclusion, there is substantial evidence that the culture experiments were conducted in a predominantly ammonia driven system. The C:N assimilation ratios from the culture grown on ammonia were acceptable (mean ratio = 8.2), however the C:N assimilation ratios from the culture grown on nitrate were unacceptably high, leading to the conclusion that this too, was an ammonia driven system.

The R.Q. values are not the reciprocal of the P.Q. values,

and though the mean R.Q. of 0.93 is acceptable, the range in R.Q. values is greater than can conventionally be accounted for. Temperature affects may be causing the wide range in R.Q. values.

# <u>Chapter 7</u>

RESULTS I.

## Chapter 7

# Results.I.

## 7.1. The 1989 BOFS field programme.

## 7.1.1.Introduction.

The Discovery cruise 182 was the first cruise of the Biogeochemical Ocean Flux Study (BOFS). The first cruise, or 'pilot study', was intended to look at the role of biological populations in the uptake of carbon dioxide from the atmosphere into the oceans. Two stations were sampled in the North-East Atlantic, 47°N 20°W and 60°N 20°W between 08/05/1989 and 08/06/1989. At the first station a rendevouz was made with the U.S.A. and German research ships allowing intercomparisons and intercalibrations of various techniques to be made.

The cruise track of Discovery 182 is shown in figure 7.1.(1).

Figure 7.1.(1)

# Crusle track of Discovery 182

North East Atlantic 1989



## 7.1.2.Experimental procedure.

Primary production rate measurements were made using four different techniques, the oxygen high precision Winkler technique (Emily Wood, Peter Williams, Carol Robinson, U.C.N.W.), the TCO2 coulometric technique (Carol Robinson, Emily Wood, Peter Williams, U.C.N.W.), the 18-0 tracer technique (Emily Wood, Michael Bender, U.R.I.), and the 15-N mass spectrometric technique (Emily Wood, Nick Owens, P.M.L.). A total of thirteen 24 hr incubations were made, nine were in vitro/in situ incubations and four were on deck incubations. Using four different forms of measurement potentially allowed a number of aspects of planktonic production to be measured: gross and net community production were measured using the oxygen and  $TCO_2$  technique, gross production was measured using the 18-0 technique and net and total production was measured from the assimilation of ammonia and nitrate using the 15-N technique.

The methodology is as described in chapter 4 for all techniques. A summary of the sampling schedule is shown in table 7.1.(a).

Parallel 14-C production measurements and chlorophyll a concentrations were made on the same water samples (Graham Savidge, Philip Boyd, Q.U.B.). 14-C incubations

were made on a separate, but adjacent, rig.

The results of the 1989 field programme have been divided into the two stations,  $47^{\circ}N$  20°W and  $60^{\circ}N$  20°W.

## Table 7.1.(a)

Sampling schedule of cruise Discovery 182 1989.

Date	Station	Rate measured	Depth (M)
13/05/89	11858/1	0 <sub>2</sub> , TCO <sub>2</sub> , 15-N	2,10,15,20,25,30
14/05/89	11858/13	0 <sub>2</sub> , TCO <sub>2</sub> , 15-N	2,10,15,20,25,30
16/05/89	11859/27	0 <sub>2</sub> , TCO <sub>2</sub> , 15-N, 18-0	2,10,15,20,25,30
21/05/89	11862/1	0 <sub>2</sub> , 15-N	10
23/05/89	11863/4	0 <sub>2</sub> , 15-N	10
24/05/89	underway	0 <sub>2</sub> , 15-N	2
25/05/89	11864/3	0 <sub>2</sub> , TCO <sub>2</sub> , 15-N,	5,15,30,45,60,80
27/05/89	11864/17	0 <sub>2</sub> , TCO <sub>2</sub> , 15-N, 18-0	5,10,15,25,35,50
29/05/89	11864/27	0 <sub>2</sub> , TCO <sub>2</sub> , 15-N, 18-0	5,10,15,20,25,35
31/05/89	11864/43	0 <sub>2</sub> , TCO <sub>2</sub> , 15-N, 18-O	5,10,15,20,25,35
02/06/89	11864/58	O <sub>2</sub> , TCO <sub>2</sub> , respiration only	5,10,15,20,25,35
04/06/89	11865/2	0 <sub>2</sub> , 18-0	5,10,15,25
05/06/89	11865/21	O <sub>2</sub> , respiration only	40,80,120,160

7.1.3.Results.

#### i. Primary production rate measurements at 47°N 20°W.

The primary production rate measurements from Discovery 182 at 47°N 20°W are assembled in table 7.1.(b). Chlorophyll a concentrations are also shown for each depth.

Two sets of measurements have been omitted (the ammonia assimilation rates, and the 18-0 production rates) on the grounds that the data was considered to be unreliable. In the case of the ammonia assimilation rates, the ambient concentrations of ammonia were not measured during the Discovery 182 cruise to a sufficient degree of accuracy in order that assimilation rates could be calculated. In the case of the 18-0 production rates, there was evidence that in certain cases the collection ampuoles had not been sufficiently vacuated (see chapter 4.).

Problems were encountered with the fixing of the samples for  $TCO_2$  production rates. Although the gross production rates as measured from the  $TCO_2$  technique, gave acceptable P.Q. values with the oxygen gross production rates, the respiration rates were very variable and often returned meaningless R.Q. values.

The gross production rate is calculated from the difference between the light and dark bottle, whereas the respi-

ration rates are calculated from the difference between the zero time and dark bottle. We suspect, therefore, that the zero time bottles were at fault. If the zero time bottles were not being fixed sufficiently, the respiration rates would be variable in value and show no correspondence to the oxygen rsspiration rates as we have found. For this reason the TCO<sub>2</sub> rates from 1989 have been omitted.

The gross production rate from the oxygen technique (table 7.1.(b)) increased over the three days sampled. Surface rates increased from 4.07 to 9.44  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup>. A subsurface maximum in gross production occurred on 13/05/1989, 14/05/1989 and 16/05/1989 at 25m, 10m and 10m respectively. The net community production rate from the oxygen technique increased from 1.6  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 13/05/1989 to 5.36  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 14/05/1989 and then decreased to 2.62  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 16/05/1989. A subsurface maximum in net community production was seen on 13/05/1989 at 15m. A surface maximum was seen on the other two days sampled. The compensation depth, where the net community production rate is equal to zero, occurred at about 30m on 13/05/1989.

The respiration rate from oxygen increased over the three days from 2.46 to 6.82  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> at the surface.

Again, a subsurface maximum in respiration at 10m was seen

on all three days.

Table 7.1.(b)

Primary production rate measurements from cruise Disc182, from oxygen, 14-C, and 15-N assimilation at station 47°N20°w.

## Ex1 Stn:11858/1 13/05/89

D	Chla	un	<b>14C</b>	<b>PgO2</b>	PcO2	RO2	<b>PQ[02/</b>	14C]	NO3
M	ug/l		ol/kg/	d umol/kg/	d umol/kg/d	umol/kg/d	PQg	PQn	umol/1/d
2	1.37	2.	85±.76	4.07 <u>+</u> .47	$1.60 \pm .43$ $1.95 \pm .33$ $2.38 \pm .16$ $1.47 \pm .44$ $0.23 \pm .26$	$2.46\pm.30 1.$	43±.41 0	.56 <u>+</u> .2	1 0.06
10	1.47	2.	24±.51	4.56 <u>+</u> .30		$2.60\pm.42 2.$	04±.51 0	.87 <u>+</u> .2	5 0.03
15	1.55	1.	86±.45	4.79 <u>+</u> .49		$2.36\pm.49 2.$	56±.68 1	.28 <u>+</u> .3	2 0.00
20	1.68	1.	41±.13	2.39 <u>+</u> .46		$0.92\pm.23 1.$	70±.19 1	.04 <u>+</u> .3	3 0.00
25	1.86	0.	88±.40	2.52 <u>+</u> .29		$2.29\pm.22 2.$	86±1.3 0	.26 <u>+</u> .3	2 0.11

## Ex2 Stn:11859/13 14/05/89

D	<b>PgO2</b>	PcO2	RO2	NO3
M	umol/kg/d	umol/kg/d	umol/kg/d	umol/1/d
2 10 15 20 25 30	$9.58\pm0.519.94\pm0.314.80\pm0.466.10\pm0.571.62\pm0.260.20\pm0.20$	$5.36\pm0.22$ $4.18\pm0.17$ $0.64\pm0.03$ $1.33\pm0.06$ $-2.70\pm0.11$ $-2.50\pm0.10$	4.22±0.18 5.76±0.24 4.16±0.17 4.77±0.20 4.30±0.18 2.66±0.11	0.09 0.15 0.11 0.09 0.07 0.01

## Ex3 Stn:11859/27 16/05/89

D	Chla	14C	<b>PgO2</b>	PcO2	RO2	PQ[O:	2/14C]	NO3
M	ug/l	umol/kg/d	l umol/kg/d	umol/kg/d	umol/kg/	d PQg	PQn	umol/1/3
2	1.75	2.19+.30	$9.44\pm.7111.13\pm.344.91\pm.542.17\pm.595.22\pm1.21.08\pm.07$	2.62 <u>+</u> .82	6.82 <u>+</u> .28	4.31 <u>+</u> .67	1.19±1	.2 0.02
10	1.83	1.84+.87		1.21 <u>+</u> .37	9.92 <u>+</u> .41	6.05 <u>+</u> 2.8	0.66±.	37 0.13
15	1.75	1.04+.15		1.64 <u>+</u> .37	3.27 <u>+</u> .27	4.72 <u>+</u> 0.9	1.58±.	42 0.01
20	1.83	0.74+.37		-2.74 <u>+</u> .50	4.91 <u>+</u> .20	2.93 <u>+</u> 1.7	-3.70±2	.0 0.0C
25	1.85	0.53+.27		-2.93 <u>+</u> .50	8.15 <u>+</u> .68	9.85 <u>+</u> 5.5	-5.53±3	.0 0.02
30	1.67	0.35+.25		-0.93 <u>+</u> .80	2.01 <u>+</u> .08	3.09 <u>+</u> 3.1	-2.66±3	.0 0.0C

Pg = gross production, Pc = net community production, R = respiration. PQ[02/14C] = Calculated PQ from 02 and 14-C (where PQg = Pg02/14-C)and PQn = Pc02 /14-C). The metabolism of oxygen with depth for the three days sampled at  $47^{\circ}N$  20°W are shown in figure 7.1.(2).

The chlorophyll a concentration with depth (table 7.1.(b)) is shown for 13/05/1989 and 16/05/1989 in figure 7.1.(3). The chlorophyll a concentration increased from 1.37 µg L<sup>-1</sup> on 13/05/1989 to  $1.75 \mu$ g L<sup>-1</sup> on 16/05/1989 at the surface. A subsurface chlorophyll a maximum was seen on both days sampled.

The 14-C production rates are shown in table 7.1.(b). The 14-C production rates with depth is shown for 13/05/1989 and 16/05/1989 in figure 7.1.(4). The 14-C production showed a different trend than seen from the oxygen measurements and the chlorophyll *a* concentrations. In place of the subsurface maximum seen in the chlorophyll *a* and gross oxygen production profiles, a surface maximum was seen on both days sampled. Instead of increasing, the production rate decreased over the time period from 2.85  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 13/05/1989 to 2.19  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 16/05/1989.

The assimilation of nitrate with depth (table 7.1.(b)) is shown in figure 7.1.(5) for the three days sampled. Nitrate assimilation rates were low on 13/05/1989 with a maximum at 25m of 0.11  $\mu$ mol L<sup>-1</sup> day<sup>-1</sup>. On 14/05/1989 the

rates had increased and the maximum assimilation rate was seen at 10m of 0.15  $\mu$ mol L<sup>-1</sup> day<sup>-1</sup>. The rates had decreased again on 16/05/1989 but still showed a maximum at 10m of 0.13 umol L<sup>-1</sup> day<sup>-1</sup>.

The depth integrated production rates are shown in table 7.1.(c).

## Table 7.1.(c)

Production rate measurements from Disc182 47°N20°W integrated from 0-25m.

Date	Chlor a mgm-2	14-C (	Pg02	PcO2 mmolm-	RO2 2day-1-	N03 -)	PQ[02 PQg	2/14-C] PQn
13/05/89	38.7	49.6	96.8	42.5	54.3	0.8	2.0	0.9
14/05/89	) –	-	181.1	61.3	119.8	2.8	-	-
16/05/89	45.0	35.0	179.1	9.4	169.8	1.2	5.1	0.3

Pg=gross production, Pc=net community production, R=respiration PQ[02/14-C] = calculated PQ from 02 and 14-C(where PQg = Pg02/14-C and PQn = Pc02/14-C).

The depth integrated gross production rates from oxygen increased from 96.8 mmol  $m^{-2}$  day<sup>-1</sup> on 13/05/1989 to 181.1 mmol  $m^{-2}$  day<sup>-1</sup> on 14/05/1989 and then decreased very slightly to 179.1 mmol  $m^{-2}$  day<sup>-1</sup> by 16/05/1989. Net commu-

nity production rates increased from 42.5 mmol  $m^{-2}$  day<sup>-1</sup> on 13/05/1989 to 61.3 mmol  $m^{-2}$  day<sup>-1</sup> on 14/05/1989 and then decreased to 9.4 mmol  $m^{-2}$  day<sup>-1</sup> on 16/05/1989. Respiration rates increased from 54.4 mmol  $m^{-2}$  day<sup>-1</sup> to 169.8 mmol  $m^{-2}$  day<sup>-1</sup> over the three days sampled.

Nitrate assimilation rates increased from 0.8 mmol  $m^{-2}$  day<sup>-1</sup> on 13/05/1989 to a maximum of 2.8 mmol  $m^{-2}$  day <sup>-1</sup> on 14/05/1989 and then decreased to 1.2 mmol  $m^{-2}$  day<sup>-1</sup> by 16/05/1989.

There was an increase in chlorophyll a from 38.7  $\mu$ g m<sup>-2</sup> to 45  $\mu$ g m<sup>-2</sup> over the three days sampled. The 14-C production rate showed a decrease over this period from 49.6  $\mu$ mol m<sup>-2</sup> day<sup>-1</sup> to 35.0  $\mu$ mol m<sup>-2</sup> day<sup>-1</sup>.



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ii. Primary production rate measurements at 60°N 20°W.

The primary production rate measurements obtained from the oxygen technique on Discovery 182 at 60°N 20°W are shown in table 7.1.(d). Chlorophyll a concentrations at each depth are shown. The production rate measurements from 14-C (Philip Boyd and Graham Savidge) are also shown and the calculated photosynthetic quotient from oxygen and 14-C, where P.Q.g is the gross oxygen production rate divided by the 14-C rate and P.Q.n is the net community production rate divided by the 14-C rate.

The nitrate assimilation rates from 15-N are also shown in table 7.1.(d).

The metabolism of oxygen with depth for the five days sampled at  $60^{\circ}N$   $20^{\circ}W$  are shown graphically in figure 7.1.(6).

On each of the five days sampled a surface maximum of gross oxygen production was evident. The gross production rate at the surface, increased over the time period studied from 3.43  $\mu$ mol kg<sup>-1</sup> day<sup>-1</sup> on 25/05/1989 to 6.38  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 31/05/1989. By 04/06/1989 the gross production rate had decreased to 5.01  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup>. The surface net community production rate increased from 2.62

 $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> to a maximum of 4.53  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 25/05/1989 and then decreased to 3.07  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 04/06/1989. A surface maximum of net community production was seen on every day sampled. The respiration rates showed less variability throughout the time period studied, the range in surface respiration rates being only from 0.77 to 1.94  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup>. They were likewise fairly uniform with depth. A slight increase in respiration rate occurred at the end of the period (04/06/1989) where the rates reached 2.3  $\mu$ mol kg<sup>-1</sup> day<sup>-1</sup> at a depth of 15m.

The chlorophyll a concentration with depth is shown in figure 7.1.(7). The chlorophyll a concentration was maximal below the surface on all but the first day sampled. The concentration ranged from 0.97  $\mu$ g L<sup>-1</sup> to 2.09  $\mu$ g L<sup>-1</sup>.

The 14-C production rate with depth is shown in figure 7.1.(8). The production rate was maximum at the surface on all the days sampled. Little variability was seen over the time period studied with the range in 14-C production rate being from 1.76  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> to 2.87  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> at the surface.
Table 7.1.(d)

Primary production rate measurements from Disc182, from oxygen, 14-C and 15-N assimilation, at station 60°N20°W.

Ex10 Stn:11864/3 25/05/89

D	Chla	14C	Pg02	PcO2	RO2	PQ[O	2/14C]
M	ug/l	umol/kg/d	umol/kg/d	umol/kg/d	umol/kg/d	l PQg	PQn
5	1.46	2.11 <u>+</u> .66	3.43 <u>+</u> .21	2.61 <u>+</u> .19	0.84 <u>+</u> .18	1.63 <u>+</u> .52	1.24 <u>+</u> .40
15	1.38	-	1.97 <u>+</u> .23	$1.00 \pm .26$	$0.98 \pm .13$	-	-
30	1.22	0.63 <u>+</u> .11	$0.64 \pm .44$	-0.94 <u>+</u> .40	$1.58 \pm .19$	$1.02 \pm .72$	-1.49 <u>+</u> .69
45	-	-	1.35+.33	$-0.04 \pm .20$	$1.31 \pm .30$		-
60	-	-	0.23 <u>+</u> .22	$-0.33 \pm .26$	$0.36 \pm .17$	-	-

Ex11 Stn:11864/17 27/05/89

Þ	Chla	14C	<b>PgO2</b>	PcO2	RO2	PQ [	02/14C]	NO3
M	ug/l	umol/Kg/d	umol/kg/d	umol/kg/d	umol/kg/d	PQg	PQn	umol/1/d
2 10 15 25 50	1.36 1.46 1.66 1.47 1.40	1.98 <u>+</u> .28 1.68 <u>+</u> .23 1.44 <u>+</u> .28 0.90 <u>+</u> .10	$5.68\pm.234.51\pm.183.58\pm.311.59\pm.270.00\pm.460.36\pm.02$	$3.93\pm.163.21\pm.172.49\pm.170.31\pm.28-0.90\pm.45-0.15\pm.19$	$1.79\pm.191.30\pm.121.10\pm.321.28\pm.190.92\pm.180.50\pm.19$	2.87±.42 2.68±.38 2.49±.53 1.77±.36 -	1.98 <u>+</u> . 1.91 <u>+</u> . 1.73 <u>+</u> . 0.34 <u>+</u> .	29 0.01 28 0.01 36 0.04 31 0.00 0.00 0.01

Ex12 Stn: 11864/27 29/05/89

D	Chla	14C	<b>PgO2</b>	PcO2	RO2	PQ [	02/14C]	NO3
M	ug/l	umol/Kg/d	umol/kg/d	umol/kg/d	umol/kg/d	PQg	PQn	umol/1/d
	1.21 1.91 1.71 1.76 1.78 2.09	2.87 - 2.59 <u>+</u> .28 2.56 <u>+</u> .37 1.85 <u>+</u> .10 1.78 <u>+</u> .26 1.15 <u>+</u> .18	$5.25\pm.114.86\pm.095.31\pm.143.76\pm.162.59\pm.240.86\pm.08$	$\begin{array}{r} 4.47\pm.10\\ 4.02\pm.15\\ 4.32\pm.14\\ 2.79\pm.17\\ 1.34\pm.26\\ 0.14\pm.08 \end{array}$	$\begin{array}{c} 0.77 \pm .08 \\ 0.84 \pm .13 \\ 0.99 \pm .09 \\ 0.97 \pm .10 \\ 1.25 \pm .14 \\ 0.72 \pm .09 \end{array}$	$1.86 - 1.88 \pm .21$ $2.07 \pm .30$ $2.03 \pm .14$ $1.46 \pm .25$ $0.75 \pm .14$	1.59 - 1.55 <u>+</u> .1 1.69 <u>+</u> .2 1.51 <u>+</u> .1 0.75 <u>+</u> .1 0.12 <u>+</u> .0	0.13 18 0.09 25 0.10 12 0.02 18 0.01 07 0.02

Ex13 Stn:11864/43 31/05/89

Þ	Chla	14C	<b>PgO2</b>	PcO2	RO2	<b>P</b> Q [ 02 /	/14C]	NO3
M	ug/l	umol/Kg/d	umol/kg/d	umol/kg/d	umol/kg/d	PQg	PQn	umol/1/d
2 10 15 20 25 35	1.26 1.35 1.24 0.97 1.14 1.24	$2.59\pm.73$ $2.13\pm.41$ $1.26\pm.72$ $0.90\pm.17$ $0.56\pm.42$ $0.53$	$6.38 \pm .27$ $3.43 \pm .24$ $2.79 \pm .35$ $1.27 \pm .21$ $0.77 \pm .11$ $0.20 \pm .31$	$4.53\pm.202.28\pm.231.67\pm.340.66\pm.120.54\pm.11-0.19\pm.33$	$1.85\pm.241.15\pm.121.12\pm.180.62\pm.190.23\pm.110.39\pm.20$	2.46 <u>+</u> .70 1.61 <u>+</u> .33 2.21 <u>+</u> 1.3 1.41 <u>+</u> .35 1.38 <u>+</u> 1.0 0.380	1.75 <u>+</u> .5 1.07 <u>+</u> .2 1.33 <u>+</u> .8 0.72 <u>+</u> .1 0.96 <u>+</u> .7	$\begin{array}{c} 0 & 0.53 \\ 3 & 0.07 \\ 0 & 0.06 \\ 9 & 0.06 \\ 5 & - \\ & 0.01 \end{array}$

Table 7.1.(d) (cont.)

Ex15 Stn:11864/69 04/06/89

RO2 Chla PcO2 PQ[02/14C] 14C Pq02 PQn ug/l umol/Kg/d umol/kg/d umol/kg/d umol/kg/d M PQq 3.07±.05 1.94±.16 2.85±.43  $1.75 \pm .26$ 1.51  $1.76\pm.26$   $5.01\pm.15$ 10 1.44 1.60<u>+</u>.09 4.35<u>+</u>.22  $2.30 \pm .21$ 2.05<u>+</u>.09 2.73<u>+</u>.21 1.44+.15 15 1.63 1.64+.35 2.30+.20 2.84+.52  $1.39 \pm .23$   $3.94 \pm .32$ 1.18+.32 25 1.50  $0.67\pm.06$   $1.43\pm.26$   $-0.15\pm.18$ 1.58±.30 2.15±.43 -0.23±.27

Pg = gross production, Pc = net community production, R = respiration.PQ[02/14C] = Calculated PQ from 02 and 14-C (where PQg = Pg02/14-C) and PQn = Pc02/14-C).

The assimilation of nitrate is shown with depth for the three days sampled in figure 7.1.(9). An increase in nitrate assimilation occurred between 25/05/1989 and 31/05/1989 from 0.01 µmol L<sup>-1</sup> day<sup>-1</sup> to 0.53 µmol L<sup>-1</sup> day<sup>-1</sup>.

The irradiance with depth is shown in figure 7.1.(10). The 1% light level is between 46m to 57m. Gross production is evident to this depth (the gross production rate is  $1.35\pm0.33 \ \mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 25/05/1989 at 45m and  $0.36\pm0.02 \ \mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 27/05/1989 at 50m. On the

other three days sampled oxygen production measurements have not been made to this depth.

The compensation depth, where the net community production rate is equal to zero, was at 25m on 25/05/1989 and 27/05/1989. It then fell to 30m on 29/05/1989, 35m on 31/05/89 and then rose again to 25m on 04/06/1989.

A natural logarithmic plot of gross production with depth for all five days sampled is shown in figure 7.1.(11). The ln plot of gross production shows a linear relationship with light suggesting light may have been a limiting factor at this station.

The integrated production rates are shown in table 7.1.(e) from 0-25m at  $60^{\circ}N$  20°W.

The integrated gross production rate from oxygen increased from 53.6 mmol  $m^{-2} day^{-1}$  on 25/05/1989 to reach a maximum of 114.5 mmol  $m^{-2} day^{-1}$  on 29/05/1989. A maximum in chlorophyll a concentration (42.2 µg  $m^{-2}$ ) was also seen on 29/05/1989 as was the production rate from 14-C (60.3 mmol  $m^{-2} day^{-1}$ ) and the net community production rate from oxygen (91.4 mmol  $m^{-2} day^{-1}$ ). The total irradiance was also maximum on 29/05/1989 being 26.3 Em<sup>-2</sup>. The assimilation of nitrate showed a maximum rate two days later on

31/05/1989 of 3.8 mmol  $m^{-2}$  day<sup>-1</sup>. The respiration rate was fairly uniform over the first four days sampled (23.1 -33.1  $\mu$ mol  $m^{-2}$  day<sup>-1</sup>) and then doubled on the last day sampled to reach a maximum of 50.2 mmol  $m^{-2}$  day<sup>-1</sup>.

Table 7.1.(e)

Production rate measurements from Disc182, 60°N20°W integrated from 0-25m.

Date	Chlor a mgm-2	14-C (	PgO2	PcO2 mmolm-	<b>RO2</b> 2day-1	NO3	PQ[02 PQg	/14-C] PQn
25/05/89	26.3	34.3	53.6	27.4	26.2		1.6	0.8
27/05/89	37.6	37.8	97.0	64.0	33.1	0.4	2.6	1.7
29/05/89	42.2	60.3	114.5	91.4	23.1	2.0	1.9	1.5
31/05/89	30.3	41.1	79.9	54.8	25.1	3.8	1.9	1.3
04/06/89	38.1	32.2	94.4	44.2	50.2	-	2.9	1.4

Pg=gross production, Pc=net community production, R=respiration PQ[02/14-C] = calculated PQ from 02 and 14-C(where PQg = Pg02/14-C and PQn = Pc02/14-C)













<u>Chapter</u> 8

RESULTS II.

#### Chapter 8

#### Results.II.

#### 8.1. The 1990 BOFS field programme

### 8.1.1.Introduction.

In 1990, primary production rate measurements were taken during two cruises, Charles Darwin 46 (CD46 (B1) 28/04/1990-23/05/1990) and Charles Darwin 47 (CD47 (B2) 25/05/1990-18/06/1990), as part of the Biogeochemical Ocean Flux Study (BOFS) Lagrangian experiment. Physical and chemical characteristics of the water column were measured on the resaerch ship, R.R.S.Discovery. A time plan of the two ships over the period of the Lagrangian experiment is shown in figure 8.1.(1). The Lagrangian experiment was designed to follow the temporal and spatial variability in the fluxes of carbon, and other associated biogenic elements, during a spring bloom in the North-East Atlantic Ocean.

Samples were taken within the vicinity of the marker buoy, (Metocean-Argos buoy #3917). Buoy #3917 was drogued at 20m depth and deployed on 26/05/1990, during the Discovery 190 cruise, within a mesoscale eddy at 49°05'N, 19°15'W. Drogue #3917 was at the centre of an array of drogues and rigs strategically located in relation to the mesoscale

eddy. Over the course of the Lagrangian experiment the marker buoy was essentially confined to an area bounded by  $48^{\circ}30$ 'N- $50^{\circ}$ N,  $17^{\circ}$ W- $19^{\circ}$ W. The drift track of the marker buoy during cruises, CD46 and CD47, is shown in figure 8.1.(2).





#### 8.1.2. Experimental procedure.

Rates of primary production and community respiration were made over 24 hr incubations in vitro/in situ. Primary production measurements were made using the oxygen Winkler technique, the  $TCO_2$  coulometric technique and the 15-N technique. Depths sampled were at 2, 10, 25, 35 and 75m, and incubations were conducted on every possible alternate day. 'True' in situ productivity rate measurements were made by calculating the change in the oxygen concentration over the day, with oxygen samples taken from selected CTD casts throughout the day and night. Table 8.1.(a) shows a detailed sampling schedule for the  $O_2$ ,  $TCO_2$  and 15-N measurements taken during CD46 and CD47.

The results of the 1990 field programme have been subdivided into the following categories;

i) Primary production rate measurements from O<sub>2</sub>, TCO<sub>2</sub> and 14-C
ii) Primary production rate measurements from 15-N
iii) Carbon to nitrogen assimilation ratios
iv) Integrated primary production rate measurements
v) Community respiration measurements from O<sub>2</sub> and TCO<sub>2</sub>

#### Table 8.1.(a)

#### Sampling schedule for crusie CD46 and CD47 1990

Sampling schedule of productivity and level 1 measurements from 02, TCO2 and 15-N for CD46 and CD47. (All oxygen measurements from CD47 analysed by Dr.D.Purdie). Date Depths sampled (M) Comments O<sub>2</sub> TCO<sub>2</sub> 15-N 01/05/90 2,10,20, 2,10,20 24hr iv/is 35,75 CTD 07:00 2-300 Level 1 02/05/90 2-300 2-300 400-Level 1 400-4000 4000 \_\_\_\_\_\_ \_\_\_\_\_ 03/05/90 2,10,20, 24hr iv/is 35,75 \_\_\_\_\_ CTD 06:00 04/05/90 2-300 09:00 13:00 17:00 21:00 05/05/90 2-300 CTD 08:00 21:00 ------06/05/90 2-300 2-300 Level 1 400-Level 1 4000 07/05/90 2,10,20, 2 24hr iv/is 35,75 ------09/05/90 2,10,20, 2 24hr iv/is 35,75 2-300 2-300 Level 1 400-400-Level 1

11/05/90 2 2 6hr on deck diel expt

4000

Table 8.1.(a). (cont.)

Date	Depths O2	sampled (M) TCO <sub>2</sub>	15-N	Comments
12/05/90	2-300			Level 1
	400- 4000			Level 1
14/05/90	2,10,20, 35,75	2		24hr iv/is
16/05/90	2,10,20, 35			12hr iv/is (intercal. Tyro)
	2-300	2-300		CTD Tyro
	400- 4000	400- 4000		CTD Tyro
17/05/90	2,10,20, 35			24hr iv/is
	2	2		6hr diel expt
CD47				
28/05/90	2,10,20, 35,75	2,10,20	2,10,20, 35,75	24hr iv/is
29/05/90	2,10,20, 35,75	2	2,10,20, 35,75	24hr iv/is
		2-300		Level 1
		400- 4000		Level 1
30/05/90	2,10,20, 35	2	2,10,20, 35	24hr iv/is
01/06/90	2,10,20, 35,75	2,10,20	2,10,20, 35,75	24hr iv/is
02/06/90	2,10,20, 35,75	2	2,10,20, 35,75	24hr iv/is
03/06/90	2,10,20, 35	2	2,10,20, 35	24hr iv/is
		2-300		Level 1

Table 8.1. (a). (cont.)

Depths sampled (M) Date Comments O<sub>2</sub> TCO<sub>2</sub> 05/06/90 2,10,25, 2 2,10,25, 24hr iv/is 35 35 2-300 Level 1 \_\_\_\_\_ 06/06/90 2 2 P/I expt \_\_\_\_ \_\_\_\_\_ 08/06/90 2,10,15, 2,10,15 2,10,15, 24hr iv/is 25,35 25,35 \_\_\_\_\_ 09/06/90 2,10,15, 2 2,10,15, 24hr iv/is 25,35 25,35 2-300 Level 1 11/06/90 2,10,15, 2,10,15 2,10,15, 24hr iv/is 25,35 25,35 P/I expt' 2 2 12/06/90 2,10,15, 2 2,10,15, 24hr iv/is 25 25 2-300 Level 1 13/06/90 2,10 24hr iv/is 14/06/90 2,10 2,10,15 2,10 24hr iv/is 15/06/90 2,10,15, 2 2,10,15, 24hr iv/is 25,35 25,35 2 2 P/I expt

iv/is = in vitro/in situ productivity experiment.

8.1.3.Results.

i. Primary production rate measurements from  $O_2$ , TCO<sub>2</sub> and 14-C.

Tables 8.1. (b) and 8.1. (c) show the primary production rate measurements for each productivity experiment from CD46 and CD47 respectively. The discreet chlorophyll a concentrations are shown for each depth. The primary production rates for oxygen and TCO<sub>2</sub> are shown with the standard error from each measurement, (where, Pg = grossproduction, Pc = net community production and R =respiration). The calculated photosynthetic quotient (P.Q.) from oxygen and 14-C is shown for both P.Q.g and P.Q.n, where P.Q.g is the gross production rate of oxygen divided by the amount of carbon consumed as measured by the 14-C technique, and P.Q.n is the net production rate of oxygen, again divided by the amount of carbon consumed. The calculated P.Q. and respiratory quotient (R.Q.) from oxygen and TCO<sub>2</sub> is also shown.

Figures 8.1.(3) to 8.1.(20) show the depth profiles from oxygen production measurements of gross production, net community production and respiration. The depth distribution of chlorophyll a is also shown for each incubation.

#### Table 8.1.(b)

Primary production rate measurements from cruise CD46, from 14-C oxygen and  $TCO_2$  with depth.

<u>Ex1</u> (01/05/1990)

De M	pth	 С1 ца/	n <b>la</b> /1	1 µm/	4C	PgO2 µmol/kg	/d	<b>PcO2</b> μmol/kg/d	μmc	802 01/kg/d		<b>PQ[0</b> 2 PQq	2/14	-C] PQn	   
  2  10  20  35	1. 1. 1. 1.	85 85 75 68	2. 1. 0. 0.	35± 89± 88± 14±	1.1 0.4 0.2 0.1	7.90 <u>+</u> 0. 5.01 <u>+</u> 0. 3.2.33 <u>+</u> 0. 7.1.07 <u>+</u> 0.	.37 .43 .51 .15	5.00 <u>+</u> 0.59 2.90 <u>+</u> 0.59 -0.57 <u>+</u> 0.69 -1.01 <u>+</u> 0.49	92. 92. 92. 92.	90 <u>+</u> 0.4 10 <u>+</u> 0.2 90 <u>+</u> 0.4	6 3. 5 2. 7 2. 7 7.	37 <u>+</u> 0.21 66 <u>+</u> 0.22 64 <u>+</u> 0.58 55 <u>+</u> 1.19	2.	13 <u>+</u> 0. 54 <u>+</u> 0. -	27 <sup> </sup> 27 <sup> </sup> 07  J
De M	 pth	 Ρς μmc	JTC	:02 'kg/	d j	PcTCO2 imol/kg/d	 	<b>RTCO2</b> μmol/kg/d	PQ[02	/TCO2] PQg	RQ	(TCO2/C R	)2]		· <sup>1</sup>
2 10 20		 7.7 4.3 1.2	70 <u>+</u> 32 <u>+</u> 26 <u>+</u>	1.9	1 9 9 -	1.60 <u>+</u> 1.70 2.17 <u>+</u> 1.60 4.22 <u>+</u> 1.40	5 5 5	6.10 <u>+</u> 1.44 2.15 <u>+</u> 1.56 5.48 <u>+</u> 1.07	1.03	)±0.31 5±0.14	2 1 1	.10 <u>+</u> 0.7 .02 <u>+</u> 0.7 .89 <u>+</u> 0.4	8		

Ex2 (03/05/90)

Depth	chla	<b>PgO2</b>	<b>PcO2 RO2</b>
	µg/l	μmol/kg/d	μmol/kg/d μmol/kg/d
<sub>2</sub>  10  20	0.62 0.62 0.60	$12.14\pm1.0911.60\pm0.542.00\pm0.400.24\pm0.22$	$5.63\pm2.02$ $6.50\pm1.70$ $9.49\pm0.76$ $2.12\pm0.54$ $0.95\pm0.49$ $1.05\pm0.28$ $-1.16\pm0.30$ $1.40\pm0.21$

Ex3 (07/05/1990)

Dep	th	Chla	140	Pg02	PcO2	RO2	PQ[02	2/14-C]
M -		μg/1	$\mu m/l/d$	µmol/kg/d	µmol/kg/d	µmol/kg/d	PQg	PQn
2	1.6	3 2.	81+0.98	11.71 <u>+</u> 0.31	7.96 <u>+</u> 0.42	3.76 <u>+</u> 0.28	4.17 <u>+</u> 0.16	2.83 <u>+</u> 0.1
10	1.5	8 2.	32+0.89	$2.13 \pm 0.45$	$-0.49 \pm 0.52$	$2.62 \pm 0.26$	$0.92 \pm 0.20$	-
20	1.6	з о.	85+1.78	$1.66 \pm 0.25$	$-0.68 \pm 0.26$	2.34 <u>+</u> 0.07	1.95 <u>+</u> 0.45	-
35	-	Ο.	$17 \pm 0.16$	2.47 <u>+</u> 0.03	$1.88 \pm 0.19$	0.59 <u>+0.</u> 19	14.4 <u>+</u> 0.86	10.99±1.23

Ex4 (09/05/1990)

Depth	Chla	14C	PgO2	PcO2	RO2	PQ[(	)2/14-C]
'M 1	μg/1	μm/1/d 	µm01/кд/а 	µmo1/Kg/a	µmo1/Kg/a	PQg	PQC
12 1.	25 4.	39 <u>+</u> 1.09	23.87 <u>+</u> 0.28	3 16.77 <u>+</u> 0.41	7.10 <u>+</u> 0.30	5.46 <u>+</u> 0.13	3.84+0.12
10 1.	40 3.	39 <u>+</u> 0.58	20.26 <u>+</u> 0.31	6.04 <u>+</u> 0.58	7.55 <u>+</u> 0.49	4.42 <u>+</u> 0.11	4.19 <u>+</u> 0.18
20 1.	31 1.	15 <u>+</u> 0.35	$12.37\pm0.09$	6.88 <u>+</u> 0.11	5.50 <u>+</u> 0.07	10.74 <u>+</u> 0.29	5.97 <u>+</u> 0.18
35 0.	66 0.	30 <u>+</u> 0.64	2.22 <u>+</u> 1.31	L -3.75 <u>+</u> 1.44	5.97 <u>+</u> 0.60	7.48 <u>+</u> 4.59	ا – لر

Table 8.1. (b) (Cont).

iDepth	<b>PgTCO2</b>	<b>PcTCO2</b>	RTCO2	PQ[02/TC02]	RQ[TC02/02]
M	μmol/kg/d	μmol/kg/d	µmol/kg/d	PQg	R
   <sup>2</sup>	17.74 <u>+</u> 0.91	8.08 <u>+</u> 1.46	9.66 <u>+</u> 1.14	1.35 <u>+</u> 0.07	1.36 <u>+</u> 0.18

Ex5 (14/05/1990)

Dej M	pt	h	C µ	<b>h1</b> g/	1	μ	1 m	40	;/	d	,	P	<b>g</b> (	<b>D2</b> 1/	kç	J/	d		P un		52 1/	kg	3/Q	 1	μ	RO	2	k	g/q	1		 1	PQ	9 9	02	/1	4	C] P(	 2ח	ا <del>۔</del> ۔ ا
2  10  20  35	2 2 1 1	.0	)0 )5 52 )1	5 4 0 0		15 38 99 21		2. 1. 0.	863	4 3 2 5	2	3. 3. 1. 3.	37 99 17 01		0000	352	9 5 1 6	1	6. 6. 3. 4.	8 ( 3 ] 2 (	- ± ± ± =	0.0.0.	40	0 ( 3 : 4 4 9 7	5. 3. 4. 7.	 57 69 18 30	±000 ±±000	).().().().().().().().().().().().().()	09 31 12 49	4 2 1 14		54 28 12 14	±0 ±0 ±0	.2 .1 .2 .4	3 4 2 0	3.	20	5 <u>+</u> ( 4 <u>+</u> ( -	).1	 17  15    '
Der	pt!	h		Pg μm		 CC 1/	- 2 k	g/	ď	 μ	Po	 >T >1	 C( /)	)2 (g	/	 I	 F 4	RT(	co 51	2 /}	 (g	 F /d	2	[02	2/9 P	rc Qg	 02	]		RQ	[7	 rC(	<b>52</b>	 /0 R	2]	ר ו ו				
1 <sub>2</sub>			1	4.	0	1 <u>+</u>	1	. 3	4	7		)8	+1	L.	75	5	6.	. 9	3 <u>+</u>	1.	. 1	2	1.	.74	<u>+</u>	0.	16			1	. (	)5 <u>-</u>	<u>+</u> 0	.0	3	ר ן				

Ex6 (17/05/1990)

,De	pth	Cl	la	14C	PgO2	PcO2	RO2	PQ[02	/14-C]
'M !		μġ	g/l	$\mu$ m/l/d	µmol/kg/d	µmol/kg/d	µmol/kg/d	PQg	PQn
,  2	3.	39	10.	85 <u>+</u> 1.77	23.06 <u>+</u> 0.15	16.84 <u>+</u> 0.2	6.23 <u>+</u> 0.14	2.13 <u>+</u> 0.03	1.55 <u>+</u> 0.03
10	3.	65	10.	24 <u>+</u> 3.76	12.19 <u>+</u> 0.48	4.05 <u>+</u> 0.78	8.14 <u>+</u> 0.62	$1.19 \pm 0.06$	0.40±0.081
20	3.	30	2.	04 <u>+</u> 1.34	0.97 <u>+</u> 0.37	-2.21 <u>+</u> 0.54	3.18 <u>+</u> 0.39	0.48 <u>+</u> 0.18	- 1
35	2.	57 	0.	30 <u>+</u> 0.18	1.21 <u>+</u> 0.56	-0.67 <u>+</u> 0.77	1.88 <u>+0.</u> 53	0.40 <u>+</u> 1.86	-

Ex7 (19/05/1990)

Dej	pt]	h Cl	la	140	Pg02	PcO2	RO2	PQ[O	2/14-C]
M		μg	'1 	µm/1/d	µmol/kg/d	µmol/kg/d	µmol/kg/d	PQg	PQn
12	2	. 24	5.	11 <u>+</u> 1.62	15.19±0.25	3.33 <u>+</u> 0.35	11.86 <u>+</u> 0.24	2.97 <u>+</u> 0.09	0.65 <u>+</u> 0.07 <sup>1</sup>
110	2.	.22	3.	91 <u>+</u> 0.94	11.46 <u>+</u> 0.75	0.34 <u>+</u> 0.47	$11.12\pm0.20$	2.93 <u>+</u> 0.20	0.09±0.12
120	2.	.10	1.	21 <u>+</u> 0.1/	$8.98\pm0.41$	$2.40\pm0.55$	$5.58\pm0.37$ 2 10±0 27	$7.42\pm0.34$	1.98 <u>+</u> 0.45
1		. / J 				-1.1J <u>-</u> 0.J2	<i>2.13<u>1</u>0.2/</i>	4.1/10.80	

Pg=gross production, Pc=net community production, R=respiration. Chla = Chlorophyll a concentrations at each depth.

PQ[02/14C] = Calculated PQ from 02 and 14-C (where PQg = Pg02/14-C and PQn = Pc02/14-C). PQ[02/TCO2] = Calculated PQ from 02 and TCO2.RQ = Calculated RQ from 02 and TCO2.

### Table 8.1.(c)

Primary production rate measurements from cruise CD47, from 14-C, oxygen and  $TCO_2$  with depth.

Ex8 (28/05/90)

Depth	Chla	14C	Pg02	PcO2	RO2	<b>PQ[O</b> :	2/14C]
M	μg/]	<b>ι μm/l/d</b>	µmol/kg/d	µmol/kg/d	µmol/kg/d	PQg	PQC
2	1.76	4.84+0.91	7.11+2.24	6.36 <u>+</u> 2.17	0.75 <u>+</u> 2.03	1.47 <u>+</u> 0.54	1.31±0.55
10	1.64	$3.52\pm0.70$	$6.75 \pm 1.83$	4.74+1.82	$2.10 \pm 0.38$	$1.92\pm0.65$	1.35±0.58
20	1.54	$0.91 \pm 0.15$	$1.11 \pm 0.57$	0.06 <u>+</u> 0.66	8.59 <u>+</u> 0.34	$1.23 \pm 0.66$	0.07±0.73
35	0.59	$0.11 \pm 0.02$	$0.10 \pm 0.37$	$0.14 \pm 0.57$	$1.59 \pm 0.43$	$0.93 \pm 3.43$	

Depth	<b>PgTCO2</b>	<b>PcTCO2</b>	RTCO2	PQ[02/TC02]	RQ[TCO2/O2]
M	μmol/kg/d	μmol/kg/d	µmol/kg/d	PQg	R
2	8.29 <u>+</u> 1.35	6.62 <u>+</u> 1.92	1.67 <u>+</u> 1.37	0.86±0.30	2.22 <u>+</u> 6.29
10	2.21 <u>+</u> 1.44	0.19 <u>+</u> 1.89	2.02 <u>+</u> 1.22	3.06±2.16	0.96 <u>+</u> 0.61

Ex9 (29/05/90)

Depth	<b>Chl 1</b>	<b>4C</b>	<b>PgO2</b>	PcO2	RO2	PQ[02/	/14-C]
M	μg/l μ	m/l/d μ	mol/kg/d	μmol/kg/d	µmol/kg/d	PQg	PQn
2 1 10 1 20 1 35 1	.70 3.0 .78 2.2 .97 0.5 .57 0.1	$\begin{array}{r} 6\pm 0.71 & 1\\ 8\pm 0.28 \\ 9\pm 0.13 & -\\ 3\pm 0.01 \end{array}$	2.08±0.97 6.12±0.87 0.09±0.78 0.37±0.23	5.68 <u>+</u> 1.28 0.75 <u>+</u> 1.07 -4.71 <u>+</u> 0.97 -0.87 <u>+</u> 0.33	6.40 <u>+</u> 0.84 4.62 <u>+</u> 0.57 4.62 <u>+</u> 0.57 1.24 <u>+</u> 0.24	$3.95\pm0.972.69\pm0.512.80\pm1.60$	1.89±0.60 0.33±0.56 -
Depth	PgTCO2	PcT	CO2 RTC	02 PQ[02	/TCO2] R(	2[TCO2/O2]	]
M	µmol/k	g/d μmo	01/kg/d μmo	1/kg/d	PQg	R	
2	10.28+	1.53 2.6	6 <u>+</u> 1.92 7.63	2 <u>+</u> 1.16 1.18	±0.20	1.19 <u>+</u> 0.24	

Table 8.1.(C) (cont.)

2	14.33 <u>+</u> 0	.45	1.00	5 <u>+</u> 0	.81	13.	26 <u>+</u> 0	).67	0.	65 <u>+</u> 0	.09	3	17 <u>+</u> 0	.94
Depth M	PgTCO2 µmol/kg	/d	PcT( µmo]	CO2 1/k	g/d	RTC µmo	0 <b>2</b> 1/kg	1/d	PQ[	02/T PQg	CO2 ]	RÇ	2[TCO2 R	2/02]
2 10 20 35	1.36 1.46 2.18 1.64	9.3 4.00 2.8 0.1	5±1. 5±0. 1±0. 5±0.	34 50 62 13	5. -0. -1. -1.	17 <u>+</u> 1 39 <u>+</u> 0 88 <u>+</u> 0 02 <u>+</u> 0	.81 .65 .80 .21	4.4	18±1 5±0 59±0 17±0	.22				
Depth M	$chla \mu g/l$	Pg( µmo)	0 <b>2</b> 1/kg	/d	Ρ μm	cO2 ol/k	g/d	R( µm(	52 51/k	g/d				

Ex11 (01/06/90)

Depth	C	hl	a	14	C				Pg	02				P	c02	2			RO	2			<b>D</b> 0	P	2[0	02	/1	(C)
	μg			μπ/ 	1/	a 	μπ 	n0.	1/M	9/	a 	μ 	mc		κg,	'a 	$\mu \pi$	01	/ Ku	3/a			PQ	?g 				PQI
2 1	. 8	3	6.	45 <u>+</u>	0.	85	12	2.4	49 <u>+</u>	0.	23	8	. 8	5 <u>+</u>	0.2	28	3.	64	±0	. 16	1.	94	±0	. 2	6	1.	37	<u>+</u> 0
20 1	.2	29	1.	$31 \pm 100$	0.	25	ć	).4	45 <u>+</u>	0.	77	-0	. 6	9±	0.8	10	1.	14	±0	. 22	0.	34	±0	.5	9	J.	/3	-
35 0 	.1	7 	<b>0.</b>	13 <u>+</u> 	:0.	02	 	).2	27 <u>+</u>	: <b>0</b> .	07	0	. 3	5±	0.1	. 6	0.	08 <u>:</u> 	±0	.14					:			
oeptn 1	ι μ	gt no	CO: 1/)	z kg/	d	PC μπ	2TC 10]	:02 [/]	r kg/	d	μı	rCO 101	2 /k	g/e	א ז	91 C	)2/ P	QG Q	02	l	кQ	ĮT	°CO	2/ R	02]		1	
:	11	.2	3±:	 1.3	5	9,	. 37	· /±2	2.7	7	1.	. 86	 ±1	.3	5 1	1	1 <u>1+</u>	0.2	24		0	.5	1 <u>+</u>	0.	 39			
.0	5	. 5	6 <u>+</u> :	1.1	.7			-					-		1	2	!7±	0.2	29				-					
0	1	. 5	1±:	1.4	5			-					-		C	).3	10 <u>+</u>	0.!	59				-					

M	μg/1	μmo	$\frac{1}{kg/d}$	$\mu$ mol	./kg/d	μπ	o1/kg/d		
2	1.31	9.9		7.67		2.	24 <u>+</u> 2.08	]	
10	1.54	3.7	72+1.99	4.28	<u>+</u> 2.37	-0.	$56 \pm 1.29$		
20	1.59	0.2	$22 \pm 1.10$	1.95	; <u>+</u> 1.35	1.	53 <u>+</u> 0.78		
35	0.14	-0.2	20±2.18	3.31	±2.19	-3.	51±0.21		
Depth M	PgTCO2 µmol/}	2 kg/d	<b>PcTCO2</b> μmol/kq	 β/d μ	TCO2	g/d	PQ[02/TC PQg	:02]	RQ[TC02/02] R

Table 8.1.(c) (cont.)

## Ex13 (03/05/90)

Dept M	ħ,	Ch	1 <b>a</b>	14C	'd um	Pg	02 a/d	Pc(	)2 1	RO2	<b>PQ</b> [	02/14C] PC
2 2 10 20	 1. 1.	23		3.98+0. 3.72+0. 1.97+0.	36 7 86 7 53 0	96+0 73+0 74+0	.21 .50 .69	0.29+: 0.66+( -4.51+(		7.67+1.1 7.07+0.2 5.25+0.6	4 2.00+0.19 8 2.08+0.50 8 0.38+0.36	0.07+0.29 0.18+0.16
Dept	1.  h	- 52  E	 PgT	 CO2 1/ka	PcTCC	52+0  )2 'ka	.94  R1 итс		PQ[	02/TCO2]	RQ[TC02/02 R	]
2		7.	21	+0.66	2.001	1.72	5.2	1+1.59	1.1	0+0.11	0.68+0.23	-

# Ex14 (05/06/90)

Depth	Chla	14C	Pg02	PcO2	RO2	PQ[	02/14C]
M	μg/l	$\mu$ mol/l	µmol/kg/d	µmol/kg/d	µmol/kg/d	PQg	PQn
2	1.34	3.41 <u>+</u> 0.60	9.78 <u>+</u> 0.70	6.38 <u>+</u> 0.38	3.40 <u>+</u> 0.61	2.87 <u>+</u> 0.55	1.87 <u>+</u> 0.45
10	1.34	2.66 <u>+</u> 0.57	7.31 <u>+</u> 0.88	4.14 <u>+</u> 0.91	3.17 <u>+</u> 0.73	2.75 <u>+</u> 0.68	1.56 <u>+</u> 0.48
25	1.32	0.46 <u>+</u> 0.10	0.50 <u>+</u> 0.45	-1.46 <u>+</u> 0.28	1.96 <u>+</u> 0.48	1.08 <u>+</u> 1.00	-
35	0.53	$0.09 \pm 0.01$	0.28 <u>+</u> 0.27	$-0.54\pm0.13$	0.82 <u>+</u> 0.28	3.11 <u>+</u> 3.02	, -

Depth	<b>PgTCO2</b>	PcTCO2	RTCO2 P	Q[02/TCO2]	RQ[TCO2/O2]
M	μmol/kg/d	µmol/kg/d	µmol/kg/d	PQg	R
2	9.32 <u>+</u> 1.10	6.33 <u>+</u> 1.69	2.99 <u>+</u> 1.28	1.05 <u>+</u> 0.14	0.88 <u>+</u> 0.41

## Ex16 (08/06/90)

Depth	Chla	140	PgO2	PcO2	RO2	PQ[0	2/14C]
M 	μg/1	$\mu m/1/d$	µmo1/kg/a	µmo1/kg/d	µmol/kg/d	PQg	PQn
2	1.05	2.81 <u>+</u> 0.42	8.13 <u>+</u> 0.60	3.25 <u>+</u> 0.53	4.88 <u>+</u> 0.28	2.90 <u>+</u> 0.49	$1.16 \pm 0.26$
10	1.18	1.70 <u>+</u> 0.2	3.54 <u>+</u> 0.54	-0.30 <u>+</u> 0.28	3.84 <u>+</u> 0.53	2.08 <u>+</u> 0.42	-
15	1.14	1.25 <u>+</u> 0.25	1.11 <u>+</u> 0.93	-1.97 <u>+</u> 0.54	3.08 <u>+</u> 0.76	0.89 <u>+</u> 0.77	-
25	1.17	0.56 <u>+</u> 0.05	0.78 <u>+</u> 1.64	-2.60 <u>+</u> 1.60	3.38 <u>+</u> 0.46	1.39 <u>+</u> 2.93	-
35	0.95	0.13 <u>+</u> 0.02	0.73 <u>+</u> 0.21	-0.14 <u>+</u> 0.11	0.87 <u>+</u> 0.19	5.62 <u>+</u> 1.79	-

Depth	<b>PgTCO2</b>	<b>PcTCO2</b>	RTCO2 1	PQ[02/TC02]	RQ[TCO2/O2]
M	µmol/kg/d	μmol/kg/d	µmol/kg/d	PQg	R
2	$5.58\pm1.40$	4.27 <u>+</u> 1.70	1.31 <u>+</u> 0.99	1.46±0.38	0.27 <u>+</u> 0.20
10	$2.63\pm1.20$	-0.04 <u>+</u> 1.61	1.67 <u>+</u> 1.07	1.35±0.65	-
15	0.08 <u>+</u> 1.72	-3.83 <u>+</u> 2.53	3.91 <u>+</u> 1.86	- 	

Table 8.1.(c) (cont.)

<u>Ex17</u> (09/06/90)

Depti M	n Chla µmol/	1 1 J	14C 1m/1	/d	Pg µmo	0 <b>2</b> 1/kg	/d	P µT	c02	2 /kg/	d	RO: umo	2 1/k	g/d		PQ	Q[02 J	/140	) PQn	
2 10 15 25 35	0.65 0.89 0.99 0.47 0.57	1.4 1.5 1.5 0.2	19±0 54±0 53±0 36±0 20±0	.49 .12 .18 .08 .02-	5.3 2.7 1.0 0.1	9±0. 0±0. 9±0. 4±0. 4±0.	26 35 42 38 28	1. 1. -3. -0.	45 <u>1</u> 24 <u>1</u> 83 <u>1</u> 64 <u>1</u> 54 <u>1</u>	0.2	4 9 8 5	3.9 3.9 1.9 0.7 0.3	4±0 4±0 2±0 8±0 0±0	.22 .14 .49 .31 .33	3. 1. 0. 0.	63±: 75±( 71±( 39±)	1.20 0.26 0.29 1.05	0.9 0.8	7±0.3 0±0.2	363
Depth M	PgTC µmol	:0 <b>2</b> /kg	1/d	PcT μmo	CO2	R' g/d j	rco µmo	2 1/k	 g/d	PQ[	02/ I	TC Qg	<b>2</b> ]	R	Q[T	CO2/ R	/02]	]		
2	7.26	±1.	48	-4.4	2 <u>+</u> 1	9 1	1.6	7 <u>+</u> 1	. 19	0.	741	0.1	16		2.9	6 <u>+</u> 0.	34			

Ex18 (11/06/90)

Depth M	Chla µg/l	PgTCO2 µmol/kg/d	PcTCO2 µmol/kg/d	RTCO2 µmol/kg/d
2	0.96	2.72+1.70	2.21+2.80	0.51+2.25
10	0.93	$1.25 \pm 0.86$	$-0.37\pm1.30$	$1.62 \pm 1.30$
15	0.91	0.57 <u>+</u> 1.25	-3.28 <u>+</u> 1.43	3.85 <u>+</u> 0.69

Ex19 (12/06/90)

Dept M	h	ф	сь 3/	1	2	μ	m,	14	с /d	. <b>-</b> . 1		μπ	<b>P</b>	gC 1/	)2 'k	g/	/d		μ	n	20 20	02 /k	: : :	/d	 µ	n M	R0	2	g	/d			P	29	2	[0	2 /	1	40	2] P(	2n	ך
2 10 15 25	0001		 79 35 37 17		2. 1. 1.	1634		00000		3	-	 5. 3. 2. 0.	780		0000		15191618		31.0		 38 33 38 74	- ± ± ± = =	).).).	 16 38 58 53	2 2 2 2 0		 36 00 12 95		).	 45 35 72 94	2 2 1	. 7 . 3 . 5	0 9 5		).:	36 65 38	1		 59 14		). ).	19 36
Dept M	Þ	1 1 4	 9 <b>g</b> 1m	 T( 0)	 cc 1/	)2 'k	 g/	'd	P H	<b>c</b> 7		02 /k		 /d	1	RT LI	- .C .O	 0 <b>2</b> 1/	- 'k	 g/	ď	PQ	)[(	D2 P	/T Qg	 C(	 )2	]	]	RQ	[T(	CO R	2/	0	2]	ן						
2		3		28	3+	1	. 2	:4	1	.7	7	<u>+</u> 1		70		1.	5	1 <u>+</u>	1	. 1	.7	1		75	<u>+</u> 0	. 6	58			0	.64	<u>+</u>	0.	5	1							

## Table 8.1.(c) (cont.)

#### <u>Ex20</u> (13/06/90)

Depth	Chla	14C	Pq02	PcO2	R02	PQ[(	02/14C]
M	µg/1	$\mu$ m/l/d	µmol/kg/d	µmol/kg/d	µmol/kg/d	PQg	PQn
2 10	0.44	1.58±0.14 2.05±0.35	4.05 <u>+</u> 0.64 3.98 <u>+</u> 0.86	0.57 <u>+</u> 0.55 0.48 <u>+</u> 0.83	3.48±0.35 3.50±0.22	2.56 <u>+</u> 0.46 1.94 <u>+</u> 0.53	0.36±0.35 0.23±0.41
Depth M	PgT( µmol,	CO2 PcT /kg/d µmol	CO2 RT /kg/d µmo	CO2 PQ[O; 1/kg/d 1	2/TCO2] R PQg	Q[TCO2/O2] R	
2	4.52	0.20 3.15	<u>+</u> 0.66 1.3	7 <u>+</u> 0.62 0.9	0 <u>+</u> 0.15	0.39 <u>+</u> 0.04	

<u>Ex21</u> (14/06/90)

Dept	h Chi	la	14C	Pg02	PcO2	RO2	PQ[	02/14C]
M	μg/1	L μπ	n/1/d	µmol/kg/a	i µmol/kg/d	µmol/kg/d	PQg	PQn
2	0.49	1.9	7+0.3	34 5.98 <u>+</u> 0.92	2 1.55 <u>+</u> 0.48	4.43 <u>+</u> 0.79	3.03 <u>+</u> 0.70	0.79±0.28
10	0.52	1.7	4+1.1	2 2.62+0.70	) -1.50 <u>+</u> 0.73	4.12 <u>+</u> 0.34	$1.51 \pm 1.05$	-
15	0.55	1.8	1+0.4	5 1.56 <u>+</u> 1.0	5 -2.43 <u>+</u> 0.35	$3.99 \pm 1.00$	0.86+0.62	' -
25	0.64	0.8	0+0.1	1-0.36+1.00	) -3.33 <u>+</u> 0.59	$2.97 \pm 0.47$	•	-
35	0.49	0.3	5+0.0	0.47+0.38	3 -1.52 <u>+</u> 0.14	$1.99 \pm 0.14$	-	-

Depth M	PgTCO2 µmol/kg/d	<b>PcTCO2</b> μmol/kg/d	RTCO2 PC µmol/kg/d	[02/TC02] PQg	RQ[TC022/02] R
2	4.32+0.59	0.81 <u>+</u> 0.84	3.51 <u>+</u> 0.60 1		0.79 <u>+</u> 0.19
10	$3.35\pm0.43$	1.25 <u>+</u> 0.58	2.11 <u>+</u> 0.40 0	.78 <u>+</u> 0.23	-
15	2.03 <u>+</u> 0.86	-2.36+1.87	4.39 <u>+</u> 1.66 0	0.77 <u>+</u> 0.61	-

Pg=gross production, Pc=net community production, R=respiration. Chla = Chlorophyll a concentrations at each depth.

PQ[02/14C] = Calculated PQ from 02 and 14-C (where PQg = Pg02/14-C and PQn = Pc02/14-C).PQ[02/TC02] = calculated PQ from 02 and TC02.RQ = calculated RQ from 02 and TC02.







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Each profile shows a surface maximum of gross production which suggests that light is not inhibiting the rate of production. The rates of gross production increase from 7.9  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 01/05/1990 to 23.87  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 09/05/1990. On 19/05/1990 the rate drops off again to 15.19  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup>. There is a fairly steady decrease in gross production over CD47, dropping to 5.98  $\mu$ mol kg<sup>-1</sup> day<sup>-1</sup> at the end of the period studied, (14/06/1990). The community respiration increases from 2.9  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> to 11.86  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> by the end of CD46, (19/05/1990). The rate then decreases again with the surface respiration rate falling to 4.43  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup>.

The compensation depth is defined as the depth at which the gross production is equal to the respiration rate, or that depth at which the net community production rate is zero. The compensation depth can be seen as the intersect between the net community production rate (Pc) and the vertical axis in figures 8.1.(3) - 8.1.(20) (given as a solid line). The compensation depth is initially at 20m on 01/05/1990 and drops to 35m on 09/05/1990. It then rises again to 20m and stabilises at 10m on 30/05/1990.

The time series of surface determined rates from oxygen,  $TCO_2$  and 14-C for CD46 and CD47 is shown in figure 8.1.(21). Gross production measurements are plotted in

figure 8.1.(21).(a) and the net production measurements are plotted in figure 8.1.(21).(b).

The time series of surface gross production measurements shows that there is a general correspondence between the  $TCO_2$  and oxygen determined rates. The agreement between the oxygen and  $TCO_2$  techniques is discussed in more detail in the discussion, section 9.2.2.

No obvious agreement is seen between the  $TCO_2$ , oxygen and 14-C techniques. This is discussed in more detail in the discussion, section 9.2.4. Not only are there problems of detail, i.e. the 14-C rates are less than the  $TCO_2$  rates of net community production, which is theoretically "impossible", but the pattern of the two rates is dissimilar. The sharp rise in the oxygen and  $TCO_2$  rates occurs in the period 01/05/1990 to 09/05/1990. A decrease in rates is then seen. The 14-C technique shows a brief maximum on 17/05/1990.

Although there is, generally, better correspondence between the chemical techniques and the 14-C technique during CD47 (e.g. the pattern of gross oxygen production rate and the 14-C rate is close), there are again problems with detail. In only 4 of the 10 surface rate measurements is the requirement met that the 14-C rate lies between the gross and net community production rate. In fact



considering the whole surface data set, in only 5 of the 16 measurements (or 31 percent of the measurements) is this requirement met.

#### 8.1.3. ii. Primary production rate measurements from 15-N.

The primary production rate measurements from the assimilation of 15-N during CD47 are shown in table 8.1.(d). The assimilation of NO<sub>3</sub> (Pnew) and NH<sub>4</sub> (Preg) is size fractionated into less than 5 $\mu$ m fraction (<5 $\mu$ m), greater than 5 $\mu$ m fraction (>5 $\mu$ m), and the total fraction (T). The total nitrogen assimilated (Pt) at each depth is shown for each size fraction. The calculated f-ratio at each depth is shown.

The percentage tracer addition made during each incubation must be taken into account when looking at the results. The mean percentage tracer addition of  $15-NO_3$  is 7.3% which is acceptable for tracer work. The mean for  $15-NH_4$ is 5.4%.

Table 8.1.(e) shows the ambient nitrate concentrations at each depth for each incubation, used to calculate the 15-NO<sub>3</sub> assimilation rates. The percentage tracer addition of  $15-NO_3$  is shown for each incubation.

Table 8.1.(f) shows the ambient  $NH_4$  concentration for each depth, used to calculate the  $15-NH_4$  assimilation rates, and again, the percentage tracer addition of  $15-NH_4$  for each incubation.

#### Table 8.1.(d)

Nitrogen assimilation data. CD47 (28/05/90-14/06/90)

**<u>Bx 8</u> (28/05/90)** 

Depth	N:	itrate		Ammonia			Tot	n1	f-ratio	
M	T	<5µm	>5µm	T	<5µm	>5µm	T	<5µm	>5µm	
2	0.226	0.070	0.154	3.211	0.790	2.420	3.437	0.860	2.574	0:07
10	1.143	0.310	0.830	0.053	0.020	0.030	1.196	0.330	0.860	0.96
20	0.572	0.550	0.025	0.080	0.020	0.060	0.652	0.570	0.085	0.88
35	0.012	0.007	0.005	0.005	0.001	0.004	0.017	0.008	0.009	0.71
I	19.81			17.62			37.43			

<u>Ex 9</u> (29/05/90)

Depth	1	Nitrat	••••••	Ammonia			To	tal	f-ratio	
M	T	<5µm	>5µm	T	<5µm	>5µm	Ť	<5µm	>5µm	
2	0.230	0.140	0.090	1.440	0.500	0.940	1.670	0.640	1.030	0.14
10	0.130	0.040	0.090	1.780	0.780	1.000	1.910	0.820	1.090	0.07
20	0.250	0.200	0.050	0.410	0.040	0.370	0.660	0.240	0.420	0.38
35	0.005	0.001	0.004	0.020	0.000	0.020	0.025	0.001	0.024	0.25
I	5.62			30.27			35.89			

### <u>Ex 10</u> (30/05/90)

Depth	Ni	trate		Ammonia	Total	f-ratio	
M 	T 	<5µm 	>5µ10a ∼−−−−	T 	T 		
2	0.404	0.149	0.255	2.372	2.776	0.15	
10	0.416	0.160	0.256	2.105	2.521	0.17	

Table 8.1.(d) (cont.)

Ex 11 (01/06/90)

Depth	N	Nitrate			Ammonia			tal	f-ratio		
M	T	<5µ <b>n</b>	>5µm	T	<5µm	>5µm	T	<5µm	>5µm		
2	0.643	0.356	0.287	0.260	0.110	0.150	0.903	0.466	0.437	0.47	
10	0.620	0.340	0.280	0.720	0.370	0.350	1.340	0.710	0.630	0.46	
20	0.020	-	-	0.080	0.040	0.040	0.280	-	-	0.20	
35	0.000	-	-	0.010	-	-	0.010	-	-	0.00	
I	9.67			9.58			19.25			0.50	

<u>Bx 12</u> (02/06/90)

Depth M	T	Nitrat <5µm	>5µm	Ammonia T	Total T	f-ratio
2	0.090	0.050	0.040	0.140	0.230	0.39
10	0.070	0.030		0.280	0.350	0.20
20	0.010	-		0.690	0.700	0.01

<u>Ex 13.</u> (03/06/90)

Depth		Nitrate		Ammonia			Tot	tal	f-ratio	
м	Т	<5µm	>5µm	T	<5µm	>5µm	T	<5µm	>5µm	
2	0.189	0.131	0.058	0.329	0.304	0.025	0.518	0.161	0.083	0.36
20	0.160	0.100	0.001	0.320	0.180	0.140	0.240	0.120	0.141	0.03
35	0.030	0.020	0.010	0.610	0.040	0.570	0.640	0.060	0.580	0.05
I	2.9			11.02			13.92			

<u>Bx 14</u> (05/06/90)

Depth	1	Nitrat		Ammonia			To	tal	f-ratio	
м	T	<5µm	>5µm	T	<5µm	>5µm	T	<5µm	>5µm	
2	0.191	0.060	0.131	0.250	0.060	0.190	0.441	0.120	0.320	0.43
10	0.360	0.280	0.080	0.680	0.320	0.360	1.040	0.600	0.440	0.35
25	0.020	0.019	0.001	0.100	0.030	0.070	0.090	0.049	0.008	0.22
35	0.006	0.003	0.003	0.010	-	-	0.009	-	-	0.67
I	5.73			10.66			16.39			

Table 8.1.(d) (cont.)

# Ex 16 (08/06/90)

Depth	1	Nitrate	••••••	 λ	mmonia			<b>Fotal</b>	f-ratio	
M	T	<5µm	>5µm	T	<5µm	>5µm	T	<5µm	>5µm	
2	0.330	0.110	0.220	0.247	0.037	0.210	0.577	0.147	0.430	0.57
10	0.300	0.100	0.200	0.390	0.090	0.300	0.690	0.190	0.500	0.43
15	0.170	0.020	0.150	0.480	0.210	0.270	0.650	0.230	0.420	0.26
25	0.080	0.060	0.020	0.150	0.060	0.090	0.230	0.120	0.110	0.35
35	0.001	0.000	0.001	0.001	-	-	0.002	-	-	0.50
I	5.99			9.32			15.31			]

Ex 17 (09/06/90)

Depth	Depth Nitrate			A	amonia		Te	stal	f-ratio	
M	T	<5µm	>5µm	Ť	<5µm	>5µm	T	<5µm	>5µm	
2	0.230	0.120	0.110	0.033	0.014	0.019	0.263	0.134	0.129	0.87
10	0.030	0.010	0.020	0.306	0.088	0.218	0.336	0.098	0.238	0.09
15	0.150	0.070	0.080	0.167	0.067	0.100	0.317	0.137	0.180	9.47
25	0.003	0.001	0.002	0.006	0.002	0.004	0.009	0.003	0.006	0.33
35	0.010	0.006	0.004	0.020	0.000	0.020	0.030	0.006	0.024	0.33
I	2.59			3.87			6.46			

# Ex 18 (11/06/90)

Depth		Nitrate			Ammonia	L	!	Total	f-ratio	
M	Т	<5µm	>5µm	T	<5µm	>5µ <b>m</b>	T	<5µm	>5µm	
2	0.070	0.040	0.030	0.280	0.090	0.190	0.350	0.130	0.220	0.19
10	0.110	0.080	0.030	0.210	0.080	0.130	0.320	0.160	0.160	0.34
15	0.030	0.020	0.010	0.230	0.090	0.140	0.260	0.110	0.150	0.11
25	0.010	0.005	0.005	0.070	0.020	0.050	0.080	0.025	0.055	0.13
35	0.050	0.030	0.020	0.004	0.003	0.001	0.054	0.033	0.021	0.92
I	1.75			5.42			7.17			

### Ex 19 (12/06/90)

Depth	Nitrate			Ammonia			Total		f-ratio	
M	T <5µm >5µm			T <5µm >5µm			T <5µm		>5µm	
2	0.436	0.274	0.162	0.393	0.011	0.382	0.829	0.285	0.544	0.53
10	0.080	0.020	0.060	1.530	1.070	0.460	1.610	1.090	0.520	0.05
15	0.080	0.040	0.040	0.400	0.110	0.290	0.480	0.150	0.330	0.17
25	0.020	0.010	0.010	0.070	0.010	0.060	0.090	0.020	0.070	0.22

Table 8.1.(d) (cont.)

#### Ex 20 (13/06/90)

Depth	Nitrate		Ammonia			T <	rot <b>al</b>	f-ratio		
M	T <5µm >5µm		T <5µm >5µm				<5µm	>5µm		
2 10	0.015 0.180	0.020	- 0.160	0.150 0.130	0.030 0.020	0.120 0.110	0.165	5 - 0.040	0.270	0.09 0.55

#### Ex 21 (14/0690)

Depth		Nitrate		Ammonia				Total f		ratio
M	T	<5µm	>5µm	T	<5µm	>5µm	T	<5µm	>5µm	
2	0.096	0.076	0.020	0.138	0.058	0.080	0.234	0.134	0.100	0.41
10	0.115	0.025	0.090	0.205	0.145	0.060	0.320	0.170	0.150	0.36
15	0.140	0.050	0.090	0.380	0.230	0.150	0.520	0.280	0.240	0.27
25	0.050	0.040	0.010	0.100	0.030	0.070	0.150	0.070	0.080	0.33
35	0.010	0.009	0.001	0.030	0.000	0.030	0.040	0.011	0.031	0.25
I	2.87			6.30			9.170			

(All 24 hr incubations) Units =µmol/1/day

```
Nitrate = NO<sub>3</sub> uptake

Ammonia = NH<sub>4</sub> uptake

T = Total uptake (<5\mum fraction + >5\mum fraction)

Total = (NO<sub>3</sub> + NH<sub>4</sub>)

f-ratio = NO<sub>3</sub>/(NO<sub>3</sub> + NH<sub>4</sub>)

I = Integrated production from 0-35M
```

# Table 8.1.(e)

Nitrate assimilation rates and ambient nitrate concentrations from CD47.

Ex	p Date	Depth M	[NO3] µm/1	spike µm/l	addtn ¥	NO3 μm/1/đ
8	28/05	2 10 20 35	0.46 0.59 2.00 5.79	0.083 0.083 0.083 0.083 0.083	18 14 04 01	0.226 1.143 0.572 0.012
9	29/05	2 10 20 35	0.23 0.23 0.36 3.71	0.017 0.017 0.083 0.250	07 13 23 07	0.230 0.130 0.250 0.005
10	30/05	2 10	0.43 0.32	0.008 0.008	02 03	0.404 0.416
11	01/06	2 10 20	0.23 0.37 3.60	0.017 0.017 0.017	07 05 .5	0.643 0.620 0.020
12	02/06	2 10 20	0.22 0.21 2.26	0.017 0.017 0.017	08 08 .8	0.090 0.070 0.010
13	03/06	2 10 20 35	0.38 0.80 1.80 0.38	0.017 0.017 0.017 0.083	04 02 01 22	0.189 0.160 0.010 0.030
14	05/06	2 10 25 35	0.63 0.55 3.72 8.80	0.017 0.017 0.083 0.417	03 03 02 05	0.191 0.360 0.020 0.006
16	08/06	2 10 20 25 35	0.50 0.47 0.46 0.33 2.34	0.017 0.017 0.017 0.083 0.417	03 04 04 25 18	0.330 0.300 0.170 0.080 0.001
17	09/06	2 10 15 25 35	0.23 0.23 0.36 0.61 4.74	0.042 0.042 0.042 0.042 0.042 0.275	18 18 12 07 06	0.230 0.030 0.150 0.003 0.010

Table 8.1.(e) (cont.)

Exp	Date	Depth	[NO3]	spike	addtn	NO3
18	11/06	2	0.08	0.008	10	0.070
		10	0.08	0.008	10	0.110
		15	0.18	0.008	04	0.030
		25	2.68	0.042	02	0.010
		35	7.82	0.250	03	0.050
 19	12/06	2	0.27	0.004	01	0.436
	,	10	0.11	0.004	04	0.080
		15	0.13	0.008	06	0.080
		25	5.57	0.083	01	0.020
20	13/06	2	0.25	0.004	02	0.015
	,	10	0.30	0.004	01	0.180
 21	14/06		0.09	0.008	09	0.096
	1.,	10	0.19	0.004	02	0.115
		15	0.18	0.004	02	0.140
		25	0.19	0.250	132	0.050
		35	1.79	0.417	23	0.010

[NO3] = ambient nitrate concentration NO3 = nitrate assimilation rate

# Table 8.1.(f)

Ammonia assimilation rates and ambient ammonia concentration data.

Ex	p Date	Depth M	[NH4] µm/1	spike µm/1	addtn ¥	$\frac{NH4}{\mu m/1/d}$
8	28/05	2 10 20 35	2.33 4.19 0.60 1.53	0.008 0.008 0.008 0.008	.3 .2 01 .5	3.211 0.053 0.080 0.005
9	29/05	2 10 20 35	1.75 2.44 0.50 0.85	0.083 0.167 0.025 0.083	05 07 05 10	1.440 1.780 0.410 0.020
10	30/05	2 10	(1.31) (1.19)	0.083	(06) (07)	2.372 2.521
11	01/06	2 10 20 35	1.31 1.19 0.85 0.32	0.083 0.083 0.017 0.033	06 07 02 10	0.260 0.720 0.080 0.010
12	02/06	2 10 20	0.57 0.76 2.95	0.042 0.025 0.008	07 03 .3	0.140 0.280 0.690
13	03/06	2 10 20 35	0.41 1.22 0.82 1.22	0.042 0.025 0.008 0.017	10 02 01 01	0.329 0.100 0.320 0.610
14	05/06	2 10 25 35	0.35 0.76 0.66 0.42	0.017 0.017 0.083 0.083	05 02 13 20	0.250 0.680 0.100 0.010
16	08/06	2 10 15 25 35	0.67 0.52 0.67 0.26 0.48	0.017 0.017 0.017 0.008 0.008	03 03 03 03 03 02	0.247 0.390 0.480 0.150 0.001
17	09/06	2 10 15 25 35	0.36 0.34 0.11 0.18 0.72	0.042 0.025 0.025 0.025 0.025 0.033	12 07 23 14 05	0.033 0.306 0.167 0.006 0.020

Table 8.1.(f) (cont.)

Exp	Date	Depth	[NH4	] spike	addtn	NH4
18	11/06	2	0.26	0.008	03	0.280
		10	0.22	0.008	04	0.210
		15	0.28	0.008	03	0.230
		25	0.61	0.017	03	0.070
		35	0.28	0.017	06	0.004
19 :	12/06	2	0.79	0.008	01	0.393
		10	0.45	0.008	02	1.530
		15	0.40	0.008	02	0.400
		25	0.57	0.025	04	0.070
20 1	3/06	2	0.26	0.008	03	0.150
		10	0.26	0.008	02	0 130
					·	
21 1	4/06	2	0.29	0.033	11	0.138
	•	10	0.34	0.017	05	0.205
		15	0.27	0.017	06	0.380
		25	0.27	0.025	10	0.100
		35	0.63	0.033	04	0.030

[NH4] = ambient ammonia concentration NH4 = ammonia assimilation rate Figures 8.1.(22) - 8.1.(29) show the depth distribution of 15-N based production rates from CD47. The size fractionated rates of Pnew and Preg (calculated from the original ammonia values) are shown with the ambient nitrogen concentrations. The consumption of nitrate is confined, mainly, to the top 20m of the water column, with a decrease in nitrate assimilation (and an increase in the ambient concentrations) seen at 35m. Nitrate assimilation is fairly evenly divided between the size fractions. Contrary to what one would expect, the overall mean percentage of nitrate assimilated by the smaller sized fraction is greater than that of the larger fraction (the percentage nitrate and ammonia assimilated by each size fraction is shown in table 8.1.(g).

The assimilation of ammonia is maximum at the surface on 28/05/1990 and sinks out through the water column, to 10m and then 35m by 03/06/1990. The maximum ammonia assimilation then remains below the surface, with an exception on 11/06/1990, where the rate is fairly uniform over the top 15m. Again the size fractionated rates are contrary to what is expected, in that the greatest percentage ammonia assimilation is in the larger size fraction (see table 8.1.(g).

### Table 8.1.(g)

Size fractionated ammonia and nitrate assimilation data as a percentage of the total assimilated. CD47

Ex	Date	Dept M	h nit: <5µm	rate >5µm	amm <5µ2	onium >5µm	
8	28/05	2 10 20 35	31 27 96 58	69 73 4 42	25 38 25 20	75 62 75 80	
9	29/05	2 10 20 35	61 31 80 20	39 69 20 80	35 44 10 0	65 56 90 100	
10	30/05	2 10	37 38	63 62	-	-	-
11	01/06	2 10 20	55 55 -	45 45 -	42 51 50	58 49 50	
12	02/06	2 10	56 43	44 57		-	
13	03/06	2 10 20 35	69 63 90 67	31 37 10 33	92 20 56 7	8 80 44 93	
14	05/06	2 10 25 35	31 78 95 50	69 22 5 50	24 47 30 -	76 53 70 -	
16	08/06	2 10 15 25 35	33 33 18 75 0	67 67 82 25 100	15 23 44 40 -	85 77 56 60	
17	09/06	2 10 15 25 35	52 33 47 33 60	48 67 53 67 40	42 24 40 33 0	58 76 60 67 100	

Table 8.1.(g) (cont.)

Ex	Date	Dept M	h nit: <5um	rate >5um	ammo <5um	nium >5um	
 18	11/0	)6 2	57	43	32	68	
		10	73	27	38	62	
		15	67	33	39	61	
		25	50	50	29	71	
		35	60	40	75	25	
19	12/0	)6 2	63	37	3	97	
	/	10	25	75	30	70	
		15	50	50	28	72	
		25	50	50	14	86	
20	13/0				20	80	
	, -	10	11	89	15	85	
 21	14/0		79	21	42	58	
	,	10	22	78	71	29	
		15	36	64	61	39	
		25	80	20	30	70	
		35	90	10	0	100	
			~~~~	<b>-</b> - 5'	. 7 .	3 3	
mea	an 8	nitrat	.e <5μ :e >5μ	m = 32	2.9 <u>+</u>	4.3	
nea	an %	ammoni	um <5	um = :	35.1	<u>+</u> 2.9	
mea	an 🖁	ammoni	um >5	um = (	57.3	± 3.1	

Figure 8.1.(30) shows the time series of surface new production, regenerated production and total production rates from the 15-NO<sub>3</sub> and 15-NH<sub>4</sub> assimilation for CD46 (Sarah Bury pers.comm.) and CD47. Total nitrogen based production increases from 1.6  $\mu$ mol L<sup>-1</sup> day<sup>-1</sup> over CD46 to reach a maximum of 2.12  $\mu$ mol L<sup>-1</sup> day<sup>-1</sup> on 14/05/1990. The rate then decreases to a minimum on 19/05/1990. There is a decrease in regenerated production and a concomitant increase in new production up until 14/05/1990 and then new production drops of. At the start of CD47 there is a

rapid increase in total production. This is due to a large increase in regenerated production rate (from 0.26 to 3.2  $\mu$ mol L<sup>-1</sup> day<sup>-1</sup>) which is effectively brought about by an increase in the ambient ammonia concentration, and not due to an increase in nitrate assimilation. The total production drops off to a minimal value by 30/05/1990. New and regenerated production rates are then both fairly low (0-1  $\mu$ mol L<sup>-1</sup> day<sup>-1</sup>) and contribute fairly equally to the total production term.

The time series of surface ambient nitrate, ammonia and nitrite is shown in figure 8.1.(31) and is consistent with the nitrate and ammonia assimilation rates. A decrease in ambient ammonia is seen over CD46, a rapid increase at the start of CD47 and then a decrease on 30/05/1990. The high nitrate levels ( $2.5 \ \mu$ mol L<sup>-1</sup>) found during CD46 are depleted and then remain at around 0.5  $\mu$ mol L<sup>-1</sup> during CD47. The large increase in ambient ammonia concentration at the start of CD47 (from 0.2 to 2.5  $\mu$ mol L<sup>-1</sup>) is probably due to contaminated ammonia values. If this is the case, the rate of ammonia assimilation will be overestimated and thus the rate of total production will also be overestimated.







13.82





Figure 8.1.(30) Surface nitrogen based production



Figure 8.1.(31) Surface inorganic nitrogen concentration



A comparison of the 1990 U.K. ambient ammonia concentrations was made with those reported by Garside (*pers.comm*) from the 1989 N.A.B.E. field programme at 47°N 20°W. The U.K. ambient ammonia concentrations were as much as ten times higher than those reported by Garside. The mean ammonia concentrations from the N.A.B.E. 1989 field programme are shown in table 8.1.(h).

#### Table 8.1.(h)

Comparison of ambient ammonia concentrations from CD46/90 (May 1990), and NABE, (May 1989).

Depth	Ammon	ium concentra	ation (µmol )	L-1)
1	CD47/90 28/05/90	CD47/90 03/06/90	CD47/90 14/06/90	NABE May 1989
2 10	2.33 4.19	0.41 1.22	0.29 0.34	0.04 0.05
15 20	0.60	_ 0.82	0.27	0.06 1.00
25 35	1.53	1.22	0.27 0.63	0.18 0.50

The high ammonia values may be due to contamination in the auto-analyser. It is not possible, owing to the limited amount of available ammonia data in this region of the ocean, to be certain as to the exact values we should expect to find. Because the ammonia assimilation rates directly reflect the ambient ammonia concentrations, it is possible, however, to assess whether the ammonia concentrations reported are acceptable by looking at the carbon to nitrogen assimilation ratios (see section 8.1.3.(iii). As discussed in section 8.1.3.(iii) the carbon to nitrogen assimilation ratios calculated from 14-C uptake and 15-N assimilation, and calculated from TCO<sub>2</sub> and 15-N assimilation, are lower than can be accounted for from the theoretical considerations and the reported values in the literature at the start of both CD46 and CD47. These low carbon to nitrogen assimilation ratios occur concomitantly with the peaks in the ambient ammonia concentrations seen at the start of CD46 and CD47.

As an exercise all the ammonia assimilation rates have been recalculated using the N.A.B.E. 1989 mean ammonia concentrations (table 8.1.(i)). The resultant carbon to nitrogen assimilation ratios were more acceptable at the start of the two cruises (see again, section 8.1.3.(iii).

#### Table 8.1.(i)

Recalculated nitrogen assimilation data.

#### <u>Ex 8</u> (28/05/90)

Depth	L 1	Ammonia			Total	f-ratio		
M	т	<5µm	>5µm	Т	<5µm	>5µm		
2	0.034	0.026	0.008	0.258	0.096	0.162	0.87	
10	0.000	0.000	0.000	1.140	0.310	0.830	1.00	
20	0.007	0.005	0.002	0.582	0.555	0.027	0.98	
35	0.005	0.001	0.004	0.017	0.008	0.009	0.71	
 I	0.295			20.09			0.99	

#### Ex 9 (29/05/90)

Depth	1 2	Ammonia			Total		f-ratio	
M	Т	<5µm	>5µm	T	<5µm	>5µm		
2	0.097	0.063	0.034	0.327	0.203	0.124	0.70	
10	0.148	0.083	0.065	0.278	0.123	0.155	0.47	
20	0.241	0.028	0.213	0.491	0.228	0.263	0.51	
35	0.003	0.003	0.000	0.008	0.004	0.004	0.63	
I	5.00			10.613	3		0.53	

#### <u>Ex10</u> (30/05/90)

Depth	1 2	Ammoni	<b></b> -		f-ratio		
M	Т	<5µm	>5µm	T	<5µm	>5µm	
2	0.181			0.585			0.69
10	0.147	-	-	0.563	-	-	0.74

#### <u>Ex 11</u> (01/06/90)

Depth	1 1	Ammonia			Total		
M	Т	<5µm	>5µm	Т	<5µm	>5µm	
2	0.027	0.016	0.011	0.670	0.372	0.298	0.96
10	0.089	0.042	0.047	0.709	0.820	0.327	0.87
20	0.012	0.006	0.006	0.032	-	-	0.63
35	0.002	-	-	0.002	-	-	0.00
I	1.19			10.86			0.89

#### Ex 12 (0206/90)

Depth M	T	<b>Ammoni</b> <5μm	<b>a</b> >5μm	T	<b>Tota</b> l <5µm		f-ratio
2	0.022			0.112	-		0.80
10	0.030	-	-	0.100	-	-	0.70
20	0.028	<b>.</b>	-	0.038	-	-	0.26

Analysis of the data from 1990 gave a standard deviation of 0.009  $\mu$ mol L<sup>-1</sup> day<sup>-1</sup> from replicate subsamples, and a standard deviation of 0.024  $\mu$ mol L<sup>-1</sup> day<sup>-1</sup> from seperate bottle replicates. The accuracy of the data is not accountable for due to the uncertainty in the nutrient data.

# Table 8.1.(i) (cont.)

# Ex 13 (03/06/90)

Depth	a 2	Ammonia			Total		f-ratio	
M	T	<5µm	>5µm	Т	<5µm	>5µm		
2	0.060	0.004	0.056	0.249	0.014	0.114	0.76	
10	0.006	0.004	0.002	0.166	0.104	0.062	0.96	
20	0.041	0.019	0.022	0.051	0.028	0.023	0.20	
35	0.033	0.032	0.001	0.063	0.052	0.011	0.48	
I	1.096			3.996			0.73	

# Ex 14 (05/06/90)

Depth	n 1	Ammonia			Total		
M	Т	<5µm	>5µm	т	<5µm	>5µm	
2	0.039	0.029	0.010	0.230	0.089	0.141	0.83
10	0.336	0.031	0.305	0.696	0.311	0.385	0.52
25	0.027	0.021	0.006	0.047	0.040	0.007	0.43
35	0.002		-	0.008	-	-	0.75
I	3.908			9.638			0.59

### Ex 16 (08/06/90)

Depth	1	Ammonia			Total	f-ratio	
M	T	<5µm	>5µm	T	<5µm	>5µm	
2	0.020	0.017	0.003	0.350	0.127	0.223	0.94
10	0.049	0.037	0.012	0.349	0.137	0.212	0.86
15	0.051	0.029	0.022	0.221	0.049	0.172	0.77
25	0.104	0.065	0.039	0.184	0.125	0.059	0.43
35	0.000		-	0.001	-	-	1.00
I	1.817			7.807			0.77

### Ex 17 (09/06/90)

Depth	1	Ammonia			Total		<b>f-ratio</b>	
M	T	<5µm	>5µm	т	<5µm	>5µm		
2	0.007	0.004	0.003	0.240	0.124	0.113	0.96	
10	0.063	0.045	0.018	0.093	0.055	0.038	0.32	
15	0.105	0.061	0.044	0.255	0.131	0.124	0.59	
25	0.007	0.004	0.003	0.010	0.005	0.005	0.30	
35	0.002	0.002	0.000	0.012	0.008	0.004	0.83	
I	1.375			3.965			0.65	

Ex 18 (11/06/90)

Depth	n 1	Ammonia			Total		f-ratio
M	Т	<5µm	>5µm	T	<5µm	>5µm	
2	0.050	0.033	0.170	0.120	0.073	0.047	0.58
10	0.054	0.033	0.021	0.164	0.113	0.051	0.67
15	0.054	0.034	0.020	0.084	0.054	0.030	0.36
25	0.023	0.016	0.007	0.033	0.021	0.012	0.30
35	0.001	0.000	0.001	0.051	0.030	0.021	0.98
 I	1.295			2.045			0.86

Ex 19 (12/06/90)

Depth	1 J	Ammonia			Total	f-ratio		
М	Т	<5µm	$>5\mu m$	Т	<5µm	>5µm		
 2	0.024	0.023	0.001	0.460	0.297	0.163	0.95	
10	0.189	0.057	0.132	0.269	0.077	0.192	0.30	
15	0.066	0.049	0.017	0.146	0.089	0.057	0.55	
25	0.023	0.020	0.003	0.043	0.030	0.013	0.47	

Ex 20 (13/06/90)

Depth	T	Ammonia			<b>Total</b>	<b>f-ratio</b>		
M		T <5μm >5μm			<5µm	>5µm		
2 10	0.027 0.020	0.024 0.015	0.003	0.042	0.035	- 0.165	0.36 5 0.90	

<u>Ex 21</u> (14/06/90)

Depth	Depth Ammonia				<b>Total</b>	1	f-ratio
M	Т	<5µm	>5µm	Т	<5µm	>5µm	
2	0.031	0.018	0.013	0.127	0.094	0.033	0.76
10	0.039	0.010	0.029	0.154	0.035	0.119	0.75
15	0.041	0.039	0.002	0.181	0.089	0.092	0.77
25	0.071	0.051	0.020	0.121	0.091	0.030	0.41
35	0.003	0.003	0.000	0.013	0.012	0.001	0.77
I	1.48			4.35			0.66

```
All 24hr incubations. Units = \mumol/l day-1
Nitrate = NO3 uptake
Ammonia = NH4 uptake
T= Total uptake (<5\mum + >5\mum)
Total = (NH4 + NO3),
f-ratio = NO3/(NO3 + NH4)
I=Integrated 0-35M.
```

Figure 8.1.(32) shows the time series of recalculated 15-N production rates. The first most obvious result of changing the ammonia values, is that the large increase, previously seen at the start of CD47, is removed. The effect of recalculating the 15-NH<sub>4</sub> assimilation rates is to reduce the total regenerated production rate (Preg), and thus reduce the total production term (Pt = Preg + Pnew). Pnew will not change, and so the percentage of Pt due to Pnew will increase. This will mean the f-ratio is increased.

If we are to make the assumption that the ambient ammonia concentrations are, in fact, closer to those reported by Garside than the U.K. data, the tracer additions of 15-NH<sub>4</sub> added during CD46 and CD47 will have been too high for tracer experiments. The recalculated ammonia assimilation rates are shown in table 8.1.(j) along with the corrected tracer additions. The high tracer additions mean that the experiments were, infact, addition experiments and not tracer experiments. A recalculation of results using a lower ambient ammonia concentration can, therefore, not satisfactorily be applied to these assimilation experiments.

# Table 8.1.(j)

Recalculated percentage 15-NH4 additions and ambient ammonia data CD47

Exp	Date	Depth M	[NH4] µmo1/1	spike µmol/l	addtn %	NH4 µmol/1/d
8	28/05	2 10 20 35	0.04 0.05 0.10 0.50	0.008 0.008 0.008 0.008	20 16 08 02	0.034 0.000 0.007 0.005
9	29/05	2 10 20 35	0.04 0.05 0.10 0.50	0.083 0.167 0.025 0.083	208 334 25 17	0.097 0.148 0.241 0.003
10	30/05	2 10	0.04 0.05	0.083 0.083	208 166	0.181 0.147
11	01/06	2 10 20 35	0.04 0.05 0.10 0.50	0.083 0.083 0.017 0.033	208 166 17 7	0.027 0.089 0.012 0.002
12	02/06	2 10 20	0.04 0.05 0.10	0.042 0.025 0.008	105 50 08	0.022 0.030 0.028
13	03/06	2 10 20 35	0.04 0.05 0.10 0.50	0.042 0.025 0.008 0.017	105 50 08 3	0.060 0.006 0.041 0.033
14	05/06	2 10 25 35	0.04 0.05 0.18 0.50	0.017 0.017 0.083 0.083	43 34 46 166	0.039 0.336 0.027 0.033
 16	08/06	2 10 15 25 35	0.04 0.05 0.06 0.18 0.50	0.017 0.017 0.017 0.008 0.008	43 34 28 4 2	0.020 0.049 0.051 0.104 0.000
 17	09/06	2 10 15 25 35	0.04 0.05 0.06 0.18 0.50	0.042 0.025 0.025 0.025 0.025 0.033	105 50 42 14 7	0.007 0.063 0.105 0.007 0.002
18	11/06	2 10 15 25 35	0.04 0.05 0.06 0.18 0.50	0.008 0.008 0.008 0.017 0.017	20 16 13 9 3	0.050 0.054 0.054 0.023 0.001

Table 8.1.(j) (cont.)

Exp	Date	Depth	[NH4]	spike	addtn	NH4
19	12/06	2 10 15 25	0.04 0.05 0.06 0.18	0.008 0.008 0.008 0.025	20 16 13 14	0.024 0.189 0.066 0.023
20	13/06	2 10	0.04 0.05	0.008	20 16	0.027 0.020
21	14/06	2 10 15 25 35	0.04 0.05 0.06 0.18 0.50	0.033 0.017 0.017 0.025 0.033	83 34 28 14 7	0.031 0.039 0.041 0.071 0.003

[NH4] = ambient ammonia concentration from NABE 1989 data. NH4 = recalculated ammonia assimilation rate.

The fact that the ammonia values have been recalculated in this way means that there are two data sets of 15-N assimilation values referred to in the thesis. Those production rates derived from the recalculated data set are differentiated by the suffix, \*.







8.1.3..iii. Carbon to nitrogen assimilation ratios.

The calculated assimilation ratios from the uptake of carbon and nitrogen are shown in table 8.1.(k). The assimilation ratios have been calculated from 14-C,  $TCO_2$  and 15-N assimilation data. The particulate organic carbon to particulate organic nitrogen ratios are also shown at each depth.

The carbon to nitrogen assimilation ratios from  $PgTCO_2$  and Pt are in the range 0.86-27.9 with a mean of  $6.59\pm1.22$ . The range of carbon to nitrogen assimilation ratios from 14-C and Pt is 0.22-17.5 (excluding the two outliers), with a mean value of  $4.75\pm0.5$ . The range of carbon to nitrogen ratios from the particulate fraction is 4-28 with a mean value of  $9.07\pm0.6$ .

The carbon to nitrogen assimilation ratios have been recalculated using the recalculated ammonia assimilation data (section 8.1.3.ii). At the start of CD46 it can be seen that the recalculated ammonia data decreases Pt and so increases the C:N ratio to a more acceptable value for both 14-C and TCO<sub>2</sub> calculated ratios. During CD46 the POC to PON ratios are fairly uniform and fall within the range of 4-7.9. 14-C to Pt ratios during CD46 are somewhat lower up until 17/05/1990. This suggests that the 14-C rate is a

### Table 8.1.(k)

# Carbon to Nitrogen assimilation ratios. CD46/CD47

Bx	Date	Depth	14C:15Pt	14C:15Pt*	14C:15Pn	TC02:15Pt	TC02:15Pt*	POC: PON
1	01/05	2 10	1.47	5,13	5,99	3.82	16.80 11.77	6.6
		20	0.60	6.07	27.50	0.86	8.69	6.4
		35	0.22	1.17	46.67	-		6.6
2	03/05	2	-	-	-	-	-	6.6
		10	-	-		-	-	4.0
		20 35	-	-	-	<b>–</b> .	-	5.0
 3	07/05	2	1.62	3.32	3.82			7.1
	•	10	3.54	17.19	44.62	-	-	7.2
		20	2.50	21.79	170.00	-		6.7
		35 	3.21	5.67	283.33			/.9
4	09/05	2	2.40	5.47	6.47	10.03	22.12	7.3
	-	10	2.83	8.09	11.11	-	-	7.3
		20	3.55	17.42	191.67	-	-	7.0
		35 	4.11	5.56	428.57			
5	14/05	2	2.43	3.38	3.94	6.62	9.83	7.3
		10	2.26	5.73	6.89	-	-	, 7.3
		20	1.18	5.10	17.07	-	-	6.4
		35 	4.38	13.04	210.00			
6	17/05	2	11.00	68.13	15.70	-	-	-
	•	10	13.67	26.60	22.91		-	-
		20	10.57	17.79	97.14	-	-	-
		35 	3.75	12.50	150.00 		-	
7	19/05	2	17.50	-	170.33	-	-	7.5
		10	10.95	-	55.86	-	-	7.9
		20	2.86	-	75.63	-	-	6.0
		35	2.69		250.00			
8	28/05	2	1.41	18.76	21.42	2.41	32.13	9.0
		10	2.90	3.09	3.08	1.85	1.94	0.8
		20	1.40	1.56	1.59	-	-	2.0
		35 	6.50	5.50	9.1/			
9	29/05	2	1.80	9.36	13.30	6.16	31.44	11.0
		10	1.10	8.20	17.54	-	-	10.8
		20	0.90	1.20	2.36	-	-	9.4
		35	5.20 	16.30	20.00		_	
10	30/05	2	-	-	-	5.20	24.29	8.3
		10		-	-	-	-	10.2
		20	-	-	-	-	-	0.9 7 A
		35	-	-			-	

# Table 8.1.(k) (cont.)

Ex	Date	Depth	14C:15Pt	14C:15Pt*	14C:15Pn	TC02:15Pt	TC02:15Pt*	POC: PON
11	01/06	2 10 20 35	7.22 3.40 4.70 -	9.63 6.38 40.94 -	10.03 7.29 65.50	10.40 4.15 5.40	13.99 7.84 47.20 -	9.3 7.7 7.9 8.4
12	02/06	2 10 20 35	- - - -			16.60 _ _ _	37.05 - - -	14.1 11.7 9.6 28.0
13	03/06	2 10 20 35	7.50 15.50 6.00 1.00	15.98 22.41 38.63 10.32	21.06 23.25 197.00 21.67	13.90 - - -	28.96 _ _ _ _	11.5 10.4 8.6 6.7
14	05/06	2 10 25 35	7.73 2.60 5.10 10.00	14.83 3.82 9.79 11.25	17.85 7.39 23.00 15.00	21.20 - - -	40.52 - - -	14.7 17.0 10.0 11.5
16	08/06	2 10 15 25 35	4.83 2.50 1.90 2.40 65.00	8.03 4.87 5.66 3.04 130.00	8.52 5.67 7.35 7.00 130.00	9.60 - - - -	15.94 7.54 0.36 , -	7.5 8.1 8.0 7.6 7.1
17	09/06	2 10 15 25 35	5.77 4.60 4.80 40.00 6.70	6.21 16.56 6.00 36.00 16.67	6.48 51.33 10.20 120.00 20.00	27.90 - - - -	30.25	7.9 8.3 8.0 7.4 7.1
18	11/06	2 10 15	-		-	7.77 3.90 2.20	22.67 7.62 6.79	8.1 8.7 8.6
19	12/06	2 10 15 25	2.53 1.00 2.80 4.90	4.63 5.93 9.04 10.23	4.89 20.00 16.50 22.00	3.95 - - -	7.13	18.9 15.3 13.5 11.0
20	13/06	2 10	9.40 6.60	37.62 10.25	105.33 11.39	26.70	140.24	12.1 13.1
21	14/06	2 10 15 25 35	8.57 5.40 3.50 5.30 8.80	15.51 11.30 10.00 6.61 26.92	20.52 15.13 12.93 16.00 35.00	18.80 10.50 3.90 -	34.02 21.75 11.22	14.1 16.2 13.9 11.3 10.7

measure of net rather than gross production and indeed the surface ratios of 14-C to Pnew are nearer to the POC:PON ratios. During CD47 the POC:PON ratios increase substantially and are in the range 6.7 - 28. The increase is also seen in the TCO<sub>2</sub> to Pt ratios and in the 14-C to Pt ratios with the exception of the 28/05/1990 and 29/05/1990 at the start of CD47. The recalculated Pt values give a more sensible C:N ratio on these days.

8.1.3.iv. Integrated primary production rate measurements.

The primary production rates measurements are shown in table 8.1.(1) integrated from 0-35M, for oxygen, 14-C and 15-N. The integrated concentration of chlorophyll *a* is also shown. The calculated integrated P.Q. and integrated f-ratios are also shown.

Table 8.1.(m) shows the integrated production rates converted into standard carbon units.

Table 8.1.(1)

Summary of integrated values (0-35M) CD46/CD47

Date	PgO2 P	c02 R02	14-C	CHlor	a Pnew	Preg	Preg*	Pt	Pt*
01/05	129.70 8	8.14 41.51	40.24	59.48	4.63	42.10	3.10	46.73	7.73
03/05	204.02 8	1.68 122.34	42.04	19.50	3.70	19.13	2.66	22.83	6.36
07/05	128.75 7	9.77 48.99	47.42	-	4.17	15.61	1.92	19.78	6.09
09/05	493.23 20	2.13 224.53	09.97	41.83	12 41	19.69	2.98	25.84	9.13
17/05	253.33 7	2.05 166.40	184.4	113.90	7.17	7.00	2.01	14.17	9.12
19/05	310.75 4	1.57 269.18	78.46	73.50	2.51	11.44	2.01	13.95	4.52
28/05	118.68 8	1.40 37.28	62.79	48.80	19.81	17.62	0.30	37.43	20.60
29/05	172.13 20	6.88 145.25	42.81	63.20	5.62	30.2	5.00	35.89	10.60
30/05	123.6 -9	9.20 132.8	-	63.80	-	-	-	-	-
01/06	136.75 7	3.51 63.24	96.25	50.69	9.67	9.58	1.19	19.25	10.86
02/06	89.50 59	9.17 30.33	-	42.06	-		-	-	-
03/06		4.38 174.68	82.54	50.41	2.90	11.02	1.10	13.92	3.96
05/06		2.70 85.23	20.24	41.74	5./3	10.00	3.91	16.39	9.64
00/00	56 08 -30	/.3/ 114.33 37	91.02	41.JI 26 04	2.33	3.92	1 39	12.31	7.01
11/06			2/.04	35.47	1.75	5.42	1.30	7.17	2.05
12/06	70.63 20	0.38 50.25	40.82	30.38	-	_	-		-
14/06	60.00 -62	2.55 122.55	43.73	18.97	2.87	6.30	1.48	9.17	4.35
Date	PQg[14C/Pg(	02] PQn[14C	/Pc02]	f-ratio	o f-rat	io*			
 01/05	3.22	2.1	 9	0.10	0.60				
03/05	4.85	1.9	4	0.16	0.58				
07/05	2.72	1.6	8	0.21	0.68				
09/05	6.30	3.1	8	0.24	0.67				
14/05	3.33	1.1	0	0.33	0.76				
17/05	1.40	0.5	0	0.51	0.78				
19/05	3.96	0.5	3 	0.18	0.56				
28/05	1.89	1.3	0	0.53	0.99				
29/05	4.09	0.6	3	0.17	0.53				
30/05	-	-	-	-	-				
01/06	1.42	0.7	6	0.50	0.89	1			
02/06	-	-		-	-				
05/06	1.58	-	1	0.21	0.73				
	2.03	1.1	0 1	0.35	0.59				
09/00	<b>b</b> //	2.9	7	0.39	0.77				
08/06	5.//			0 40	0 66				
08/06 09/06 11/06	2.01	-		0.40	0.65				
08/06 09/06 11/06 12/06	2.01 - 1.73	-	9	0.40 0.24	0.65 0.86				

(Units= $\mu$ moll<sup>-1</sup>m<sup>-2</sup>) \* = nitrogen assimilation rates from recalculated ammonia data.

#### Table 8.1.(m)

Integrated values of Carbon Oxygen and nitrogen converted into standard C units (mmol C m-2 d-1)

Date	Pg02	PcO2	RO2	14-C	Pt	Pt*	Pnew			
01/05	103.76	70.51	33.21	40.24	308.42	51.02	30.56			
03/05	163.22	65.34	97.87	42.04	150.68	41.98	24.42			
07/05	103.00	63.82	39.1 <del>9</del>	47.42	130.55	40.19	27.52			
09/05	394.58	161.70	179.62	69.97	170.54	60.26	40.59			
14/05	202.66	61.34	141.40	76.03	270.67	118.40	89.83			
17/05	206.72	73.64	133.12	184.40	93.52	60.59	47.32			
19/05	248.60	33.26	215.34	78.46	92.07	29.83	16.57			
28/05	94.94	65.12	29.82	62.79	247.04	132.59	130.75			
29/05	137.70	21.50	116.20	42.81	236.87	70.03	37.09			
30/05	98.88	7.36	106.24	-	-	-	-			
01/06	109.40	58.81	50.59	96.25	141.37	71.68	52.07			
02/06	71.60	47.34	24.26	-	-	-	-			
03/06	104.24	-35.50	139.74	82.54	91.87	26.40	19.14			
05/06	118.34	50.16	68.18	56.24	108.17	63.62	37.82			
08/06	69.58	-21.90	91.48	41.02	101.05	51.55	39.53			
09/06	44.86	-31.50	76.36	27.84	42.64	26.20	17.09			
11/06	-	-	-	39.31	47.32	11.55	13.53			
12/06	56.50	16.30	40.20	40.82	-	-	-			
14/06	48.00	-50.04	98.04	43.73	60.52	28.71	18.94			
1										
Conve	Conversion : $PQ[02/C02] = 1.25$ (Laws, 1991)									
		ROLCOS	$\frac{1}{2} \frac{1}{2} \frac{1}$	U.O K K						
		6.4.	$\frac{1}{2} = \frac{1}{2}$	0.0 	Han dad	+ <b>a</b>				

The time series of integrated production rates from oxygen, 14-C and 15-N measurements from CD46, and CD47 are shown in figure 8.1.(33). The rate measurements have been divided into those that are measuring indices of total production, figure 8.1.(33).(a), and those that are measuring indices of new production, figure 8.1.(33).(b).


Figure 8.1.(33) Time series integrated production







The total production rate estimates are from  $PgO_2$ ,  $P_t15$ -N and 14-C (assuming 14-C is measuring gross production). High rates of Pt at the beginning of CD46 and CD47 reflect the spuriously high ammonia assimilation rates on these days. The general pattern shown is one of a peak in gross oxygen production (09/05/1990) proceeded by a peak in nitrogen assimilation (14/05/1990) and then a peak in 14-C production rate (17/05/1990). The production rate falls to less than 100  $\mu$ mol C L<sup>-1</sup> day<sup>-1</sup> during CD47. Ignoring the high Pt values at the start of CD47, the rates from PgO<sub>2</sub> and Pt are very similar and show the same trend.

The new production rate estimates are from  $PCO_2$ ,  $P_{new}15-NO_3$  and 14-C (now assuming 14-C measures net primary production). The new production rate estimates show an apparent disagreement during CD46. The depth integrated net community production rate from oxygen peaks 8 days before the depth integrated 14-C production rate, as seen in the discreet data. The Pnew rate estimates from 15-N are of the same order of magnitude as the oxygen rate measurements, but the peak in production from Pnew lies midway between that of  $PCO_2$  and 14-C during CD46. The increase in Pnew from 15-N at the start of CD47 is not reflected in the oxygen or 14-C measurements either. During CD47 the oxygen technique returns negative values of net community production on four days which can not be measured from the 14-C or 15-N techniques.

The mean daily rate of carbon assimilation from gross oxygen production is 132  $\mu$ mol C L<sup>-1</sup> day<sup>-1</sup> assuming a P.Q. of 1.25. The total carbon assimilated from 15-N measurements is 143.3  $\mu$ mol C L<sup>-1</sup> day<sup>-1</sup> assuming a C:N ratio of 6.6. The amount of carbon available for export or the new production rate (measured from the net community production of oxygen) gives a mean daily rate of 36.5  $\mu$ mol C L<sup>-1</sup> day<sup>-1</sup> and from the 15-N measured assimilation of nitrate, 39.1  $\mu$ mol C L<sup>-1</sup> day<sup>-1</sup>. The mean daily production rate from 14-C lies between that from PgO<sub>2</sub>and PcO<sub>2</sub>, 63.1  $\mu$ mol C L<sup>-1</sup> day<sup>-1</sup>. The mean PQ[PgO<sub>2</sub>/14-C] is 3.25±0.39. The mean PQ[PcO<sub>2</sub>/14-C] is 1.42±0.25.

Table 8.1.(n) shows the integrated assimilation ratios of carbon to nitrogen. The 14-C to Pt ratios are in the range 0.86-16.18 with a mean value of  $4.00\pm0.96$ . The range of 14-C to Pnew is 3.17-31.26 with a mean value of  $13.29\pm2.18$ .

Table 8.1.(n)

Integrated carbon:nitrogen assimilation ratios (0-35M) CD46/CD47

Date	14C:15Pt	14C:15Pt*	14C:15Pn	POC: PON
01/05	0.86	5.21	8.69	5.80
03/05	1.84	6.61	11.36	4.90
07/05	2.40	7.79	11.37	7.14
09/05	2.71	7.66	11.38	7.19
14/05	1.85	4.24	5.59	6.81
17/05	16.18	20.09	25.72	
19/05	5.62	17.36	31.26	6.35
28/05	1.68	3.13	3.17	6.49
29/05	1.26	4.03	7.60	6.59
01/06	4.49	8.86	12.12	6.69
03/06	5.93	20.64	28.50	6.22
05/06	3.43	5.83	9.82	10.29
08/06	2.68	5.25	6.85	5.81
09/06	4.31	7.01	10.75	9.45
11/06	-	-	-	7.12
12/06	-	-	-	11.49
14/06	4.77	10.05	15.24	11.19

# 8.1.3.(v). Community respiration measurements from oxygen and $TCO_2$

Community respiration rates from oxygen and  $TCO_2$  are shown in tables 8.1.(b) and 8.1.(c) for CD46 and CD47.

The time series of surface respiration rates from oxygen and  $TCO_2$  are shown in figure 8.1.(34) The respiration rate due to the bacterial component of the community, from the incorporation of thymadine (Stirling, pers.comm.), is also shown. The scale is ten times smaller.

The  $TCO_2$  and oxygen respiration rates are in good agreement with the exception of three high  $TCO_2$  respiration rates. Neglecting these points, the trend in respiration shows an increase during CD46 and a gradual decline over CD47. The bacterial component of the respiration is a factor of 10 times smaller than that due to the total community respiration rates as measured from oxygen and  $TCO_2$ . The trend is, however, the same, showing an increase during CD46. The initial increase in respiration during CD47 is also reflected by the bacterial component. There are no bacterial measurements over the end of the period.

The change in R.Q. with time over CD46 and CD47 is shown in figure 8.1.(35).



# <u>Chapter 9</u>

# DISCUSSION

#### Chapter 9.

## Discussion

# 9.1. The BOFS 1989 field programme.

# 9.1.1.Primary production rate processes at 47° N20°W

Primary production rate measurements were made at the southerly station,  $47^{\circ}N$  20°W on three days, 13/05/1989, 14/05/1989 and 16/05/1989 on Discovery 182. A diatom bloom had commenced in the area at the end of April, when the mixed layer depth had shoaled to 40-50m and the winter nitrate levels of around 6  $\mu$ mol L<sup>-1</sup> had started to be depleted (Ducklow *et al.*, 1990). The water column was well stratified, with a temperature difference of 1.5°C between the surface and the subsurface. Gradually the diatom population was superseded by a predominantly flagellate population and by 13/05/1989, when Discovery 182 was on station, the diatoms were only to be found at depth (Boyd *et al.*, 1990). The phytoplankton community at this time was comprised of 40 to 55 % of microphytoplankton by biomass (Boyd *et al.*, 1990).

At the onset of the bloom the bacterial abundance was 15 percent of the total particulate organic carbon (Ducklow et al., 1990). By the end of May the bacterial abundance

had risen to 30 % of the total POC.

Discovery 182 was, therefore, on station at 47°N 20°W in a post-diatom bloom situation, where the population was predominantly microflagellates, and the heterotrophic community was increasing. The ambient nitrate levels had dropped from 6  $\mu$ mol L<sup>-1</sup> but were still high for post bloom conditions at a concentration of between 1 and 2.5  $\mu$ mol L<sup>-1</sup>. It would appear that the bloom conditions were being sustained by a large flux of new nitrogen from below the euphotic zone, mixing upwards along isopycnal surfaces (Garside *et al.*, 1990). A large heterotrophic activity at depth kept the ammonia concentrations sufficiently high for nitrate assimilation to be inhibited, and was thus available at the surface for the autotrophic population.

The DOC to DON ratios of the total dissolved organic matter (Suzuki et al., 1990) were low in the surface water and high in the deeper water, with carbon to nitrogen ratios from the dissolved organic matter of 7 to 8.

In situ measurements of  $TCO_2$  and ambient nitrate concentrations were positively correlated with each other, the slope of the regression giving a carbon to nitrogen ratio of 8 (Robinson et al., 1990).

The gross production rates of oxygen showed a subsurface maximum on all three days sampled. This was also seen in the chlorophyll *a* concentration, but not in the 14-C rates which all showed a surface maximum.

On 13/05/1989 the maximum gross oxygen production rate was at 15m  $(4.79\pm0.49 \ \mu\text{mol Kg}^{-1} \ \text{day}^{-1})$ . Gross production normalised to chlorophyll *a* was highest at 10m  $(3.1 \ \mu\text{mol})$  $O_2 \ \mu\text{g}$  Chlor *a*  $^{-1} \ \text{day}^{-1}$ ). Gross production rates had increased by the following day, with a rate at 10m of 9.94  $\mu\text{mol kg}^{-1} \ \text{day}^{-1}$ . The oxygen gross production rates increased further by 16/05/1989 to reach a maximum of  $11.13\pm0.34 \ \mu\text{mol kg}^{-1} \ \text{day}^{-1}$  at 10m. Gross production normalised to chlorophyll *a* reached 6.08  $\mu\text{mol } O_2 \ \mu\text{g}$  chlor  $a^{-1}$  $\text{day}^{-1}$  at this depth.

The depth integrated gross production and net community production rates, measured from the oxygen technique, were maximum on 14/05/1989, with the rates being 181.1 and 61.26 mmol  $O_2 m^{-2} day^{-1}$  respectively. The nitrate assimilation rates were also highest on this day, (2.75 mmol N<sub>2</sub>  $m^{-2} day^{-1}$ ), as was the PAR at 23.7Em<sup>-2</sup>.

The community respiration rates from the oxygen technique showed little variability with depth on 13/05/1989 or 14/05/1989. A slight subsurface maximum was evident at

10m. The respiration rates were more variable with depth on 16/05/1989 showing a large subsurface maximum at 10m, and a secondary maximum at 25m. The respiration rates increased quite substantially over the time period studied, from 2.46  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 13/05/1989 to 4.22  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 14/05/1989, and then to 6.82  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 16/05/1989. The integrated respiration rates also showed the same increase over the three days, with the rates increasing from 54.3 to 169.77 mmol m<sup>-2</sup> day<sup>-1</sup> over this period. This is in accordance with the increase in the bacterial abundance seen over this period (Ducklow et al., 1990).

The R/Pg ratios for the depth integrated rates were 0.560.66, and 0.95 for the three respective days.

The southerly station was also visited by the German research ship, Meteor. Experiments were attempted to separate the microplankton respiration rates into the autotrophic and heterotrophic components using a selective inhibitor (MS-222, Ethyl-m-aminobenzoate) (Reitmeier, 1990). They report respiration rates from the total microplankton community as 60 to 123 mmol  $m^{-2}$  day<sup>-1</sup> (which are in the range reported by Discovery 182), with the heterotrophic component comprising some 45.3 to 77.3 % of the total respiration.

The net community production rate averaged over the three days gave a figure for the available downward flux of carbon of 26.9 mmol C m<sup>-2</sup> day<sup>-1</sup>, having applied a P.Q. of 1.4, (assuming nitrate is the main nitrogen source to the phytoplankton).

The U.S.A. research ship, Atlantis II, occupied the southerly station at  $47^{\circ}N$   $20^{\circ}W$  after Discovery 182. On 22/05/1989, they found the ambient nitrate concentrations were still above 1  $\mu$ mol L<sup>-1</sup> at the surface (Garside et al., 1990). Nitrate assimilation rates were greater than ammonia assimilation rates in the top 25m, thus f-ratios of greater than 0.5 were occurring in the top 25m. Below 25m, ammonia was being preferentially utilised. Over the period of 22/05/1989 to 31/05/1989 the ambient surface nitrate concentrations were exhausted.

During the period preceding this, when Discovery 182 was on station, it can, therefore, be assumed that nitrate was being preferentially assimilated in the top 25m, and that the surface f-ratios were all greater than 0.5, as the ambient nitrate concentrations were between 1 and 2.5  $\mu$ mol L<sup>-1</sup>.

By converting the production rate measurements from the oxygen technique into carbon units, the production rates from the oxygen and 15-N assimilation experiments can be

compared. The oxygen flux is converted into a carbon flux by the application of a photosynthetic quotient. A P.Q. of 1.4 is assumed, (typical for phytoplankton assimilating nitrate) (Laws, 1991). Assuming a carbon to nitrogen assimilation ratio of 7:1, the contribution of the observed nitrate assimilation rates to the total nitrogen assimilated may be estimated. The nitrate assimilation rates from the 15-N measurements are then 8 %, 15 %, and 6 % of the total nitrogen assimilated for 13/05/1989, 14/05/1989 and 16/05/1989 respectively. This conclusion is at variance with the U.S.A. observations which give fratios of 0.5-1.0, and at present there is no explanation for this.

#### 9.1.2. Primary production rate processes at 60°N 20°W.

The northerly station at  $60^{\circ}N$   $20^{\circ}W$  was sampled between 25/05/1989 and 04/06/1989. During this time, five 24hr incubation experiments were carried out *in situ* for measurements of primary production.

Arrival at 60°N 20°W was to a pre-bloom situation where the nutrient levels were still high, with nitrate concentrations ranging from between 9-11  $\mu$ mol L<sup>-1</sup>. The *in situ* TCO<sub>2</sub> levels were indicative of an early bloom situation with concentrations ranging from 2,105 to 2,120  $\mu$ mol Kg<sup>-1</sup> whereas levels of between 2,050 to 2,070  $\mu$ mol Kg<sup>-1</sup> had been found at 40°N 20°W, suggestive of recent photosynthetic activity at the more southerly station (Robinson *et al.*, 1990).

The phytoplankton community was dominated by the nanophytyoplankton over the period studied. Only on, and after, 04/06/1989 did the microphytoplankton establish dominance (Boyd *et al.*, 1990). This was accompanied by a rapid decline in the surface silicate levels. The nitrate levels, however, remained high - not decreasing to levels lower than 6  $\mu$ mol L<sup>-1</sup>.

The gross oxygen production rate ranged from 3.43 to 6.38  $\mu$ mol kg<sup>-1</sup> day<sup>-1</sup> at the surface. Each depth profile showed

a surface maximum, with the exception of 29/05/1989, where a subsurface maximum was found at 15m. The irradiance was at its highest on this day at 25.27  $\mu$ E m<sup>-2</sup> and the chlorophyll a concentrations were enhanced. The surface samples may have experienced light inhibition on this day, (this was not, however, evident in the 14-C rates which showed a surface maximum on every day). Surface gross production, normalised to chlorophyll a ratios, ranged from 2.05  $\mu$ mol O<sub>2</sub>  $\mu$ g chlor a day<sup>-1</sup> (25/05/1989) to 5.06  $\mu$ mol O<sub>2</sub>  $\mu$ g chlor a day<sup>-1</sup> (31/05/1989).

Net community production rates varied from 2.61 to 4.53  $\mu$ mol kg<sup>-1</sup> day<sup>-1</sup> at the surface. Again, the net community production rate was maximum at the surface and decreased with depth. The compensation depth, where the net community production rate is equal to zero, occurred at 22.7m on 25/05/1989, dropped to 27.6m on 27/05/1989, 30.6m on 29/05/1989, and 32.4m on 31/05/1989. It then rose again to 24.2m on 04/06/1989.

Rates of net community respiration ranged from 0.77 to  $1.94 \ \mu$ mol kg<sup>-1</sup> day<sup>-1</sup> in the surface samples. An increase in respiration was seen at 30m on 25/05/1989 and at 25m on 29/05/1989. A subsurface maximum was also evident on 04/06/1989 at 15m.

The integrated gross production rates were lower than that found at the southerly station. The rates were 53.55 mmol  $m^{-2} day^{-1}$  to 114.54 mmol  $m^{-2} day^{-1}$ , the maximum being on 29/05/1989, coincident with the maximum available irradiance, and also the maximum integrated chlorophyll *a* concentrations. The net community production rate was also maximum on this day (91.41 mmol  $m^{-2}$ ). The 14-C production rate was also maximum on 29/05/1989 at 60.29 mmol  $m^{-2}$  day<sup>-1</sup>.

The assimilation of nitrate increased over the time period at  $60^{\circ}N$  20°W, with the maximum observed rate on 31/05/1989 of 3.78 mmol m<sup>-2</sup> day<sup>-1</sup>. High nitrate concentrations meant that nitrate was at no time limited at this station.

As seen at 47°N 20°W, the respiration rate reached a maximum after the gross production rate, increasing from 26.17 mmol m<sup>-2</sup> day<sup>-1</sup> on 25/05/1989 to a maximum of 50.23 mmol m<sup>-</sup>  $^{2}$  day<sup>-1</sup> on 04/06/1989. The heterotrophic component did not develop to such a substantial degree as at 47°N 20°W, with the bacterial production being about 10 percent of the integrated production rate (Ducklow et al., 1990). Respiration measurements of Reitmeier, (1990) made in the same area were in the range of 36 to 170 mmol m<sup>-2</sup> day<sup>-1</sup> with the heterotrophic rate comprising between 9.3 to 69.7 %.

The gross oxygen production rate can be compared to the

nitrate assimilation rate, first by the application of a P.Q. to convert the oxygen flux into a carbon flux. By dividing by a P.Q. of 1.4, (assuming nitrate is being assimilated due to its high ambient concentrations) the gross oxygen production rate gives a measure of a carbon flux. The ratio of carbon to nitrogen assimilation is assumed to be 7. The f-ratios from the assimilation of nitrate are then 0.04 on 27/05/1989, increasing to 0.17 on 29/05/1989 and 0.46 by 31/05/1989.

The net community production rate from oxygen averaged over the five days sampled gave a mean value of 56.33 mmol  $m^{-2}$  day<sup>-1</sup>. In situ rates of production were calculated from the change in oxygen and pCO<sub>2</sub> in the water column with time (Robertson et al., in press), between 29/05/1989 and 03/06/1989, in the same water mass. The daily averaged, net community production rates from oxygen ranged from 46 to 61 mmol O<sub>2</sub>  $m^{-2}$  day<sup>-1</sup>. The mean daily averaged net community production from pCO<sub>2</sub> was 41.5 mmol C  $m^{-2}$  day<sup>-1</sup>. A P.Q. of 1.36 converts the in vitro net community production rate from the Winkler oxygen technique into the *in situ* net community production rate

Despite the fact that the total integrated gross production rates were higher at the  $47^{\circ}N$  20°W, (the average

gross production rate being 152.3 mmol  $O_2 m^{-2} day^{-1}$  at 47°N 20°W and 87.9 mmol  $O_2 m^{-2} day^{-1}$  at 60°N 20°W), the net community production rate was lower. That is, at the northerly station the export flux was 56.3 mmol  $O_2 m^{-2} day^{-1}$  as opposed to 37.7 mmol  $O_2 m^{-2} day^{-1}$ .

The higher value at the northern station may be attributable to the lower respiration rates found at this station, and also due to the fact that the heterotrophic community had not developed to such an extent at this station. This is evident from the fact that the total depth integrated respiration rates, as measured using the oxygen Winkler technique, are less at  $60^{\circ}N$  20°W, being 50.23 mmol  $O_2$  m<sup>-2</sup> day<sup>-1</sup>, as opposed to 169.8 mmol  $O_2$  m<sup>-2</sup> day<sup>-1</sup> at 47°N 20°W. The bacterial abundance was also less at  $60^{\circ}N$  20°W (Ducklow *et al.*, 1990) and the oxygen measurements of Reitmeier, (1990) gave a lower percentage contribution from the heterotrophic community to the total respiration term at  $60^{\circ}N$  20°W than at 47°N 20°W.

9.1.3. The relative rates of oxygen and carbon metabolism during the 1989 field study.

During the 1989 field study, the metabolism of oxygen was measured using the high precision Winkler technique. Parallel measurements of the metabolism of carbon dioxide were made, using the 14-C technique (Graham Savidge, Philip Boyd, Q.U.B.). The results from the two stations sampled, 47°N 20°W and 60°N 20°W, are shown in tables 7.1. (b) and 7.1. (d) respectively.

An apparent photosynthetic quotient was calculated from the oxygen and 14-C rate measurements, where P.Q.g is the gross production rate of oxygen divided by the 14-C production rate, and P.Q.n is the net community production rate of oxygen divided by the 14-C production rate. The quotient values are also shown in table 7.1.(b) and table 7.1.(d).

At  $47^{\circ}N$  20°W gross production from oxygen showed a subsurface maximum on all three days sampled. A subsurface maximum was also evident on the first day (13/05/1989) in the net community production rate. For the two days on which parallel 14-C rate measurements had been made, a surface maximum in production was seen. On 13/05/1989 calculated P.Q.g values ranged from 1.43±0.41 to 2.86±1.3. The ratio of respiration to gross production ranged from

0.38 to 0.60 with the exception of the deepest measurement, where respiration was 91 % of gross production (this being coincident with the highest P.Q.g value of  $2.86\pm1.3$ ). On 16/05/1989 the range in P.Q.g values was much greater with values from  $2.93\pm1.7$  to  $9.85\pm5.5$ . The respiration rates were greatly increased on this day, with the ratio of respiration to gross production ranging from 0.67 to 2.26. The high respiration rates may be causing an increased discrepancy between the oxygen and 14-C rate measurements. This may be attributable to an increased heterotrophic contribution to the community, which is not measured by the 14-C technique.

This can be seen from the depth integrated data. The P.Q.g values calculated from the depth integrated rate measurements are shown in table 7.1.(c). On 13/05/1989 the P.Q.g is 2.0 and the integrated ratio of respiration to gross production is 0.56. On 16/05/1989 the P.Q.g is 5.1, and the ratio of respiration to gross production is markedly higher at 0.95.

At  $60^{\circ}N$   $20^{\circ}W$  the range in P.Q.g was from  $1.63\pm0.52$  to  $2.87\pm0.42$  at the surface, and from 0.38 to  $2.87\pm0.42$  for all depths sampled. At this station the range in P.Q.g values is much smaller and can, considering the error on the quotient, be more easily constrained by the

theoretical limitations on the P.Q. The respiration rates were lower than at  $47^{\circ}N$  20°W (the mean respiration rates being 50.2 mmol m<sup>-2</sup> day<sup>-1</sup> and 169.8 mmol m<sup>-2</sup> day<sup>-1</sup> at the two stations respectively), with the heterotrophic component playing a smaller role (Ducklow et al., 1990; Reitmeier, 1990). The depth integrated P.Q.g values are shown in table 7.1.(e) and range from 1.6 to 2.9. The respiration to gross production ratios, from the depth integrated oxygen rates, range from 0.2 to 0.53, with the greatest value corresponding with the highest P.Q.g of 2.9.

Figure 9.1.(a) shows the frequency histogram of P.Q.g values from all the 1989 field data. The quotient values have been subdivided into those that are acceptable, that is those values that are greater than twice the standard error on the quotient, and those that are not, and are therefore unnacceptable. Of the acceptable quotient values, those which correspond to a respiration to gross production ratio of greater than 0.5 have also been differentiated. Removing these values, the range in P.Q.g values is narrowed down considerably to 1.25 to 3.0, and are evenly distributed. The range of quotient values is greater than the theoretical range of 1.1-1.4 (Laws, 1991), but the deviation is not great, with no values being less than, or greater than, twice those set by the theoretical constraints.



A frequency histogram of P.Q.n is shown in figure 9.1. (b) for all the 1989 data. The quotient values become meaningless when we consider negative net community production rates. When the quotient values are removed, whose standard error is greater than half the quotient value, the range is narrowed down to between 0 and 2. The values less than 1.1 can be explained by the 14-C production rate being in between the net community production rate and gross production rate, as measured from the oxygen technique.

The quotient values greater than the acceptable range of 1.1-1.4 are not so easy to explain, as they inply that the 14-C net primary production rate is less than the net community production rate. However, consideration of the deviation of the P.Q. values from the acceptable values, reveals that the error is not large (the maximum difference being only 30 %). The depth integrated P.Q.n values are from 0.3 to 1.7, with the maximum deviation from the upper limit of the P.Q. being only 18 %.

We can conclude that during the 1989 field study the 14-C production rates were approximating to a value between the net and gross community production rates as measured by the oxygen technique. The greatest deviations from the gross production rate were seen when the ratio of

respiration to gross production was high, suggesting that discrepancies between the two flux measurements can be attributable to the relative magnitude of the community respiration. 9.1.4. Critical depth measurements.

The 1989 oxygen gross production and respiration rates have been used in an attempt to measure critical depths as an indication of the advancement of a phytoplankton bloom.

Sverdrup, (1953) defined the critical depth as that depth where the total depth integrated photosynthesis exactly equals the total depth integrated respiration. From Sverdrup, (1953), the gross production decreases logarithmically with depth, corresponding to the logarithmic decrease in light intensity. Assuming that the plankton are well mixed throughout the surface mixed layer, a second assumption is made that respiration is linear with depth throughout this layer. The depth where the gross production rate is equal to the respiration rate, is the 'compensation depth', where the net community production is equal to zero. Net growth of the phytoplankton community will only occur if the plankton spend more time in an area where net production is positive, rather than where net production is negative. The depth of the mixed layer must be above this critical value (the critical depth) in order for a bloom to be able to develop.

From using the oxygen rate measurements, the compensation depth and critical depths have been estimated. That the

compensation depth occurs at the 1 percent light level has often been assumed, following the early work on compensation depths of Jenkins (1934) in the English Channel. Using the oxygen measurements, this assumption need not be made. That respiration is linear with depth need not be assumed either, as a rate of net community respiration from the oxygen technique can be measured with depth. The compensation depth is discussed in more detail in chapter 2.9.1. and the critical depth is discussed in chapter 2.9.2.

The compensation depth (Zcomp) is calculated from the natural logarithmic plot of gross production (Pg) and respiration (R), where;

and where;

Pgo = the gross production rate at the surface Ro = the respiration rate at the surface Kp = the slope of the gross production curve Kr = the slope of the respiration curve

The critical depth (Zcrit) is calculated from the

integrated gross production and respiration rate values. The integrated Pg and R values are calculated with depth, where;

A graph of these values enables the critical depth to be found, where the critical depth is such that;

$$\{_{z} Pg = \{_{z} R$$

The compensation depth and critical depth calculations outlined above have been applied to the oxygen data at the northern station  $60^{\circ}N$   $20^{\circ}W$ . By looking at the critical depth values over the period spent at this station, an insight into the development of the bloom may be gained.

Figures 9.1.(2) - 9.1.(4) show the critical depth calculations for 27/05/1989, 31/05/1989 and 04/06/1989 respectively. The figures all show the plots of gross production

and respiration with depth (a), the natural logarithmic plots of gross production and respiration (b), and the curve of the integrated gross production and respiration rate (c); from which the compensation depth and critical depth are calculated.

Over the time period spent at 60°N20°W, the depth of mixing rose quite substantially from 100m to 35m. As the mixing depth shallows, the phytoplankton will spend more time in the illuminated surface waters, and as such will be able to increase in population. It is noticeable that the compensation depth is substantially higher than the 1 % light level - this is because the oxygen technique is measuring a community compensation depth, which we would expect to be higher than an algal compensation depth due to the respiration term from the heterotrophic component of the community.

The critical depth is 43m below the depth of mixing on 27/05/1989 and though it shallows, it remains well below the depth of mixing.

The critical depth cannot be a deciding factor in this scenario for the development of the bloom. At no time will the plankton population be mixed below the critical depth. This is also evident from the fact that there is an available export flux (or positive net community production rate) on every day sampled at this station.







#### 9.2. The BOFS 1990 Lagrangian experiment.

9.2.1. The physical characteristics during the Lagrangian experiment.

The BOFS 1990 Lagrangian experiment involved two ships, the R.R.S.Charles Darwin, and the R.R.S.Discovery, in the N.E. Atlantic between 46-50°N 14-22°W. Sampling was carried out between 18/04/1990 and 25/06/1990 adjacent to a Lagrangian buoy drogued at 30m. The hydrographical and physical processes were characterised from box grid surveys carried out at the beginning and end of the time series, and from underway measurements made on the two ships.

The reference drogue was deployed in the centre of an anticyclonic eddy (Savidge et al., in press). The drogue moved anticyclonically around the eddy, before exiting after the first 13 days and becoming entrained in a discontinuity zone located between discrete warmer and cooler water bodies. The drogue then tracked S.E to the end of the time series, grazing the margins of the warmer and cooler water bodies (Savidge et al., in press).

### 9.2.1.i.Time series observations.

At the start of the time series vertical stability was low, with no evidence of the development of the seasonal thermocline. The time series of temperature is shown in figure 9.2.(1). Surface temperatures were 12°C, with only a 1°C decrease over the top 250m. On 11 May a discrete lobe of cooler water (<11.0°C) intruded at around 200m, forcing a rapid shoaling of the 11.5°C isotherm. This was interpreted as the stage when the drogue left the eddy (Savidge et al., in press). The intrusion was developed further on the 26<sup>th</sup> May, and lasted through to the 7<sup>th</sup> June. The 11.0°C isotherm shoaled to 160m. The thermocline was intensified and the mixed layer depth shallowed to 10m from an average of 20m. After the 7<sup>th</sup> June the 11.0°C isotherm deepened again.

The time series of the mixed layer depth is shown in figure 9.2.(2). This is taken as the depth of a 0.2°C temperature change (Savidge, *pers comm.*) and as an average over each day.

The mixed layer depth started at around 50m, rising to 20m on the 11<sup>th</sup> May with the intrusion of the cooler water. By the 17<sup>th</sup> May, when the intrusion of cooler water had intensified the thermocline, the mixed layer depth was at





10m. Over the rest of the time series, the mixed layer depth deepened to between 20 and 35m, with an exception on the 13<sup>th</sup> June where it shallowed to 15m.

The major features shown in the temperature time series are reflected in the time series of salinity distributions (figure 9.2.(3)). The 11.5°C isotherm showed a close congruence with the 35.6psu isohaline.

In general the salinity changes over the time series were small. Surface values were in the range 35.5 - 35.6 psu. The intrusion of cooler water at depth was associated with salinities of less than 35.4 psu.

Chlorophyll a concentrations, throughout the time series, were generally greater than 1  $\mu$ g l<sup>-1</sup> at the surface decreasing with depth (figure 9.2.(4)). Chlorophyll a concentrations increased up until the 13<sup>th</sup> May, when a maximum developed, with levels of greater than 3  $\mu$ g l<sup>-1</sup> recorded over the 16<sup>th</sup> and 17<sup>th</sup> May. The 1.0  $\mu$ g l<sup>-1</sup> chlorophyll a isoline deepened progressively over the time series in parallel with the vertical deepening of the mixed layer. On the 13<sup>th</sup> June, the isoline shallowed slightly, which was parallelled by a rise in the mixed layer depth.




Nitrate concentrations are shown in figure 9.2.(5). The slow decrease in nitrate seen over the initial time period from 6  $\mu$ mol L<sup>-1</sup> to 4  $\mu$ mol L<sup>-1</sup> coincided with the increase in chlorophyll *a*. The maximum chlorophyll *a* concentration on the 13<sup>th</sup> to 17<sup>th</sup> May was accompanied by a rapid depletion of nitrate to less than 0.5  $\mu$ mol L<sup>-1</sup>. Nitrate was being introduced at depth, with the cold water intrusion on the 12<sup>th</sup> to 13<sup>th</sup> May being reflected in increased nitrate levels at these depths.



9.2.2. Primary production rate measurements from the oxygen and  $TCO_2$  technique - and the calculated photosynthetic quotient.

During the BOFS 1990 field programme production measurements were made with both the oxygen and the  $TCO_2$  techniques. The time series, from between 01/05/1990 to 19/06/1990, of the surface gross production rates, from both techniques, are shown in figure 9.2.(6).

The two chemical techniques both showed the same general trend in gross production rate with an increase from 8  $\mu$ mol Kg<sup>-1</sup> day<sup>-1</sup> on 01/05/1990 to a maximum of 23.87  $\mu$ mol O<sub>2</sub> Kg<sup>-1</sup> day<sup>-1</sup> and 17.74  $\mu$ mol C Kg<sup>-1</sup> day<sup>-1</sup> on 09/05/1990. By the start of the second cruise, 28/05/1990, both rate measurements showed a gradual decline in gross production to 7.11  $\mu$ mol O<sub>2</sub> Kg<sup>-1</sup> day<sup>-1</sup> and 8.29  $\mu$ mol C Kg<sup>-1</sup> day<sup>-1</sup>. The gross production rate then increased, and then showed a gradual decrease, to the end of the period studied.

A more rigorous and informative analysis is achieved by looking at the photosynthetic quotient  $(PQ[O_2/TCO_2])$  derived from the two measurements.

The photosynthetic quotient is discussed in more detail in chapter 2.5. The P.Q. is controlled by the state of



oxidation of the nitrogen source assimilated and should, ideally, reflect the relative importance of ammonia and nitrate as a source of nitrogen to the phytoplankton. The accepted range for the quotient value is 1.1-1.4 from theoretical considerations (Laws, 1991) with nitrate and ammonia as the different nitrogen sources respectively. The experimental range in quotient values has frequently been found to be much larger than can be explained by theory. Explanations of the wide range of P.Q. values have included the possible production of exotic cellular metabolites by phytoplankton, or the utilisation of different nitrogen species. However more recently Irwin, (1991) using the same two chemical techniques, namely the oxygen and  $TCO_2$  techniques, found the experimental range of the quotient could be narrowed down to 1.07-1.84. This is in contrast to earlier P.Q. values obtained from the two chemical techniques (Johnson et al., 1983) which, being less than unity required the production of nonconservative gases, such as methane, carbon monoxide or hydrogen. Williams and Robertson, (1991) again using the same two chemical techniques, showed that, providing an error analysis was made, all the P.Q. values they derived from their observations could be contained within the range 1- 1.36.

A frequency histogram of the P.Q. values derived from

oxygen and  $TCO_2$  is shown in figure 9.2.(8) for all the 1990 in vitro/in situ and on deck, incubations. The histogram shows a modal distribution of 1-1.5 with a mean value of 1.23. Of the 27 points, only 5 points cannot be conventionally accounted for, two of which are suspect quotient values, as the value is less than twice the value of the standard error on the quotient. The frequency histogram fails to give account of the variance in the observations, which can be a source of apparent discrepancy. This is better seen as a plot of gross production rate from oxygen, against the gross production rate from  $TCO_2$ . The gross production rates from oxygen and TCO<sub>2</sub>, plotted against each other, show the regression line of the P.Q. (figure 9.2.(9)). Regression analysis (where n=19) reveals a correlation coefficient between the two rate measurements of 0.846, with an r squared value of 71.54 percent. The slope is 1.16 with an intercept of 0.16. As can be seen from this figure, the quotient values are within the constraints set by nitrate and ammonia metabolism.

A time series plot of the change of P.Q. with time is shown in figure 9.2.(7), with the time series of gross production rate measurements from oxygen and TCO<sub>2</sub>. The initial increase in P.Q. value from 1.03 at the start of the period (01/05/1990) to 1.36 on 09/05/1990 parallels the increase in gross production rates. This would suggest





that the plankton are switching from ammonia to nitrate as a source of nitrogen, as the rate of gross production increases. The P.Q. values are around unity during the second cruise. This suggests that the nitrate has been depleted, and ammonia is being assimilated as the nitrogen source.

As discussed earlier in chapter 2.5 the total P.Q. is composed of a carbon controlled P.Q. and a nitrogen controlled P.Q. The relative effect of the two components of the P.Q. will be dependent on the carbon to nitrogen assimilation ratio of the cell. For high C:N assimilation ratios, the carbon P.Q. will dominate the quotient value, and the source of nitrogenous nutrient will have little effect on the total P.Q. For low C:N assimilation ratios the nitrogen P.Q. will have a proportionally greater effect on the overall P.Q. and in such a situation a shift in nitrogen assimilation from ammonia to nitrate should show up as a detectable change in the P.Q. value. A relationship in the results has, therefore, been sought between the P.Q. and the source of nitrogen utilised, taking the C:N assimilation ratios into consideration. The form of nitrogen assimilated is reflected in the f-ratio (the ratio of new to total production - discussed in chapter 2.6).

9.2.3. The relationship between the photosynthetic quotient (PQ[O2/TCO2]) and f-ratio.

The photosynthetic quotient, or P.Q., is discussed in chapter 2.5. The f-ratio is discussed in chapter 2.6.

The photosynthetic quotient, or molar ratio of oxygen produced to carbon dioxide consumed during photosynthesis, can be divided into a carbon and nitrogen controlled P.Q. The carbon P.Q. will be influenced by the ratio of carbon to oxygen in the end products of photosynthesis.

The state of reduction of the nitrogen source utilised during photosynthesis will influence the nitrogen component of the P.Q. The nitrogen component of the P.Q. (PQn) is composed of the nitrate, nitrite and ammonia components,  $PQ_{NO3}$ ,  $PQ_{NO2}$  and  $PQ_{NH4}$  respectively, where;

If we assume nitrite assimilation is negligible, PQn is controlled by the amount of nitrogen assimilated that is

nitrate. PQn is, therefore, related to the f-ratio, the fratio being the ratio of nitrate assimilated to total nitrogen assimilated;

(assuming  $\mathrm{NO}_3$  and  $\mathrm{NH}_4$  are the main sources of nitrogen to the plankton.)

The relative influence of the PQc and PQn on the overall P.Q. will be reflected in the C:N assimilation ratio of the cell where the C:N assimilation ratio is the ratio of carbon assimilated to total nitrogen assimilated;

Combining equations 1, 4 and 5;

 $PQn = 2 \times (NO_3)$  C  $PQn = 2 \times f - ratio(NO_3 + NH_4)$  C

from 5,  $C = C:N(NO_3 + NH_4)$ 

$$PQn = 2 \times \frac{f - ratio(NO_3 + NH_4)}{C:N(NO_3 + NH_4)}$$

$$PQn = 2 \times \frac{f - ratio}{C:N}$$

The P.Q. is therefore directly related to the f-ratio and related to the reciprocal of the C:N ratio. For a given value of PQc we should expect to find a direct relationship between the P.Q. and the f-ratio and an inverse relationship between the P.Q. and the C:N ratio.

The theoretical relationship between the P.Q. and f-ratio has been plotted in figure 9.2.(10) for different values of C:N assimilation ratio. By looking for a relationship between the P.Q. and f-ratio in this way, a value for the C:N assimilation ratio must be assumed. Considering the 1990 experimental measurements, there are two data sets of f-ratio values to choose between (the f-ratio derived from 15-N measurements using the U.K. BOFS nutrient data; and the f-ratio\* derived from 15-N measurements using the U.S. NABE nutrient data). There are also two data sets for the C:N assimilation ratios calculated from TCO<sub>2</sub> and 15-N productivity data, again depending on the nutrient data used in the calculations.

The theoretical P.Q. values calculated from the experimentally derived f-ratios and C:N assimilation



ratios have been compared to the experimentally derived  $PQ[O_2/TCO_2]$  values. By fixing the C:N assimilation ratio at a mean value of 6.6, the effect on the theoretical P.Q. value of changing the calculated f-ratio from that calculated using the U.K. nutrient data, to that calculated using the U.S.A. data, can be assessed.

Figure 9.2.(10) shows the theoretical relationship between the P.Q. and f-ratio. The relationship between the experimentally derived P.Q. ( $PQ[O_2/TCO_2]$  and f-ratio is also shown in (a) as is the relationship between the  $PQ[O_2/TCO_2]$  and f-ratio\* in (b).

Regression analysis of the relationship between the  $PQ[O_2/TCO_2]$  and the f-ratio shows a correlation coefficient of 0.789 with an r squared value of 62.24 percent, (removing two obvious outliers). The regression line is such that the PQc (where the f-ratio is equal to zero) is 0.75. The graph shows the regression line assuming PQc = 1.1. For this relationship to hold a C:N assimilation ratio of 3.13 is required. The regression of  $PQ[O_2/TCO_2]$  and f-ratio\* is poor, with a correlation coefficient of 0.556 and an r squared value of 30.9 percent, (even after removing the one most distinctive outlier). Assuming a PQc of 1.1 the regression line is shown in figure 9.2.(10) and can only be explained by a

carbon assimilation ratio of 0.36. Such a simple relationship cannot be assumed to hold if, as is the case, we have a change in C:N assimilation ratios.

The experimentally derived P.Q. values agree statistically better with the experimentally obtained f-ratios calculated using the U.K. nutrient data, rather than that calculated using the U.S.A. NABE data.

The most obvious difficulty, in seeking a relationship between the P.Q. and f-ratio from the 1990 data, is the choice of assumptions to make about the ammonia data. However, from equation 1, the P.Q. can be related directly to the rates of nitrate and carbon assimilation and thus avoid the use of any ammonia data. The theoretically derived P.Q. values calculated from the assimilation of 15-NO<sub>3</sub> and PgTCO<sub>2</sub>, and the experimentally derived PQ[O<sub>2</sub>/TCO<sub>2</sub>] values, are shown in table 9.2.(a).

The mean standard deviation of the experimental P.Q. values from that of the theoretical P.Q. values is 0.3. The mean standard error on the experimental P.Q. values is 0.27. Figure 9.2.(10a) shows the relationship between the experimental and theoretical P.Q. values. It can be seen that 78 % of the values agree within twice the value of their standard error, and 56 % agree within their standard error.



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of the P.Q. values donained fine for expand and TO techniques are estimin the second even of th theoretically calculated P.G. Willow Table 9.2.(a).

Relationship betwen the experimentally derived P.Q. and the theoretically calculated P.Q.

PQ[02/TC02]	PQ(T) *
$\begin{array}{c} 1.03\pm0.31\\ 1.16\pm0.14\\ 1.35\pm0.07\\ 1.74\pm0.16\\ 0.86\pm0.32\\ 1.18\pm0.20\\ 0.65\pm0.09\\ 1.11\pm0.24\\ 1.27\pm0.29\\ 2.20\pm0.80\end{array}$	1.23 1.33 1.18 1.29 1.16 1.15 1.16 1.19 1.32
$\begin{array}{c} 2.39\pm0.89\\ 1.10\pm0.11\\ 1.05\pm0.14\\ 1.46\pm0.38\\ 0.74\pm0.16\\ 1.75\pm0.68\\ 0.90\pm0.15\\ 1.39\pm0.28\\ 0.78\pm0.23\\ \end{array}$	1.15 1.14 1.22 1.16 1.37 1.11 1.14 1.17

#### where;

 $PQ[O_2/TCO_2] = experimentally derived P.Q. from O_2 and TCO_2 rate measurements <math>PQ(T) * =$  theoretical P.Q. calculated from experimental 15-NO<sub>3</sub> assimilation rates and TCO<sub>2</sub> gross production rates.

It can be concluded that the experimentally derived P.Q. values can be compared directly with the theoretically derived P.Q. values, by using only the nitrate and carbon assimilation rates, and thus avoiding the use of the ammonia assimilation data. Considering all the data, 56  $\pm$  of the P.Q. values obtained from the oxygen and TCO<sub>2</sub> techniques are within the standard error of the theoretically calculated P.Q. values.

9.2.4. A comparison of the 14-C technique with the oxygen and  $TCO_2$  techniques.

During the BOFS 1990 field study, productivity measurements were made using three different techniques, namely the 14-C technique, the oxygen technique and the TCO<sub>2</sub> technique. Before attempting to compare the rate measurements made from these three techniques it is necessary to address the relative precision of each of the techniques, and to assess the compatibility of the sampling strategies employed, and the incubation procedure followed by each of the techniques.

#### 9.2.4.i. Analytical precision of the 14-C technique.

The instrumental precision of the oxygen technique is discussed in chapter 4.3.1.vii, and the accuracy of the productivity rate measurements made during the BOFS study is discussed in chapter 5.1.

The instrumental precision of the TCO<sub>2</sub> technique is discussed in chapter 4.3.3.vii, and the accuracy of the productivity rate measurements made during the BOFS study is discussed in chapter 5.2.

The 14-C technique for measuring productivity was intercalibrated in the 1990 BOFS field programme between the

U.K and the Dutch research ships. Common samples were taken from 6 depths and transported between the ships. Parallel *in situ* incubations were done and 14-C stock solutions were exchanged between both research groups. The efficiency of the liquid scintilation counters, used by the Dutch and U.K. researchers, was also tested. Integrated rates of primary production from 14-C fixation were estimated at 1013 mg C m<sup>-2</sup> over a 9hr incubation period by the U.K. and 976 mg C m<sup>-2</sup> by the Dutch; a difference of only 4 % between both rate estimates (Joint *et al.*, *in press*).

# 9.2.4.ii. Compatibility of the sampling strategy.

The sampling procedure for the oxygen technique and the  $TCO_2$  technique is discussed in chapter 4.1.1. and 4.1.3. respectively. The sampling strategy for the 1989 and 1990 BOFS field programme was such that primary production rate measurements by the different techniques employed could be compared. The procedure for 1989 and 1990 differed slight-ly and are outlined below.

## a. The 1989 sampling strategy.

Water was collected in acid cleaned 30L Go-Flo sampling

bottles. The first sample was for the 14-C rate measurements. The water was siphoned by silicon rubber syphon into the 60ml polycarbonate bottles for the 14-C incubations. The remaining water was then siphoned by silicon rubber siphon into a 50L polycarbonate carbuoy, and then mixed thoroughly with undue aeration being avoided. The water was then taken from the carbuoy for the oxygen production measurements, and then for the  $TCO_2$  production measurements. It was necessary to obtain the water for the 15-N incubations from a separate Go-Flo sampling bottle cast.

Samples were processed for all the techniques immediately. The maximum time from sample collection to the fixation of the samples and deployment of the incubation rig was an hour. The short time between sample processing and manipulation made it unlikely that sample manipulation could have had a significant effect on any of the comparisons between the rate measurements.

The fact that the bottles for each productivity technique were filled sequentially (14-C, oxygen,  $TCO_2$ , respectively), an intercomparison between the rate measurements may have been affected by differential particle settling occurring within the sampling bottle. Most of the delay in the filling procedure was incurred from the time that the 14-C bottles were filled to the time the oxygen and  $TCO_2$ 

samples were taken, when the common water sample was transferred from the Go-Flo sampling bottle to a 50L carbouy. Particle settling was reduced in the carbuoy by gentle swirling of the carbouy. If, however, particle settling did produce a sampling artifact it is likely that it would bias the production comparisons in one direction, as the experimental procedure was the same for each incubation.

#### b. The 1990 sampling strategy.

During 1990 the water samples for the productivity rate measurements were all collected from the same Go-Flo sampling bottle. The sampling bottle, for each of the techniques was filled sequentially. The order of filling was, oxygen,  $TCO_2$ , 14-C and then 15-N. All four techniques, therefore, sampled the same body of water. The time delay between the surfacing of the Go-FLo sample bottle, and the filling of the incubation bottles was not considered to be sufficient to allow settling of the particles within the Go-Flo sampling bottle. Again, as the bottles were filled in the same order on each incubation, if any settling was occurring it could only effect the rate comparisons by biasing the comparison

between the rate measurements in one direction.

## 9.2.4.iii. Compatibility of the incubation procedure.

In the 1989 and 1990 programme, the oxygen, TCO2 and 15-N samples were incubated on the same rig, with the 14-C incubations on a separate rig. The fact that different rigs were used may have produced a bias in the oxygen and 14-C rate comparisons. This bias would only occur if the rigs were constructed in such a way that they were actually incubating at different depths. The rigs were carefully measured before deployment to ensure that the correct depths were being used. In 1990 the oxygen production rig was remeasured after the CD46 cruise and again after the CD47 cruise. No expansion, or contraction, of the ropes had occurred and therefore the depths incubated at had remained the same. Both the oxygen and the 14-C rigs were weighted, and constructed to minimise surface shading. The fact that the oxygen rig was supported by two round buoys, and the 14-C rig by a doughnut buoy, could only cause a bias between the surface rate measurement comparisons, (and only if differential shading effects were occurring). Again, the only effect of having different incubation rigs would be to bias the comparison in one direction.

9.2.4.iv. The photosynthetic quotient derived from 14-C and oxygen rate measurements.

The two most common methods of measuring primary production is by the 14-C tracer technique (Steeman Nielsen, 1952) and the Winkler titrimetric technique (Williams and Jenkinson, 1982). A direct comparison of the two techniques, is not possible as one measures a carbon flux and the other an oxygen flux, but the two may be compared by the application of the photosynthetic quotient or P.Q.

The two techniques are discussed in more detail in chapter 3.3 (the oxygen technique) and 3.4 (the 14-C technique). A comparison of the two techniques is discussed in chapter 3.8. The photosynthetic quotient is discussed in more detail in chapter 2.5.

The photosynthetic quotient is the molar ratio of the rate of carbon dioxide consumption to oxygen production. Theoretical boundaries for the P.Q. are set using stoichiometric calculations derived from the composition of photosynthetic products, and the state of reduction of the nitrogen source assimilated. Theoretical boundaries are from 1.1 to 1.4 (Laws, 1991). Using chemical techniques the P.Q. has been observed to be within this range in natural populations (Williams and Robertson,

1991) but using the oxygen and 14-C technique, the reported range in the literature is much more variable. Some investigators report good agreement between 14-C and the gross oxygen production rate, (e.g. Williams et al., 1983; Davies and Williams, 1984; Irwin, 1991), while others report elevated values of 1.5-2.0 (Williams et al., 1979; Raine, 1983; Holligan et al., 1984), and others as great as a three fold variation in values (Burris, 1980; Smith et al., 1986; Grande et al., 1989a).

The uncertainty as to whether 14-C is measuring a gross, production rate will have contributed to the or net, variation in observed P.Q. values. However, often the observed quotients can not be conventionally accounted for, even if the 14-C technique is approximating to a net primary production rate. Explanations for the elevated that have been observed have included the P.Q. values extension of the theoretical limits of the quotient by the production unconventional metabolites, of or the utilisation of more reduced forms of nitrogen. These ideas have been comprehensively discussed by Williams and Robertson, (1991) and have been discredited as being of any major significance.

The production of such metabolites as methane, for example, will yield elevated P.Q. values and our knowledge

of this process is far from satisfactory at the present. However comparison between the chemical techniques, namely the  $TCO_2$  and the oxygen techniques, suggests that we are not looking to stretch the quotient values but rather for a reason for the apparent incompatibility between the oxygen and 14-C techniques.

During the 1990 BOFS Lagrangian experiment primary production rate measurements were made using both the oxygen and the 14-C technique. A comparison of the rate estimates yielded P.Q. values of a much greater variability than can conventionally be accounted for, with values ranging from less than, to greater than, five times the theoretical P.Q. values. 9.2.4.v. A comparison of the time series of oxygen and 14-C rate measurements.

The time series of integrated production rates of gross production from oxygen  $(PgO_2)$ , net community production from oxygen (PcO<sub>2</sub>) and 14-C during cruises CD46 and CD47 is shown in figure 9.2.(11). That a disparity exists between the two techniques is evident, in that, during the initial stage of the study, the 14-C measurements are less than the net community production measurements from the oxygen technique. The most concerning disparity is evident on the 9<sup>th</sup> of June where the rate of gross production as measured by the oxygen and  $TCO_2$  is at a maximum, while the rate of production obtained from the 14-C technique peaks on the 17<sup>th</sup> of June. During the second cruise the techniques are in better agreement, with the 14-C production rates falling in between that of gross and net community production as measured by the oxygen technique. A reason must exist for the disparity production rates found at the onset of the period in studied, and in particular for the large difference observed on the 9th and 17th of June.

The process of integrating data may often mask variation seen in the discreet data points; it is therefore appropriate to examine the discreet data set as well. In this way the 14-C data can be directly compared with the TCO<sub>2</sub>



data.

Figure 9.2.(12) shows the time series of surface rate measurements of  $PgO_2$ ,  $PcO_2$  and 14-C from CD46 and CD47. The same disparity is still evident as in the integrated data, in that, during the initial period, 14-C returns a production rate less than the net community production measurement by the oxygen technique. Again, a large disparity is evident on the 9<sup>th</sup> and 17<sup>th</sup> of June.

Before attempting to reconcile the two rate measurements it is necessary to decide whether the 14-C rates are approximating to a net, or gross, production rate.

The cited P.Q. values for gross production measurements are 1.1 and 1.4 for ammonia and nitrate based production respectively (Laws, 1991). The mean P.Q. value obtained from  $PQ[O_2/TCO_2]$  in 1990 was 1.23. The frequency distribution of P.Q. values obtained from  $PgO_2$  and 14-C is shown in figure 9.2.(13). The range of P.Q. values is from 1.47 to 5.46, with a mean value of  $3.11\pm0.26$ . Theoretically none of the observed P.Q. values can be explained by the 14-C measuring a gross production rate. The frequency distribution of  $PcO_2$  and 14-C is shown in figure 9.2.(14). The range of P.Q. values from  $PcO_2$  and 14-C is from 0.07 to 3.84, with a mean value of 2.66 $\pm$ 0.53. The large range in values, and the high mean







value, shows that the 14-C production rate is often less than the net community production rate as measured by the oxygen technique.

- It should be noted here that a quotient value from a net community production measurement  $(0_2)$  and a net primary production measurement (14-C) can only be used as an indicator of whether 14-C is approximating to a net production rate measurement, and cannot, therefore, be treated in the same way as a gross production quotient value. Measurements of net community production rates are inclusive of both the autotrophic, and heterotrophic, contribution to the production rates. Net primary production, on the other hand, only considers the contribution from the autotrophic component of the community. To use a quotient from these two indices of production requires the assumption to be made that the heterotrophic contribution is negligible.

From calculations on the depth integrated production rates, a mean P.Q. from  $PgO_2$  and 14-C of  $3.25\pm0.39$  is obtained. The mean P.Q. from  $PcO_2$  and 14-C is  $1.42\pm0.25$ . The frequency distribution of P.Q. values suggests that the 14-C production rate, averaged through the water column, is approximating to a net productivity rate, rather than a gross production rate.

The 14-C rate measurements can be compared to the oxygen rate measurements only by the application of a theoretically derived P.Q. value. A comparison of the 14-C rate measurements with those made by the  $TCO_2$  technique removes the necessity to invoke a quotient value as both techniques are measuring a carbon flux directly.

9.2.4.vi. A comparison of the 14-C and TCO<sub>2</sub> productivity rate measurements.

The 14-C rate measurements should approximate to the  $TCO_2$ rate measurements without the necessity of applying a chosen P.Q. value, as both techniques are measuring a carbon flux directly. There is, however, one major difference. The  $TCO_2$  technique measures the change in carbon due to the total community, inclusive of both the heterotrophic and the autotrophic components, whereas the 14-C technique only measures the change in carbon due to the autotrophic component of the community. While both techniques are able to measure gross production, the distinction must be made that the  $TCO_2$  technique can only measure net community production (Pc), while the 14-C technique approximates to a rate of net primary

production (Pn) that does not incorporate the heterotrophic component of the community.

Another important difference between the two techniques is that any excretion of organic carbon that may occur during an incubation will affect the results from the 14-C technique, but not from the TCO<sub>2</sub> technique. The TCO<sub>2</sub> technique measures the total change in carbon in the dissolved inorganic carbon pool. During photosynthesis, carbon is transferred from the inorganic carbon pool into the organic pool, and, during respiration, is released back into the inorganic pool. The excretion of organic carbon that may occur during an incubaton will be from the particulate organic carbon pool within the plant, to the dissolved organic carbon pool. The concentration of carbon in the inorganic carbon pool will, therefore, not be affected by excretion, thus the resultant photosynthetic rates from the TCO2 technique will not be affected. The 14-C technique, however, measures the increase in particulate organic carbon during an incubaton. Any losses from the particulate organic cabon pool to the dissolved particulate organic carbon pool which will occur during excretion, will, therefore, affect the production rates from the 14-C technique.

We therefore have two different scenarios, which may cause
a discrepancy in production rates measured by the  $TCO_2$  and 14-C techniques; one, the effect of the heterotrophic component of the community, and two, the effect of the excretion of organic carbon.

# a. The effect of the heterotrophic community on TCO<sub>2</sub> and 14-C rate comparisons.

If the 14-C technique is returning a gross production rate, it should be directly comparable with the rate of gross production as measured by the  $TCO_2$  technique. If 14-C is measuring a net production rate we would expect it to give a higher value than the net community production rate as measured by the  $TCO_2$  technique ( $PcTCO_2$ ), as net primary production it is a measure of gross production minus the respiration of the autotrophs (Ra), and net community production, is a measure of gross production minus the respiration of both the autotrophs and heterotrophs (Ra + Rh). The difference between the two rates can, therefore, be taken as the respiration due to the heterotrophic component of the community. Where;

Pn(14-C) = Pg - Ra

 $Pc(TCO_2) = Pg - (Ra + Rh)$ 

 $Pn(14-C) - Pc(TCO_2) = Rh$ 

If the situation is such that  $PgTCO_2 > 14-C > PcTCO_2$ , by the application of these calculations it is should be possible to divide the community respiration rate, as measured by the  $TCO_2$  technique, into that due to the autotrophic and heterotrophic components. The calculated heterotrophic term (Rh) is simply subtracted from the total respiration term (RTCO\_2), with the resultant value being the autotrophic term (Ra).

 $R(TCO_2) - Rh = Ra$ 

If the autotrophic respiration term is then added to the 14-C net primary production rate, the 14-C rate should equate to the gross production rate as measured by TCO<sub>2</sub>.

 $Pn(14-C) + Ra = Pg(14-C) = Pg(TCO_2)$ 

During the 1990 field programme, 13 incubations made in which both  $TCO_2$  and 14-C surface rate measurements were run simultaneously. The situation such that  $PgTCO_2 >$ 14-C >  $PcTCO_2$  was fulfilled, only occured in 6 of the incubations. The above calculations have been applied to this data. Table 9.2.(b) shows the recalculated respiration rates subdivided into autotrophic and

heterotrophic respiration.

### Table 9.2.(b)

Respiration rates from the oxygen technique recalculated into autotrophic and heterotrophic components.

Exp	Date	<b>R</b> [-μmo]	Rh 1/Kg/0	<b>Ra</b> layj	<b>%Rh</b> ]	%Ra	R:Pg
1 9	01/05/90 29/05/90	6.10 7.62	0.75	5.35	12 5	88 95	79.2 74.1
13 17 19	03/06/90 09/06/90 12/06/90	5.21 11.67 1.51	1.98 5.91 0.36	3.23 5.76 1.15	38 51 24	62 49 76	72.3 160.7 46.0
21	14/06/90	3.51	1.16	2.35	33	67	81.3

where; R = total respirationRh = heterotrophic respiration rate R:Pg = ratio of total respiration to gross production

The percentage of the total respiration due to each component is also shown. The ratio of respiration to gross production from  $TCO_2$  is also shown for each experiment.

b. The effect of excretion on the comparison of TCO2 and 14-C rate measurements.

For 7 of the incubations made in 1990, where both TCO2 and 14-C rate measurements have been made simultaneously, the 14-C rate is less than the net community production rate

as measured by  $TCO_2$  (PcTCO<sub>2</sub>). A possible explanation for the 14-C technique returning lower rates than PcTCO<sub>2</sub> is that excretion of organic carbon is occurring. The difference between PcTCO<sub>2</sub> and 14-C can be taken as the excretion rate of organic carbon. However, to make this assumption the second assumption must be made, that net primary production is equal to net community production (Pn is equal to Pc, or, Rh = 0).

Table 9.2.(c) shows the calculated excretion rates needed to reconcile the 14-C rate with  $PcTCO_2$ . The percentage of net production that is required to be excreted is shown, and the ratio of respiration to gross production is also shown.

It is immediately obvious that there is a major problem with the presentation of these results. In the one case (table 9.2.(b)), excretion is ignored and in the other (table 9.2.(c)), the heterotrophic respiration rate is ignored. Two different scenarios are therefore required, into which, idealistically, the data can be sorted; one situation occurring where the heterotrophic component is making a major contribution to the respiration term, and excretion rates are low, and the other, where the heterotrophic contribution is negligible but excretion is significant.

Table 9.2.(c)

Calculated excretion rates required to reconcile 14-C rates with PcTCO<sub>2</sub>.

Ex	pt	Date	Excretion	% of Pc	R:Pg
4	09	/05/9	0 3.69	45.7	54.5
5	14	/05/9	0 1.93	27.3	49.5
8	28	/05/9	0 1.78	26.9	20.1
11	01	./06/9	0 2.92	46.1	16.6
16	08	/06/9	0 1.46	34.2	23.5
20	13	/06/9	0 1.57	49.8	30.3

where;

Pc = net community production from  $TCO_2$ R:Pg = ratio of respiration to gross production from  $TCO_2$ 

#### c. The ratio of respiration to gross production.

By looking at the ratio of respiration to gross production (R/Pg), investigators have reported that the relative contribution of the autotrophic and heterotrophic components to the community can be assessed. Iriarte et al., (1991) found that at low R/Pg ratios the community structure was that of a predominantly algal population, with high net community production, and that, as the R/Pg ratio increased, the percentage due to the heterotrophic component appeared to increase. Grande et al., (1991) also found that the heteretrophic component made a greater contribution to the respiration term at higher R/Pg ratios than at the lower ratios.

The two sets of data obtained from the 1990, 14-C and  $TCO_2$  rate comparison, can be divided according to their R/Pg ratios. The range of R/Pg ratios is higher in table 9.1.(b) than table 9.2.(c). The higher range of ratios (46 to 160.7) occurs in the situation where 14-C is greater than  $PcTCO_2$  and the contribution of the heterotrophic community to the respiration term has been used to explain the apparent disparity in rates. The lower range of ratios (16.6 to 54.5) occurs in the situation where excretion is assumed significant, and the heterotrophic contribution is assumed negligible. This is, ofcourse, only a tentative separation of the data.

9.2.4.vii. A comparison of 14-C and oxygen productivitiy measurements.

The same principals that applied when comparing 14-C and TCO<sub>2</sub> production rate measurements, apply when comparing oxygen and 14-C production rate measurements, except for the important difference that the oxygen technique measures an oxygen flux and not a carbon flux, necessitating the use of a quotient value when comparing the two rate measurements.

As before, if the 14-C technique is measuring a net production rate (Pn14-C) it should be greater than the net community production rate as measured by the oxygen technique (PcO<sub>2</sub>), as Pn14-C is equal to gross production minus the respiration of the autotrophic community, and PcO<sub>2</sub> is equal to gross production minus the respiration due to both the autotrophic and heterotrophic community.

Because a quotient value is needed for the conversion of an oxygen flux into a carbon flux, it is more appropriate to compare the gross production rate from the oxygen technique, with the 14-C production rate, rather than, comparethe net community production rate from the oxygen technique with the 14-C production rate.

The calculation is, therefore;  $Pg^{C} = Pn^{C} + Ra^{C}$   $\frac{Pg^{O}}{PQ} = Pg^{C}$   $\frac{Pg^{O}}{PQ} - Pn^{C} = Ra^{C}$ PQ

where superscript c denotes a carbon flux, and superscript o denotes an oxygen flux, and:

 $R^{O} \star R.Q. = R^{C}$ 

 $R^{O} \star R.Q. - Ra^{C} = Rh^{C}$ 

The above calculations have been applied to the depth integrated data set from the 1990 field programme. Table 9.2.(d) shows the recalculated respiration rates from the oxygen technique, converted into carbon units and divided into the respiration rates due to the autotrophic and heterotrophic components of the community. The percentage of the total respiration due to each component is shown, and the ratio of R to PgO<sub>2</sub> is also shown in the table.

When converting from oxygen into carbon units a quotient value must be assumed. The P.Q. and R.Q. values used in the calculations were the mean values obtained from each respective cruise, from the oxygen and  $TCO_2$  gross production rate measurements. For CD46, P.Q. = 1.35, R.Q. =

1.48. For CD47, P.Q. = 1.2, R.Q. = 1.13.

It can be seen that all the integrated production rates from 14-C and oxygen can be equated by the use of these calculations. It is also evident that the ratio of R to Pg increases, as the calculated percentage of the total respiration that is due to the heterotrophs increases, (there is an exception on 03/06/1990).

#### Table 9.2.(d)

Recalculated respiration rates divided into autotrophic and heterotrophic components.

Exp	t date	R [	Rh mg C 1	<b>Ra</b> m-2]	%Rh	*Ra	R:Pg
1	01/05/90	61.44	3.42	58.02	6	94	32.0
2 3	03/05/90	72.51	68.54 22.39	50.12	38 31	62 69	59.9 38.1
4 5	09/05/90 14/05/90	332.30 261.59	28.61 145.70	303.69	9 56	91 44	45.5 69.8
6	17/05/90	246.27	234.91	11.36	95 61	5	64.4
			241.43	130.90			
8 9	28/05/90 29/05/90	42.13	6.02 63.50	36.11 100.63	14 39	86 61	31.4 84.4
11 13	01/06/90	71.46	53.75 171.35	17.71	75 87	25 13	46.2
14	05/05/90	96.31	29.27	67.04	30	70	57.6
17	09/06/90	129.22	97.72 88.97	31.46 18.89	82	24 18	131.5 170.2
19 21	12/06/90 14/06/90	56.78 138.48	38.74 132.21	18.04 6.27	68 95	32 5	71.1 204.3

where;

R = total column integrated respiration rate Rh = heterotrophic respiration rate Ra = autotrophic respiration rate R:Pg = ratio of total respiration to gross production From these calculations it is apparent that when comparing production rates obtained from oxygen and carbon flux measurements, the respiration term is of vital importance. Heterotrophic respiration will affect any comparison of oxygen and 14-C rate estimates that are made, in accordance with the magnitude of the percentage contribution it is making to the total respiration rate. More importance must, therefore, be assigned to the respiration term, and subsequently to the R.Q. values, when production rate measurements from the 14-C technique are compared with measurements from the oxygen or  $TCO_2$ techniques. By the appropriate application of respiration rates and R.Q. values, it is evident that apparently discrepant data sets may be reconciled.

The calculations that have been applied to reconcile the oxygen and 14-C measurements, have produced a method of subdividing respiration rates between that of the autotrophic and heterotrophic components of the community. Few attempts have been made to look at the relative contribution of these components to overall respiration rates. In fact, that the heterotrophic component has any significant contribution to make has only been thought of relatively recently (Williams, 1981). It has now been suggested that the bacteria may be one of the major

consumers of photosynthetically fixed carbon (Azam et al., 1983; Linley et al., 1983).

It has been reported that at low chlorophyll a concentrations (low algal biomass), the correlation between chlorophyll a and the respiration rate will be weak (Packard, 1979, Iriarte *et al.*, 1991) as the microheterotrophs are making up a major contribution to the community. Iriarte *et al.*, (1991) observed that a significant correlation was only found at chlorophyll a concentrations above 5  $\mu$ mol L<sup>-</sup> 1. Lake studies of Schwaerter *et al.*, (1988) lend support to this idea, as, at low chlorophyll a concentrations, they found that the bacterial component, accounted for 70 % of the total respiration, whereas at higher chlorophyll a concentrations they only accounted for 20 %.

A correlation between the respiration rates measured by the oxygen technique, and as calculated from the oxygen and 14-C comparisons, was sought against the oxygen gross production rate. A better correlation is expected from the autotrophic respiration rate than from the total respiration rate. Figure 9.2.(15) shows this relationship from the 1990 depth integrated data. The autotrophic respiration rate (b) is significantly correlated to gross production, (79.88 %), whereas the total respiration rate shows a correlation of 65.84 % where n = 16 for both cases.







The relationship of depth integrated respiration to depth integrated gross production normalised to chlorophyll *a* is also increased when differentiating autotrophic respiration from the total respiration (figure 9.2.(16)). The correlation increases from 25.67 % to 65.04 % using the total respiration rate and the autotrophic respiration rate respectively, with n = 16 for both situations. 9.2.5. Carbon to nitrogen assimilation ratios.

From oceanographic observations and stoichiometric calculations, Redfield, (1934) concluded that the cellular composition of phytoplankton should fall in a narrow range of carbon to nitrogen to phosphorous ratios, and over the life time of the cell, the assimilation ratios of carbon to nitrogen should approximate to this ratio (6.6).

In the 1990 BOFS field programme the C:N assimilation ratios of the plankton community have been calculated by three different methods. Primary production measurements from  $TCO_2$  and 14-C give a measure of carbon assimilation. Nitrogen assimilation was measured from the uptake of 15-NO<sub>3</sub> and 15-NH<sub>4</sub>. Thus C:N assimilation ratios were calculated from  $TCO_2$  and 15-N and from 14-C and 15-N rate measurements. The ratio of particulate organic carbon to particulate organic nitrogen was also calculated (Kennedy, pers comm).

The  $TCO_2$  technique can be analysed to provide values of both gross and net community production. It is therefore possible to calculate both gross, and net, assimilation ratios. The gross or 'total' assimilation ratios are calculated from PgTCO<sub>2</sub> and Pt(15-N). The net, or 'new,' assimilation ratios are calculated from PcTCO<sub>2</sub> and

Pnew(15-NO<sub>3</sub>), assuming a meaningful comparison can be made between new and net community production, (see section 9.1.4.(v). The C:N assimilation ratios can be used as an indicator of whether 14-C is approximating to a gross, or net, production rate by comparing the 14-C ratios with those from PgTCO<sub>2</sub> and PcTCO<sub>2</sub>.

The observed range of C:N ratios, from the particulate fraction in 1990, is 4-28 with a mean value of  $9.07\pm0.6$ , which is within the range of quoted values for oceanic particulate matter (Goldman, 1980).

Total production assimilation ratios are shown in figure 9.2.(17). The ratio of gross oxygen production to total nitrogen assimilation has been calculated. By introducing a photosynthetic quotient the O:N ratio can be treated in the same manner as a C:N ratio. A typical C:N ratio of 6.6 will translate as an O:N ratio of 5.28 assuming a P.Q. of 1.25. The modal range of O:N ratios is between 2.5-7.5. The gross TCO<sub>2</sub> production to total nitrogen assimilation ratios are shifted up, as we would expect, with a modal range of 2.5-12.5. The 14-C to total nitrogen assimilation ratios are at the lower mode, which suggests that the 14-C is nearer to a net assimilation rate than a gross assimilation rate. The mean value for the C:N assimilation ratio from 14-C:Pt(15-N) of 4.75+0.5 is

Figure 9.2.(17)

10098

10.14

Total production assimilation ratios



Assimilation ratio of PgO2 to Pt(15-N)



Patcoz Pt



Assim at on ratio of PgTCC2 to Pt(15-N)

almost a factor of two lower than that cited for open ocean oligotrophic situations - the value being 8.9 (Perry, 1976). The  $TCO_2$  gross production assimilation ratio mean value is  $6.59\pm1.22$  which is that value cited by Redfield, (1934). The fact it is lower than what is typical of an open ocean situation is possibly due to the presence of a large heterotrophic component in the plankton community which will effectively reduce the C:N ratios, as bacteria have a typical assimilation ratio of 3-4 (Billen, 1984).

The recalculation of 15-N assimilation rates, from the use of the recalculated ammonia data, causes a shift up in the distribution of C:N assimilation ratios. A larger percentage of erroneously 'high' ratios are, however, introduced.

New production assimilation ratios are shown in figure 9.2.(18). A modal distribution is still seen around 2.5-7.5 from net oxygen production assimilation ratios and from net  $TCO_2$  production assimilation ratios. The 14-C:Pnew(15-NO<sub>3</sub>) ratios are all increased and show a modal distribution of 2.5-7.5 with a greater spread in higher ratios than from the total production ratios. If assuming higher C:N ratios are to be expected, the observed increased ratios can be used as an indicator that the 14-C technique is measuring a new production rate.



The time series of the surface C:N assimilation ratios from gross TCO<sub>2</sub> production, and total nitrogen assimilation, is shown with the particulate C:N ratios for 1990 in figure 9.2.(19). Although one is measuring a growth property (PgTCO2:Pt), and the other a standing stock (POC:PON), a similarity in the ratios is to be expected. The ratio of C:N in the particulate fraction should be preceded by a C:N assimilation ratio of the same dimension. Both ratios, indeed, show the same trend over the time series with little change occurring during B1 and a rapid increase seen at the start of B2. The lower ratios at the onset of the period may be due to higher growth rates occurring in this period, as nitrate is being rapidly consumed and production is increasing. At the start of B2 the production rate has dropped and a more oligotrophic situation is moved towards. The relationship however between C:N ratios and growth is reported as being "one=many" in that there is no one unique interpretation for a particular C:N ratio (Tett et al., 1985). The influence of other factors, such as light, must be considered. The various fractions that make up the community will also influence the C:N ratio, for example the influence of zooplankton and bacteria.



9.2.6. In situ  $O_2$  versus In vitro  $O_2$  net community production rate measurements.

The BOFS 1990 field programme was an attempt at a Lagrangian experiment, meaning a single body of water was tracked and sampled from, over a period of 41 days. During the first cruise (CD46) the water column was sampled with a CTD cast every six hrs. The hydrographical, physical and chemical properties of the water column were, therefore, monitored four times over every 24hrs. The CTD oxygen electrodes were regularly calibrated, using the Winkler oxygen titrimetric technique. The frequency of the CTD sampling regime has enabled *in situ* measurements of the net community production of oxygen.

The in situ rates can be directly compared to the in vitro production rates. There are problems involved in trying to compare the two different rate measurements, this has been discussed in more detail in chapter 3.2. That the Lagrangian experiment was truly successful, in that a single body of water was tracked, is discussed in a BOFS publication (Savidge in press). For the sake of this comparison assumptions have to be made that the same body of water was sampled in both the in vitro and in situ rate measurements.

9.2.6.i. Calculation of in situ net community oxygen production.

To calculate the change in oxygen concentration in situ, the change over 24hrs due to the transfer of oxygen to the atmosphere, and the net change of oxygen in the water column, must be calculated. The sum of these two rates can be assumed to be the rate of net community oxygen production. The depth of the mixed layer is ascertained from the profiles of salinity and temperature. The *in situ* rates are calculated from the average change in oxygen from four depth profiles over the 24hr period.

The transfer of oxygen to the atmosphere  $(O_2T)$  is calculated from the transfer velocity (k) multiplied by the average oxygen saturation  $(O_2sat)$  and divided by the depth of the mixed layer (Z).

 $O_2 T = K \times O_2 sat$ 

where;

k = transfer velocity, (m day<sup>-1</sup>) and k = (2.53 x w - 13.09) x 0.96 for w > 9.5 m s<sup>-1</sup> k = (1.11 x w + 0.35) x 0.96 for w < 9.5 m s<sup>-1</sup> when;

w = 24-hourly averaged wind velocity data, corrected for ships heading and velocity (m s<sup>-1</sup>)

 $O_2$ sat = the average oxygen saturation over 24 hrs (µmol  $L^{-1}$ ) calculated from the mean oxygen concentration minus the mean oxygen concentration at 100 percent saturation (the mean excess oxygen). The mean value from each profile is calculated down to the mixed layer depth (Z) and then averaged over the 24 hr period from the four CTD profiles.

The net in situ oxygen change is calculated from the change in the mean excess oxygen (the mean oxygen concentration minus the mean oxygen concentration at 100 % saturation) over the 24 hr period, by calculating the change between the dawn oxygen profiles.

The net community oxygen production is then calculated from the addition of the oxygen transfer to the atmosphere and the net *in situ* oxygen change over the 24hrs. This is then multiplied by the depth of the mixed layer to give an integrated rate ( $\mu$ mol m<sup>-2</sup> day<sup>-1</sup>), which can then be compared directly to the integrated *in vitro* production rate.

9.2.6.11. Results of the in vitro and in situ oxygen production comparison from the 1990 BOFS Lagrangian experiment.

Table 9.2.(e) shows the calculated average wind velocity, transfer velocity, the mixed layer depth, the average oxygen saturation, oxygen transfer, in situ oxygen change and the net community production rate for each date sampled.

Table 9.2.(e).

Date	Av.Wind velocity	Transfer velocity	Mixed layer depth	Average oxygen saturtn.	Oxygen transfer	In site oxygen change	u Net in situ oxygen change
	(V) E s <sup>-1</sup>	(k) m day-1	(5) R	(0 <sub>2</sub> sat) (*)	(02T) (*)	(**)	(***)
===== 04/05	7.36	8.11	25 e	16.76	5.44	0.10	190.75
05/05	9.18	10.12	25	19.20	7.77	0.15	276.50
06/05	9.05	9.98	20	8.80	4.39	-9.67 -	-184.80
07/05	7.33	8.10	25	3.80	1.54	0.93	86.45
08/05	2.50	2.98	30	4.38	0.44	0.30	25.90
09/05	6.66	7.66	20	9.03	3.46	3.45	241.85
10/05	4.73	5.33	30	18.84	3.35	5.45	308.00
1/05	7.86	8.64	20	12.04	5.20	-1.80	119.00
12/05	9.08	10.32	20	9.08	4.69	1.20	206.15
13/05	7.82	8.59	35	9.91	2.43	-0.36	72.45
14/05	4.81	5.63	40	9.35	1.30	0.16	51.10
16/05	3.58	4.27	25	9.83	1.68	-0.47	42.35

\*\* =  $\mu mol O_2 L^{-1} day^{-1}$ \*\*\* =  $mmol O_2 m^{-2} day^{-1} (0-35m)$ 

The cumulative net community production rate is shown in figure 9.2.(20) from 04/05/1990 to 16/05/1990.



The *in vitro* net community oxygen production rates and 14-C production rates have been added cumulatively as shown in figure 9.2.(20), in order that the different rates may be compared. For the days on which *in situ* measurements have not been made, the assumption has been made that the production rate is linear between the days sampled.

Good agreement between the *in situ* and *in vitro* oxygen net community production rates have been found in accordance with such workers as Davies and Williams, (1984) and Williams and Purdie, (1991). The initial higher *in situ* rate on the 4th is followed by a day of negative production on the 5th, which keeps the two oxygen rate measurements within the same order of magnitude as each other. The 14-C rate is consistently lower. The cumulative *in vitro* oxygen production rate measurements average 86 % of the cumulative *in vitro* oxygen rate measurements, the 14-C rate measurements average 45 %.

For the days sampled concomitantly, the *in situ* and *in vitro* oxygen net community production rates show considerably good agreement, the *in vitro* rate is 92.3 % of the *in situ* rate on 07/05/1990, 83.6 % on 09/05/1990 and 150.1 % on 14/05/1990.

The results from this comparison give support to the accuracy of the *in vitro* oxygen production rates measured during the 1990 BOFS field programme.

<u>9.2.7. Critical depth calculations - predicting the onset</u> of a phytoplankton bloom.

"The annual ecological succession of the phytoplankton can only proceed if excess production is invested into the community" (Margelef, 1967). The process of annual succession is, therefore, linked to an increased stability in the water column.

In 1953, Sverdrup outlined a model whereby the phytoplankton succession (or 'bloom'), could be predicted from the relationship between the calculated depth of mixing, the compensation depth and the critical depth in the water column. The definitions of the compensation depth, the critical depth, and the mixed layer depth, and how they are calculated, is discussed in chapter 2.9.1, chapter 2.9.2, and chapter 9.1.3 respectively.

In order that the phytoplankton are able to "bloom", it is necessary that the production of organic matter by phytoplankton exceeds the destruction by respiration (Sverdrup, 1953). The phytoplankton must, therefore, spend more time in an area of positive net production, than negative net production in order that net growth can occur. A depth must exist whereby, 'blooming' is only able to occur if the mixed layer depth is shallower than this critical

value. By looking at the relationship between the critical depth and the depth of mixing, it should, therefore, be possible to ascertain whether a 'bloom' is able to occur.

The critical depths have been calculated for the 1990 BOFS N.E.Atlantic Lagrangian experiment from the oxygen production rate measurements for the time series between 01/05/1990 and 16/06/1990. The calculated critical depths, calculated compensation depths and the depths of mixing are shown in table 9.2.(f).

The change of the mixed layer depth, and the critical depth with time, is shown in figure 9.2.(21). Initially the critical depth was deeper than the depth of mixing, suggesting that at the beginning of the study net phytoplankton growth was able to occur. The critical depth lay at between 50 and 150m until day 150 (30/05/1990) when it shallowed to 27m, only 7m below the depth of mixing. For the rest of the time series the critical depth was equal to, or shallower than, 50m. On day 154, (03/06/1990) the critical depth was shallower than the depth of mixing. On days 160 and 165 (09/06/1990 and 14/06/1990 respectively), the critical depth was shallower than the mixed layer depth.

If the relationship of the critical depth to the mixed

## Table 9.2.(f)

Date	Zmđ N	Zcomp M	Zcrit M
			65 71
01/05/1990	20	20.03	-
02/05/1990	20	24.45	137.78
03/05/1990	50		-
05/05/1990	40	-	-
06/05/1990	28	-	-
07/05/1990	32	23.80	(-)
08/05/1990	29	-	-
09/05/1990	38	24.64	78.00
10/05/1990	24	-	-
11/05/1990	20	-	-
12/05/1990	27	-	-
13/05/1990	25	-	-
14/05/1990	25	10.73	48.18
15/05/1990	28	-	-
16/05/1990	25	-	<i>-</i>
17/05/1990	10	12.07	(-)
18/05/1990	15	-	
19/05/1990	10	18.33	(-)
20/05/1990	15	-	
			20 00
28/05/1990	15	12.20	50 00
29/05/1990	12	12.34	27.14
30/05/1990	20	9.50	-
31/05/1990	20	27.86	(-)
01/06/1990	20	9.79	48.18
02/06/1990	20	6.45	14.71
03/06/1990	25	-	-
04/06/1990	25	13.89	50.00
06/06/1990	35	-	-
07/06/1990	-	-	-
08/06/1990	25	11.02	36.07
09/06/1990	25	7.19	22.50
10/06/1990	35		-
11/06/1990	35	-	- ł
h2/06/1990	25	22.75	(-)
13/06/1990	15	-	-
14/06/1990	30	4.41	9.79
15/06/1990	30	-	-
			<b>.</b>
Zmd = Depth	of	mixing,	taken as a
0.2° change	in	tempera	ture.
Zcomp = Com	pens	ation d	epth,
Zcrit = Cri	tica	l depth	L

Critical depth calculations from oxygen rate measurements



layer depth does in fact determine the development of a phytoplankton bloom, a positive net community production rate should occur on the days when the critical depth is deeper than the mixed layer depth. Likewise, a negative net community production rate should be evident when the mixed layer depth is deeper than the critical depth.

Figure 9.2.(22) shows the time series of net community production from the oxygen technique, integrated through the water column from 0-35m. Only the days on which critical depth calculations have been able to be made, have been included in the time series.

From day 121 to 149 the net community production rate was positive, and the critical depth was deeper, than the mixed layer depth. On day 150, when the critical depth had shallowed to only 7m below the mixed layer depth, the net community production rate fell to near zero. On day 153 the critical depth sank and the net community production rate increased. On day 154 the critical depth rose again to higher than the mixed layer depth, and the net community production rate became negative. On day 155 the net community production rate became positive again and the critical depth dropped. On days 160 and 165 the net community production rate was negative and the critical depth had shallowed to above the mixed layer depth.

It is apparent that there is a close correspondence between the relationship of the critical depth to the mixed layer depth and the net community production rate. All the days on which the critical depth is below the mixed layer depth, the depth integrated net community production rate is positive, and on all the days on which the compensation depth is above the mixed layer depth, the depth integrated net community production rate is negative.

Figure 9.2.(23) shows the relationship between the critical depth and the mixed layer depth and the depth integrated net community production rate. When the critical depth rises above the zero line it is shallower than the mixed layer depth. When the net community production rate falls below the zero line, it is negative.

A time delay between the relative depth of the critical depth to the mixed later depth and the net community production rate should be expected of the order of a day or so, therefore the analysis cannot be that exact.

The time series of chlorophyll a concentrations (figure 9.2.4.) is supportive of the critical depth calculations. Chlorophyll a concentrations increased over the time series to a maximum on 13/05/1990 (day 133). The critical depth was deeper than the mixed layer depth over this



time, allowing a net growth of the phytoplankton community to occur. During the second half of the time series the chlorophyll *a* levels decreased, as the mixed layer depth decreased, falling below the critical depth and stopping a net growth of the community from being able to occur.
## Chapter 10

## General discussion and conclusions.

A comparative study has been made between the productivity measurements from the oxygen high precision Winkler technique and the  $TCO_2$  coulometric technique. A comparison between the productivity rates from these two techniques is at present, the most accurate means of assessing the community photosynthetic and respiratory quotients. Both techniques should be influenced by the microheterotrophic community in the same way, resulting in comparable rates. The protocol for *in vitro* and *in situ* incubations is the same for both techniques (chapter 3.4.1.), as are the assumptions for the calculation of the productivity rates (Chapter 4.3.1.vi and 4.3.3.vii).

Simultaneous measurements of the assimilation of nitrogen from the 15-N technique, has enabled a relationship to be sought between the calculated P.Q. values and the source of nitrogen utilised by the phytoplankton. A relationship between the P.Q., the f-ratio, and the carbon to nitrogen assimilation ratio has been sought in both a cultured (Chapter 6.4) and open ocean environment (Chapter 9.1.3 and Chapter 9.2.3).

Before any relationships between the different flux measurements are discussed, the validation of the techniques needs to be considered. The precision of each technique employed is outlined in the methodology (Chapter 4). The accuracy of the techniques has been examined by inter-comparison of the rate measurements. The application of the *in vitro* methodology has been assessed by comparison of *in vitro* rate measurements with *in situ* rate measurements.

During the 1989 and 1990 BOFS field programmes concomitant 14-C production rates were made (Savidge, Boyd and Pomroy) allowing a comparison to be made with the oxygen and  $TCO_2$  production rates. The results and nature of these comparisons are discussed.

Productivity rate measurements from the oxygen technique can be used in the calculation of critical depths (Sverdrup, 1953). A comparison of the critical depths and the calculated depths of mixing can be used as a means of assessing the advancement of a phytoplankton bloom. Such comparisons have been made in this study, and the findings compared to the net export flux, or depth integrated net community production rates as measured from the oxygen technique.

10.1. Validation of the techniques used for measuring primary productivity.

The precision of the oxygen Winkler technique is discussed in chapter 4.3.2.vi. An inter-comparison of the oxygen technique made during this study was indicative of the general accuracy of the technique.

A comparison of productivity rates from the oxygen technique was made between the U.K. and the Dutch research ships during the 1990 field programme. The Dutch measurements were coordinated by Dr.Kraay. The mean difference in gross production rate was  $0.62 \ \mu mol \ Kg^{-1}$  $9hr^{-1}$ , a value less than the average standard error on the Dutch gross production values, of  $0.79 \ \mu mol \ Kg^{-1} \ 9hr^{-1}$ . The respiration rates differed by a mean value of  $0.42 \ \mu mol \ Kg^{-1} \ 9hr^{-1}$ , again less than the standard error on the Dutch respiration rates, of  $0.66 \ \mu mol \ Kg^{-1} \ 9hr^{-1}$ . The difference in production rates between the two ships was, in all cases, less than twice the value of the combined standard error (Chapter 5.2, Table 5.2.(a)).

The precision of the TCO<sub>2</sub> coulometric technique is discussed in chapter 4.3.3.vii. The comparative accuracy of the technique has been assessed from an intercomparison of the technique made during the 1990

programme. The HP86 computer controlled coulometer, model 5010 used in this study and the IBM PC computer controlled coulometer, model 5011 (Carol Robinson, UCNW) were compared. The mean difference in the measured  $TCO_2$  concentrations from the first inter-comparison made, was 7.6  $\mu$ mol Kg<sup>-1</sup>, a mean difference of 0.4 %. The second parallel set of measurements differed by a mean value of 10.5  $\mu$ mol Kg<sup>-1</sup>, a mean difference of 0.5 %.

The application of the *in vitro* methodology was assessed by a comparison of the *in vitro* rates obtained, with *in situ* production rates. During the 1989 BOFS field study, the *in vitro* net community production rates from the oxygen technique, averaged over the 5 days sampled at  $47^{\circ}N$  20°W gave a mean value of 56.33 mmol O<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup> (Chapter 7.1, Table 7.1.(c)). This was within the range of values from the *in situ* oxygen measurements made over the same period, of 46 - 61 mmol O<sub>2</sub> m<sup>-2</sup> day<sup>-1</sup>.

The in situ net community production rate from  $pCO_2$ measurements (Robertson et al., in press) was 41.5 mmol C m<sup>-2</sup> day<sup>-1</sup>, A net photosynthetic quotient of 1.35 is therefore obtained from the *in situ* carbon and *in vitro* oxygen measurements.

In the 1990 BOFS field programme, cumulative net community production rates from the oxygen technique, averaged 86 %

of the cumulative in situ net community production rates (Chapter 9.2.6). For the days sampled concomitantly, the in vitro and in situ oxygen production rate measurements showed good agreement, with in vitro rates of net community production being 92.3 % of the in situ rate on 07/05/1990, and 83.6 % of the in situ rate on 09/05/1990.

The instrumental precision of the 15-N mass spectrometric technique has been discussed in chapter 4.3.4.v. The calculations required in measuring nitrogen assimilation rates are discussed in chapter 3.7.2. The major difficulty in the calculation of ammonia assimilation rates, in this study, was due to the measurement of ambient ammonia concentrations. This has been discussed in chapter 9.2.3.

It can be concluded that the comparative accuracy of the rate measurements obtained from the oxygen and TCO<sub>2</sub> techniques are at the limits of the relative precision of the two respective techniques.

Comparison of *in vitro* and *in situ* rate measurements have shown that the *in vitro* methodology employed in this study is not an overriding cause for concern. This is in accordance with such workers as Davies and Williams, (1984) and, Williams and Purdie, (1991), who both found that there was no evidence for consistent or major

discrepancies between in vitro and in situ determined rates of gross production as measured by the oxygen technique. Earlier reported discrepancies were as high as ten fold, but were mainly from comparisons between in vitro 14-C production rates and in situ oxygen production rates (Gieskes, et al, 1979; Schulenberger and Reid, 1981). Daneri, (in press) compared in vitro and in situ oxygen production rates and found, however, that in vitro rates underestimated in situ rates by as much as 50 %.

10.2. The PQ[O2/TCO2] and its relationship to the f-ratio and C:N assimilation ratio.

The photosynthetic quotient was calculated from oxygen and TCO<sub>2</sub> rate measurements on both a culture of the diatom *Thalassiosira Weissfloggi* (Chapter 6.4) and on an open ocean community (Chapter 9.2.2).

The distribution of the P.Q. values obtained from the culture studies, is shown in figure 10.2.(1) as a plot of gross production from the oxygen technique against gross production from the  $TCO_2$  technique. The P.Q. values can be seen to all fall (with one exception) within the theoretical range of P.Q. values, of  $1.1\pm0.1$  to  $1.4\pm0.1$ 



(Laws, 1991). The P.Q. values from the culture ranged from between  $1.13\pm0.18$  to  $1.55\pm0.09$  with a mean value of 1.3.

In the 1990 BOFS field study, P.Q. values were calculated from 24hr in vitro oxygen and TCO<sub>2</sub> production rate measurements. The modal value from the frequency distribution of the P.Q. values was for the range 1-1.5, with a mean value of 1.23 (Chapter 9.2.2., Figure 9.2.(8)). Only 5 of the 27 values obtained could not be accounted for by conventional P.Q. values, in the case of two of these, the standard error exceeded 50 % of the quotient. Regression analysis between the *in vitro* determined gross production rates measurements made during the 1990 study (Chapter 9.2.2., Figure 9.2.(9)), gave a correlation coefficient of 0.846 with and r squared value of 71.54 %.

The theoretical constraints on the P.Q. are set by the end products of photosynthesis (Rabinowitch, 1945) and the source of nitrogen utilised by the cell (Myers, 1949). The P.Q. can be separated into two components a carbon P.Q. (PQc) controlled by the state of reduction of the end products of photosynthesis and a nitrogen P.Q. (PQn) controlled by the state of reduction of the nitrogen source (Williams *et al.*, 1979). The PQn will, therefore, be related to the f-ratio (Chapter 9.2.3), the ratio of nitrate assimilated to total nitrogen assimilated (Chapter

2.7). The relative contribution of the carbon and nitrogen P.Q. to the overall P.Q. will be reflected in the carbon to nitrogen assimilation ratio of the cell.

Simultaneous measurements of the assimilation of nitrogen by the 15-N technique, and productivity rate measurements from the oxygen and  $TCO_2$  techniques, should enable much of the uncertainty between calculated and observed P.Q. values to be accounted for.

This was attempted with the culture studies of Thalassiosira Weissfloggi. However, the data was not consistent with expectations from theory (Williams et al., 1979; Laws, 1991; Williams and Robertson, 1991). Evidence from the calculated carbon to nitrogen assimilation ratios and the theoretically calculated P.Q.values (Chapter 6.4.) suggested that the culture was growing predominantly on ammonia, and so no shift in P.Q. values due to nitrate assimilation resulted.

Theoretical P.Q. values were calculated from the  $15-NO_3$ assimilation and TCO<sub>2</sub> production rates. The deviation of the experimental P.Q. values from the theoretical P.Q. values was 0.01 to 0.28, these values lying at the limit of the obtainable precision of the combined analytical techniques.

During the 1990 field programme, a relationship was sought between the calculated P.Q. from the oxygen and TCO<sub>2</sub> gross production rate measurements and the experimentally derived f-ratio from the 15-N production measurements. The P.Q. was plotted against the f-ratio (Chapter 9.2.2., Figure 9.2.(6)) and regression analysis gave a correlation coefficient of 0.789 with an r squared value of 62.24 percent. The regression line was such that the PQc (the P.Q. when f-ratio = 0) was equal to 0.75. The relationship between the f-ratio and the P.Q. gave a calculated carbon to nitrogen assimilation ratio of 3.13. Such a situation cannot, however, be assumed to hold if, as is the case (Chapter 9.2.5), we have a change in C:N assimilation ratios.

The major problem in comparing the P.Q. values from the 1989 and 1990 data to the f-ratios calculated from 15-N incubations, was in the assumptions made about the ammonia concentration. Theoretical P.Q. values can be calculated, however, from the carbon to nitrate assimilation ratios obtained from the TCO<sub>2</sub> and 15-NO<sub>3</sub> data (Chapter 9.2.3). P.Q. values were calculated in this way and compared with the experimentally obtained P.Q. values from the oxygen and TCO<sub>2</sub> production rates (Chapter 9.2.3, Table 9.2.(a)). In this way, the C:N assimilation ratio need not be assumed a constant value either.

The mean standard deviation between the experimental and the theoretical P.Q. values was 0.3. By plotting the theoretical P.Q. against the experimental P.Q. values (Figure 9.2.(10a), it was shown that 78 % of the experimental values agreed with the theoretical values within twice the value of their standard error, and 56 % agreed within their standard error.

Gross carbon production to total nitrogen assimilation ratios were calculated from the production data obtained during the 1990 field study (Table 8.1.(k)). Total C:N assimilation ratios were calculated from gross production from the TCO<sub>2</sub> technique and total production from the 15-N technique. The range in values was large and was most likely due to the problems encountered with measuring the ammonia assimilation rates. The mean value was  $6.59\pm1.22$ , however, and 66 % of the values were within the range of 2.5-12.5, with the quoted range of oceanic C:N ratios being 5.3-14 (Goldman, 1980).

The surface carbon to nitrogen assimilation ratios calculated from the  $TCO_2$  and 15-N production rates gave comparable values to the carbon to nitrogen ratios from the particulate organic fraction (Kennedy pers. comm) (Chapter 9.2.5., Figure 9.2.(19)) and the two sets of measurements showed the same trend over the period studied.

10.3. A comparison of the production rates obtained from the 14-C, oxygen and TCO<sub>2</sub> techniques.

The 1989 and 1990 BOFS field programmes provided an opportunity to compare the oxygen and  $TCO_2$  techniques with the 14-C technique. The problem of the incompatibility between the techniques is discussed in chapter 3.8. It is argued in chapter 9.2.4, that it is justifiable to use the two sets of observations for comparative purposes even though the study was not set up solely for the purpose of an intercomparison.

Direct comparisons of  $TCO_2$  and 14-C rate measurements are possible without the necessity to invoke quotient values as both are measuring a carbon flux. The 14-C technique, if measuring a gross production rate, should return a rate equal to  $PgTCO_2$ . At the other extreme, if it measured net primary production rates, the 14-C rate should be greater than  $PcTCO_2$ , the difference between the two rates being equal to the heterotrophic respiration rate (Chapter 9.2.4).

During the 1990 field programme, 13 experiments were conducted in which surface 14-C and  $TCO_2$  production rates were made (Table 8.1.(b) and Table 8.1.(c)). The situation

where  $PgTCO_2 > 14-C > PcTCO_2$  only occurred, however, in 6. This data can be analysed in a straight forward manner. In the other 7 experiments the 14-C production rate was less than the  $PcTCO_2$  rate.

The offered explanation for the 14-C production rate to be less than the net community production rate is from the effect from the excretion of organic carbon. This would effectively reduce the measured production rate from the 14-C technique, but would have no effect on the rate measured by the TCO<sub>2</sub> technique (Chapter 9.2.4.vi). Excretion rates of up to 49.8 % need, however, to be invoked in this situation.

An attempt to explain the observed discrepancies between the  $TCO_2$  and 14-C rates has been an arduous task. The explanation arrived at, was to divide the set of comparison into two different scenarios; one where the heterotrophic component is making a major contribution to the respiration rates and resulting in 14-C > PcTCO<sub>2</sub>, and the other where the heterotrophic respiration rates are negligible, and the excretion of organic carbon is substantial.

I can provide no evidence for the excretion rates of carbon being this high, and tentative calculations from the CD46 cruise, (Boyd pers. comm.) suggest excretion rates

were in the order of 10%. However, it was found that the two sets of comparisons could be separated on the basis of the respiration to gross production ratios as calculated from the oxygen technique. The first set all had associated high R:Pg ratios ranging from 46 to 160.7 % (Table 9.2.(b)). The second had low R:Pg ratios ranging from 16.6 to 54.5 % (Table 9.2.(c)). This implies that where R:Pg is high, 14-C < Pn and where R:Pg is low, Pg > 14-C > Pn. The latter case would be due to a mature population containing both autotrophs and heterotrophs. That a community with a high R:Pg ratio, contains a larger percentage of heterotrophs than a community with a low R:Pg ratio is supported by such workers as Iriarte et al., (1991), and Grande et al., (1990). The former case would be due, to a predominantly algal population with no associated heterotrophic population.

The 14-C production rates from the 1990 field study were also compared to the oxygen production rates. To compare the oxygen production rates with the 14-C production rates the oxygen rates must first be converted in to a carbon flux, by the application of a P.Q. and R.Q value. The quotient values used to convert the oxygen rates into a carbon flux were those calculated from the oxygen and  $TCO_2$  production rates (Chapter 9.2.4.vii).

By integrating the production rates through the water

column, it was found that apparent discrepancies between the techniques were reduced. A comparison of the depth integrated 14-C production rates with the depth integrated oxygen production rates showed that for all the days sampled the situation was such that  $PgO_2 > 14-C > PcO_2$ .

If the difference between the 14-C rate and the PcO<sub>2</sub> rate was taken as the heterotrophic respiration rate, then a method of dividing the respiration rate from the oxygen technique into its autotrophic and heterotrophic components is obtained (Chapter 9.2.4.vii). The percentage contribution of the calculated heterotrophic respiration rate to the total respiration rate was seen to increase as the ratio of total respiration to gross production increased (Table 9.2.(d)).

To support the subdivision of the respiration rates in this manner, a correlation was sought between the respiration and gross production rates. The correlation was shown to increase when comparing the calculated autotrophic respiration rate, to the gross production rate normalised to chlorophyll *a*, as opposed to the total respiration rate to the gross production rate normalised to chlorophyll *a*. The correlation coefficients increased from 25.7 % to 65.0 % (Figure 9.2.(16)).

It can be concluded that when comparing oxygen,  $TCO_2$  and 14-C rate measurements the respiration term is of vital importance. Heterotrophic respiration will effect the comparison in accordance with the magnitude of its percentage contribution to the total respiration rate. More importance must be attributed to the respiration term when making such comparisons.

The situation where the 14-C production rate was less than the net community production rate was observed. Richardson et al, (1984) found that when comparing the disappearance of inorganic carbon form infrared gas analysis, to 14-C incorporation rates, on algal cultures, that the 14-C technique returned a rate less than net primary production at low respiration rates. The relationship differed between different algal species.

They concluded that the relationship between 14-C incorporation and net primary production is not constant between species or even within the same species grown under different conditions. This would result in disparate field observations, as seen in this study, when comparing 14-C production rates with net primary production rates as measured from chemical techniques.

10.4. Critical depth calculations.

The oxygen production and respiration rate measurements made during the 1989 and 1990 BOFS field study have been used in an attempt to calculate the critical depths (Sverdrup, 1953), as an indication of the advancement of a phytoplankton bloom.

In the 1989 study, at 60°N 20°W the calculated depth of mixing rose quite substantially from 100m to 35m (Chapter 9.1.4). The critical depth was initially 43m below the depth of mixing, and though it too shallowed, it remained below the depth of mixing throughout the time spent on station. At no time therefore were the plankton mixed below their critical depth. This implies that over the 24hr period, the net photosynthetic rate should be positive, and that there is the potential for an export flux. On each day sampled the depth integrated community production rate was indeed positive.

During the 1990 field programme a more comprehensive study of the critical depths was undertaken (Chapter 9.2.9), with critical depths being calculated from the oxygen production rate measurements for every day sampled between 01/05/1990 and 16/06/1990. The relationship of the critical depth to the mixed layer depth was plotted

against time (Chapter 9.2.7, Figure 9.2.(22)).

The depth of the critical depth from the mixed layer depth showed an inverse relationship with the daily depth integrated net community production rate. On the days on which the critical depth was below the mixed layer depth the net community production rate was positive and on the days when the critical depth had risen above the compensation depth, the net community production rate was negative. The concentration of chlorophyll *a* over the time period studied also corresponded well to the change in the critical depth, with concentrations increasing during the period that the critical depth was below the mixed layer depth and decreasing when the critical depth had risen above the mixed layer depth.

We can conclude that the production rate measurements from the oxygen technique can not only be used to measure the net available export flux of carbon, but can be used to assess the critical depths from which the development of a phytoplankton bloom can be studied. That a relationship holds between the net community production rate and the critical depth is evident, with positive net community production, or net phytoplankton growth, only being able to occur when the critical depth is below the depth of the mixed layer.

## 10.5. Conclusions.

This study has led to the following conclusions:

1) Comparisons of *in vitro* and *in situ* rate measurements have shown that the *in vitro* techniques for the measurement of the fluxes of oxygen and carbon dioxide methodology as employed in this study, are not subject to serious error.

2) The two chemical techniques for oxygen and total  $CO_2$  for the measurement of primary production, can be used to assess the community P.Q. values, which for the most part, can be contained within the boundaries set from theoretical considerations, both in cultured and open ocean environments.

3) The experimental observations agree with the theoretical relationship found between the P.Q., f-ratio and C:N assimilation ratio, but one is operating at the limits of the combined precision of the analytical techniques employed.

4) Comparisons of rate measurements obtained from the 14-C technique, with those obtained from the oxygen or  $TCO_2$  techniques, will be affected by the heterotrophic

respiration rate in accordance with its percentage contribution to the total respiration rate.

5) The productivity rate measurements from the oxygen technique can be used to assess the critical depths. The temporal pattern of critical depth is consistent with the state of the phytoplankton bloom: the depth integrated net community production rate is only positive when the critical depth is below the depth of the mixed layer.

7) More than any other single factor, the analysis of open ocean water for its ammonia concentration limits our ability to resolve the exact relationship between nitrogen, carbon and oxygen metabolisms.

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