Stable isotopic insight into pelagic carbon cycling in Loch Lomond: a large, temperate latitude lake.

By

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Declaration

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This work is based on individual research carried out by myself, and any published or un-published material not of my own has been fully acknowledged in the text.

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Glossary and abbreviations:

Allochthonous: Material produced in the catchment area and imported to the aquatic system.

Autochthonous: Material produced within the aquatic system.

BP: Bacterial production, defined as the sum of bacterial biomass production (BBP)and bacterial respiration (BR).

BBP: Bacterial biomass production, defined as carbon processed by bacteria for synthesising biomass.

BR: Bacterial respiration, defined as the carbon utilised by bacteria for energy production and cell maintenance.

DIC: Dissolved inorganic carbon, defined as the sum of inorganic carbon species in a solution. Depending on pH the dissolved inorganic carbon content is a varying balance between dissolved carbon dioxide, bicarbonate and carbonate ions.

DO: Dissolved oxygen, defined as the sum of dissolved oxygen in solution.

DOC / M: Dissolved organic carbon / matter, defined as that which passes through a pore size between 0.45 and 0.7 μm.

Epilimnion: The top most layer of a thermally stratified lake, separated from the hypolimnion below by a thermocline. The epilimnion is usually well mixed and can freely exchange gases with the atmosphere.

Hypolimnion: The bottom most layer in a thermally stratified lake, separated from the above epilimnion by a thermocline and is generally isolated from wind mixing.

PP: Primary production, the catabolic process of producing organic compounds from inorganic substrates and energy, most commonly photosynthesis which uses CO_2 and sunlight and is mainly carried out by phytoplankton in aquatic systems.

Stratification: The separation of a water column into two main layers, the epilimnion and hypolimnion, separated by a thermocline. Stratification is usually caused by significant temperature differences between layers during the spring / summer and breaks down as temperatures become similar in the winter.

TDS: Total dissolved solids, the sum of organic and inorganic substrates that pass through a pore size of between 0.45 and 0.7μm.

TDN: Total dissolved nitrogen, the sum of organic and inorganic nitrogen that passes through a pore size between 0.45 and 0.7 μm.

Thermocline: An area between the epilimnion and hypolimnion of rapid temperature change with depth, otherwise known as the **metalimnion**.

Abstract

Lakes play an important role in biosphere carbon dynamics. Though proportionally they constitute a small surface feature on the planet, in many cases lakes are subject to significant subsidies of organic material from their catchments. This input of allochthonous organic material, in addition to autochthonous organic material, has shown that lakes, particularly in temperate and boreal zones, can be heterotrophic systems and as such are net producers of CO_2. Thus, understanding the magnitude of fluxes of carbon through these limnetic systems is important if their contribution to ecosystem / global carbon dynamics is to be elucidated. In this research two separate field campaigns were undertaken with the goal of understanding if, and exactly how significant secondary (bacterial) production utilising allochthonous carbon is to overall pelagic production in Loch Lomond, Scotland.

Stable isotopic composition of dissolved inorganic carbon (DIC), dissolved oxygen (DO), dissolved organic carbon (DOC) and total dissolved nitrogen (TDN), along with their respective concentrations, were measured in a temporal and spatial survey. Range in [DIC] and $\delta^{13}C_{DIC}$ was consistent with that predicted by the shifting balance between autotrophic and heterotrophic pathways. [DIC] peaked in the summer / autumn (0.27 ± 0.09 and 0.17 ± 0.05 mM, south and north basins respectively), reflecting a period when bacterial processing of allochthonous material is high, and thus so is CO_2 production. This effect was more pronounced in the mesotrophic south basin of the lake, compared to the oligotrophic north. Surface waters in the south, middle and north basins were generally saturated in CO_2 beyond atmospheric equilibrium and thus sources of CO_2 to the atmosphere.

$\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ exhibited seasonal and spatial variability, probably also a result of changing metabolic balance and inflow characteristics. Spring / summer peaks in $\delta^{13}C_{DIC}$ (-5.1‰ epilimnion maximum) are indicative of photosynthetic incorporation, and vice versa in the autumn / winter (-13‰ hypolimnion minimum) points towards respiratory dominance. $\delta^{18}O_{DO}$ is enriched during respiratory utilisation and peaks in the autumn / winter months. Depletion in $\delta^{13}C_{DIC}$ coupled to concurrent enrichment in $\delta^{18}O_{DO}$ observed with increasing depth (particularly during lake stratification) is assumed to again be a result of a shift in metabolic process dominance from autotrophic to heterotrophic (Myrbo and Shapley 2006). Spatial variability was consistent with the varying trophic states between basins, e.g., most enriched $\delta^{13}C_{DIC}$ was recorded in the more productive south basin compared to the middle or north.

Dissolved organic carbon concentration also changed with position in the lake. Highest concentrations in the south basin were linked to a shallow gradient catchment, draining base rich soils and agricultural land, compared to the steep sloped, base-poor

catchment in the north. The greater quantities of dissolved organic carbon in the south suggested that if bacterial processing of allochthonous material was significant it would likely be most prevalent in the south.

During the spatial survey consistent and significant heterogeneity in DIC, DO and DOC was recorded. Although the same degree of variability may not be associated with other, more mophometrically / hydrologically simple lakes, this work has shown consideration of this possibility is advisable.

The second field campaign used direct measurements of algal and bacterial productivity, using labelled stable isotope incorporation methods, to elucidate the balance between autotrophic and heterotrophic processes. Primary production (PP) followed a predictable seasonal pattern, peaking in the spring and remaining relatively high until autumn. During this period primary production generally exceeded bacterial production (BP) per litre. During the winter this pattern was reversed.

Using integrated estimates of both PP and BP this work showed that BP exceeded PP in the pelagic zone for the majority of the year, and over much of the lake's extent. Even in the epilimnion BP was regularly the more significant process through the water column, and thus it is concluded Loch Lomond is a heterotrophic system and a likely source of CO_2 to the atmosphere. The PP: BP ratio ranged from 0.6 – 0.8 in the north basin, and 0.4 to 0.6 in the south. On average for the whole lake, bacterial production exceeded primary production by between 2,700 and 4,400 kg C day^{-1}. In total it was estimated that PP processes approximately 970 tonnes of carbon per year and BP between 2,300 and 2,800 tonnes of carbon per year.

The proportion of total pelagic production fuelled by bacterial utilisation of allochthonous carbon changed throughout the year. During peaks of PP in the spring and summer much of the bacterial carbon demand was met by autochthonous supply. During the autumn / winter allochthonous carbon utilisation dominated pelagic production and regularly contributed over 90% of total pelagic production. Combining estimated quantities of allochthonous carbon utilised in the north and south basins per m^2 (the middle basin taken as an intermediate between the two) and combining it with GIS data on lake volume, the total quantity of terrestrially derived carbon processed in Loch Lomond was estimated at approximately 3,300 ± 2,100 kg C_{allo} day^{-1}.

Both spatial and temporal surveys of natural abundance stable isotope ratios, along with concurrent measurements of algal and bacterial production, have provided substantial evidence for the importance of allochthonous carbon in Loch Lomond. Even minimum estimates imply a system dominated by bacterial production, fuelled by a proportionally high quantity of terrestrial material, thus producing excess CO_2, and potentially fluxing CO_2 to the atmosphere.

Chapter 1

Introduction

In my Ph.D research I set out to examine the sources and sinks of carbon in a mid latitude lake, the overall target being to determine how much terrestrial carbon is added to the system, the balance between photosynthesis and respiration across time and space, and how much of the terrestrially derived carbon may be utilised and made available to higher trophic levels; through direct and concurrent measurements of the phytoplankton production (PP), bacterial biomass production (BBP) and bacterial respiration (BR), I will delineate whether this lake is, and to what extent net heterotrophic. In this context, the introduction will detail the following aspects relevant to this goal, and essential to understanding further discussions throughout this thesis:

1.1 Photosynthesis in aquatic ecosystems.

- The photosynthetic pathways.
- Factors controlling photosynthesis in aquatic systems.

1.2 Respiration in aquatic ecosystems.

1.3 The importance of heterotrophy in lakes.

1.4 The limnetic inorganic carbon cycle.

1.5 The limnetic oxygen cycle.

1.6 Dissolved organic matter: The microbial loop and the organic carbon cycle.

1.7 Stable isotopes and their applications in aquatic ecosystem research.

- Background and principles.
- Notation and terminology.
- Fractionations during aquatic metabolism.

1.8 Geographic Information Systems (GIS).

1.9 Loch Lomond, Scotland.

1.10 Thesis aims.

1.1) *Photosynthesis in aquatic ecosystems.*

1.1.1) *The photosynthetic pathways.*

Photosynthesis and respiration occur in all aquatic ecosystems. They are metabolic processes by which inorganic nutrients are transformed to organic compounds and back again for the production of energy. Simplistically, photosynthesis is the utilisation of inorganic nutrients, using light energy, to produce organic compounds (Falkowski and Raven 1997). This process utilises carbon dioxide (CO_2) and produces oxygen (O_2). Conversely, respiration is the breakdown of reduced organic compounds to release chemical bond energy (del Giorgio and Williams 2005), during which O_2 is utilised and CO_2 produced.

Figure 1 shows a diagrammatic representation of the photosynthetic process in algal cells. Unless otherwise stated, the following description of the photosynthetic pathways is taken largely from Falkowski and Raven (1997), although there are many other detailed descriptions and publications on the process available.

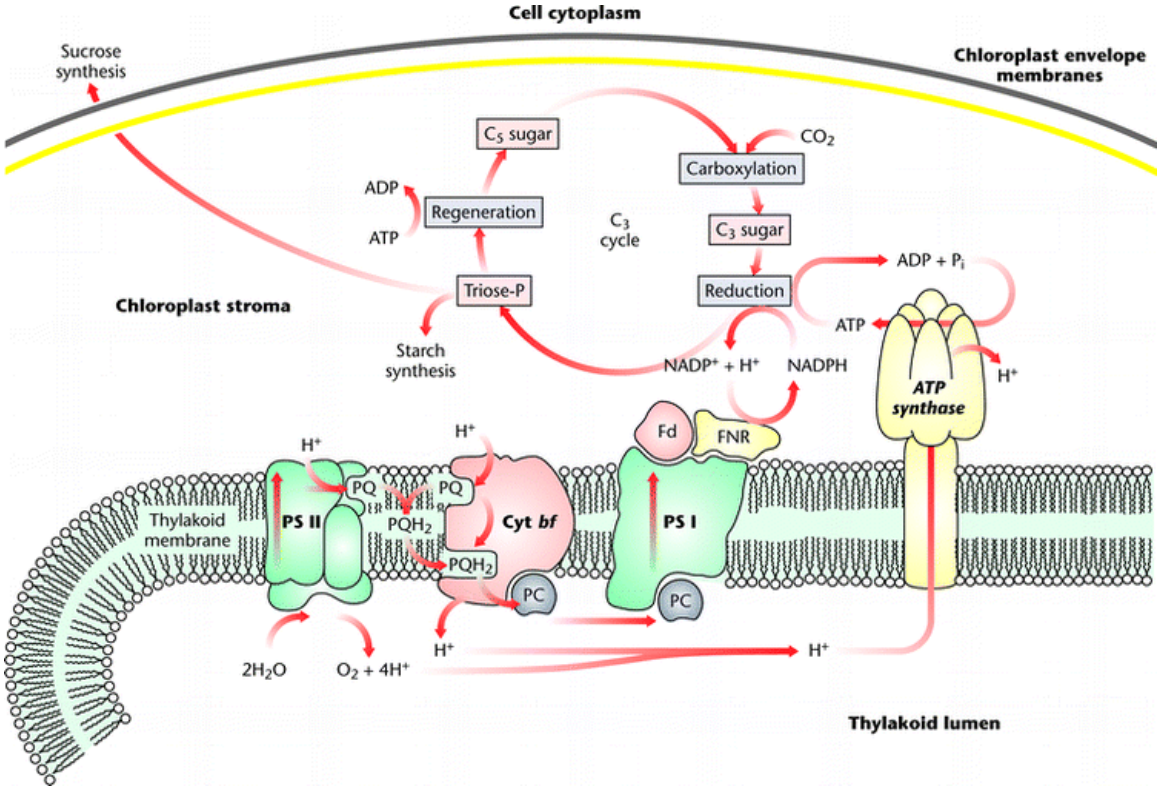

Figure 1: Schematic representation of the photosynthetic pathways. Taken from the University of Arkansas, botany web page (www.ualr.edu/botany/botimages.html)

Photosynthesis is comprised of light and dark reactions. In the light dependent phases of photosynthesis, light is utilised by photosynthetic pigments (most commonly chlorophyll a) to excite the electrons of a magnesium atom to a higher energy level. This produced energy is then transported via various electron acceptors and donors, producing adenosine-tri-phosphate (ATP) and nicotinamide-adenine-dinucleotide-phosphate (NADPH). It is during the light stages that water is split and oxygen is evolved. The reactions in the light dependent phase are collectively known as photophosphorylation. During anaerobic conditions, electrons created during initial excitation in photosystem II (labelled PSII in Fig. 1) are cycled back to the same system in cyclic photophosphorylation (e.g., Wintermans 1955, Avron and Neumann 1968).

In the dark stages of photosynthesis (the Calvin Cycle) atmospheric CO_2, or dissolved CO_2 species in aquatic systems, is used to form simple carbohydrates. The process uses the ATP and NADPH produced in the light dependent phases. The product carbohydrates are then exported from the stroma of the chloroplast.

The light reactions take place in, or are associated with, some kind of membrane, depending on the photosynthetic organism. In cyanobacteria these reaction centres are arranged in sheets or lamellae (Bryant 1994); in eukaryotes, thylakoid membranes containing embedded proteins and functional groups are the site of reaction, with the membrane itself contained in specific photosynthetic organelles, chloroplasts (Singer and Nicolson 1972). The dark reactions generally occur in the centre of cells or the stroma of the chloroplast.

1.1.2) *Factors controlling photosynthesis.*

Many different factors influence the rate of photosynthesis achieved by aquatic algae. A thorough review of factors influencing phytoplankton photosynthesis can be found in Fogg (1991). The rate of capture of light energy can influence photosynthetic rates. This depends on both the adsorbing power of the photo pigments and the intensity of the light reaching the alga (Kirk 1994). As the duration and intensity of light increases, so does the photosynthetic rate, until a maximum point is reached at which time all photosynthetic enzymes are functioning at maximum capacity. If light levels exceed this functional maximum it becomes inhibiting, known as photoinhibition. The effect of photoinhibition can be significant in surface layers of natural waters, particularly systems with good clarity (e.g., Marra 1978, Belay 1981). When no light is available respiration exceeds photosynthesis and an algal cell is net heterotrophic. During light conditions, when the rate of energy production via

photosynthesis exceeds energy consumption by respiration, photosynthetic organisms are net autotrophic. Although this relationship may seem simple, the point of photosynthetic saturation (maximum attainable photosynthetic rate of organism) and the effect of light intensity vary markedly between species, in response to inorganic carbon concentrations and temperature, and in turn this influences the rate of photosynthetic carbon fixation.

The effect of CO_2 depends largely on the species involved and the environment considered. Free CO_2 is the preferred carbon source for aquatic plants (Kirk 1994), but the ability to utilise bicarbonate and carbonate sources is important for some species. This can be especially important in relatively high pH systems where the majority of inorganic carbon is in the form of HCO_3^- and free CO_2 is thus limited. The ability to utilise other forms of inorganic carbon varies within different groups and species, with diatoms, dinoflagellates, chlorophytes and cyanobacteria having variable efficiencies of HCO_3^- utilisation, along with variation within each group (e.g., Allen and Spence 1981, Raven 1970, Maberly and Spence 1983).

CO_2 limitation for algae depends on the enzymatic uptake of CO_2 by the enzyme ribulose 1-5 bisphosphate carboxylase/ oxygenase (RUBISCO), and the theoretical explanation of its behaviour by Michaelis-Menten enzyme kinetics. In general it is hypothesised that inorganic carbon availability can limit photosynthetic rates in natural aquatic systems (Kirk 1994), and that total inorganic carbon concentrations can give indications of photosynthetic patterns, with lower concentrations indicative of high photosynthetic utilisation. Temperature has also been shown to affect the rate of photosynthesis (e.g. Platt and Jassby 1976, Malone 1977, Reynolds 1984, Robarts and Zohary 1987). Enzymatic processes usually proceed quicker at higher temperatures, and in some cases the rate of photosynthesis can increase exponentially with temperature to maximum values between 25 - 40^{o}C. Again however, the response is variable between species and environments.

The RUBISCO enzyme acts as an oxygenase as well as a carboxylase. This means that RUBISCO is oxygenated as well as reduced in photosynthesis. The ratio favours the use of CO_2 over oxygen (Siedow *et al* 2000) at a ratio of approximately 3 carboxylations for every oxygenation, although oxygenation does occur often in RUBISCO. This photorespiration pathway is energetically more costly than photosynthesis, producing no ATP and acts to decrease the overall net gain from photosynthesis. This can be more influential when the concentration of oxygen is relatively high in the water column.

Other factors can influence photosynthetic carbon fixation, for example, in all but the very calmest of conditions there will be circulation in a water column. Even in

stratified conditions the epilimnion will circulate. This can be beneficial to algae, keeping them away from inhibiting intensities of light at the surface, but can be detrimental if they are circulated below the depth at which net photosynthesis can occur. The depth of this mixed layer will be negatively related to the total community photosynthesis, and if on average the whole population spend more time below a certain depth net respiration will result causing mortality of much of the algal community. This critical depth was first defined by Braarud and Klem (1931).

The optical clarity of water also affects the photosynthetic potential of aquatic algae. The negative effects of reduced water clarity are seen in lakes of high coloured substance such as humic lakes (discussed in section 1.6). Dissolved organic matter (DOM) is known to have a detrimental effect on the amount of incident radiation that penetrates through a water column. Indeed, Cole and Cloern (1984, 1987) showed that phytoplankton abundance in estuaries, at least in part, could be explained by observed changes in optical quality, with a negative relationship between algal abundance and increased suspended material.

Temporal variability of photosynthesis in aquatic ecosystems can be significant. If all or some of the previously described constraining factors reach optimal conditions, photosynthetic organisms can become highly abundant. The limiting factors tend to increase and decrease over time.

Temporal variability can occur both on diel and seasonal timescales. Diurnal variation is the more straightforward, as no photosynthesis is carried out at night. The diurnal pattern of photosynthesis in a water column tends to follow the cycle of illumination, beginning at dawn and ending at dusk. This simple concept is complicated by photoinhibition, which can reduce photosynthetic rates in surface waters during the day, and by the observed active migration of some algal species (mainly dinoflagellates) to deeper waters to avoid the highest light intensities (e.g., Tilzer 1973).

In temperate areas there is also a pronounced seasonal variation in the quantity of aquatic photosynthesis (Fig. 2). The general pattern is that very little to no photosynthesis occurs in the winter months. Both light and temperature levels are generally low in winter months. Low temperatures coupled with a high level of water column mixing caused by rough winter weather means the stratification breaks down and phytoplankton are regularly mixed below the critical depth for net photosynthesis. The formation and breakdown of stratification is of significance in deep lakes, and less so in shallow systems where stratification is usually temporary and easily broken down. As spring approaches the weather stabilises, temperatures rise and thermal stratification occurs. After the phytoplankton blooms of the previous year and during

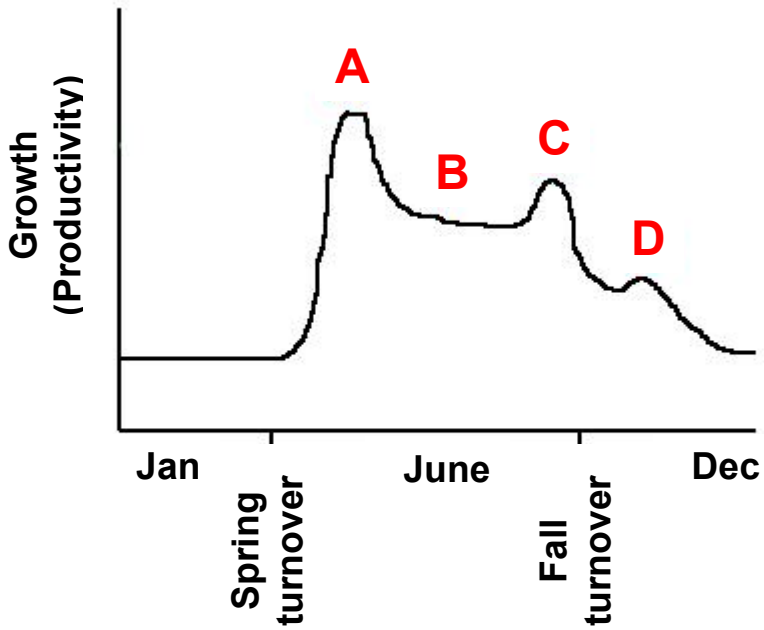

Figure 2: Generalised annual cycle of primary productivity in temperate aquatic systems showing A) Rapid growth during the spring bloom, B) stationary summer productive phase limited by nutrient availability and C) / D) secondary summer blooms caused by autumnal turnover of the thermocline and nutrient availability.

winter, bacterial breakdown of the remaining organic matter has been dominant, which combined with loading from the catchment, leads to high nutrient levels in the water column. In deeper lakes stratification prevents mixing below the upper levels, which along with increased duration and quantity of irradiation, and the high nutrient availability causes a bloom in phytoplankton numbers, photosynthetic rates and sedimentation. The bloom is often short lived (rarely over a month) as zooplankton species rapidly graze the blooming algae, and nutrient limitation often occurs. In some systems rougher autumnal weather can stir nutrients from below the thermocline and cause secondary blooms in the autumn. The secondary bloom ends when the thermocline breaks down completely.

This is the general pattern but is by no means universal. In lakes a relatively shallow thermocline can readily be disturbed by rough weather throughout the year and can lead to higher productivity levels than in deeper systems. This along with unpredictable influxes of nutrients from the watershed can increase or decrease rates of photosynthesis. Also, littoral zones are often not deep enough to be below critical

depth so photosynthesis can occur year round, instead being limited by temperature changes (Williams and Murdoch 1966).

Photosynthesis in Loch Lomond will be dependent on and influenced by many of the discussed factors. Day length and temperature vary on the usual temperate zone cycle, but other factors such as morphometry of different basins and variable nutrient loadings may influence algal productivity (see section 1.8 for more detail). For example, the shallowness of the south basin means even during unstratified conditions its possible much of the algal community can remain above the critical mixing depth. Temporal and spatial measurements of primary productivity and related parameters will help elucidate any possible patterns in variability.

1.2) *Respiration in aquatic ecosystems.*

In classical limnology the main focus of energy dynamics research has been on the productive pathways such as photosynthesis, with much less attention on respiration at the ecosystem level. It is only in recent times that the importance of catabolic processes in water column dynamics has been realised (e.g., del Giorgio and Williams 2005).

Respiration is oxidation of organic molecules such as glucose, amino acids and lipids, to give energy. Being an oxidation reaction, oxygen is required as an electron acceptor, although some organisms can respire using other terminal electron acceptors instead (anaerobic respiration), such as bacteria of the *Clostridium* genus, and methanogenic bacteria. Respiration occurs in all organisms (except obligate fermenters). Respiration at the cellular level has been extensively studied, what follows is a brief summary.

The process of respiration can be divided into three pathways. Glycolysis, the tri-carboxylic acid cycle (TCA / Krebs cycle) and the electron transport chain. Glycolysis is the breakdown of glucose to form pyruvate, releasing ATP and NADP in the process. After decarboxylation of pyruvate, acetyl-CoA enters the Krebs cycle, where it undergoes a series of enzymatic changes releasing energy, electrons and CO_2. The electrons enter the electron transport chain in cell mitochondria, which involves the passing of electrons via multiple electron acceptors to the final acceptor, oxygen. This releases energy in the form of ATP and water. A simple schematic representation the Krebs cycle is shown in Figure 3.

Nearly all organisms are responsible for respiration in aquatic ecosystems, although protists, photolithotrophs (phytoplankton, photosynthetic bacteria) and zooplankton carry out the bulk. Like photosynthesis there are various factors that

affect the rate of respiration in the various different respiring organisms in aquatic ecosystems. These range from the availability of oxygen and its uptake rate, (or the equivalent electron acceptor, e.g., NO_3^-, NO_2^-, Fe_3^+, SO_4^{2-}) (e.g., Fenchel and Finlay 1983, 1995, Fenchel 2005), temperature variation (Caron *et al* 1990), availability of organic material for breakdown and respiration in relation to body size.

Oxygen concentration and temperature limit the respiratory rates in aquatic organisms in much the same way that CO_2 and temperature affect photosynthetic rates. The process of oxygen uptake requires concentration gradients to diffuse across multiple membranes, be they cell membranes in bacteria or epithelial cells in fish gills. There reaches a point where oxygen tension is too low and diffusion will not be efficient enough to support respiration. The oxygen tension required by different organisms varies, with larger aquatic animals requiring greater than 10% of atmospheric saturation, whereas for aerobic bacteria this can be lower than 0.1% (Fenchel 2005). Temperature acts as expected by enzymatic kinetics in poikilotherms, which respond significantly to environmental temperatures. As temperatures rise the respiratory rate increases to an upper threshold, which indicates the limit of tolerance for the particular organism (Caron *et al* 1990).

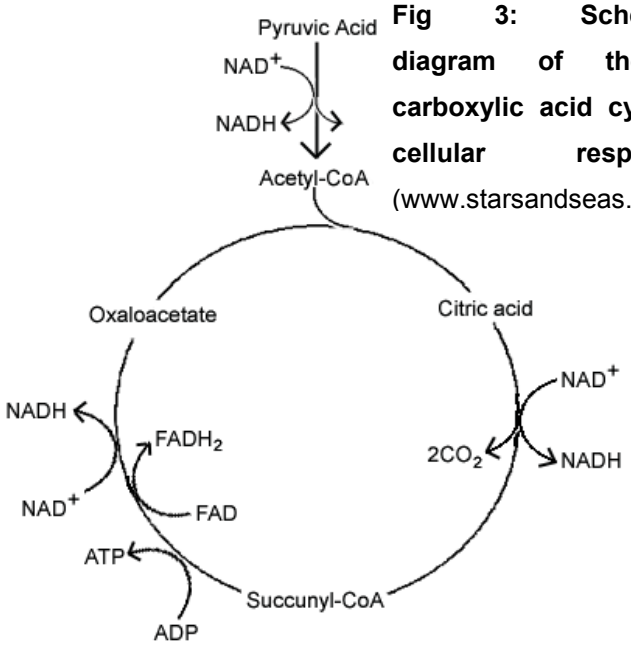

Fig 3: Schematic diagram of the tri carboxylic acid cycle in cellular respiration (www.starsandseas.com)

The effects of nutrient deprivation on aquatic organisms has been thoroughly explored (e.g., Hamburger and Zeuthen 1971, Humphry *et al* 1983, Fenchel and Finlay 1983). In times of low availability of organic substrates, oxygen consumption tends to decrease along with respiratory rates. This is believed to be in a mechanism to prolong life during stressful conditions. A reduction in bacterial cell size (Humphrey *et al* 1983) and a reduction in mitochondria numbers in protists due to internal breakdown (autophagy) has been observed under low organic substrate availability (Trinci and Thurnston 1976). The relationship between respiratory rates and organism size is influenced by numerous factors such as cell stress, temperature, oxygen availability, position in the cell cycle, and type of organism (e.g., Fenchel 1991, Hansen *et al* 1997). The general

pattern however is that, as body weight/volume increases, so does the respiratory rate of the organism.

The importance of respiration as a process separate from photosynthesis in the energy dynamics of aquatic systems came from the realisation that the two processes are not coupled as strongly as initially believed. The original concept was that respiration would directly follow photosynthesis in aquatic systems as it relied on the former for organic compounds. This to an extent is correct, but in reality there are deviations from this coupling that vary in both time and space. All aquatic ecosystems receive organic material and export it, no system is entirely closed. As such respiration can proceed in aquatic systems, even in the absence of photosynthetic organisms in the same space.

The overall balance of respiration to photosynthesis in lakes is of importance in terms of our understanding of how lakes process, store and release nutrients. In lakes respiratory microorganisms have two potential sources of nutrition, from organic material produced in the lake (autochthonous), and that transported from the watershed (allochthonous) (see section 1.6). The level of respiration can significantly affect the net balances of carbon in the lake. Many lakes are now known to be net heterotrophic systems. i.e., respiration is exceeding gross primary production and the lakes are net sources of CO_2 to the atmosphere and sinks of O_2. For example; Cole *et al* (1994) found 87% of 1835 lakes with worldwide distribution were supersaturated with CO_2, implying net heterotrophy; Cole *et al* (2004) found 4 experimental lakes in Wisconsin too to be naturally heterotrophic systems; Urabe *et al* (2005) found Lake Biwa in Japan to be often largely dependent on allochthonous carbon and net heterotrophic. The phenomenon is now widely accepted in the aquatic science community.

Pace and Prairie (2005) examined literature values to consider patterns influencing lake planktonic respiration. Three main driving factors behind planktonic respiration were elucidated: temperature, lake trophic condition and organic matter loading. Carignan *et al* (2000) described the relationship between temperature and planktonic respiration. This has rarely been done, as most studies tend to limit investigation to epilimnetic water, during small time periods. Carignan *et al* (2000) found a positive log-log relationship between temperatures of 11 - 22.5^{o}C. Whether this holds at lower temperatures has still to be adequately investigated.

Planktonic respiration was also found to be positively correlated with chlorophyll *a* concentration (del Giorgio and Peters 1993) and dissolved organic matter / carbon (DOM / C) concentrations (McManus *et al* 2003). The relationship recorded with chlorophyll (and phosphorus) suggests that planktonic respiration is strongly linked to

autotrophic production via photosynthesis, thus lakes of higher trophic status should support greater levels of respiration. However, numerous studies have shown that although total respiration rates may be lower in oligotrophic systems, the relative importance of respiration compared to primary production can be far higher (e.g., del Giorgio and Peters 1993, del Giorgio *et al* 1997, Biddanda *et al* 2001). Whilst changes in [DOC] may describe respiratory rates, as DOC can be of allochthonous or autochthonous origin, means that it is of limited help in identifying flow of carbon through the entire ecosystem. However, [DOC] has been shown to be an important driver in community metabolism in many recent studies (e.g., del Giorgio and Peters 1994, Cole *et al* 2000, Hanson *et al* 2003), such that the concept that lakes with high [DOC] are net heterotrophic, whilst those with low DOC are net autotrophic has gained increasing support (Hanson *et al* 2003). There is now evidence that autochthonous DOC can be the predominant source of carbon to heterotrophs in low DOC environments, and allochthonous carbon may be more significant in eutrophic systems. Hanson *et al* (2003) demonstrated this by showing in general low DOC systems have comparable values for gross primary production (GPP) and respiration (R) rates. This suggests the two processes are linked and carbon utilisation is balanced by its production, with no external subsidies. They also showed however that as DOC concentration increases, this close coupling begins to breakdown: respiration rates increase beyond that possible by autochthonous DOC utilisation alone, suggesting an allochthonous contribution to the organic material being respired. This supported work done by Prairie *et al* (2002) who put the threshold at which photosynthesis and respiration remain balanced at a DOC concentration of approximately 6 mg / L, below which a system will be net autotrophic and above which heterotrophic.

1.3) *The importance of heterotrophy in lakes.*

Only in recent times has the importance of bacterial respiration in unproductive lake systems ecosystem metabolism in been realised (e.g., Kling *et al* 1991, 1992, del Giorgio *et al* 1997). Early evidence such as the prevalence of carbon dioxide (CO_2) super saturation in temperate latitude lakes (Kling *et al* 1991, Cole *et al* 1994), and the proportional relationship between higher CO_2 emissions and increased terrestrial input of organic matter (e.g., Kling *et al* 1991, 1992, del Giorgio *et al* 1997, Sobek *et al* 2003) suggested that bacterial utilisation of terrestrial dissolved organic matter may be an important, if not a dominant process in many limnetic systems. This is of particular interest in boreal and temperate zones, which are potential carbon

sinks (Apps *et al* 1993) as they have undergone rapid growth in vegetation since the last ice age and large amounts of CO_2 are stored in the trees / soils / peat etc. However, most calculations of total carbon loss/gain in these areas fail to include the possible importance of lakes (Algesten *et al* 2003).

Early indicative studies that terrestrially derived subsidies of particulate organic material (POM) is an important limnetic energy source have subsequently been supported by accumulating evidence that terrestrial subsidies of organic carbon to lakes are fuelling bacterial respiration and a subsequent flux of carbon from the land, to lake, to atmosphere (Hanson *et al* 2004). The super-saturation of lake surface waters with CO_2 is now thought to be a common phenomenon (Hope *et al* 1996, Striegel *et al* 2001, Sobek *et al* 2003), and flux of CO_2 from lakes to the atmosphere has also been measured (e.g., Riera *et al* 1999).

However, quantification of the effect lakes have on total carbon flux in the biosphere is in its infancy and many unanswered questions still remain. Although it is known that allochthonous subsidies of carbon occur in limnetic systems, the proportionate contribution they make is less well defined and may be variable between systems. Importantly, the proportion of this terrestrially derived material that is utilised by bacteria once within the lake basin is of significance. Thus questions then arise about how much extra bacterial production / respiratory activity occurs in lakes as a result of these subsidies and therefore, how much of this subsidiary carbon is potentially available for higher trophic levels via microbial loop pathways?

In order for more accurate estimates to be made of net ecosystem CO_2 exchange (NEE), a greater understanding of the bacterial/algal production balance is required, along with a detailed understanding of quantities and fluxes of terrestrially and aquatically derived organic carbon. Despite the fact that estimates suggest bacterial respiration contributes 30-60% of bulk phytoplanktonic production (Jones *et al* 2001), there have been only a few studies using direct measurements of bacterial respiration in aquatic ecosystems (Hansell *et al* 1995, Jahnke *et al* 1995, del Giorgio 1997). However, an understanding of its importance is now being realised (del Giorgio and Williams 2005).

1.4) *The inorganic carbon cycle in lakes.*

Inorganic carbon is mainly present in aquatic systems as dissolved CO_2 or bicarbonate. The general equation for the disassociation of CO_2 in aquatic systems is:

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2H^+ + CO_3^{2-} \quad \text{Eq. 1}$$

This carbonate equilibrium is pH dependent (Fig. 4). At high pH the reaction tends to be shifted towards the right, and low pH to the left (Falkowski and Raven 1997). As a general rule, freshwater ecosystems at pH 5 and below will be dominated by dissolved CO_2, between 6 and 9 will be mainly HCO_3^-, and above 11 will mainly be CO_3^{2-}. CO_2 is approximately 200 times more soluble in water than oxygen. CO_2 saturation in water is approximately 1.1 mg/L at 0 ^oC, 0.6 mg/L at 15 ^oC and 0.4 mg/L at 30 ^oC (Wetzel 2001).

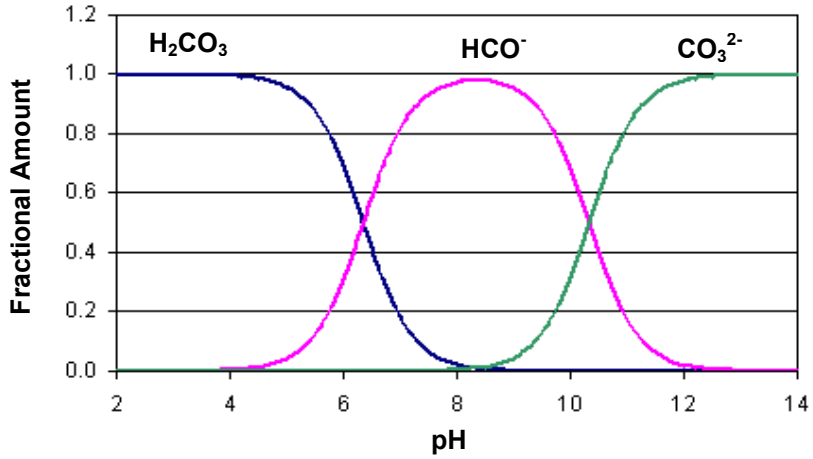

Fig 4: The Carbonate Equilibrium in aquatic systems. Taken from Wetzel (2001)

Together respiration and photosynthesis have a significant effect on the amount of inorganic carbon in lakes, and as such, variation in CO_2 exchange between water and the atmosphere cannot be explained by simple pressure differences alone. Photosynthesis relies on inorganic carbon to proceed and has been shown for some time to increase the flux of CO_2 from the atmosphere to surface waters (Weiler 1974, Emerson 1975). Inorganic carbon is taken up, and, via the processes described in section 1.1, transformed into more complex organic carbon compounds. These carbon compounds are then available to fuel metabolism within the rest of the ecosystem (Wetzel 2001). Supersaturation of surface waters in CO_2 has been widely described (Cole *et al* 1994). Photosynthesis and atmospheric draw-down can not explain this phenomenon alone, thus this is widely regarded as evidence many lakes are net heterotrophic. This general pattern is accepted for many lakes, although trophic variations and observed seasonal variations complicate interpretations (e.g., Kling *et al* 1991).

The importance of inorganic carbon both as a source of nutrition to photosynthesis and a by-product of respiration have lead to significant work describing patterns in concentration both temporally and spatially. Hanson *et al* (2006) examined multiple

studies to look at drivers affecting both dissolved inorganic carbon (DIC) and dissolved oxygen (DO). Multiple factors can affect the variability measured in DIC. As well as the biological influence previously mentioned, both physical and chemical factors play a role. Temperature and pH have been shown to influence the carbonate equilibrium (e.g., Stumm and Morgan 1981), and the loading of external carbon has been shown to affect the DIC balance in numerous systems (e.g., Graneli *et al* 1996; Klug and Cottingham 2001). Hanson *et al* (2006) examined variability over three different timescales. Over diel timescales metabolism is the main driving force behind DIC concentration changes. Metabolism and variation in the air-water gas exchange in spring/autumn overturn had similar effects on DIC variation on seasonal and annual scales. Over decadal scales and beyond metabolism has little driving power with variability in solute inputs being the main controlling factor. Other work has also shown the significant effect metabolic balance in an ecosystem has on the concentration of DIC, supporting many of the conclusions drawn by Hanson *et al* (2006) (e.g., Maberly 1996, Talling 1976, Heaney *et al* 1986). Given the importance metabolic processes (photosynthetic and respiratory) have been shown to have on lake DIC and DO, a temporal and spatial survey of dissolved inorganic carbon and oxygen in Loch Lomond was undertaken (Chapter 3), to infer if spatial variability in dominant metabolic pathways existed. From this representative sample sites were chosen for direct measurement of primary/secondary production and the interpretations of chapter 3 reconsidered (chapter 5).

The spatial distribution of DIC in lakes changes on a seasonal scale under the influence of physical changes in the water column and changes in photosynthetic/respiratory rates. Horizontal distributions are variable from system to system, but patterns in depth variability have been observed and described (Wetzel 2001). The biggest changes with depth occur when lakes are stratified. When lakes are circulating completely, during winter periods then the total inorganic carbon (TIC) is distributed evenly throughout the water column.

During stratification the variability seen in TIC depends on the trophic state of the water body. In oligotrophic waters there tends to be a slight increase in the TIC concentration below the thermocline, which is matched by a slight drop in pH (Wetzel 2001). However, recent work has shown that even in stable conditions, the production of TIC can be greater in the epilimnion in oligotrophic lakes (Aberg *et al* 2007). This vertical variability is dependent on photosynthesis: respiration. Increased primary production can use more inorganic carbon in surface waters and lead to the drop in concentration. However, in oligotrophic systems, because photosynthesis is usually low, this epilimnetic depletion is rarely seen in open waters (Wetzel 2001).

Lack of epilimnetic depletion in oligotrophic waters contrasts with eutrophic systems where primary production in the surface waters can be significant and the TIC concentration can be significantly depleted, and the pH will rise in response. Below the thermocline, TIC concentration, especially HCO_3^-, rises as CO_2 production by respiration approaches and exceeds CO_2 utilisation in the epilimnion (e.g., Heaney *et al* 1986).

Other processes create more complicated depth distributions. Often there will be an area of high [TIC] just above the sediments where respiration is high, and also nitrification and sulphide oxidation can raise TIC levels and decrease pH, while denitrification and sulphate reduction can do the opposite (Wetzel 2001).

As already stated, metabolism has a significant effect on the inorganic carbon cycle. If metabolic processes did not operate, TIC concentrations would easily be described by a combination of CO_2 partial pressures and solubility coefficients (Maberly 1996). Algae and submersed macrophytes require a source of inorganic carbon for photosynthetic utilisation. Multiple studies have shown that when photosynthesis dominates a system, a drop in TIC concentration and a rise in pH are observed (e.g., Schindler and Fee 1973, Talling 1976, Hesslein *et al* 1990, Maberly 1996). This is in contrast to when respiration dominates, either in the water column or the watershed, where TIC concentrations are seen to rise and pH to fall (e.g., Norton and Henriksen 1983, Sutcliffe and Carrick 1988, Cole *et al* 1994).

1.5) *The aquatic dissolved oxygen cycle.*

Oxygen is essential to all aerobic organisms in aquatic systems. It is used in aerobic respiration as the final electron acceptor, combining with hydrogen to give water (see section 1.2). Due to the importance of oxygen, to the majority of life in aquatic systems, study of its spatial and temporal variability dates back many years (e.g., Sale and Skinner 1917), and oxygen remains the most measured parameter in aquatic systems (Barth *et al* 2004). As such the dissolved oxygen cycle has been described in detail in many papers and text books. The information that follows is from Wetzel's review (2001) unless otherwise stated.

There are two main processes by which oxygen is added to aquatic systems; diffusion from the atmosphere, and active addition via the photosynthetic pathways. The solubility of oxygen, like CO_2 is temperature dependent and more O_2 can be absorbed in colder waters (see Benson and Krause 1980). Photosynthesis and respiration act on O_2 in the opposite way to CO_2: photosynthesis produces oxygen, while respiration utilises it. Thus, particularly in eutrophic systems with high levels of

bacterial respiration acting on high levels of organic material, dissolved oxygen in the epilimnion is often supersaturated, while the hypolimnion can be anaerobic.

The rate of diffusion from atmosphere to a lake is generally slow, and needs good water circulation in order to reach equilibrium. Therefore, during winter turnover, equilibrium can be reached and oxygen distribution is uniform. However, in deep lakes such circulation takes longer and equilibrium can be delayed or not occur at all.

There are two idealised distributions of dissolved oxygen in lakes of different trophic state (Fig. 5). In reality few lakes will follow the specific patterns set out here, but will be somewhere between the two. The orthograde distribution (Aberg and Rodhe 1942) describes oligotrophic systems, with variation mainly due to physical characteristics during summer stratification. As water temperatures in the epilimnion rise, the concentration of oxygen decreases, however oxygen remains at 100% saturation, set by the temperature of the water. This system is rarely found as most lakes have some degree of microbial processing of organic matter in the hypolimnion, particularly near the sediments, which can deplete oxygen levels below saturation.

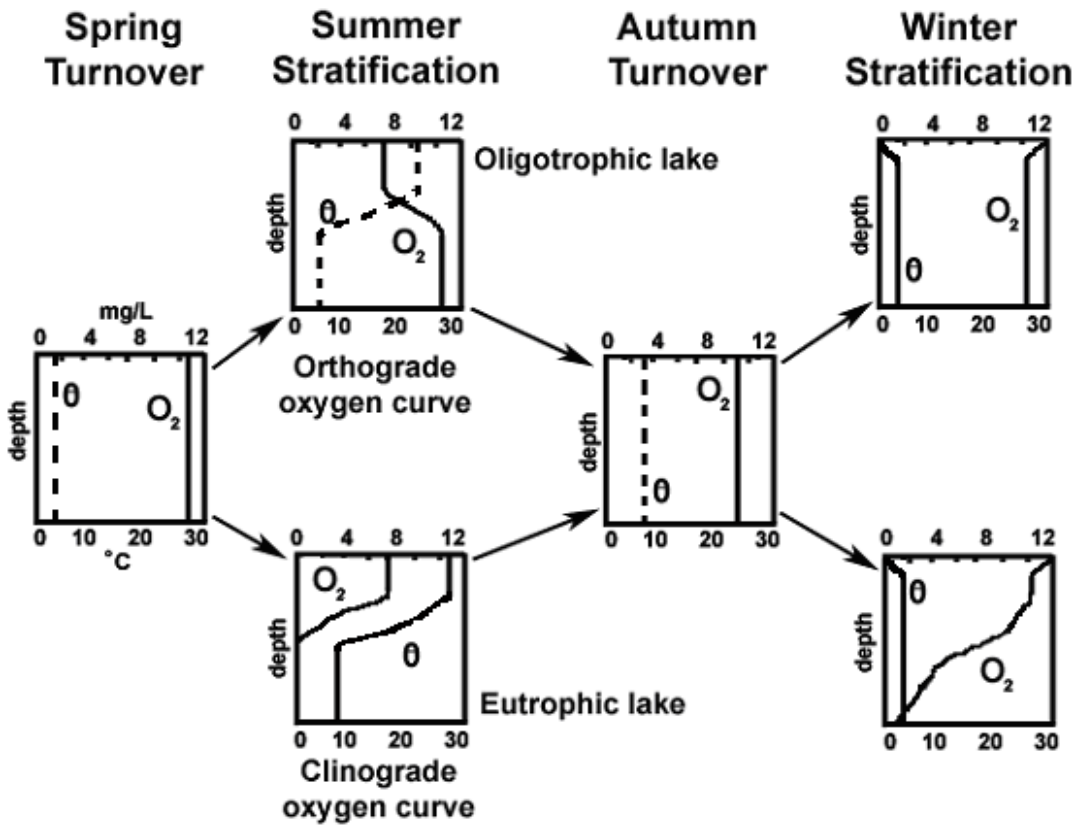

Figure 5: Orthograde and clinograde seasonal depth profiles of dissolved oxygen in and oligotrophic and eutrophic system respectively. Taken from Wetzel (2001).

Clinograde systems are more complicated and include variations brought by metabolic processes. When the lake is not stratified, dissolved oxygen profiles are constant through the water column and dictated by diffusion rates. As stratification occurs, photosynthesis in the epilimnion can cause super-saturation of oxygen, which will decrease with depth. However, this is complicated by surface floating macrophytes that in some cases can rapidly deplete epilimnetic oxygen levels through respiration at night (Caraco *et al* 2006). Oxygen concentrations will remain relatively constant through the well-mixed epilimnion, dropping steeply as the hypolimnion is reached and heterotrophic breakdown of organic matter dominates over primary production (e.g., Seto *et al* 1982).

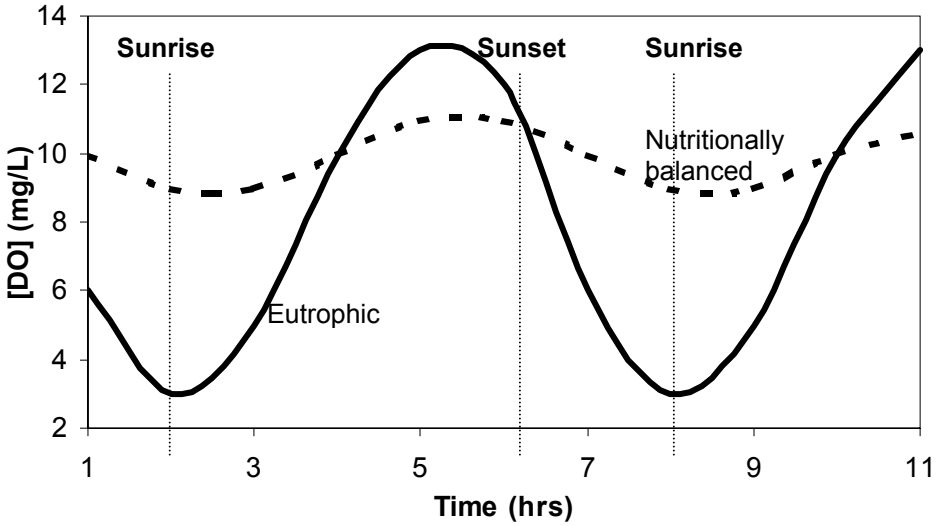

Fig 6: The diurnal cycle of dissolved oxygen concentration in a eutrophic and in a nutritionally balanced water body. (Based on data from Gower, 1980)

Interpretation of oxygen concentration is further complicated by both diel and horizontal variation in many lakes. Diel cycles occur (Fig. 6) as photosynthesis ceases in the dark and respiration by both algae and bacteria consumes oxygen. Diel variation will be influenced by the trophic level of the water body, with high nutrient systems undergoing larger relative changes than low nutrient (Fig. 6). At diel timescales metabolism is the driving force behind both TIC and O_2 concentrations (Odum 1956, Schindler and Fee 1973, Hanson *et al* 2006). Horizontal variation is seen in many lakes of variable morphometry (e.g., Welch and Eggleton 1932, Lind 1987). Often separate bays are subject to different environmental and biological conditions, and thus operate as separate functional units.

1.6) *Dissolved organic matter (DOM): The microbial loop and the organic matter cycle.*

Dissolved organic carbon (DOC) constitutes part of dissolved organic matter and is the largest pool of reduced carbon in aquatic ecosystems (Volk *et al* 1997) and aquatic ecosystems house the largest pool of organic carbon on the planet (Tulonen 2004). In all of these aquatic systems it is DOC, which makes up the majority of the TOC (total organic carbon) pool (Kortelainen 1999). It is generally accepted that dissolved carbon and dissolved matter is that which passes through filters with a pore size between 0.45μm and 0.7μm. The carbon / material that is retained by the filter is defined as the particulate fraction. Wetzel (1984) quantified the relative amounts of carbon in aquatic systems, and found that the refractory (not readily utilised by microbes) organic carbon was the most significant element (75% average), with 50-90% of this pool being humic substances. This, along with excreted organic carbon and labile organic carbon made up the DOC pool. The distinction between labile and refractory DOM/DOC has great biological significance. Labile DOC (usually dissolved free amino acids, carbohydrates, fatty acids, vitamins, nucleotides and steroids (Munster *et al* 1999)) is that which is readily available for microbial utilisation, refractory DOC/M is less readily available to bacteria, consisting of molecules difficult to break down enzymatically.

DOM and POM can be of two different sources. Autochthonous is produced within the aquatic system. Allochthonous sources of DOM / POM are imported to the aquatic system from a terrestrial origin. Allochthonous sources are generally considered a larger source of carbon than autochthonous sources in oligotrophic lacustrine aquatic systems: Although allochthonous material has generally undergone many degradation steps via bacterial utilisation of the labile components before it reaches the lake / river system, and is considered to be mostly refractory at this point, it may still be of significance to respiring organisms when autochthonous supply is limited.

Autochthonous sources of DOM are readily used by heterotrophic organisms and have been shown to directly affect the activity and composition of pelagic microbial communities (Pomeroy 1974; Azam and Cho 1987). Heterotrophic microorganisms gain a large amount of substrate from photosynthetic production and release of DOM. Photosynthetically-derived DOM is from algae, macrophytes and to a lesser extent, photosynthetic bacteria. It is the algae that have received most attention to date (Munster 1993), although macrophytes can be the dominant photosynthetic organism in shallow systems or lakes with a high proportion of littoral zone. The dominant algae

in lentic (still water) systems are the phytoplankton (Bertilsson and Jones 2003), with periphyton tending to dominate lotic (flowing-water) systems. Although the overall contribution of autochthonous DOM is relatively small in most systems compared to allochthonous, it is because of its generally labile nature it assumes such significance.

Algae contribute to the DOM pool via multiple pathways. Many algae will exude a high proportion of their photosynthate during times of nutrient stress, which can be particularly important at the end of a bloom event (Lancelot 1983, Baines and Pace 1991). The algal cells themselves add a source of both POM and DOM upon death. Also, breakdown of algal cells by grazers will release DOM either directly as cells are broken during feeding (Jumars *et al* 1989), excreted from the grazer, or returned from the sediments.

The transfer of nutrients from the phytoplankton to heterotrophic microorganisms can be a very important process. Average estimates vary from 32% to 46% of gross primary production being directly processed through microbial degradation in aquatic environments (Duarte and Cebrian 1996; Bertilsson and Jones 2003), although the numbers that gave these averages showed high variability. This variability is likely enhanced by other factors affecting DOM quality, such as UV radiation which can damage and reduce functionality of photosynthetic apparatus in near surface waters (e.g., Goes *et al* 1996).

Since the early 1980's as the microbial loop was being alluded to, DOM from phytoplankton was known to be a good substrate for bacterial utilisation (Cole *et al* 1982). This is clear from the very rapid turnover of DOM released from phytoplankton (Petit *et al* 1999) and the elevated bacterial numbers associated with bloom events. This elevation in bacterial production is even more significance in areas of little terrestrial or littoral nutrient input. Current estimates of DOM exudation by phytoplankton are likely to be underestimates of true values, due to use of the DOM by bacteria immediately upon production. However, it is clear that bacterial utilisation of phytoplankton DOM exudation is significant. Bertilsson and Jones (2003) reviewed a number of studies and found on average 46% of phytoplankton exudation is incorporated into bacterial biomass in marine systems. The input of DOM into bacterial biomass could have significant effects on the microbial food web and thus to the metazoan food web also (Weiss and Simon 1999).

Macrophytes also provide an important source of autochthonous DOM to bacteria and thus the microbial loop. The supply from macrophytes is of particular importance in coastal marine environments, and in lake systems where the littoral zone can be extensive. Macrophyte DOC production is likely to be little in Loch Lomond as large

portions of the lake have very steep drop offs with little littoral zone. Further, when studying lentic water bodies, the catchment must be taken into consideration. Many will drain wetlands and various other environments, which support macrophytes production, allowing high production and exportation of allochthonous DOM from the drainage basin. Research on macrophyte production of DOM is less extensive than for the algae, but from what is known it appears that exudation is both less common and more variable than in phytoplankton. Wetzel and Manny (1972) found between 4 - 10% of net primary production by macrophytes was released via exudation. As with phytoplankton, bacteria rapidly use the DOM released from macrophyte exudation. 12% of DOM created by *Spartina alterniflora* was recorded to be up taken by bacteria in the first 16 hours after production, this increased to 30% after 30 days (Moran and Hobson 1989). Thus it would seem that autochthonous DOM produced by sea grass exudation is labile in nature, and hence autochthonous sources of DOM were potentially important in this aquatic system. The relative importance of algae or macrophyte production to autochthonous DOM supply will vary between systems.

Allochthonous DOM and POM is that which is imported into aquatic ecosystems and has a terrestrial production origin. Allochthonous DOM is produced as precipitation moves through the vegetation, infiltrates the soil organic horizon and then percolates down through soil mineral horizons. Thus DOM is both gained and lost during this transportation. Significant proportions of the DOM will be lost along the way, either through carbon utilisation in biological pathways (Yano *et al* 2000), or by DOC adsorption to soil particles. The further along the pathway from precipitation to stream/lake/ocean water the lower the percentage of high molecular weight (HMW) DOC and thus the low molecular weight DOC increases, suggesting microbial breakdown of HMW DOC during its passage to the final water body (Cole *et al* 1984).

Allochthonous DOM can undergo numerous changes of concentration and fluxes through precipitation, throughfall and soil organic and mineral horizons (Aitkenhead-Peterson *et al* 2003). The most direct path of DOM to the final water body will be precipitation directly into that water body. Neff *et al* (2002) and Willey *et al* (2000) reviewed work conducted on concentration fluxes of DOC and DOM. DOC and DON in precipitation water are most likely to have been derived from pollen and organic dust particles in the atmosphere. But mixed phase atmospheric reactions can convert precursors such as peroxyacetyl nitrate to DON and DOC. Urea can also be an important source of nitrogen to precipitation DON (Cornell *et al* 1995).

Precipitation water will pick up DOM as it travels through vegetation, (Aitkenhead-Peterson *et al* 2003), due to dissolution of dry organic material on leaf and other

surfaces. Throughfall DOM is defined as precipitation which passes over vegetative material and falls directly into the water body. This throughfall water will generally include pollen, dust and insect exudates and will typically have a far higher concentration than that of precipitation. Throughfall itself contributes little DOM to surface waters, with most water passing over vegetation will drop to the ground and enter the soil horizons.

Water which lands on the forest floor from vegetation run off enters the soil horizons, of which the organic horizon is on the surface. DOM from the forest floor and organic soils make a high contribution to surface water concentrations. The concentration of DOC on forest floors and organic soils can vary greatly, depending on depth of the forest floor and the organic soil horizons. Measured concentrations have been shown to vary between 7.2 ± 4.0 mg/L in cool grasslands to 36.9 ± 23.3 mg/L in cool coniferous forests (Aitkenhead-Peterson *et al* 2003).

Mineral soil contribution of DOM will depend on the slope of the watershed, antecedent soil moisture, depth of the water table and barriers to organic soil solutions infiltration of the mineral soil (Aitkenhead-Peterson *et al* 2003). If the water from the organic soil enters the mineral horizon it will undergo a drop in DOM before entering the aquatic system. Nearly all mineral soils have the ability to adsorb a significant amount of DOC from the organic soil solution as it passes through. Hydrophobic fractions particularly are easily adsorbed to mineral soils and labile fractions will be readily taken up by soil microbes en route (Yano *et al* 1998), resulting in either a net sink for DOC or storage until later release by bacterial breakdown.

Each of the above processes and their interactions result in a wide variation in the amount of DOM delivered to surface waters. The balance between these processes will be reflected in the quantity and quality of DOM in stream water. Variations in flow path and thus the contributions of various altering processes can lead to a large (five fold) change in DOC concentration, over a very short timescale of hours to days (Boyer *et al* 1996).

Loch Lomond was studied to examine possible differences in DOM/C loading across time and space, as well as to try and elucidate whether the majority is autochthonous or allochthonous in origin. This information was used to see if DOM quality and quantity influences levels of primary and secondary productivity (see chapters 3 and 7).

The microbial loop (Fig. 7) is the remineralisation of nutrients by bacterial utilisation that were otherwise thought lost in the classical food chain (Azam *et al* 1983). Bacteria utilise the DOM and other organic material released by

phytoplankton / zooplankton / vertebrates etc in heterotrophic breakdown. Heterotrophic nanoflagellates are capable of grazing on bacteria directly (Fenchel 1982). Ciliates then graze the flagellates and are subsequently grazed by mesozooplankton, re-entering the classical food chain.

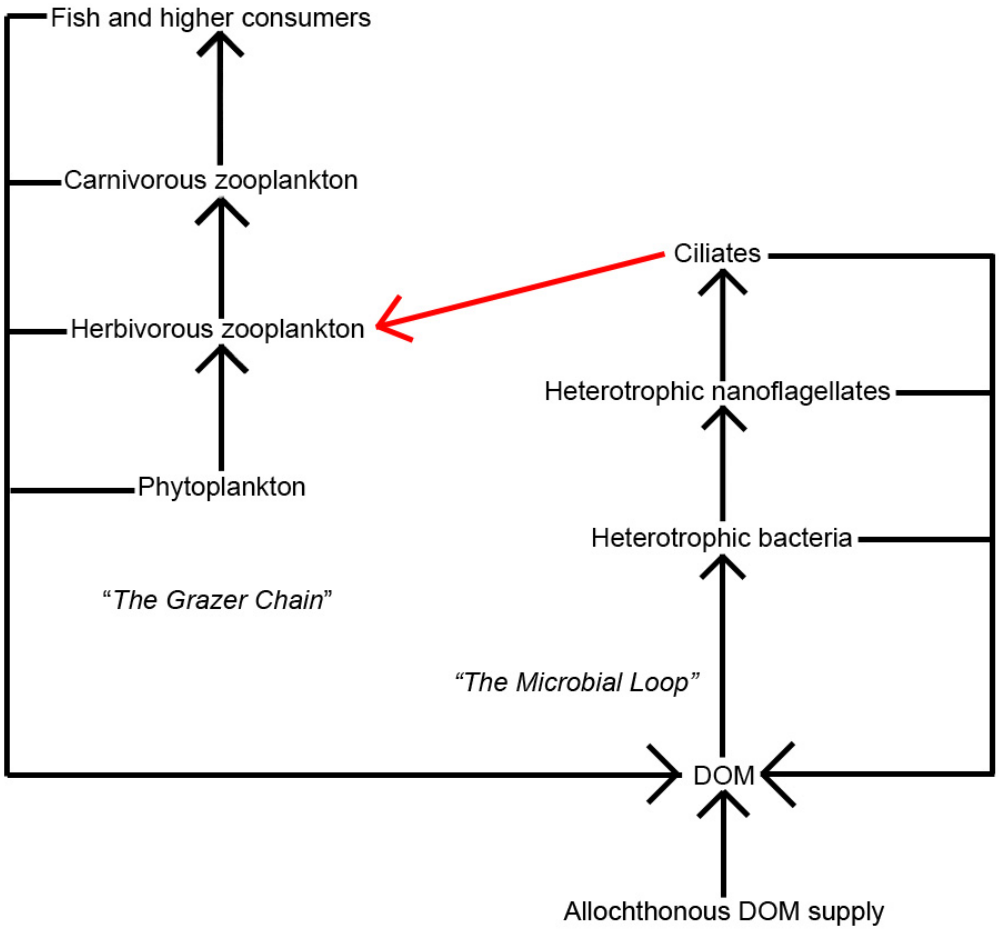

Fig 7: The microbial loop is the utilisation of DOM by bacteria and the grazing of these bacteria by nano flagellates, and their consumption by ciliates. All steps produce DOM that re-enters the pool for bacterial utilisation completing the loop. The red arrow represents grazing of ciliates by zooplankton and the incorporation of energy from the microbial loop into the classical grazer chain. This microbial link is now thought to be proportionally significant in systems with little phytoplankton production and high allochthonous loading.

This is an oversimplification of the microbial loop. In reality it is a complex web, with various facets carrying out different and multiple roles. The importance of the microbial loop in limnetic systems has the potential to be high. Many lakes have large allochthonous inputs of both POM and DOM as previously discussed which are available for bacterial utilisation. The energy they produce is then available for higher

trophic levels in absence of an algae dominated food chain. The relationship between bacterial biomass and DOM concentration has been seen for some time (e.g., Ford 1993, Volk 1997). Whether this relationship exists and to what extent in Loch Lomond was one of the purposes of this research (see chapter 7).

1.7) Stable isotopes and their applications in aquatic ecosystem research.

1.7.1) Background

Stable isotope research can offer insight into numerous biological problems. In this research both natural abundance and experimental tracer additions have been used to elucidate patterns in the biogeochemistry of Loch Lomond. Due to the importance their use has taken in this research a background on the principles and applications is discussed.

The phenomenon of isotopic variation has been known for some time (Soddy 1914). It was theorised that the place occupied by a certain element in the periodic table could accommodate more than one kind of atom. These different types of atoms were termed isotopes. Isotopes of an element vary in having a different number of neutrons to the most common form. For example, Carbon has 12 protons and 12 neutrons in its most abundant form; however, it can also exist with 13 neutrons, known as ^{13}C, or "heavy carbon". Two types of isotope exist;

1. Radioactive forms which are subject to decay at statistically predictable rates to give daughter atoms. Daughter atoms will often be stable.
2. Stable forms, which do not decay. It is possible that they show some level of decay but over a timescale undetectable through current methodologies.

There are more than 2500 elemental isotopes known, most of which are radioactive, although most elements are known to have at least two stable forms. The lighter of the two (at least) isotopes will tend to be the most abundant. Generally the more abundant stable isotope makes up approximately 99% of the isotopic composition for the element (Table. 1).

Element	Isotope	Atomic Weight (u)	Abundance (atom %)
Carbon (Z=6)		**12.011**	
	^{12}C	12.00000	98.90
	^{13}C	13.00335	1.10
Nitrogen (Z=7)		**14.0067**	
	^{14}N	14.003074	99.63
	^{15}N	15.000109	0.37
Oxygen (Z=8)		**15.9994**	
	^{16}O	15.994915	99.76
	^{17}O	16.999131	0.04
	^{18}O	17.999160	0.20

Table 1: Atomic weights and abundances of stable C, N and O isotopes used in this research (Walker *et al* 1989). Z is the number of protons in the most common state and U represents the unified atomic mass, defined as one twelfth the mass of an unbound atom of ^{12}C in its ground state.

The values in table 1 are averages, as actual abundance varies from sample to sample. This variation implies that the isotopic ratios vary from sample to sample also, known as isotopic fractionation. There are equilibrium and non-equilibrium effects that cause this observed fractionation (Following taken from Criss 1999).

Equilibrium Effects tend to affect atoms which form covalent bonds (O'Neil 1986). These bonds undergo strong vibrational and rotational motions strongly linked to mass, and thus to isotopic form. Bonds which rely on electrostatic forces (metallic, ionic) show little of this variation.

Non Equilibrium Effects is fractionation accompanying dynamic processes. Several processes can cause this.

1. **Diffusion.** The diffusional variation is based on the principal that heavier isotopes have slower velocities than light ones. For example, if a gas is diffusing through an opening then the lighter isotope will diffuse through faster than heavier ones.
2. **Evaporation.** During evaporation lighter isotopes tend to form vapour faster than heavy ones. For example, water left to evaporate will become enriched in the heavier water isotopes as lighter isotopes evaporate first.
3. **Kinetic isotope effects**. In reactions which have a clear rate determining step, if that step is isotopically dependent then a kinetic isotope effect will be seen. For example, if a reaction depends on breaking a bond and a lighter isotope is easier to break the reaction will go slower for the heavier isotopes.

4. **Metabolic effects**. Biological systems often show isotopic preferences. Respiration in humans for example favours ^{16}O, while ^{17}O and ^{18}O become more abundant in the lungs as they're not taken up at the same rate, a consequence of enzymatic processes affected by the factors mentioned above.

1.7.2) *Notation and terminology*

During mass spectrometry the relative difference between a sample and a known standard is measured, rather than that of the absolute isotope ratio of the sample, which is generally very small. These relative differences can be measured more accurately than the absolute ratio (O'Neil 1986). The relative difference between sample and standard is expressed in the δ (delta) notation and is expressed by the equation:

$$\delta_x = 1000 \times \left(\frac{(R_x - R_{std})}{R_{std}} \right) \qquad \text{Eq. 2}$$

R is the atomic ratio expressed as the ratio of the heavy to the light isotope, R_x is the atomic ratio of the sample and R_{std} is the atomic ratio of the standard.

The reference standard used for carbon is a calcium carbonate known as Pee Dee Belemnite (PDB) (Craig 1957). The standard was a limestone fossil of *Belemnitella americana*. The original PDB source is no longer available but its R-value has been set at 0.0112372 (Craig 1957).

Oxygen was originally reported in relation to the SMOW (Standard Mean Oceanic Water) standard (Craig 1961). Subsequently a large reservoir of water produced in Vienna has become the recognised standard. V-SMOW is now the internationally recognised standard for both oxygen and hydrogen.

The reference standard used for nitrogen is AIR, referring to atmospheric air.

1.7.3) *Fractionations during aquatic metabolism*

The field of stable isotope applications in aquatic research is so broad and extensive that a thorough review of all work would be impossible in this thesis. Thus I

will focus on the isotopes of carbon, oxygen and nitrogen and their relation to aquatic metabolic processes.

During utilisation and flux through the biosphere, fractionation of stable isotopes occurs, i.e., there is a selective partitioning of one isotope in either catabolic or anabolic processes. Fractionation occurs due to thermodynamic properties of the element which depends on the mass of the atom. Equilibrium isotope effects are common where chemical exchange occurs between two molecules (Peterson and Fry 1987) and involve heavier isotopes concentrating in the molecule where bond strengths are greatest. For example, dissolved CO_2 in exchange with ocean bicarbonate will result in ^{13}C-enriched bicarbonate as it has the greater bond strength (Mook *et al* 1974). Non-equilibrium effects are common in biological processes where most reactions are more complex than a simple equilibrium, and invoke a kinetic isotope effect. In enzymatic reactions for example, the bonds created by the lighter isotope will be weaker and easier to break. Therefore the lighter isotope reaction rate will proceed faster than the heavier and the product can be depleted in the heavier isotope.

Stable isotopes of carbon ($^{12}C/^{13}C$, ~98.9 : 1.1% abundance) are fractionated during photosynthetic pathways (e.g., Park and Epstein 1960, Troughton *et al* 1974). It was found that terrestrial C3 vegetation had an isotope signature of –27.8‰, compared to source CO_2, which was –7.7‰. Similar depletions can be seen in aquatic systems, although variability in source CO_2 makes interpretation more complicated. Many studies have considered the carbon signature of dissolved inorganic carbon (DIC) under the assumption that as photosynthesis favours the lighter carbon isotope, the remaining DIC pool will become ^{13}C-enriched (e.g., Quay *et al* 1986, Herczeg 1987, Hollander and McKenzie 1991, Wang and Veizer 2000). The baseline for lake DIC is set by its surrounding geochemical characteristics, but variation around this baseline can give indications of photosynthetic processes (Bade *et al* 2004). Respiration acts in the opposite direction, producing more depleted CO_2 during catabolism. Where respiration inputs of DIC are high (e.g., eutrophic systems at night) $\delta^{13}C_{DIC}$ can approach –20‰ (Rau 1978).

Oxygen ($^{16}O/^{18}O$, ~99.7 : 0.2% abundance) in the atmosphere (23.8‰) diffuses into aquatic systems, where there is a small enrichment such that O_2 dissolved in water is approximately 24.2‰. Although not used to the same extent as $\delta^{13}C_{DIC}$ values, $\delta^{18}O_{DO}$ responds in a similar way to metabolic processing. Deviation from the average of 24.2‰ in a positive direction is indicative of lower photosynthetic rates and higher respiratory ones (Wang and Veizer 2000). The preferential use of both

isotopes simultaneously can give more insight into metabolic processes in aquatic systems.

The most common use of stable nitrogen isotopes ($^{14}N/^{15}N$, ~99.6 : 0.4% abundance) in biological research has been in assigning trophic levels, on average the consumer is 3.4‰ more enriched in ^{15}N than its diet (e.g., Minagawa *et al* 1984). Compared to carbon cycling in aquatic systems nitrogen cycling is less well understood (Goericke *et al* 1994). Phytoplankton have been shown to preferentially incorporate ^{14}N during nitrate assimilation, thus leading to ^{15}N enrichment of the nitrate pool (Fogel and Cifuentes 1993). Nitrogen fixation of atmospheric nitrogen to more reduced compounds, carried out by many cyanobacteria can also have significant effects on aquatic systems, mainly in tropical regions. The $\delta^{15}N$ of atmospheric nitrogen is zero, and as such nitrogen fixation produces inorganic nitrogen compounds, which tend towards this value (Cline and Kaplan 1975). Therefore, in general, primary productivity and associated uptake of inorganic nitrogen leads to an enrichment of surrounding dissolved nitrogen, whereas nitrogen fixation leads to an inorganic pool approaching an atmospheric value of zero.

1.8) *Geographic Information Systems (GIS).*

Analytical approaches on spatial variability in this work will often refer to the use of Geographic Information Systems (GIS), so as such a short definition of the principles is desired. GIS systems are a set of computer hardware, software and geographical data that is used for the purpose of managing, analysing and displaying geographical data sets (www.GIS.com).

GIS has countless practical applications that are not explored at this juncture. However, in my work spatial distribution of data points of various measured parameters have been interpolated using GIS techniques to interpret whole lake distributions and estimate values between measured data points. Further details on this method can be found in chapter 2, where the model used is explained more fully.

1.9) *Loch Lomond: Morphological, hydrological and biological characteristics.*

Loch Lomond is one of the most comprehensively studied lakes in Scotland. Most recently the Loch was one of four lakes included in the EU funded ‘Eurolakes’ project. Much of the following information is derived from the public Eurolakes reports (www.eurolakes.com).

Loch Lomond is located in the Trossachs national park, at approximately 56˚80'N, 4˚40'W. The Loch has the largest surface area of any lake in the mainland United Kingdom and is the third deepest, with a maximum depth of ~200 m. The loch drains a catchment area of 696 km^2 with an average population of 19 people / km^2.

Loch Lomond consists of three basins caused by the varying bathymetry and local geology (Fig. 8). The southern basin (~ 28 km^2) is largely separated from the other basins by an archipelago of islands, caused by the Highland Boundary Fault, a geological fault line. The basins show clear physical and physiochemical differences. The southern basin is broad and shallow reaching 8.8 km in width and between 5 and 30 m deep. This contrasts with the northern basin (~ 16.5 km^2), which is long, and fjord like, reaching 1.5 km in width and up to 200 m depth. The northern basin drains a mountainous, base poor, rocky catchment with little anthropogenic input, whereas the southern basin has a catchment mainly of lowland, base rich, agricultural land. A middle basin (~ 27 km^2) can also be discerned that is an intermediary in physical and physiochemical characteristics.

The total catchment of the lake is ~767 km^2, which includes the natural catchment of 696 km^2, and a lake surface area of 71 km^2. There are two main and numerous smaller inflows into the northern basin. The River Falloch and the Inveruglas comprise the two main sub-catchments above the Highland Boundary Fault. They drain the high altitude (mean = 300 m) catchments, which includes mountains over 900 m in height, such as Ben Lomond. The geology of these catchments consists mainly of base poor Dalradian metamorphic schist's, schistose grit and slate. Only 3% of the catchment is base-rich. This leads to soils, which are base poor in the northern sub-catchment. The soils can be divided into three types, which are separated by their respective altitudes. Humus-Iron Podzols, which are largely acidic and nutrient deficient, dominate the lower ground; peat and peaty clays dominate intermediate altitudes, and are acid-rich and have very poor water retention capabilities; alpine soils and rankers make up the highest elevation. Due to the soil types much of the area around the northern catchments is unsuited to forestry and agriculture and is dominated by grassland and heather moorland. However, where the soil type allows, forested areas are a significant part of the overall catchment (approx 8%). The human population of the northern sub-catchment is small at 2.3 people / km^2.

The southern sub-catchments show marked differences to the northern. The two main sub-catchments are the Fruin and the Endrick. These two sub-catchments are found below the Highland Boundary Fault where the topography is more lowland in nature, with a mean altitude of 181m. The lower altitude, shallower slopes than the

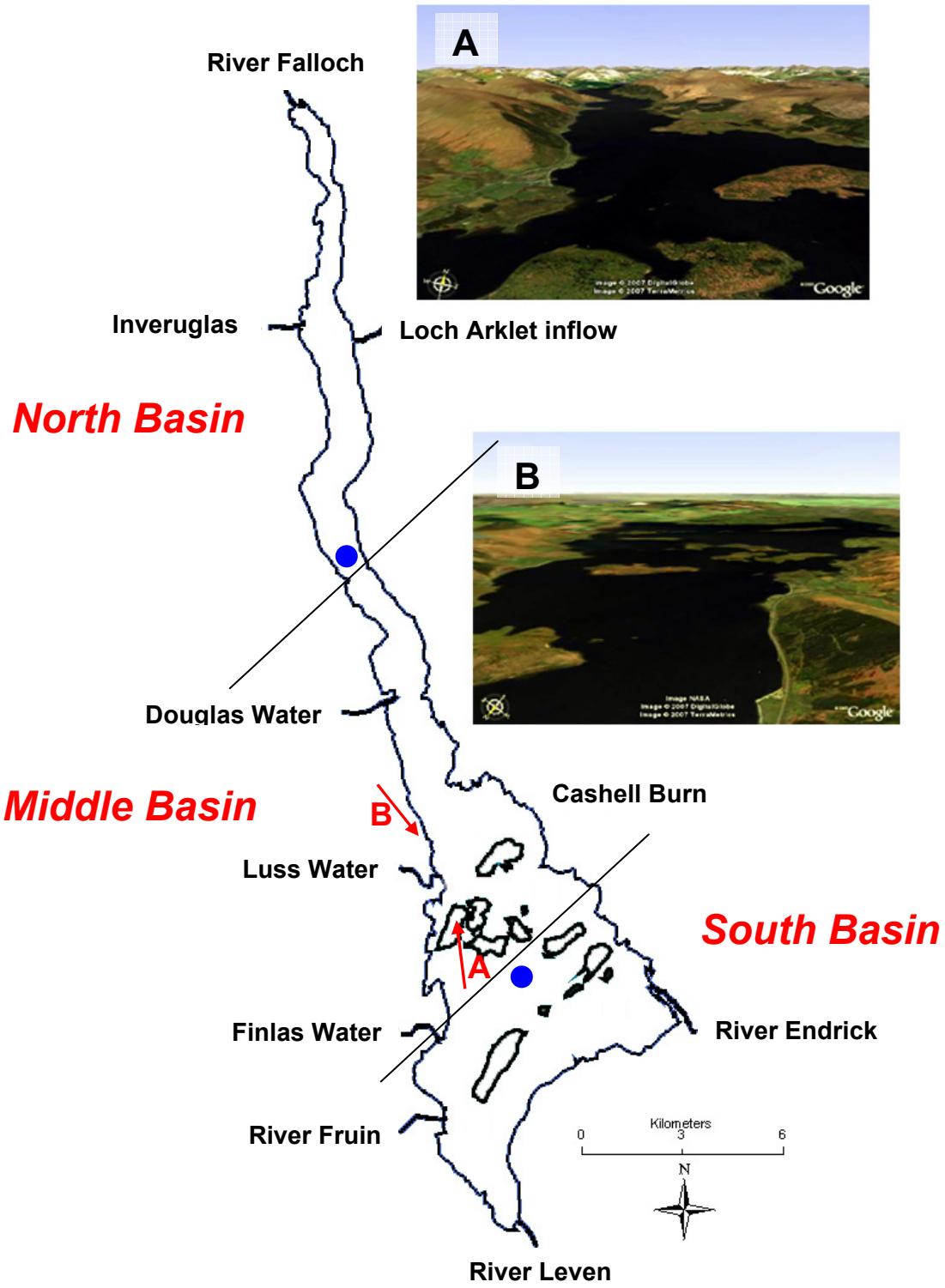

Fig 8: Map of Loch Lomond, showing islands and major inflows and outflow (R. Leven), and basin divisions used in this work. Images A and B show digital representations of the terrain looking towards the north basin (A) and the south basin (B) illustrating differences in catchment morphology. Arrows represent the approximate position and direction of the views A and B. Sample sites for incubation experiments are shown as blue dots.

northern catchments mean base-rich sedimentary rocks are more abundant in the southern sub-catchments, making up 35% of the Fruin and 98% of the Endrick catchments. The rocks are mainly Devonian Old Red sandstones and carboniferous cementstones. Due to the different geology, the southern sub-catchments show a relative abundance of base-rich soils. Again the soil type varies with altitude, with the lower grounds having extensive areas of brown forest gleys, and non-calcareous gleys in areas of poor drainage. On the higher ground peat and peaty gleys dominate. There is a higher level of arable farming in the Endrick and Fruin catchments (26%) compared to the northern areas of the Lomond catchment, although large areas are still used for rough moorland and forestry (56% and 13% respectively). The human population in the southern sub-catchments is also higher than the north at 28 people / km^2.

Loch Lomond is located in west-central Scotland, in the temperate north Atlantic. The climate is wet, windy and cool. Winters and summers rarely reach extreme temperatures. Mean air temperature in January and July are 4.5˚C and 14.5˚C respectively. The overall climate of the loch can be spatially variable. In one season the north basin Inveruglas catchment experienced 3008 mm of rain, and in the same time Ballindalloch in the south had 1372 mm (Curran and Poodle 1992).

The average annual rainfall for the entire loch is between 1300 mm minimum (recorded near Dryman in the Endrick basin) to a maximum of 3600 mm on the slopes of Ben Ime. This average rainfall has been shown to be increasing in recent times. Curran and Poodle (1992) recorded a significant increase of 25 ± 7% between 1972 and 1990. However, since 1990 this trend appears to be reversing with rainfall levels dropping compared to the average (Hansom and McGlashan 1999). While this will have an effect on the overall lake volume, water level is generally controlled by an artificial barrage in the Leven outflow. This barrage was built in 1971 to ensure a reservoir of drinking water. Thus it has stabilised the frequency distribution of water levels, and increased the overall level of the loch by around 10cm. When the water level in the loch exceeds a certain maximum level, the barrage is fully opened and the loch can drain freely into the Clyde estuary.

Loch Lomond is a warm, monomictic lake. Monomictic lakes have one period of complete mixing each year, which is separated, by one period of stratification. In Loch Lomond stratified conditions will begin around May and continue throughout the summer. They are most significant in the northern basin where the epilimnion of approximately 14˚C sits on top of the hypolimnion at approximately 6˚C. As autumn approaches the thermocline breaks down.

Wind plays an important role in overall lake mixing. Time for complete mixing of the water column is around 29 days with a wind speed of 10 m / s. Wind is also very important with regard to waves as the lake experiences no swell and little fetch. Loch Lomond is dominated by waves, which show small heights and large frequencies.

The water quality in Loch Lomond is categorised as class 1, i.e., water quality has exhibited little to no detrimental effect through the activities of man. Eutrophication can be of concern in the southern basin where the Endrick alone can input 8350 kg of phosphorous per year into the lake. Run-off from agriculture land is the most significant diffuse source (the others were point sources). It is possible that the sediments in the Loch are accumulating P at a rate of 34 kg / day, although this doesn't take into account usage of phosphorus by lake biota. At certain times the southern basin rises into the mesotrophic category, not oligotrophic, which is believed to be its natural state.

Water clarity has a strong seasonal fluctuation. SEPA recorded Secchi disc measurements regularly between 1997 and 2005. The lowest values were recorded in September, with March showing the highest. Moreover, a slight decrease in Secchi disc depth was found between 1997 and 2005 in the north basin, although not enough to change the trophic level classification. No such decrease was found in the south. Surface water temperature fluctuates seasonally with maxima in July-August and minima in February. The southern basin shows a greater range in temperature variation than the north, from 2^{o}C to 20^{o}C.

A diatom-desmid community dominates the lake algal flora. *Melosira,* and *Asterionella* dominate in the autumn with *Staurodesmus, Scenedesmus* and *Tabellaria* dominating summer. Cyanobacteria blooms can also occur during the summer months. Long term measurements of primary productivity suggest oligotrophic conditions in the northern basin (2-3 µg chl *a* / L average summer biomass) and mesotrophic the southern (4-6 µg chl *a* / L average summer biomass).

20% of known UK zooplankton species are found in Loch Lomond, including several rare species. Biologically, Loch Lomond can be said to have an unusually high diversity of species when compared to other lakes at similar latitudes. This is due to the wide variety of habitats that the lake provides.

The mean residence time of the Loch is around 2 years. This original estimate is calculated by the ratio of mean lake volume and mean river inflow. This description is a simplistic view of the whole system; more relevant to hydrological research in this lake is considering residency times in terms of different lake regions and for different inflowing waters. A previous study attempted to model residency times in different lake sectors using methods detailed in Eurolakes report D24.

The models used approximately 10^6 marked water particles in a computer simulation. Two avenues of investigation were pursued. The first was to mark the whole water body of the lake with marked particles at a certain date, the second to mark the incoming water from one of the tributaries at a certain date. These parcels of water were followed until they left the loch via the Leven.

A total of five regions were defined in the study: the three main basins and subsequent splitting of the two deeper basins (middle and north) into < 30 m and > 30 m. According to the model the five regions show significantly different residence times. The lowest recorded value was found in the south basin where water left 6 months after entering. Residence times in the > 30 m northern basin could reach 4 years. Residency times in Loch Lomond have been shown to be significantly variable depending on location which needs to be considered when examining lake elemental cycling.

1.10) *Thesis aims:*

I) To describe the temporal and spatial characteristics of DIC and DO in Loch Lomond. Designed to examine possible relationships and dependencies of metabolic balance to both [DIC] and $\delta^{13}C_{DIC}$, along with $\delta^{18}O_{DO}$. Using this data expected patterns in productivity can be elucidated.

II) To describe temporal and spatial characteristics of DOC and TDN in Loch Lomond. DOC and TDN are expected to influence bacterial production levels and as such knowledge of there seasonal and spatial patterns will help elucidate predicted effects on the bacterial community.

III) Use the above data sets to model distributions of [DIC], [DOC], [TDN], $\delta^{13}C_{DIC}$, $\delta^{13}C_{DOC}$ and $\delta^{15}N_{TDN}$ throughout the lake, with the goal of assessing the validity of single point sampling procedures in large inland water bodies.

IV) Assess the possibility of using molar C:N of POM and TDS to estimate the contribution of allochthonous material to the lake.

V) Using survey work (above) chooses representative sites from the south and north basin and use isotope tracer methodology to quantify algal and bacterial production to elucidate the relative contributions to pelagic production. Is Loch Lomond heterotrophic and does this change between basins?

VI) Utilise the data obtained by isotope tracer methodology to estimate the contribution of bacterial production fuelled by allochthonous carbon makes to total pelagic production, and thus estimate how much allochthonous carbon is processed in Loch Lomond on a seasonal and annual basis.

Chapter 2

Dissolved inorganic carbon (DIC) and dissolved oxygen (DO): A dual isotope approach to examine temporal and spatial variation of lake production in a trophically variable system, Loch Lomond, Scotland.

2.1) Introduction

Dissolved inorganic carbon (DIC) and dissolved oxygen (DO) are two nutrient pools linked in nature through metabolic processing (Hanson *et al* 2006). The respective concentrations of these two pools have been used to examine production/respiration balances in aquatic ecosystems for some time (e.g., Juday 1935, Schindler and Fee 1973). The use of isotope ratios of carbon (^{13}C / ^{12}C) and oxygen (^{18}O / ^{16}O) for DIC and DO respectively have been used in aquatic systems (e.g., Quay *et al* 1986, 1995), but to a lesser extent, and combining the two in a dual isotope approach is still relatively rare.

DIC is the primary source of carbon for photosynthetic organisms in aquatic environments, used for the utilisation of energy and production of organic material (Wetzel 2001). This utilisation of DIC is generally met by CO_2 production via the respiratory pathways of most organisms, as well as influxes from other sources such as drainage basins and the atmosphere (Wetzel 2001).

The concentration of dissolved inorganic carbon in freshwater systems is far more variable than oceanic. In oceanic water DIC concentration rarely drifts far from 2 mmol C / L, whereas in freshwater concentrations can range from 50 µmol C /L to 10 mmol C / L. DIC has influence on both gaseous and nutrient availability depending on its concentration and form, and as such the study of its properties, in variable inland systems is of importance (Wetzel 2001).

DO is essential for respiratory organisms, and consumption is offset by its production during photosynthetic pathways and influx from atmospheric dissolution. (Wetzel 2001). In oligotrophic lakes dissolved oxygen concentrations with depth are largely dictated by the physical/hydrological processes of the lake during stratification (largely assuming the effects of metabolism in these systems is insignificant). For large oligotrophic lakes the DO concentration during periods of complete mixing will usually be close to 100% saturation (Wetzel 2001). The onset of stratification is

accompanied by a decrease in O_2 concentration in the epilimnion as temperatures increase (for solubility is reduced with increasing temperature).

Factors which affect the concentrations of DO and DIC in lakes can differ from system to system. In large, deep lakes concentrations may mainly be mediated by water column bacteria and their breakdown of autochthonous and allochthonous carbon. In shallower systems with high inflow loading, the breakdown of allochthonous carbon and benthic particulates may dominate (Melack and Fisher 1983).

During photosynthesis (e.g., Park and Epstein 1960) and respiration (e.g., Kroopnick 1975) ^{13}C and ^{18}O are respectively utilised less readily than ^{12}C and ^{16}O. Thus during times of high photosynthesis the $\delta^{13}C_{DIC}$ signature of surrounding lake water will be enriched. This will particularly be pronounced during stratification at times of high irradiance and water column stability (e.g., Myrbo and Shapley 2006). Lake DIC often has $\delta^{13}C_{DIC}$ values around -5‰, but during stratification hypolimnion values can become more depleted and epilimnion values more enriched (Quay *et al*. 1986; Keough *et al*. 1996) reflecting increased relative importance of primary production in epilimnetic waters and increased relative importance of respiration in the hypolimnion. If the photosynthetic rate decreases or the respiratory rate increases the $\delta^{13}C_{DIC}$ signature will become more negative (Quay *et al* 1986). As well as the metabolic fractionation discussed, influx of atmospheric CO_2 also drives the $\delta^{13}C_{DIC}$ to more-enriched values (Bade *et al* 2004). The describable behaviour of $\delta^{13}C_{DIC}$ in response to metabolic processes allows the testing of certain hypotheses, further detailed in the discussion (section 2.4). In order to differentiate which process (respiratory or photosynthetic) is responsible for any observed isotopic fractionation, simultaneous measurement of $\delta^{18}O_{DO}$ and $\delta^{13}C_{DIC}$ is useful (Wang and Veizer 2000). There is a fractionation of $\delta^{18}O_{DO}$ as oxygen passes from air to water from ~23.5 to 24.2‰ (Kroopnick and Craig 1972). Variation from this can reflect metabolic processes. In the opposite way to $\delta^{13}C_{DIC}$, increased photosynthesis leads to more depleted $\delta^{18}O_{DO}$ and increased respiration leads to $\delta^{18}O_{DO}$ enrichment.

By using the relative changes in isotopic compositions and comparing them in the same space and time, insight can be gained on the balance between photosynthesis and respiration in this lake.

In this chapter there are three primary hypotheses.

i) $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ will show opposite seasonal and spatial patterns as each responds differently to metabolic balance. Hanson *et al* (2006) examined a twenty-year time series on 7 lakes to elucidate the main drivers of DIC and DO concentrations, and found that metabolism was the most important factor influencing both concentrations over diel and seasonal timescales. If isotopic composition and concentration are linked, as they are theorised to be, and as both are dependent at least partly on metabolic rates, can metabolism also be the main driver of isotopic composition?

ii) Both $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ will be spatially heterogeneous in this morphometrically and hydrologically complex system. Studies have shown for some time DIC and DO dynamics may vary across relatively small spatial scales, particularly in lakes with varying bathymetry (Wetzel. 1966; Lind. 1987), but the majority of lake studies still use single sampling points to represent an entire system. The second hypothesis can be used to assess the validity of such sampling strategies.

iii) The flux of DIC through the lake will vary significantly between basins, and over time, related to both hydrological and biological functioning. Due to a lack of DO concentration data (a result of equipment failure) $\delta^{18}O_{DO}$ values will be used mainly to substantiate assumptions made from [DIC] and $\delta^{13}C_{DIC}$ distributions.

2.2) Materials and Methods

2.2.1) Sample sites

For the temporal and spatial survey of various parameters in Loch Lomond 21 sites were selected across the loch (Fig. 9). The sample sites were designated as being upper/north basin (U), middle basin (M) or south/lower basin (L). For each of the defined basins (boundaries shown in chapter 1, figure 1) three main sample sites were designated (1, 2 and 3). These sites were used to assess large-scale spatial variation (mean distance between sites 4.73 ± 1.27 km). Around one of the three main sampling sites per basin (U3 in north, M3 in middle and L1 in south) four more sites were sampled in close proximity (mean distance from main site 0.49 ± 0.43 km) to assess smaller scale spatial variation. These sites are included in all subsequent spatial analyses. At each of the 21 sample sites three depths were sampled: surface water, a middle depth and approximately 3-5 m from the lakebed. The only exceptions to this were certain sites of the north basin where depths exceeded the

limits of our sampling equipment. When this was the case bottom samples were the maximum depth we could reach (100 m). GPS positions were recorded for each site and depth (see appendix 1).

Nine of the major inflows into the lake were also sampled (Fig. 9), together covering over 80% of the catchment area. The sampling procedure carried out was the same at these sites but only surface water was measured. Water was collected far enough up stream to be confident the water was of stream origin and not mixed with lake water.

2.2.2) *Dissolved Inorganic Carbon (DIC)*

A new method for analysing DIC concentration ([DIC]) and carbon isotopic composition was used in this study.

DIC samples were collected in 12 ml glass containers fitted with a screw cap holding piercable septa (Exetainer brand). Prior to use all exetainers were acid washed with boiling (105%) phosphoric acid (H_3PO_4) for 24 hours for first use and effectively acid washed each subsequent time they were used for DIC analysis. Exetainers were then rinsed with distilled water and dried at 60^{o}C. 200 µl of H_3PO_4 were then added to each exetainer, before capping and evacuation on a vacuum line for 35 - 45 minutes to minimise contamination from water or atmospheric CO_2.

Water samples were collected using a Van Dorn sampler. A plastic disposable syringe was first rinsed with loch water three times, and then filled under the water in the Van Dorn water sampler to a 10 ml volume. While still underwater the sample was added to each exetainer by piercing the septa. Due to the vacuum, the sample was drawn into the exetainer with no application of pressure. This also made assessing any exetainer that had lost vacuum clear, as they would not draw in the sample. Any sample where the vacuum was less good were recorded, but run as normal, and justifiably rejected later if there was poor agreement with other replicates. After the sample was taken into the exetainer and the needle withdrawn, the sample was removed from the Van Dorn water, shaken, and stored upside down to await analysis. Exetainers were stored upside down, with the headspace away from the septa to limit CO_2 ingression or egression. Samples were taken in triplicate, with duplicates first being run together, and the third after on a separate run if prior agreement between the first two was poor. Samples were then analysed using a continuous flow isotope ratio mass spectrometer (2.2.4).

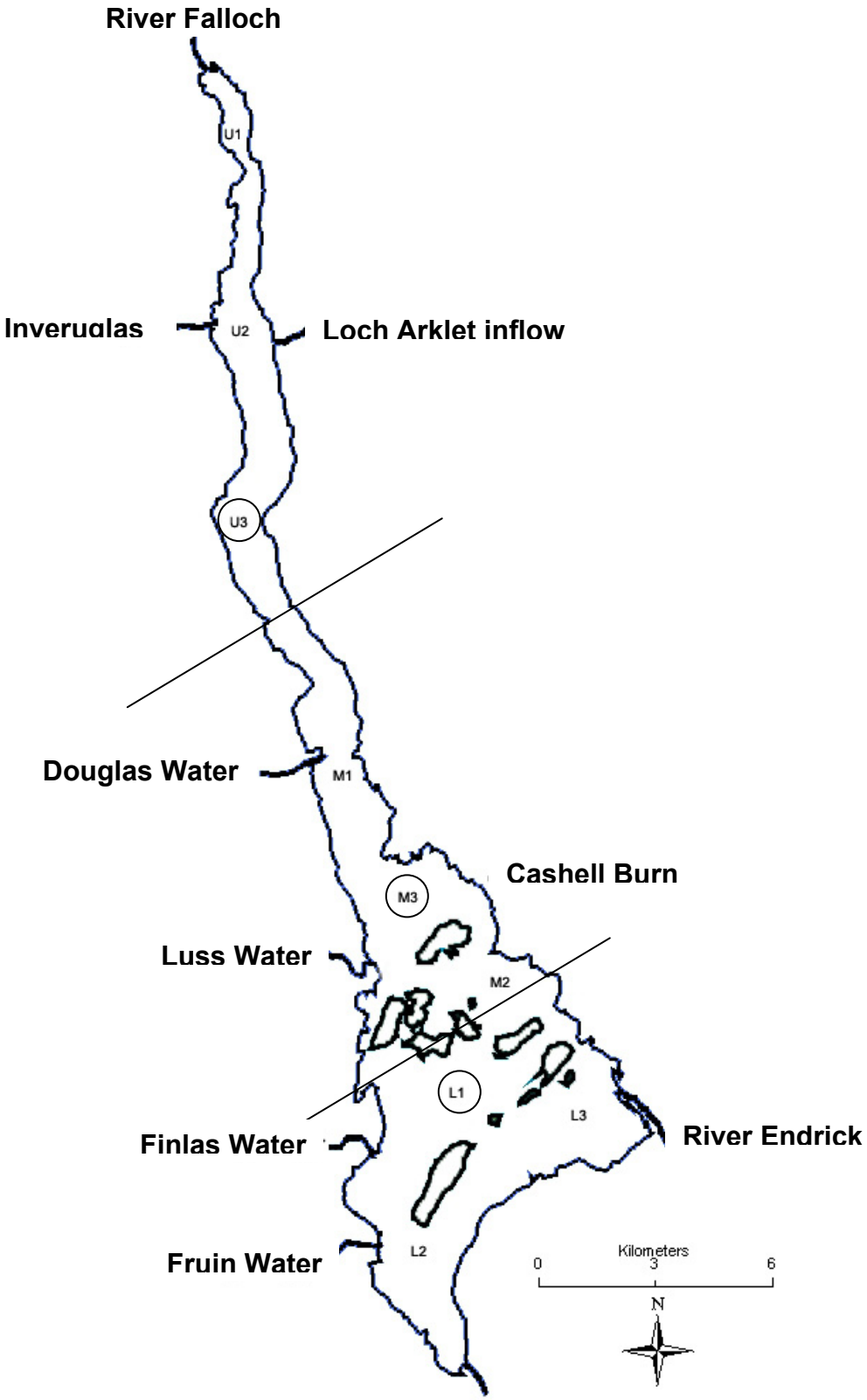

Fig 9: Diagram of Loch Lomond, Scotland. Primary sample sites are shown (U1 – L3), as are major sampled inflows. Solid lines indicate boundaries between The North, Middle and South basins defined in this study. Circled sites represent the primary sites that had four patchiness sites in close proximity.

2.2.3) *Dissolved oxygen*

DO analysis was following the method described by Barth et al (2004).

DO samples were collected in 12 ml exetainers. Exetainers were pre-washed with distilled water and dried at 60^{o}C. To each exetainer 100 µl of saturated mercuric chloride ($HgCl_2$) was added. This ensured that all respiratory activity would be stopped when the sample was added and thus could not cause any secondary fractionation effects on the $\delta^{18}O$ value.

In the field using a plastic syringe, the exetainers were completely filled with loch water (again from the Van Dorn reservoir) and capped. Due to the toxic nature of $HgCl_2$ extreme caution was used during this procedure and rubber gloves were worn always. Full exetainers were stored in a refrigerator to await analysis. Samples from November 2004 were lost as the refrigerator was too cold, and the majority froze and cracked the exetainers.

Field dissolved oxygen concentrations were recorded, but due to inconsistent reproducibility and values seemingly lower than is biologically feasible, were subsequently rejected. Efforts were made to correct the data but were unsuccessful.

2.2.4) *DIC and DO mass spectrometry*

Measurement of [DIC], $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ was undertaken at the Scottish Universities Environmental Research Centre, Scotland.

[DIC] and $\delta^{13}C_{DIC}$ were measured by an automated continuous flow isotope ratio mass spectrometer (IRMS). The system used an AP gas preparation interface linked to a VG Optima IRMS. Aqueous DIC standards were prepared of known concentrations in order to correct the unknown samples via linear regression. Stability during the runs was generally good, with linearity effects on $\delta^{13}C_{DIC}$ requiring correction. Thus various sizes of standard were prepared, ranging from ~0.025 mM to 0.30 mM. Three standards with different isotopic signatures were used (one $NaHCO_3$ and two $CaCO_3$). A concentration calibration curve derived from these standards was used for measurement of sample [DIC]. Precision on these replicate standards is better than ±0.1‰. Values of $\delta^{13}C_{DIC}$ in freshwaters ecosystems generally range from -5 to -13‰ (Jones et *al*. 1998; Meili *et al*. 1996). Crucially, the range in $\delta^{13}C_{V\text{-}PDB}$ of the standards (-24.5 to 2.5‰) was greater than that predicted in our sample site.

Consistency of the reference gas input and electronic drift was monitored by use of a fourth internal control standard, a Na/Ca-CO_3 mixture, at least every 10 samples throughout the run.

Standards were made up by the method described by Waldron and Scott (in prep). Standards were weighed on a Mettler Toledo balance accurate to 0.001 mg. Standard was added to small, acid washed glass beakers for weighing. Material was then transferred into the exetainer, and the beaker was reweighed to calculate the mass in the exetainer. Exetainers were immediately capped with fresh septa and evacuated on a vacuum line for 45-60 minutes. Once evacuated 10 ml of boiling water, acidified to ~pH 1 by addition of H_3PO_4, was added using a needle and syringe. Water was boiling to ensure complete dissolution of $CaCO_3$ and to reduce the blank from dissolved CO_2 and N_2.

Standards and samples were mixed thoroughly using a Whirlmixer and arranged for analysis. Standards were run before samples and the first 8 analyses were 'conditioners' (usually previously run samples) used to check the system was working properly. Standards were ordered in sequence of lowest to highest concentrations and then samples were randomly added to the run, with the fourth, drift control standard placed every 10 samples. A new collection of linearity standards was run at the start of each individual run.

All samples/standards were then left for 24 hours to equilibrate between liquid and headspace (DeNiro and Epstein 1989, Salata *et al*. 2000; Torres *et al*. 2005).

$\delta^{18}O_{DO}$ was measured on an AP2003 mass spectrometer and preparation unit, supplied by a XL222 Gilson autosampler. Air was used as the standard for these analyses. 4 ml exetainers were first greased around the seal and capped to minimise any exchange with the atmosphere. The capped exetainers were then flushed with He to purge air and thus O_2, after which a known amount of air was added to the exetainers thus providing oxygen as a standard. Atmospheric oxygen was used, as the isotopic signature is globally quite constant. Linearity standards ranged from 200 µl added to 700 µl added, with repeating 400 µl standards used to correct for drift.

Before sample analysis preparation of a sample headspace was required. The first step was creation of a headspace as exetainers were filled to capacity in the field. For this, headspace creation mode on the mass spectrometer was used. In this mode the autosampler needle has two holes, one 3 mm and one 15 mm from the needle tip. The higher side hole was connected to a He flow at 10 ml / min, the lower side hole connected to a plastic tube running into a polyethylene bottle to collect sample displaced by He inflow. 4 ml of sample was displaced per exetainer.

Samples were shaken for one hour on a wrist shaker at ~250 strokes per minute. This was to ensure mobilisation of oxygen into the headspace and equilibration. Samples and standards were then arranged into run order. Usually a suite of standards was run before and after the approximately 145 samples, with drift standards run every 10 samples throughout.

2.2.5) *Additional measured parameters.*

Along with DIC concentration, $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$, other measurements were taken. At each site and each depth, temperature was recorded using an YSI 550 DO probe. From four of the six sites per basin, small (200 ml) plastic bottles were filled, underwater for alkalinity titrations and pH measurement. Due to time constraints between sampling trips for analysis only surface and deep depths were sampled for gran alkalinity. Samples for dissolved organic carbon concentration [DOC], $\delta^{13}C_{DOC}$, total dissolved nitrogen concentration [TDN]; $\delta^{15}N_{TDN}$, molar C:N and $\delta^{18}O_{H2O}$ were also collected. The results for these parameters will be discussed in more detail in other chapters and only here if relevant.

2.2.6) *Spatial and statistical analysis.*

Spatial analysis was undertaken using ArcGIS version 9.1. Data was entered onto an outline of Loch Lomond and Inverse Distance Weighted interpolation was used to estimate values between data points and construct contour maps of the loch. IDW acts by explicitly implementing the assumption that points which are close are more likely to have similar values than points far apart. In Loch Lomond, although unknown, values in the far north would be expected to be most dissimilar to values in the far south, so this method has been chosen. This however does not apply to construction of a lake depth profile, which used a Triangular Irregular Network (TIN) conversion. Using a TIN depth profile, as opposed to IDW, was decided by comparing outputs to chart datum, and seeing which corresponded more closely. In all calculations, depth profiles created by TIN formation have been used in conjunction with spatial distributions by IDW. The following information is taken from the ArcGIS help pages.

The most common and simplest form of IDW is defined by the equation:

$$F(x,y) \equiv \sum_{i-1}^{n} W_i F_i \qquad \text{Eq. 3}$$

Where n is the number of scatter points, W_i is the weight function assigned to the scatter point and F_i is the unique function value of the scatter point (in this case the data collected). The weight function is expressed as:

$$W_i \equiv \frac{h_i^{-P}}{\sum_{j-1}^{n} h_i^{-P}} \qquad \text{Eq. 4}$$

Where P is the power parameter (usually P = 2), and h_i is the distance of the scatter (sample) point to the interpolation point, which is more accurately defined by the equation:

$$h_i = \sqrt{(x - x_i)^2 + (y - y_i)^2} \qquad \text{Eq. 5}$$

Where X, Y give the location of the interpolation point and Xi, Yi give the location of the scatter (sample) points.

Due to the difficulties associated with varying basin depths, when comparing distributions across the loch this section will focus on epilimnetic water only. SEPA used Secchi disc measurements to calculate the thickness of the epilimnion as follows:

Epilimnion depth = 2.0 – 2.5 x Secchi disc depth Eq. 6

This gave depths of 5.7 - 7.2 m in the south and 8.4 - 10.5m in the north. This, along with anecdotal accounts (Adams pers comm), based on regular seasonal measurements of the thermocline, support the interpretation of an epilimnion usually between 7 - 13 m. Thus for the purposes of comparing epilimnetic distribution, [DIC] was converted to g / m^3 and then scaled up to a depth of 13 m. Epilimnetic spatial

analysis of $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ underwent no such multiplication as it is assumed values should be relatively constant in the well-mixed surface layer. The epilimnion was not truly defined in this study by temperature measurement for ease of modelling purposes. i.e., modelling the likely different epilimnion depths between locations was beyond my expertise.

All statistical analysis was carried out on SPSS version 13. Data was analysed using multi factorial analysis of variance and linear regression models.

2.3) *Results*

2.3.1) *Influence of basin, depth and season on:*
a) Physical parameters

Water temperature in Loch Lomond showed a seasonal cycle (Fig. 10). A maximum of 17.5 ^{o}C was recorded in south basin surface waters in June, and a minimum of 4.5 ^{o}C in the south basin epilimnion in March. A three-way analysis of variance shows basin, month and depth all have a significant relationship with temperature ($P < 0.001$ for all three). However, more detailed analysis shows a more complex pattern. Surface water temperatures show significant difference between basins in November, March and September ($P < 0.001$), although no significant difference was observed in June ($P = 0.098$). Middle and deep (hypolimnion) depths show intrabasin differences with all months ($P < 0.001$. Although the three way AVOVA shows significant effects of depth on temperature, this effect varies between basin and over time. No significant difference in temperature between depths in March was observed in any basin ($P = 0.591$-1.000). In November only the south basin showed no significant effect of depth ($P = 1.000$). All three basins showed significant differences in temperature between depths in June and September.

pH was only recorded in March, June and September. pH showed variation on a seasonal scale (Fig. 10) in all three basins ($P <0 .001$). Values varied from 5.85 (south basin deep water in June) to 7.45 (south basin surface water in September). Whole lake average pH maximum was recorded in March, of 7.17 ± 0.19 ($N = 29$) and a minimum of 6.19 ± 0.20 ($N = 30$) in June. The seasonal pattern is the same for the three basins but with slightly different ranges. Consistent differences are observed with depths in all three basins. Significant differences in pH between epilimnion and hypolimnion water were observed in June in the mid basin ($P = 0.001$), and June and September in the north basin ($P = 0.046$ and 0.027 respectively). All other sites showed no significant effect of depth. As with temperature profiles, the greatest difference in pH between surface and deep sites is in the north basin.

It must be noted that pH variability during a diel cycle can match and exceed what has been measured in this study (e.g., Maberly 1996, Waldron *et al* (in press)). Thus it can not be discounted that the observed seasonal variation is a reflection of diel variability.

b) [DIC], $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$

[DIC] was lowest in the north basin, ranging from ~0.08 mM in the hypolimnion, to a maximum of 0.16 mM in the epilimnion (Fig. 11). The south basin has the highest concentrations, with lowest values occurring mid-depth in March (~0.16 mM) and highest values of 0.27 mM occurring in June surface waters. The middle basin shows less seasonality than either south or north basin, remaining relatively constant around the 0.16 mM level.

Seasonal patterns of [DIC] in south and north basin are similar, with a maximum value in June surface waters of 0.27 ± 0.09 mM and 0.17 ± 0.05 mM respectively. Minimum values were recorded in March of 0.17 ± 0.02 mM (south basin, mid depth) and 0.08 ± 0.01 mM (north basin, deep water).

Month and basin each have a significant effect on [DIC], although depth has no significant effect (P = 0.993). Post hoc analysis shows that all three basins are significantly different. September and March are not significantly different from each other but are different from June and November.

$\delta^{13}C_{DIC}$ shows significant variability with depth, basin and month (Fig. 11). Most enriched values were recorded in the epilimnion of the middle basin in September 05 (mean = - 5.1 ‰) although the means for north and middle basin epilimnetic water in both June and September were above – 6 ‰. The most depleted values recorded were in the deep and middle water of the north basin, where signatures were between –11 ‰ and –13 ‰ in November and March. Values similar to these were also found in middle basin deep/mid water in September.

Each basin showed a degree of seasonality in the isotope signature, although the pattern is variable between basins. North basin, epilimnion shows the largest range, with a difference of over 6 ‰ between March (mean $\delta^{13}C_{DIC}$ = -12.2 ‰) and June (mean $\delta^{13}C_{DIC}$ = -5.8 ‰). In the north basin patterns for $\delta^{13}C_{DIC}$ closely match seasonality in temperature (Fig. 11).

$\delta^{18}O_{DO}$ values varied from 22.3 ‰ (March, mid-basin, mid-water) to 26.7 ‰ (September, mid-basin, mid-water) (Fig. 11). $\delta^{18}O_{DO}$ is significantly affected by depth, basin and month. In all three basins there is a significant increase in $\delta^{18}O_{DO}$ between March and September with a more enriched signature in the hypolimnion compared to the surface. Whole lake averages (ignoring basin and depth) show a significant increase from 23.2 ± 1.5 to 25.4 ± 1.0 ‰ between March and September ($P < 0.005$). Overall basin effect is significant ($P < 0.005$) but further analysis

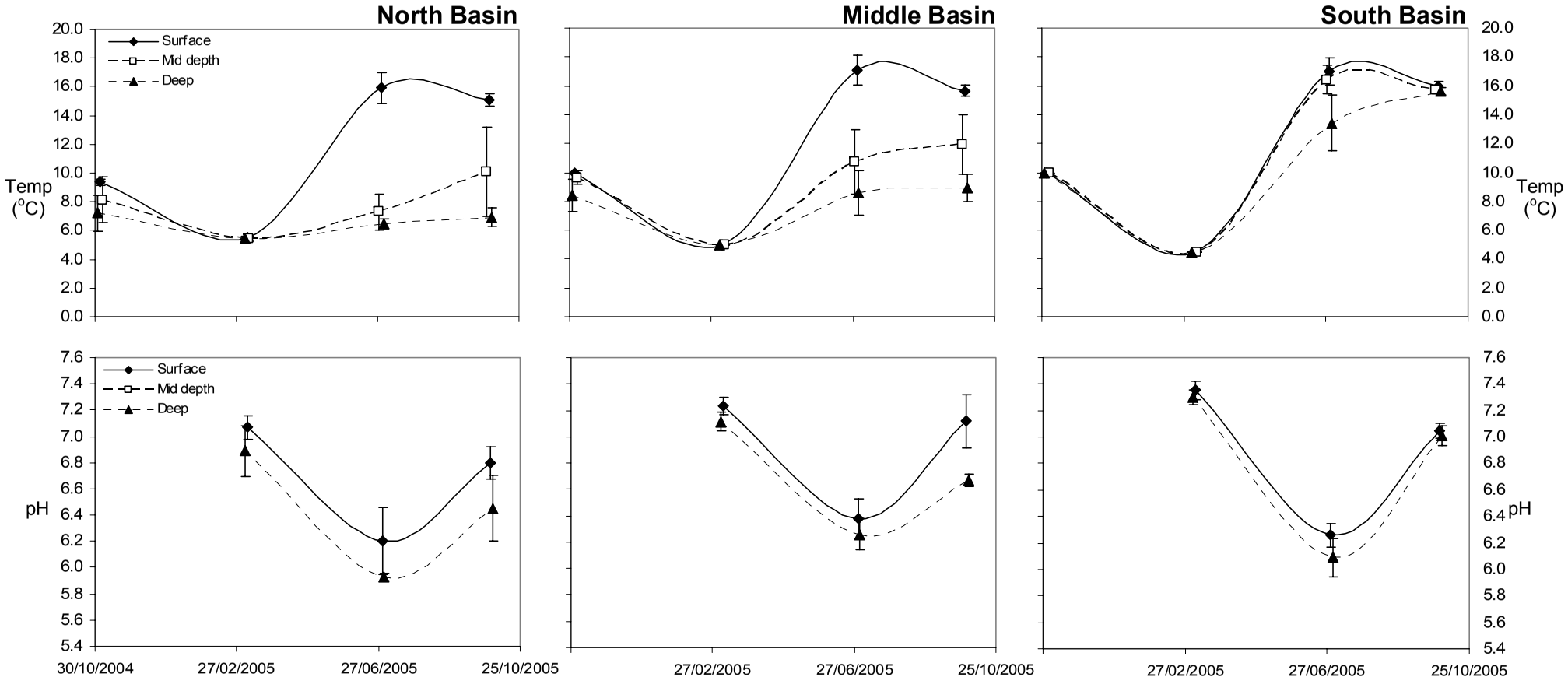

Figure 10: Seasonal temperature (°C) and pH variation in Loch Lomond. Loch divided into basins (North, middle and south) and depths surface, middle and deep). In general, middle and deep values represent the hypolimnion water, and surface values the epilimnion. Data points plotted one day apart to allow resolution between error bars. Error bars represent ± 1 SD.

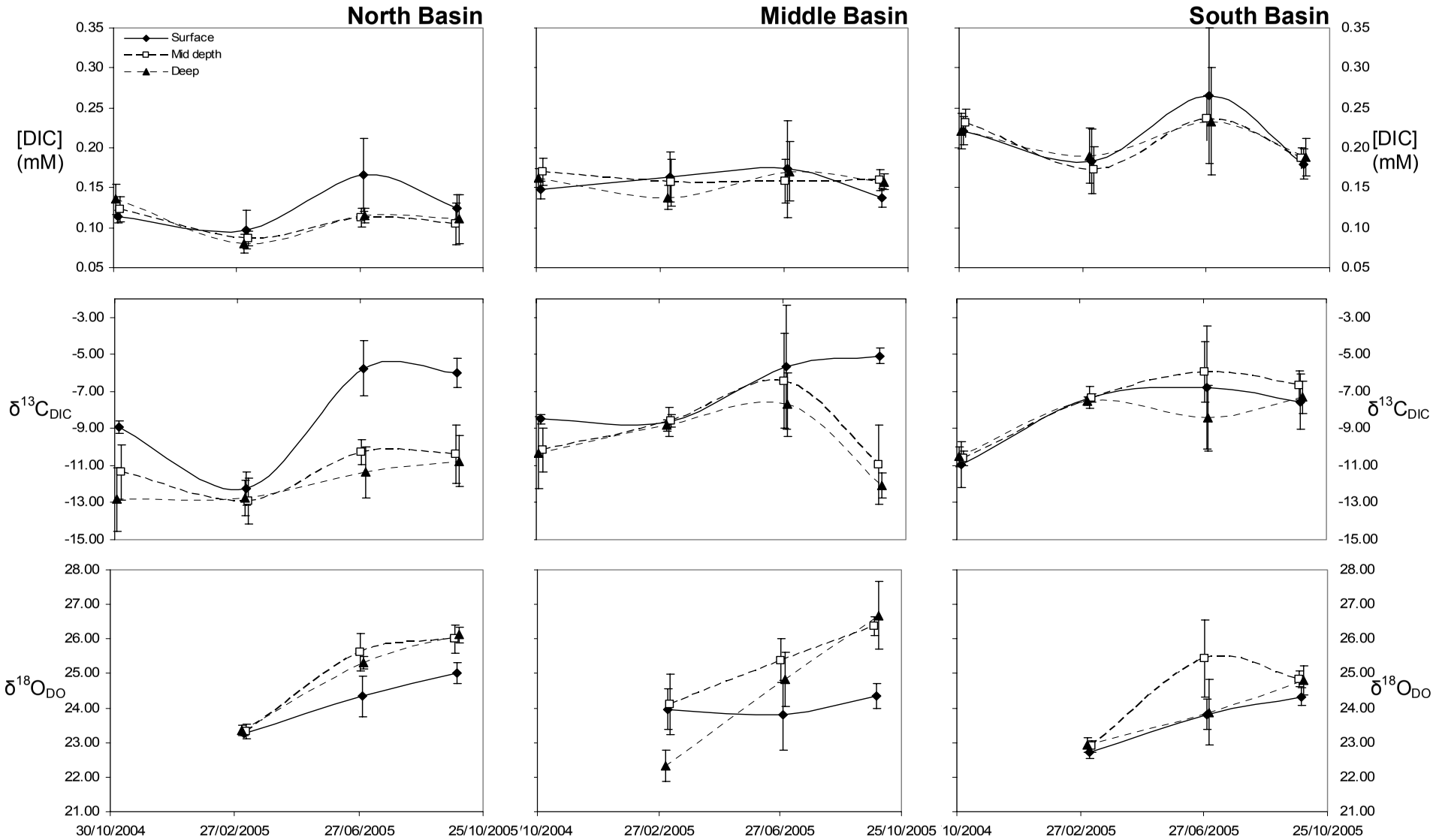

Figure 11: Seasonal [DIC], $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ in Loch Lomond. Loch divided into basins (North, Middle and South) and depths (Surface, middle and deep). In general, middle and deep values represent the hypolimnion water, and surface values the epilimnion. Data points plotted one day apart to allow resolution between error bars. Error bars represent ± 1 SD.

shows the middle and north basin are not statistically different from each other (P = 0.341).

2.3.2) Controls on [DIC], $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$.

When comparing various parameters for inter-relationships the loch has not been divided into basins to simplify interpretation and presentation of the connected water body. A more detailed analysis of intra basin spatial patterns is found in section 2.3.3.

DIC concentration showed a significant relationship with temperature in all four months sampled (Fig. 12a). The significance of the interaction varied between months. In March temperature explained much of the variation seen in [DIC] (R^2 = 0.638, P < 0.001). Temperature explained less of the variation seen in November, June and September, but all relationships were significant (all months P<0.001).

pH was found to be significantly related to [DIC] in March (R^2 = 0.379, P = 0.001) and September (Fig. 12b). June showed a significant relationship but only a small amount of the variation in [DIC] was actually explained by pH (R^2 = 0.015, P = 0.025).

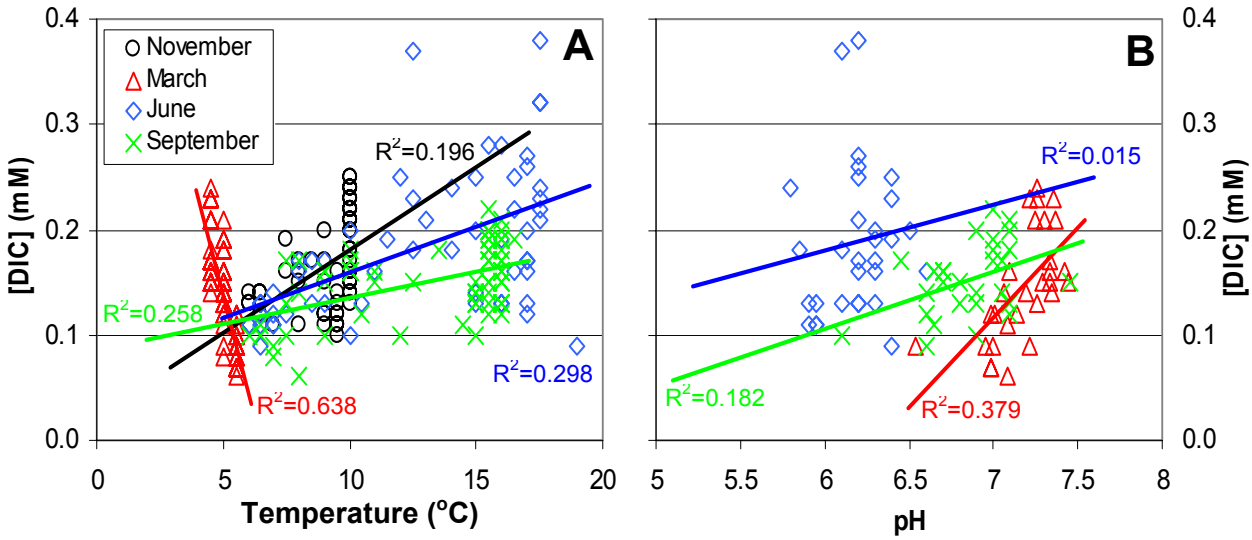

Fig 12: [DIC] against a) temperature and b) pH for four and three sampling dates respectively. Alkalinity titrations were not carried out in November, thus lack of pH data for this month.

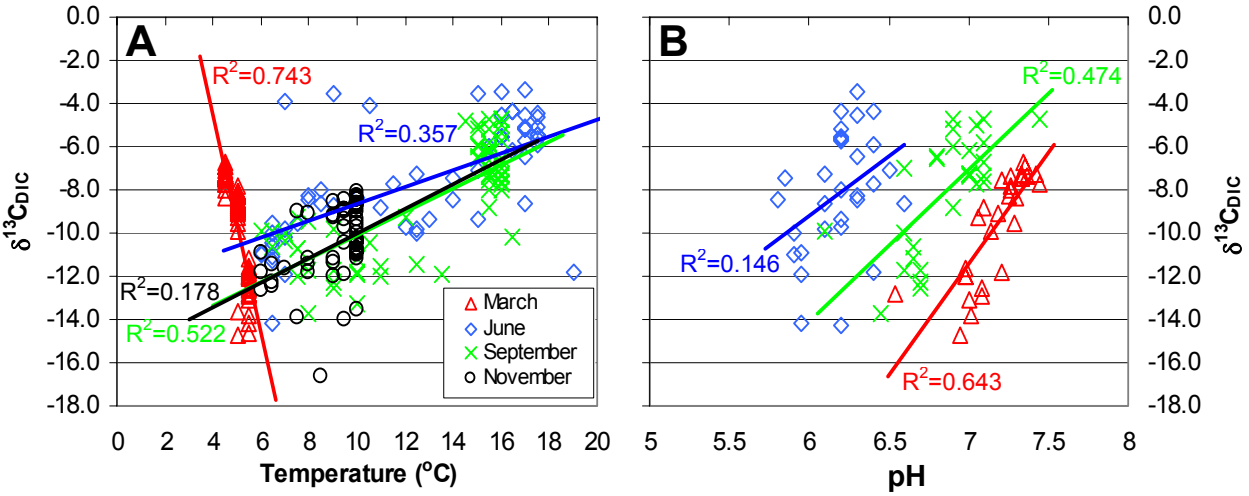

Fig 13: $\delta^{13}C_{DIC}$ against a) temperature and b) pH for the four sampling dates.

As with [DIC], $\delta^{13}C_{DIC}$ showed significant linear relationships with temperature (Fig. 13a) in all four sampling months. March showed the strongest correlation with temperature explaining 74.3% of variation seen in $\delta^{13}C_{DIC}$ (R^2 = 0.743, P < 0.001). The weakest correlation was in November 04 with an R^2 of 0.178, but again this was a significant relationship (P < 0.001). November, June and September all show a positive correlation between $\delta^{13}C_{DIC}$ and temperature with March showing the only negative correlation.

$\delta^{13}C_{DIC}$ was positively and significantly correlated with pH in all months sampled. March and September showed the strongest correlations each with an R^2 greater than 0.470. June had a weaker correlation (R^2 = 0.146) but the relationship was still statistically significant (P = 0.037).

Chlorophyll *a* analysis was undertaken as part of the spatial and temporal survey, using ethanol extraction of the GF/F filter papers followed by UV-spectrophotometery. However, likely due to repeated freezing of the samples (at least twice) results obtained were unreliable and the data proved of little use. Chlorophyll concentration has been shown to be of significance in other studies in relation to DIC dynamics and as such could not be ignored. A limited amount of data was obtained from SEPA who regularly sample certain sites on the Loch. Figure 14 shows chlorophyll data from SEPA matched to the closest possible sites sampled in this study ([DIC] and $\delta^{13}C_{DIC}$) and the closest dates. Unfortunately exact matches were not available, so relevant chlorophyll data from October was plotted against my data from November, July against June and September against September. Four to five sites were matched

against SEPA locations and only surface water was considered as deeper water was not sampled by SEPA.

Although caution must be applied when interpreting the data, as neither exact times nor locations are plotted against each other, some relationships may be inferred. From the data used chlorophyll *a* seems to have a significant influence on both [DIC] and $\delta^{13}C_{DIC}$ for all sampled times of year. Chlorophyll a shows a positive linear relationship with [DIC] for all sampling times. $\delta^{13}C_{DIC}$ has a negative linear relationship with chlorophyll a concentration. Data from September and November show the most significant relationships, with less variation explained by linear regression in June.

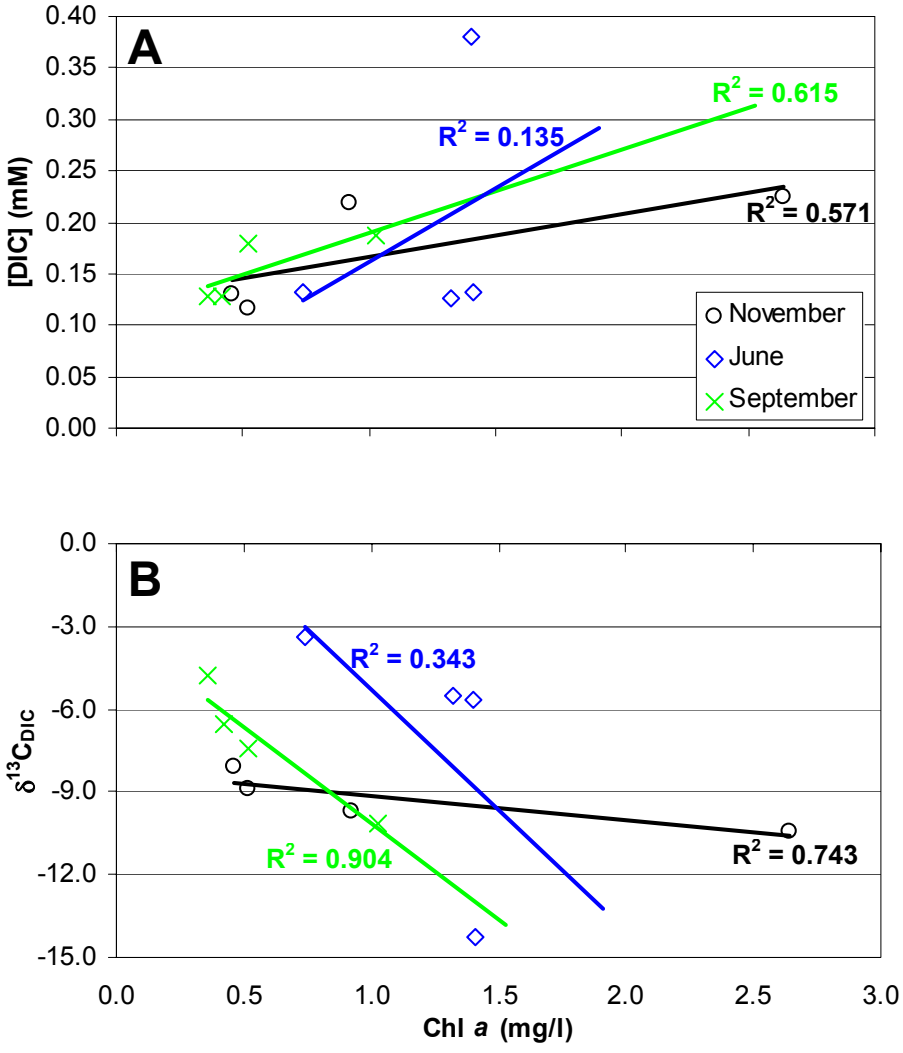

Figure 14: Chlorophyll a data supplied by SEPA plotted against a) [DIC] and b) $\delta^{13}C_{DIC}$ measured in this study.

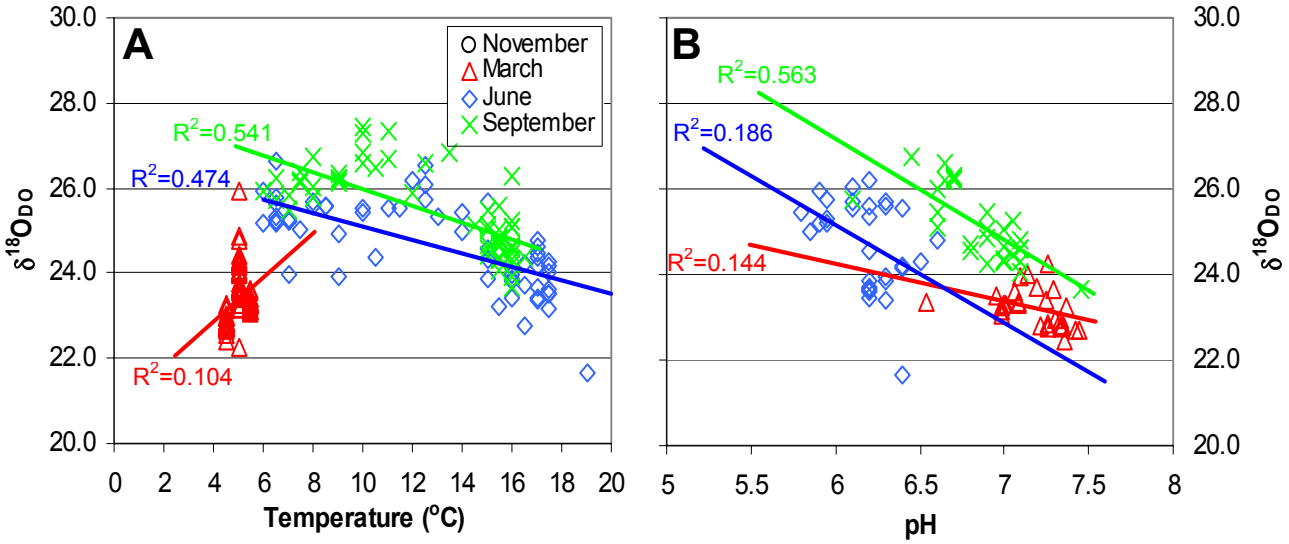

Fig 15: $\delta^{18}O_{DO}$ against a) temperature and b) pH for March, June and September 2005.

Temperature described a significant amount of variation seen in $\delta^{18}O_{DO}$ in March, June and September (Fig. 15a) ($P = 0.009$, < 0.001 and < 0.001 respectively). pH variation had a significant relationship with $\delta^{18}O_{DO}$ in all sampling periods, describing the most variation in September ($R^2 = 0.563$, $P < 0.001$), but still significant in March and June also ($R^2 = 0.146$, $R^2 = 0.186$ respectively).

The relationship between $\delta^{13}C_{DIC}$ and [DIC] varies between months in Loch Lomond (Fig. 16). In March, when the water column is well mixed and, during our sampling period at least, relatively stable, the concentration of DIC explains the variation in $\delta^{13}C_{DIC}$ well ($R^2 = 0.631$, $P < 0.001$). The two variables show less significant relationships in the other sampling periods. Like March, in June the relationship was significant ($P = 0.005$), but far less of the variation seen in $\delta^{13}C_{DIC}$ is explained by [DIC]. Both November and September show no significant relationship.

Figure 17 illustrates the interaction between $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$, spilt into the three different sampling months where complete data was available. There are significant linear relationships between $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ in both June and September ($R^2 = 0.721$, $P < 0.001$ and $R^2 = 0.531$, $P < 0.001$ respectively). Both follow a similar pattern: as the $\delta^{13}C_{DIC}$ signature becomes depleted by ~15‰ there is a corresponding enrichment of $\delta^{18}O_{DO}$ by ~4‰. Data collected in March, when the water column is well mixed and considered unproductive, showed no such significant relationship ($P = 0.455$) due to relatively wide variation in the $\delta^{18}O_{DO}$ signatures when the DIC signature is enriched (between –5‰ and –10‰).

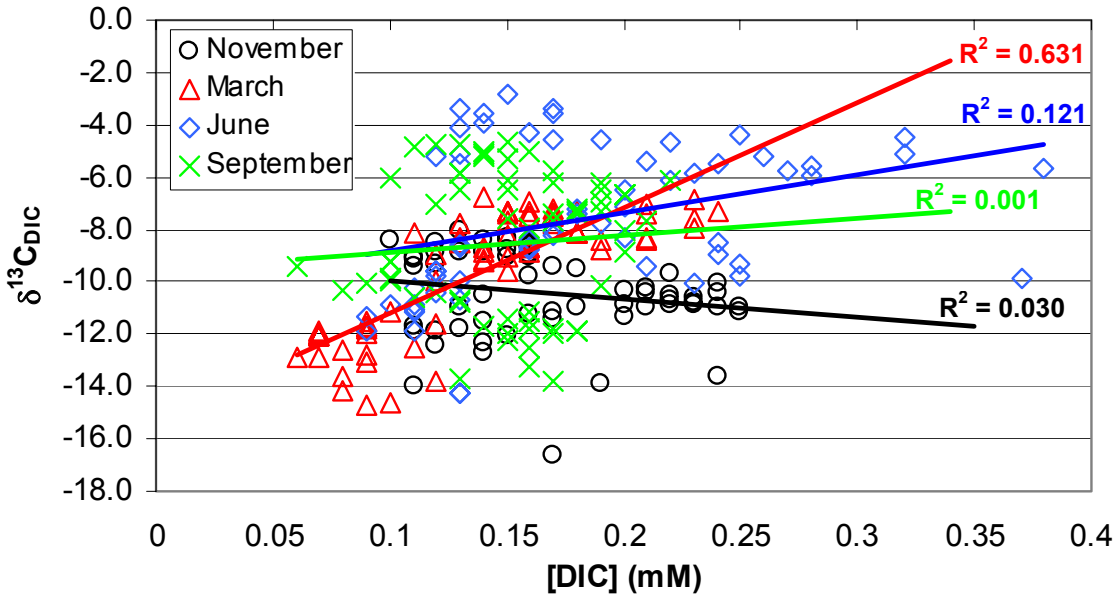

Fig 16: Relationship between $\delta^{13}C_{DIC}$ and [DIC] for all sampling periods.

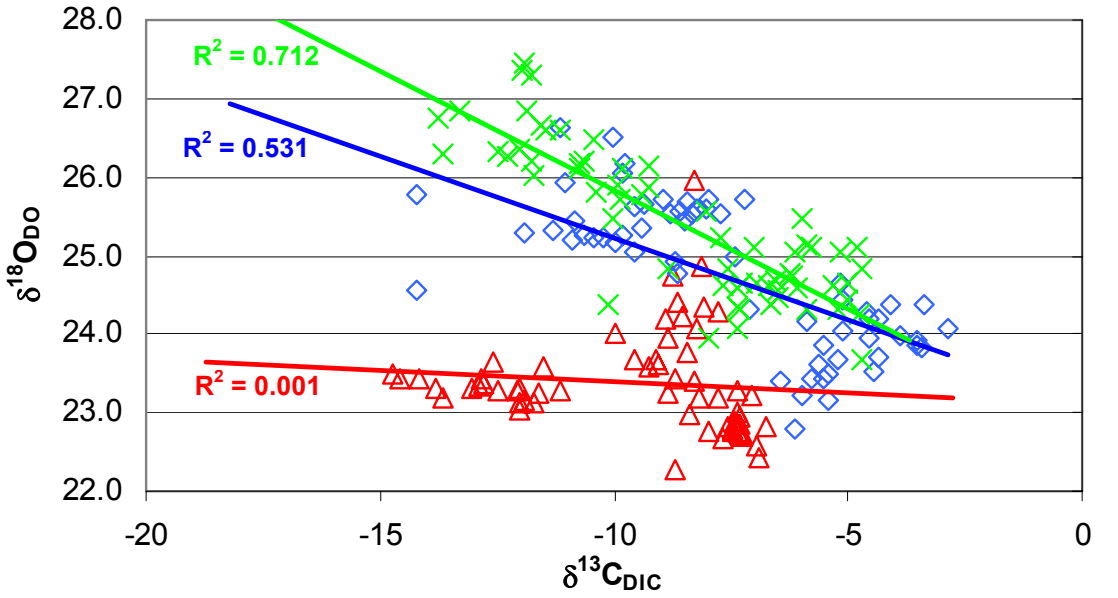

Figure 17: Relationship between $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ for March, June and September 2005.

2.3.3) *Excess partial pressure of CO_2 ($EpCO_2$).*

Using measured data on temperature, pH and [DIC] (see Appendix 1) the excess partial pressure was calculated. Figure 18 shows $EpCO_2$ for each basin in the surface and deep waters. A threshold value of 1 represents the transitional period between a net sink of atmospheric CO_2 ($EpCO_2 < 1$) and a net source of CO_2 ($EpCO_2 > 1$).

All three basins exhibited the same general seasonal trend, with lowest $EpCO_2$ in the winter and peak values in the summer. The North basin hypolimnion was measured to have the lowest $EpCO_2$ values consistently, averaging from 1.07 ± 0.48 in March 05 to 3.44 ± 1.72 in June 05. The north basin showed significant deviation between surface and deep water in June, likely reflecting the period of stratification. In June epilimnetic water in the north is supersaturated with respect to atmospheric CO_2 concentrations, and while the hypolimnion does not reach the same levels it is still over-saturated also. Only hypolimnetic water in March in the north basin had an average $EpCO_2 < 1$ (= 0.097 ± 0.34).

South basin $EpCO_2$ is generally the highest and shows no detectable difference between surface and deep water, even in June when stratification was observed. Minimum values of 1.02 ± 0.34 were estimated in March and a maximum of 8.98 ± 2.93 in June. Average $EpCO_2$ in the south basin never dropped below 1 in our sampling periods and as such CO_2 egression from water to atmospheres could be predicted as the general pattern.

In all three basins the highest $EpCO_2$ corresponds to the most enriched $\delta^{13}C_{DIC}$ values in June (see Fig. 11). In September however, the epilimnion of the north and middle basin, as well as both epi and hypolimnion in the south, $EpCO_2$ was measured to be significantly lower than in June, yet the $\delta^{13}C_{DIC}$ remained roughly the same.

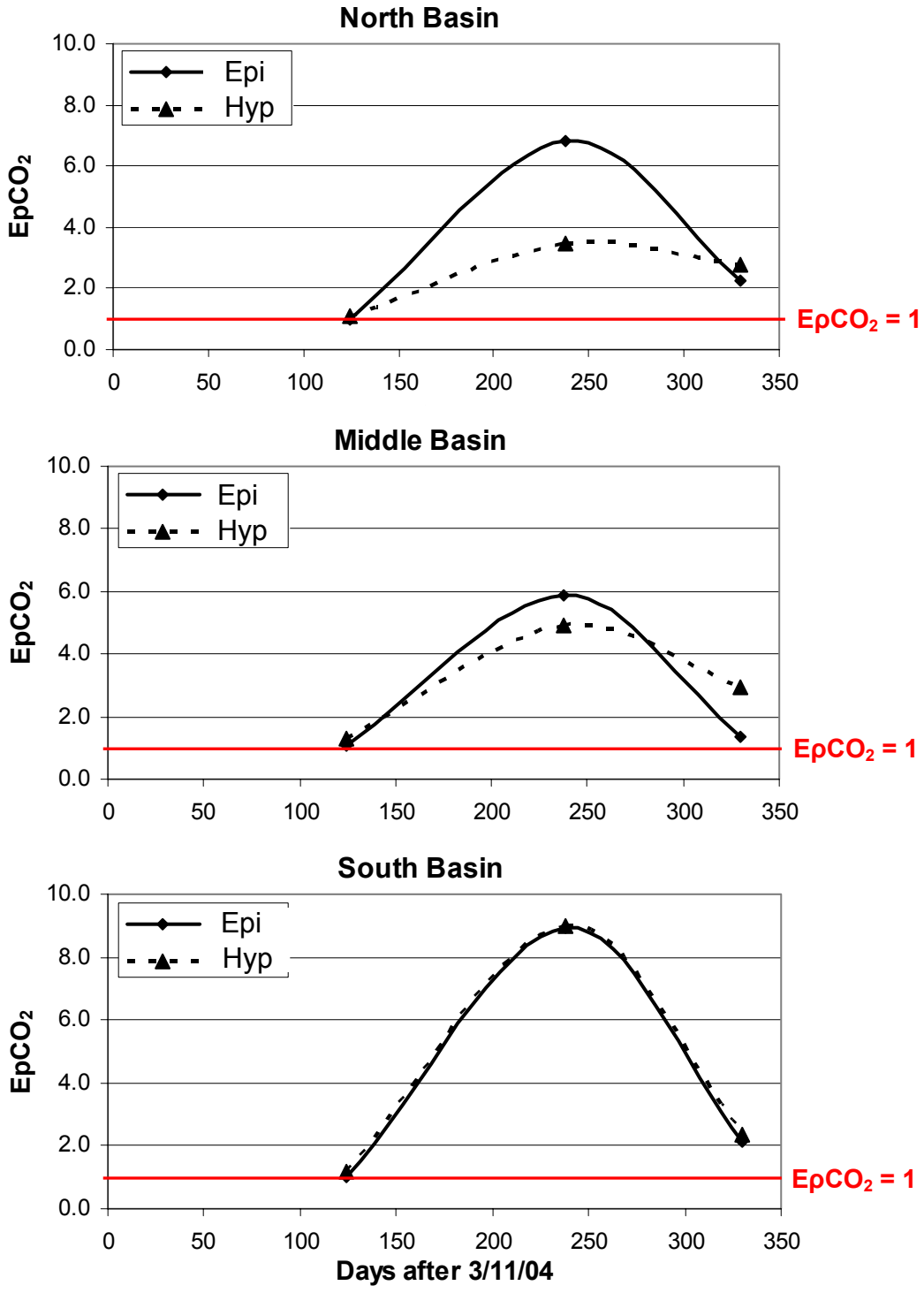

Figure 18: $EpCO_2$ temporal variability, divided into basin (north, middle and south) and depth (epilimnion / hypolimnion). Y = 1 represents the point at which [CO_2 (aq)] and [CO_2 (atm)] are in equilibrium.

2.3.4) *Spatial analysis of epilimnetic [DIC], $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$.*

Figure 19 shows the distribution and variation in epilimnetic DIC concentration. In all four months the Loch shows a latitudinal gradient of decreasing DIC concentration with distance north. In November and September the highest concentrations (~37 - 41 g / m^2) are found in the southeast corner near the inflow of the River Endrick (see Fig. 8). In March the higher concentrations are found slightly further west in the middle region of the south basin. June shows the both the highest concentrations of DIC and the greatest variation across the loch. As in March the highest concentrations are found in the middle and western areas of the south basin, where concentrations reach 55-57 g / m^2. The south east corner near the River Endrick mouth is an area of lower concentration. Further north the concentration falls again, but unlike the other three periods, increases again on approach to the mouth of the River Falloch in the north basin.

$\delta^{13}C_{DIC}$ show significant spatial variability in the epilimnion of Loch Lomond. In November the south basin has the most depleted signature with enrichment occurring throughout the middle and north basins (Fig. 20). This contrasts with March where the opposite pattern is observed. Here the most enriched values (-6.5‰ to –5.5‰) occur in the south with a general enrichment further north. June shows both the most enriched $\delta^{13}C_{DIC}$ signatures recorded (whole loch mean = -6.3 ± 1.8‰) and the most complex patterns in spatial variability. $\delta^{13}C_{DIC}$ reaches –4.9‰ to –3.5‰ in the upper middle and lower north basins and the entire loch is consistently more enriched than –7‰. The pattern in June is more complex although due to local areas of relative depletion. In the southeast corner there is an area of significantly more depleted values. There is also a similar area of depletion in the middle basin on the east side near the Cashell Burn. As in March, there is also depletion further north, but to a lesser extent. September shows a similar spatial pattern to June, with the most enriched values in the middle section of the lake, with more depleted values in the far north and south. There is no area of significant depletion in the southeast corner in September; instead the most depleted values were recorded in the southwest near the outflow into the River Leven. As with June and March $\delta^{13}C_{DIC}$ became more depleted close to the River Falloch inflow.

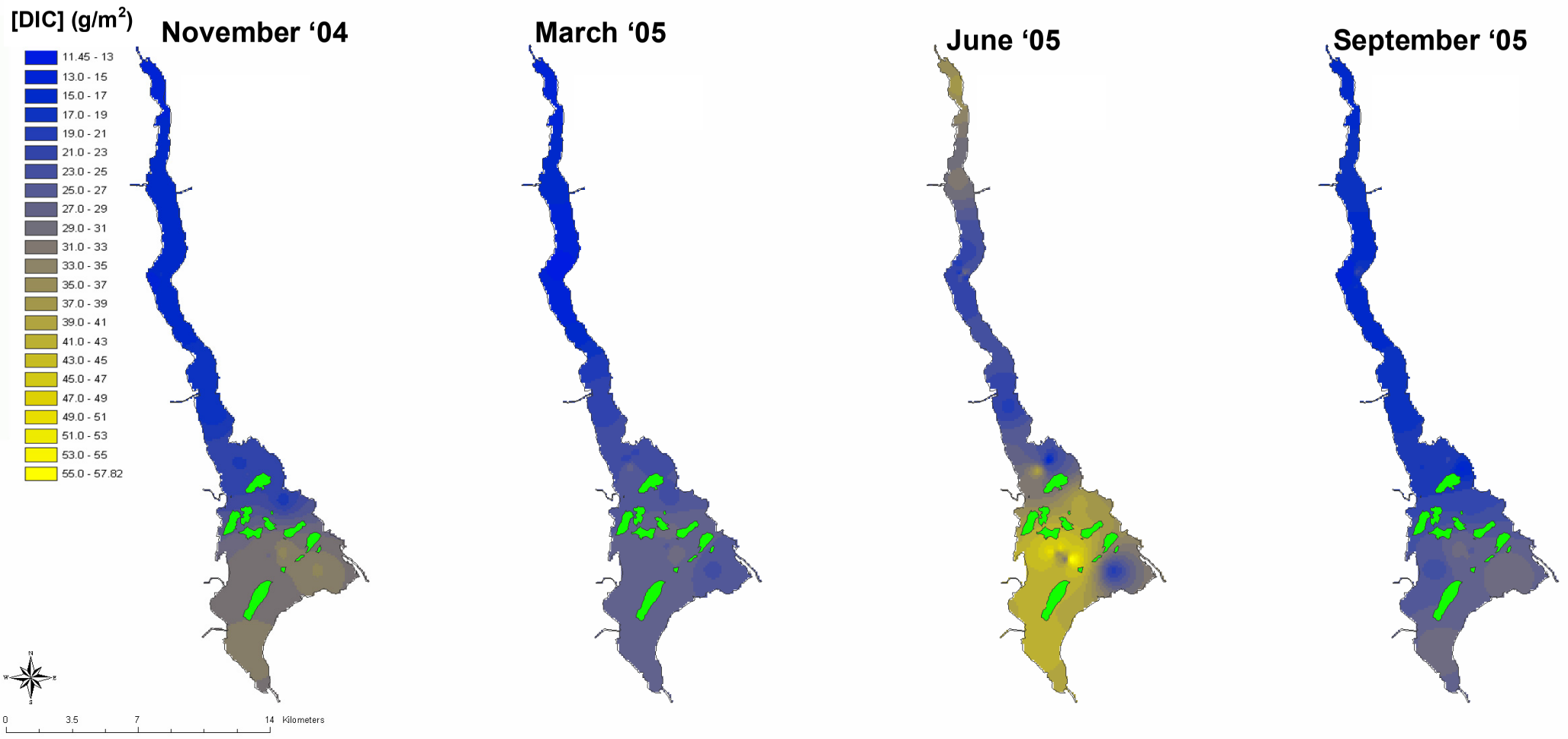

Figure 19: Epilimnetic (top 13m) distribution of dissolved inorganic carbon in Loch Lomond (g/m^2) for all four months sampled. Lighter colours indicate higher concentrations. Islands are shown in green.

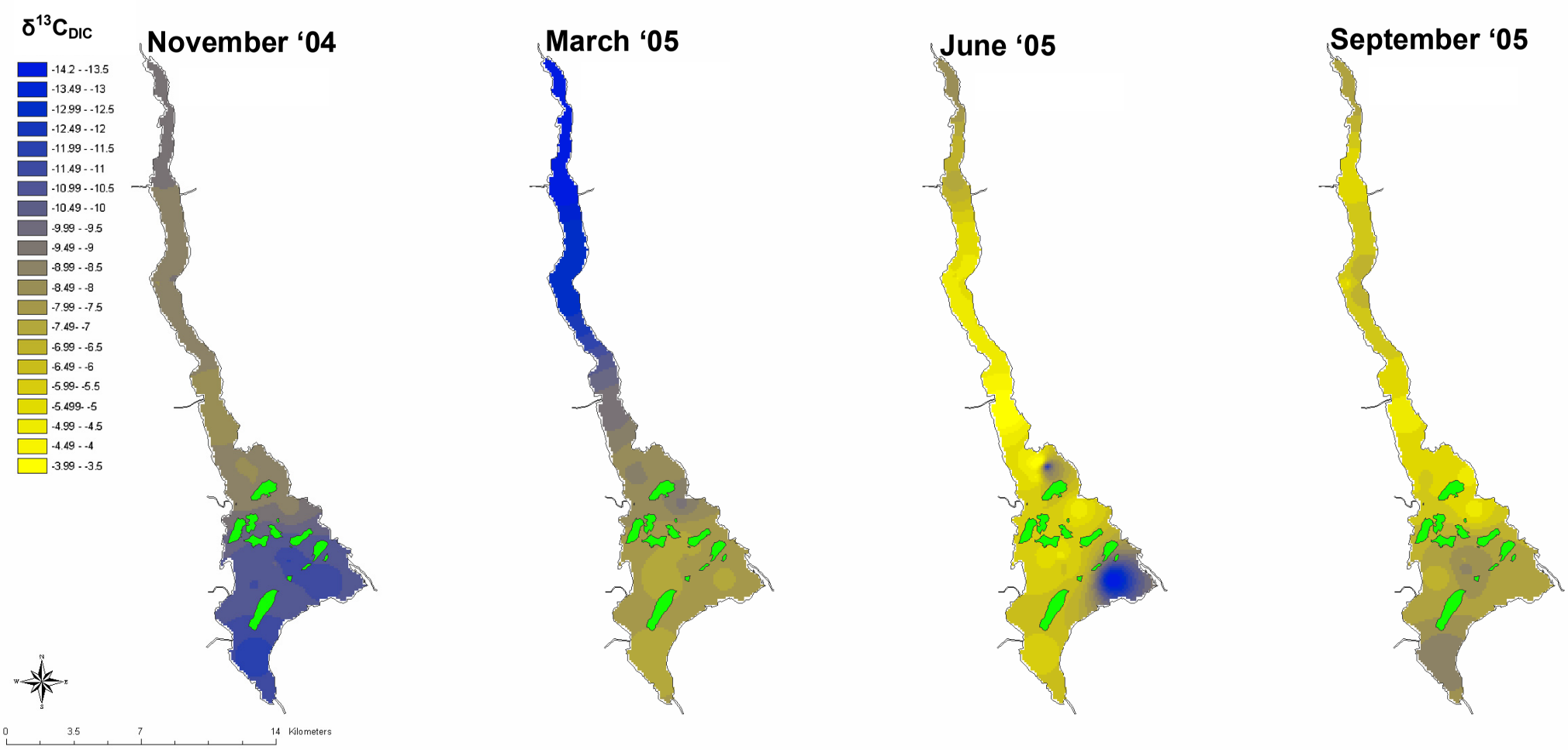

Figure 20: Epilimnetic distribution of $\delta^{13}C_{DIC}$ isotope signatures (‰) in Loch Lomond for all four months sampled. Lighter colours indicate areas of more enriched values. Islands are shown in green.

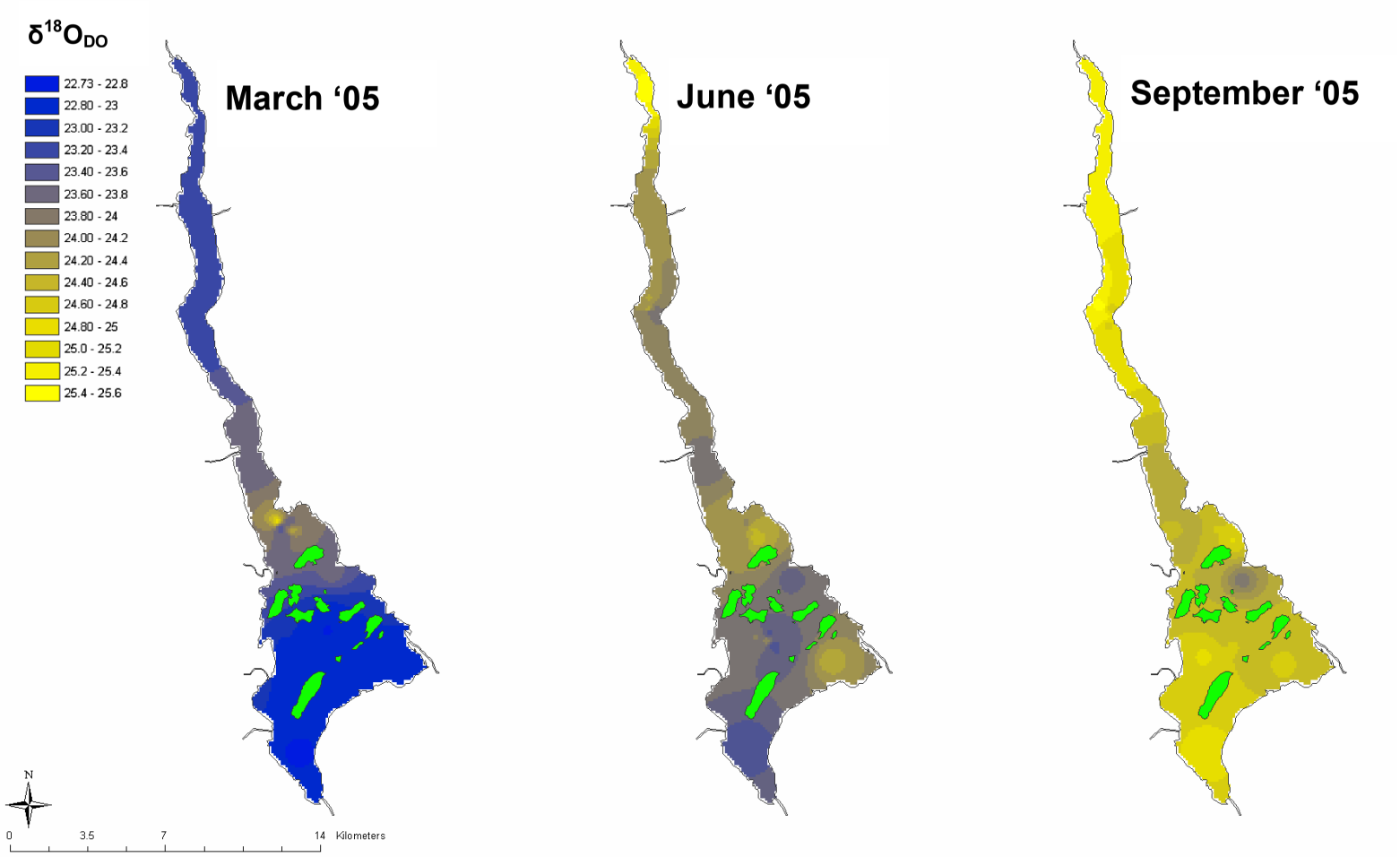

Fig 21: Epilimnetic distribution of $\delta^{18}O_{DO}$ isotope signatures (‰) for three sample months where data was available. Lighter colours indicate areas of more enriched values. Islands are shown in green

Figure 21 shows the spatial distribution of $\delta^{18}O_{DO}$ for March, June and September. The maps clearly show the overall enrichment in isotopic composition from March to September, but also smaller scale spatial variability. In March the south basin has a uniform distribution with little variation. The oxygen isotope signature then rises in the middle basin before lowering again approaching the north basin. The distribution of more enriched values expands in June. The south basin shows enriched areas in the southeast corner, as well as areas of the middle basin. The $\delta^{18}O_{DO}$ value remains quite constant at ~ 23.8‰ for the north basin until there is significant enrichment at site U1 (Fig. 9) near the mouth of the River Falloch. September has consistently the most enriched values with particular areas of high enrichment in the southwest corner of the south basin and the far north of the north basin. $\delta^{18}O_{DO}$ values for the middle basin are less enriched than the north and south basin, but interestingly, vary little from March or June. Indeed Epilimnetic averages for the middle basin do not significantly differ between months ($P < 0.001$).

2.3.5) *Generalised and localised flux of DIC in Loch Lomond*

By using concentration distributions and multiplying by the epilimnetic and non-epilimnetic depths total amounts of DIC in different sections of the lake can be estimated.

kgs DIC	November	March	June	September
Lake total	3714.8	3033.2	3907.9	3268.0
Epilimnion total	1454.0	*Not stratified*	1781.0	1314.8
Hypolimnion total	2260.8	*Not stratified*	2126.9	1953.3
South basin epi	722.7	*Not stratified*	827.6	597.3
South basin hypo	130.7	*Not stratified*	140.8	108.7
South basin total	853.4	694.1	968.4	706
Middle basin epi	489.1	*Not stratified*	608.8	451.1
Middle basin hypo	675.6	*Not stratified*	673.8	632.6
Middle basin total	1164.7	1102.9	1282.6	1083.7
North basin epi	242.2	*Not stratified*	344.7	266.5
North basin hypo	1454.4	*Not stratified*	1312.2	1212.0
North basin total	1696.6	1236.2	1656.9	1478.5

Table 2: Mass in kilograms of DIC in different sections of Loch Lomond. Lake has been divided into basin and epilimnion / hypolimnion.

Although DIC concentration decreases from the south basin to the north, the absolute quantity of DIC increases along with the greater volumes along the same gradient. In general the total amount of DIC in the north basin is approximately 100% greater than that in the south basin.

In all four periods sampled the hypolimnion contains the bulk of the DIC. The magnitude of the difference between hypolimnion and epilimnion varies between seasons, although not to a significant amount. The average proportion of DIC contained in the hypolimnion for all sampling months is 58.4% ± 3.4%. This is not representative of each basin however where the depth range changes and as such the ratio of epilimnion to hypolimnion alters also. The south basin has a high proportion of epilimnetic (<13 m) water, whereas the north basin, regularly being greater than 100 m in depth is mainly hypolimnion. Reflecting this variation in depths, the epilimnion of the south basin contains 84.91% of the DIC in the whole basin, with little variation from season to season (standard deviation of 0.47%). The percentage of the total DIC in the epilimnion decreases with distance north as the hypolimnion begins to dominate the total volume, the middle and north basin epilimnions containing 43.70 ± 3.27% and 17.70 ± 3.27% respectively.

Using the data available on total concentrations of DIC in the lake, fluxes, both absolute and relative can be calculated (Table 3). Assumptions made are that between sampling dates the concentration changes by an equal amount per day, (though this is unlikely I have no details here to assess otherwise). Rates of change were calculated using the following formula:

$$change/day_{DIC} = \frac{\left(DIC_B - DIC_A\right)}{B - A} \qquad \text{Eq.7}$$

where DIC is expressed in kilograms, a represents the date of the first sampling period, and b the date of the second sampling period. Assuming that values in November of the first year would be comparable to the second year, a rate between September and November is also estimated (although November ’05 was not sampled). For the purposes of comparing March data with other sample periods, the lake is divided into depth ranges although stratification was not present. For this the average values for epilimnion / hypolimnion discussed previously on this page were applied to each basin. For example, it is assumed that 84.91% of the DIC in the south basin is in the epilimnetic layer.

Total epilimnetic DIC reaches a minimum in March at 183,554 g DIC and peaks in June at 258,570 g DIC. This translates as a cycling rate of 658.03 g DIC produced per day. Between November and March, June and September cycling rates are ~218.18 g DIC and 762.40 g DIC consumed/lost per day respectively.

Absolute (kg) and % change	Sept - Nov	Nov - March	March - June	June - Sept
Total lake DIC	12.76 *(0.14%)*	-5.50 *(0.18%)*	7.67 *(0.29%)*	-6.95 *(-0.16%)*
South basin epilimnion	3.58 *(0.21%)*	-1.08 *(-0.18%)*	2.09 *(0.40%)*	-2.50 *(-0.28%)*
Middle basin epilimnion	1.09 *(0.08%)*	-0.06 *(-0.01%)*	1.11 *(0.26%)*	-1.71 *(-0.26%)*
North basin epilimnion	-0.69 *(-0.09%)*	-0.19 *(-0.10%)*	1.10 *(0.58%)*	-0.85 *(-0.23%)*
South basin hypolimnion	0.63 *(0.20%)*	-0.21 *(-0.20%)*	0.32 *(0.34%)*	-0.35 *(-0.23%)*
Middle basin hypolimnion	1.23 *(0.07%)*	-0.44 *(-0.08%)*	0.46 *(0.09%)*	-0.45 *(-0.06%)*
North basin hypolimnion	6.93 *(0.20%)*	-3.53 *(-0.30%)*	2.59 *(0.29%)*	-1.09 *(-0.08%)*

Table 3: Absolute and *percentage* change per day of DIC for the entire lake, as well as basin and depth specific values.

Data presented in table 3 represents minimum losses and gains of DIC in these time periods. In reality the DIC pool will have undergone numerous losses and gains in these periods and total quantities cycled could thus be greater.

The rate of change in the DIC pool varies with both time and space in Loch Lomond. Considering each time period individually there is significant variability between basins. Between November and March the epilimnion of the south basin loses DIC at a rate of 1.08 kg / day, or 0.18 % / day. The north basin loses DIC at a comparable rate (0.1 % / day) but the middle basin loses very little by comparison at only 0.06 kg / day, or 0.01% of its total stock. The hypolimnion presents a similar scenario where the middle basin flux of DIC as a percentage (0.08 %) is significantly lower than both south and north (0.2 and 0.3 % respectively).

Between March and June, during the spring period the amount of DIC increases in all basins, both epilimnion and hypolimnion. The greatest estimated accumulation rate is in the hypolimnion of the north basin, again due to its large volume, with 2.59 kg of DIC being added per day. The rate of accumulation in north basin deep water is closely followed by the epilimnion in the south, where 2.10 kg is added per day. This is a far bigger relative increase in the south epilimnetic waters as the volume is far smaller than the north, this represents an addition of 0.40 % the total DIC pool per day, compared to 0.29 % in the north hypolimnion. In this time period the biggest relative increase is observed in north basin surface waters where the amount of DIC rises by 0.58 % per day.

In the period from June to September, corresponding with the summer bloom there is a drop in DIC in all parts of the lake. DIC is most readily lost from the epilimnion of the south basin (0.28% per day), although surface water DIC loss across the entire lake is of comparable relative magnitude (0.26 and 0.23% per day fro middle and north basins), as is the hypolimnion of the south (0.23% per day).

In general between September and November there is an increase in DIC concentration, however, the surface waters of the north basin continue the loss seen between June and September at approximately 0.09% per day. This contrasts with the hypolimnion of the north, which accumulates at 0.20% per day. The south basin shows consistently the highest accumulating flux between these seasons as both the epilimnion and hypolimnion gain DIC at a rate of 0.20% and 0.21% per day respectively (3.59 and 0.63 kg / day).

2.4) *Discussion*

Loch Lomond is a monomictic lake system. It undergoes one period of complete mixing with a time of stratification during the summer months (approx May-November). During stratification the middle and north basins will both form stable thermoclines. The south basin however, stratifies temporarily in stable climatic conditions but breaks down in rough weather. In this project the south basin was only noticeably stratified in June. The north and middle basin were stratified in November, June and September.

Dissolved inorganic carbon is the primary source of carbon for photosynthetic utilisation. Previous work has shown that [DIC] can be closely associated with photosynthetic production (Heinn 1997, Jones *et al* 2001), especially when studying short (diel-seasonal) timescales, where metabolism is believed to be the driving force for both DIC and dissolved oxygen concentrations (Hanson *et al* 2006). [DIC] variation was between 0.07 and ~0.25 mM / L (Fig. 11, page 47), consistent with other lake studies (e.g., Schindler *et al* 1973, Heinn 1997 and Hanson *et al* 2006). Concentrations of DIC peaked in the summer months, which correspond to the expected peak primary production (Fig. 11, page 47). This is in contradiction to some other work (e.g., Hanson *et al* 2006) where in all of the seven lakes sampled maximum [DIC] was reached in the winter months. This may suggest that in Loch Lomond primary production could be at least partly controlled by DIC availability and not vice versa, partly supported by the observed saturation and thus loss of CO_2 to the atmosphere in Figure 18. The sampling frequency of this study may have been to low to detect the periods of maximum and minimum concentrations also. Very high respiratory rates in bacteria along side high primary production may account for the elevated [DIC]; it has indeed been shown that in cases where [DIC] is low, a decline can inhibit photosynthetic production (Heinn 1997). This could be the case in Loch Lomond for the middle and north basins especially. [DIC] in the hypolimnion (mid and

deep samples) follow a similar pattern to the epilimnetic water but with generally lower concentrations.

Water levels are continuously measured on Loch Lomond throughout the year (SEPA data). A possible explanation for [DIC] change may be dilution by increased inflow volume and subsequent lake volume. The [DIC] showed a minimal change between November and March, with a decrease of ~16%, and a maximal change between March and June with an increase of ~28%. Neither of these whole lake increases can be explained wholly by the corresponding 1.6% decrease in lake volume between November and June, or the 0.5% increase between March and June. Dilution alone cannot explain the observed changes in [DIC].

The south basin generally has the highest concentration of DIC. Two major inflows join the loch in the south basin, the Rivers Endrick and Fruin (Fig. 9). These two alone account for 47.7% of total inflow into the loch (Maitland 1981, Smith *et al* 1981), and represent the two inflows highest in DIC. Average concentration of DIC for the Endrick and Fruin were measured at 0.939 ± 0.54 mM (n = 9) and 0.443 ± 0.21 mM (n = 7) respectively, possibly accounting for the high concentrations in the south. Figure 11 clearly shows the higher concentrations of DIC in the south basin.

It has been known for some time that rates of metabolism in lakes play an important role in affecting the isotope signature of DIC (Oana and Deevey 1960), e.g., during photosynthesis the selective uptake of ^{12}C by enzymatic processes leads to enrichment in the $\delta^{13}C_{DIC}$ pool of surrounding water. If $\delta^{13}C_{DIC}$ is linked to rates of primary production by photosynthesis, and heterotrophic respiration, three hypotheses can be constructed.

i) The isotope signature will be enriched at times of high primary productivity, namely the spring / summer months;

ii) The magnitude of this temporal difference will be different between basins, with the smallest difference being found in the oligotrophic north basin, and the largest in the mesotrophic south. This would be expected as the south basin supports higher levels of primary and secondary production than the north, but this doesn't consider the greater volume and thus potentially influential area of the north;

iii) The epilimnetic waters will become ^{13}C enriched relative to deep waters (Myrbo and Shapley 2006).

$\delta^{13}C_{DIC}$ shows significant variation between the seasons in Loch Lomond (Fig. 11, page 47). Epilimnetic waters in the north basin have the largest range in isotopic values, although the pattern in each basin is similar. Previous studies have shown increases in the $\delta^{13}C_{DIC}$ signature of the epilimnion in summer months caused by higher photosynthetic activity (Herczeg 1987, Hollander and McKenzie 1991, Wang and Veizer 2000), so this would also be expected in Loch Lomond. Although other studies have recorded this pattern the magnitude of change in a lake of Loch Lomond's size is significantly more than other, smaller systems. Quay *et al* (1986) showed the range in $\delta^{13}C_{DIC}$ in the epilimnion of lake Washington to be 3.2 ‰. In the small Minnesota and Montana lakes (all less than 325 Ha and 17.5 m deep) $\delta^{13}C_{DIC}$ changed by a maximum of approx 8 ‰. Here a range over 11 ‰ through the seasons was observed. All basins reach a peak $\delta^{13}C_{DIC}$ value in June. The enrichment observed in the summer months could be related to the potentially higher amounts of photosynthetic activity at that time of year. Bade *et al* (2004) used various statistical models and showed that although the "potential" $\delta^{13}C_{DIC}$ for a lake is set by the geochemical characteristics of the watershed, metabolism can give significant variation around this baseline. This range in the data suggests $\delta^{13}C_{DIC}$ variation in Loch Lomond is caused by a combination of metabolic and inflow variability.

Contrary to the second hypothesis the magnitude of variation in $\delta^{13}C_{DIC}$ was similar in both the north and south basin epilimnetic water (Fig. 11) If the level of enrichment is directly related to rate of primary production, this suggests primary production could be similar in both north and south? We know from other data related to PP (Total phosphorus, chl *a*, etc) that PP is highest in the south, especially during the summer months (SEPA report, 2006). However, during incubation experiments carried out in 2006 / 07, no significant difference was observed in primary production levels (chapter 5). Likely more temporal resolution would be required to ascertain for certain any variability. The situation of equal ranges in $\delta^{13}C_{DIC}$ becomes more understandable when considering production to respiration ratios as opposed to just PP. Although PP causes enrichment in the DIC pool, respiration (as well as acting on the DO pool, discussed later) has the opposite effect. The amount of dissolved organic matter / carbon (DOM / C) in a system has consequences for both autotrophic and heterotrophic organisms. Heterotrophic bacteria use labile forms of DOM as a direct source of carbon for respiration (Pomeroy 1974, Azam 1983), but at the same time, high concentrations of DOM can inhibit photosynthetic activity in the water column (Jones 1998) most probably by light attenuation. Patterns of DOM distribution in Loch Lomond show the south basin receives considerably more from its inflows than the north. Mean DOC concentration in the north for June is 2.98 ±

1.25 mg/l, considerably lower than the south basin (4.63 ± 1.30 mg/l). It could be suggested that although photosynthetic rates in the south may be higher, the greater level of bacterial breakdown of available allochthonous DOM may be limiting the enrichment of the DIC pool and they appear similar. It was observed the following year via direct bacterial production estimates that the south basin supported significantly more heterotrophic activity than the north basin, and could likely be a contributing factor to similar $\delta^{13}C_{DIC}$ range (see chapter 5).

Patterns in $\delta^{13}C_{DIC}$ in the hypolimnion vary between basins (Fig. 11). In Figure 11 surface values represent epilimnetic values, with middle and deep representing hypolimnetic. The south basin has no significant difference between epilimnion and hypolimnion except in June when there was a temporary thermocline. The north basin however, has a very stable signature in the hypolimnion, showing only a slight enrichment in the summer months. This may possibly be due to export of more enriched organic matter from the more productive epilimnion, but it suggests respiration is the dominant process for the majority of the water column in the north.

Changes in carbon isotope composition with depth support the idea that, at least in part, metabolic processes control the $\delta^{13}C_{DIC}$ signature. Whenever the lake was stratified there is a significant depletion from epilimnion to the hypolimnion, reflecting photosynthetic export of ^{12}C from the epilimnion and its subsequent remineralisation in the hypolimnion, caused by bacterial processing of phytoplanktonic biomass enriched in ^{12}C during the photosynthetic pathways (Myrbo and Shapley 2006). Measurement of $\delta^{13}C_{DIC}$ here is of particular help as no significant change in [DIC] was observed with depth, but $\delta^{13}C_{DIC}$ often decreases with increasing depth as respiration begins to dominate.

The calculation of partial pressure of CO_2 in the lake (Fig. 18, page 54) provides further evidence that $\delta^{13}C_{DIC}$ is driven by metabolic processes and not the physical consequences of diffusion. In-flux of atmospheric CO_2 into a water body will drive the $\delta^{13}C_{DIC}$ towards 0‰ (Deuser *et al* 1967). The opposite would thus be predicted as CO_2 egression occurs. In this study the highest $EpCO_2$ values occurred in June (Fig. 18), implying the highest rates of CO_2 egression from the water column. If $\delta^{13}C_{DIC}$ was driven by this process this should correspond to the most depleted signatures, which is opposite to the measured variability. The consistent saturation of the Loch Lomond water column is the first direct evidence that heterotrophic breakdown of allochthonous matter may be of significance (Cole *et al* 1994).

In general if photosynthesis is the dominant process over respiration $\delta^{18}O_{DO}$ will tend to be less than 24.2‰ (Hanson *et al* 2006), and if respiration is dominant $\delta^{18}O_{DO}$ will tend to be greater than 24.2‰ (Quay *et al* 1995). In this work there is no direct

data suggesting this threshold to be applicable, and as such is not strictly adhered to. Rather more enriched values are used to infer respiratory dominance and more depleted values to infer photosynthetic dominance.

The similarities in responses to biological processes, but different responses to physico-chemical processes in $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ (Hanson *et al* 2006) allow more confidant conclusions to be drawn about the lake biogeochemistry. If the observed changes in $\delta^{13}C_{DIC}$ (more enriched in the summer, more depleted with depth) were caused by metabolic activity and a shift between photosynthetic and respiratory dominance, we would expect the opposite pattern in the $\delta^{18}O_{DO}$ signatures (more depleted in summer, more enriched with depth). $\delta^{18}O_{H20}$ showed no significant variability throughout the lake (Appendix. 1) so is likely not a cause for much of the observed $\delta^{18}O_{DO}$ variability.

$\delta^{18}O_{DO}$ becomes more enriched over the summer months, reaching a peak in September, three months after the peak in $\delta^{13}C_{DIC}$ (Fig. 11). Respiration increases along with photosynthesis as the phytoplankton supply a valuable source of autochthonous, labile dissolved organic material, (via excretion, exudation, lysis, etc), which heterotrophic organisms readily break down during such bloom events (Lancelot 1983, Jumars et al 1989). Thus I hypothesise that the $\delta^{18}O_{DO}$ and therefore the relative importance of respiration could continue to increase after the autotrophic peak has subsided. Even after the bloom event large quantities of organic material may remain from the dead / dying autotrophs that supply a food source for heterotrophs. This accompanied by the return of autumnal weather, with storms and the fall of leaves etc bringing more labile organic material into the loch may fuel high respiratory rates. Indeed, the south basin epilimnion in particular showed a significant peak in bacterial production in September (see chapter 5).

Depth has a significant influence on $\delta^{18}O_{DO}$ with values becoming more enriched with the transgression from epilimnion to hypolimnion. This enrichment supports the conclusion that depth related changes in both isotopes are caused by a change in the photosynthesis to respiration ratio. $\delta^{18}O_{DO}$ becomes more enriched as respiration rises and becomes relatively more important. $\delta^{13}C_{DIC}$ does the opposite. This is the pattern observed with increasing depth at all sites when not completely mixed (as in March, Fig. 11). This $\delta^{13}C_{DIC}$ – $\delta^{18}O_{DO}$ interrelationship suggests that metabolism and not other physical factors, is the driving force behind the observed isotopic variation. Indeed when plotting $\delta^{13}C_{DIC}$ against $\delta^{18}O_{DO}$ (Fig. 17) clear significant relationships ($P < 0.001$) are observed at all times except mid winter when metabolism is at its lowest.

The epilimnetic distribution of $\delta^{18}O_{DO}$ values vary between month and basin. Mean epilimnetic $\delta^{18}O_{DO}$ in the middle basin remains statistically homogenous during the

study period. One interpretation of this pattern could be it is the only basin lacking a large inflow so not subject to widely varying nutrient or DOM inputs, unlike the south and north basins, thus production may not peak and trough in response to fluxes of nutrients and their subsequent utilisation. In general the south basin is more ^{18}O-depleted than the north basin. This may be a reflection of higher PP in the south basin, an assumption that was tested by primary productivity measurement (chapter 5) and shown to be false, although again sampling frequency may have been to infrequent to catch bloom events in each basin and there may still be different productivity values. The north basin, is at most an oligotrophic system, and regularly ultraoligotrophic in the winter months. Primary production is limited to effectively non-existent at these times. Respiration is the dominant process for most, if not all of the year, particularly when considering depth-integrated values due to the large areas of hypolimnion.

The pattern of spatial distribution in the epilimnion of $\delta^{13}C_{DIC}$ is regularly mirrored by $\delta^{18}O_{DO}$. In June for example, the areas of $\delta^{13}C_{DIC}$ depletion in the southeast corner and the east coast of the middle basin, are accompanied by local areas of enrichment in the $\delta^{18}O_{DO}$ signature. A similar response is also observed in the southwest corner, near the River Leven outflow in September. This supports the idea that $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ are each influenced by metabolic processes, and respond in opposite fashions to photosynthetic and respiratory dominance. Thus it may be possible to utilise these two, biologically linked pools to ascertain metabolic balance and its variability.

DIC distribution and stable isotope composition has been studied for some time to elucidate patterns in metabolism over temporal scales. Patterns in vertical distribution through the water column have been classified thoroughly. However, many studies have, and still do use single point sampling to represent what are, in some cases, large water bodies. There has been much evidence in the past that planktonic, horizontal distributions can vary due to wind (George and Edwards 1976), edge effects (Laybourne-Parry *et al* 1990, Laybourne-Parry and Rogerson 1993) and variable catchment characteristics (George and Jones 1987). Work on Loch Ness in 1993 (Jones *et al* 1995), and in Lake Windermere (2004) showed wind again to be the driving force behind this heterogeneity in water column plankton distribution. Although, as shown, a good body of work exists looking at plankton distributions, to my knowledge this is the first, detailed spatial survey over a combined horizontal and vertical gradient, of stable carbon and oxygen isotopes in a water body of this size.

Significant spatial heterogeneity was observed in the $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ signatures in Loch Lomond (Fig. 11, 20 and 21) Changes with depth were as predicted by previous work, and have been discussed already. Significant variability in both $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ was also observed at different sites. Due to the different trophic status of the north, middle and south basins we would expect latitudinal variation as productivity and the production: respiration (P: R) changes. This pattern has been described previously; with $\delta^{13}C_{DIC}$ becoming more depleted further north and $\delta^{18}O_{DO}$ the opposite. This change was shown to be significant in both cases for all months except March, when as previously discussed the loch is well mixed and relatively unproductive. However, figures 19 to 21 reveal that a simple latitudinal gradient doesn't explain the variation observed. The south basin shows significant variability at different sites. For example, water around the mouth of the Endrick in June is over 6‰ more depleted than the rest of the south basin water (likely a result of the addition of depleted water from the river). This is the most extreme case of relatively small-scale variability, but other cases are clearly visible.

Predicting the variation shown is not possible with the data set currently available. More detail on both the temporal and spatial scale would be needed for this. Loch Lomond is a complex water body and numerous factors affect the hydrological as well as biological patterns. The north basin is relatively simple in structure, much like Loch Ness, being a deep, narrow trough. Water enters mainly through the Falloch inflow and drains south. Predicting patterns in isotope change for this basin may be possible as the inflowing water from the Falloch is likely the driving force behind the isotope signatures for much of the time, and this isotopic signature (either enriched or depleted depending on time of year) seems to spread south, being diluted as it does. South basin spatial variation is complicated by other factors. The islands in the south basin lead to complex hydrological patterns. Coupled with varying wind direction, water flow directions can changes significantly.

In conclusion it has been observed that Loch Lomond exhibits both temporal and spatial variation in [DIC], $\delta^{13}C^{DIC}$ and $\delta^{18}O_{DO}$.

Temporal patterns match that observed in other studies (e.g., Quay *et al* 1986, Bade *et al* 2004, Myrbo and Shapley 2006) for $\delta^{13}C_{DIC}$, reaching the most enriched values in the summer months and the most depleted in the winter. This is believed to be mainly due to varying levels of primary productivity and the selectivity of the enzymatic processes in photosynthesis. Temporal changes in $\delta^{18}O_{DO}$ showed a similar pattern, becoming more enriched in the summer months as respiration rates increase due to higher temperatures and dissolved organic material availability. The

fact that $\delta^{18}O_{DO}$ values are consistently over 24.2‰ suggests respiration is often as/more important than photosynthesis in lake nutrient cycling.

Spatial distributions show heterogeneity across the loch. This ranges from changes between the epilimnion and hypolimnion (seen in $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ but not [DIC]) which has been described in previous studies, to complex and variable distributions of these isotopes in the epilimnion of the lake. Hydrological patterns as well as biological processes vary isotopic compositions significantly between areas of the loch. The largest and most consistent pattern is with latitude. In the south basin however, there is significant variation in both carbon and oxygen isotopes that is latitude independent. Local areas of enrichment and depletion are found, varying by as much as 6‰ in $\delta^{13}C_{DIC}$ over relatively small scales. The variation we see here in all but the most stable periods (very calm preceding weather in March) suggest that single point sampling for Loch Lomond would risk statistically significant errors. Whether this applies to smaller lakes with simpler hydrological regimes is unclear but we suggest consideration of this fact at least.

Both temporal and spatial variability in [DIC], $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ have revealed a potentially, biologically complex system, likely dictated by a combination of watershed characteristics, hydrological cycles and metabolic balance. The potential variability in the balance between phytoplanktonic and bacterial production pathways means elucidating patterns in an overall metabolic balance that may be dynamic. In order to provide more insight into these questions other parameters are subsequently considered, the first being variability in dissolved organic matter / carbon and what its concentration, isotopic composition and stoichiometry can tell us about lake functioning, and what further insight they can provide to conclusions drawn in chapter 2.

Chapter 3

Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) dynamics in Loch Lomond. Implications for heterotrophic microbial processes

3.1) Introduction

Dissolved organic matter (DOM) is often the largest pool of reduced carbon and nitrogen in freshwater aquatic ecosystems, and therefore the largest source of carbon and reduced nitrogen to microbial communities (Azam *et al* 1983, Hobbie and Wetzel 1992, Kaplan and Bott 1983, Volk 1997, Ziegler and Fogel 2003). The largest elemental component of DOM is often carbon and as such DOC will mainly be considered from now on. The importance of DOC to driving aquatic carbon/nitrogen cycles means that ever more detailed understanding of sources, fate and flux is desirable. The complexity in organic carbon cycles in freshwater systems stems from the fact there can be numerous sources of DOC to the system. In the majority of the oceans autochthonous DOC (produced in the water column by bacteria, phytoplankton and higher trophic levels) is the dominant source (Hedges 1992). In littoral and lacustrine systems however, DOC can be derived from autochthonous and allochthonous (from the drainage basin) sources. In catchments of high peat content and base-rich soils contribution of DOC from terrestrial sources can be significant (Aitkenhead-Peterson *et al* 2003).

In this work, although acidification procedures were carried out to remove inorganic carbon, other inorganic substances remained. As such total dissolved solids (TDS) were extracted, not DOM, though much of the discussion will relate to both.

Often large amounts of DOM can be transported into aquatic systems, for example rivers transport between 0.4 - 0.9 Pg / C / Yr to the oceans (Schlesinger and Melack 1981, Degens 1982, Degens *et al* 1991). Such quantities render terrestrial influx important when considering metabolism in aquatic environments. External input of dissolved material provides a supply of nutrients for heterotrophic bacteria to utilise for respiration and biomass production. It is now largely accepted that this external input of DOM is responsible for many lakes in the boreal and temperate zones being net heterotrophic environments (e.g., del Giorgio *et al* 1997), although the majority of

DOM imported (up to 75%) is often refractory and not readily useable by lake bacteria (Wetzel 1984).

Autochthonous sources of DOM are the most readily available and utilised by aquatic bacteria. Sources vary from algae and macrophytes (Munster 1993), from bacteria, and from higher trophic levels (e.g., zooplankton, fish, etc). Although macrophytes can be a significant contributor to the DOM pool in shallow lake systems, the general deep depths and small percentage of littoral zones in my study site is such that their contribution will be minimal and not considered further. It is therefore assumed that pelagic algae will be the dominant supplier of autochthonous DOM to the system.

Algae synthesise organic material from inorganic constituents (Chapter 1, section 1.1). There are three direct pathways of DOM from algae to the water column. Phytoplankton often exudate a significant proportion of their photosynthate in times of either high productivity, or nutrient stress (Lancelot 1983, Baines and Pace 1991). As much as 60% of all organic material synthesised can be lost to the water column in this way (Bertilsson and Jones 2003). After death the aging and decay process (senescence) of phytoplankton cells releases organic matter to the water column. Destruction of phytoplankton cells via zooplankton grazing could also lead to a significant supply of DOM to the surrounding water and the bacterial communities (Jumars *et al* 1989). More detail on sources and fluxes of DOM is described in chapter 1, section 1.6.

In the open ocean and in lakes with little terrestrial input, the contribution of autochthonous DOM can make up the bulk of DOM inputs. However, for the majority of lakes the supply of terriginous (allochthonous) DOM is far greater in quantity. When it comes to DOM as a source for bacterial utilisation however, quality is as, if not more important than quantity (Goes *et al* 1996). DOM is picked up as rain falls on the land, passes through vegetation, infiltrates the soil organic horizon and percolates through the soil mineral horizons (Aitkenhead-Peterson *et al* 2003). During this passage DOM is both added and lost, but in general, the further down stream the more refractory DOM becomes. Cole *et al* (1984) recorded the concentration of high molecular weight (HMW) DOM decreasing and low molecular weight (LMW) DOM increasing with distance from the stream source. Interpreted to represent bacterial breakdown during transportation. This biological breakdown coupled to adsorption to soil particles can limit the amount of usable DOM that reaches a lake (Yano *et al* 2000). Processes which change the proportion of labile / refractory DOM change with

varying characteristics of the catchments, such as soil type, land use, slope of the watershed, height of the water column, etc, such that both the quality and quantity of DOM to a system can vary significantly over both time and space. Loch Lomond presents an ideal site to consider DOM characteristics due to varying basin/catchment characteristics (Chapter 1, section 1.8). Organic material transported through northern basin catchments is liable to be exposed to significantly different degradation/addition steps than that of the south.

With the significance DOM / C has in aquatic systems, a more detailed understanding of its cycling and functioning is of importance. As such this work will address three main hypotheses:

i) As with the dissolved inorganic carbon / oxygen work (chapter 2), DOC / TDN will show variability on a temporal and spatial scale. Peak DOC / TDN concentrations will likely be recorded in the productive seasons and autumn, related to biomass production and increasing allochthonous input respectively.
ii) The balance between allochthonous and autochthonous sources of TDS will vary over time. Considering initial evidence of supersaturation of CO_2 in Loch Lomond (chapter 2), TDS sources will mainly be of allochthonous origin.
iii) DOM concentration and isotopic composition will vary on a spatial scale reflecting the significant morphological and hydrological variability observed between the basins of Loch Lomond.

3.2) *Materials and Methods*

3.2.1) *Pre-Field DOM.*

DOM samples were collected in 2-Litre Tetrapak polyethylene bottles. Before each bottle was used they were acid washed for a minimum of 24 hours in 5 M nitric acid (HNO_3) to remove any organic material. Each bottle was then rinsed with copious amounts of distilled water and dried for ~ 6 hours at 60^{o}C, when they were capped with an acid washed polyethylene screw cap.

3.2.2) *In-Field DOM.*

Once water was collected for DIC, dissolved oxygen (DO) and water ($\delta^{18}O_{H2O}$) analysis the remaining water from the water sampler was decanted into the pre-washed polyethylene bottles. Whilst on the boat, to try and minimise any effect temperature may have on rates of metabolism, and thus potentially the parameters

later analysed, bottles were stored under a dark, damp cloth to minimise heat loss / gain before landing. Once landed bottles were immediately frozen between –20 and –40^{o}C to cease any metabolic activity, and stored like this until the following stage of processing.

3.2.3) *Post-Field DOM.*

DOM samples were defrosted by submersion in hot water for approximately 30 minutes. Often the sample was allowed to begin defrosting overnight in a refrigerator. The defrosted sample was filtered through a Millipore Sterefil Aseptic System under vacuum (Buchi V-500 vacuum pump, pressure ~20 mg / Hg) loaded with a pre-combusted (6 hours at 450^{O}C) GF/F filter (nominal pore size 0.7µm). The filtrate was transferred to a 2L acid washed glass flask, for use on a rotary evaporator for concentration. As with the collection bottles, filtration units were acid washed prior to use. GF/F filter papers were pre-combusted before use to eliminate any organic matter. The filter paper containing the particulate material was immediately removed and frozen until processed for chlorophyll analysis.

DOM was concentrated by rotary evaporation, using Buchi Rotavapor R-200, controlled by a V-800 vacuum controller and heated in a B-490 heating bath. Vacuum pressure was maintained at 72 mbar and water temperature at 60^{O}C. For the first two trips (Nov and Mar), the significance of DIC in the sample was not realised and as such the entire sample was rotary evaporated as one. However for the last two trips (Jun and Sept), samples were split and one half was acidified, to remove DIC from the sample (which affected the DOC isotopic signatures). To the acidified half, was added 0.1M sulphuric acid, until a pH of ~ 4.0 was reached. To obtain more accurate [DOC] value for November 2004 and March 2005, the average % composition DIC made up of the entire carbon pool in incubation experiments (Appendix 4) was subtracted from bulk DOC values.

Once rotary evaporation was complete, DOM concentrate was pipetted into an acid washed, pre-weighed glass beaker, and covered with a pre-combusted GF/A filter paper (to prevent contamination). The beaker was frozen prior to freeze-drying to a powder, usually a 48 hour process. Samples were freeze dried as this yielded an easily manageable substance for mass spectrometry preparation and the sample could be indefinitely stored in a desiccator with little chance of physical / chemical alteration. Samples collected in November ‘04 and part of March ‘05 were freeze-dried at SUERC in a Christ Alpha 1-4 freeze dryer. Subsequent samples were freeze-dried in Glasgow University using a Christ Alpha 1-2LD-freeze dryer connected to a vacubrand 2.5 pump.

Following freeze-drying the beaker containing TDS isolate was weighed, homogenised (ground with a spatula for 30 seconds) and scraped into 6 ml glass vials for storage, which were sealed with lab sealant whenever not being used to limit any moisture addition. ~2 mg of each homogenised sample was weighed into a 5 x 7 mm tin cup for stoichiometric and isotope analysis at SUERC.

Isotope ratios were determined by a Finnigan Delta Plus mass spectrometer linked to a Carlo Erba NA1500 elemental analyser (EA), by a Finnigan ConFlowII interface. The EA operates based on a flash combustion method in which the tin capsules containing sample are dropped into a combustion furnace at 1020 ^{o}C. A pulse of ultra high purity oxygen, which raises the temperature further to > 1700 ^{o}C is passed. The gases created in the combustion are then passed into sequential oxidation and reduction columns (~ 650^{o}C) in the EA's furnace. The gases created (CO_2 and N_2) are separated in the gas chromatography (GC) column after passing through a Nafion water trap with a helium carrier flow at ~60 ml min^{-1}. The helium vent is attached to the ConFloII interface via a stainless steel line. The ConFloII interface controls the introduction of gases, both sample and reference, into the ion source of the mass spectrometer via a fused silica capillary.

In preparation for DOC stable isotope analysis, different concentrations of gelatin were analysed at the start of the run to correct for linearity effects, and then at least every 10 samples to correct for drift. An internal control standard was analysed separately to assess accuracy, and a series of different concentrations of tryptophan were run as a second check and for stoichiometric measurements. Results were accurate to ± 0.1 ‰ for carbon and ± 0.3 ‰ for nitrogen.

3.3) Results

3.3.1) *Influence of Basin, Depth and Season on TDS.*

Total dissolved solid samples were collected at four intervals between November 2004 and September 2005.

Average [TDS] shows significant ($P < 0.001$) variation with basin and season, but not with depth ($P = 0.189$) (Fig. 22). For all three basins the concentration of TDS increases throughout the year from November '04 to September '05. Peak concentrations are reached in the hypolimnion of the south basin in September at 53.73 mg/L with minimum levels observed in middle depth water of the north basin in November (18.94 mg/L). Insignificant difference between depths is observed in every

sampling trip, except September in the south basin, where [TDS] is significantly different greater in the epilimnetic, compared to hypolimnetic water (P = 0.016).

If the percentage of DOC in the TDS remains constant then we would expect its relationship with depth, basin and season to be the same also. [DOC] was found to vary significantly with depth and basin (P = 0.031 and P < 0.001 respectively), but not with season (P = 0.298) (Fig. 22). These results show that in the loch, although the concentration of TDS increases during the summer / autumn months, the concentration of DOC does not, suggesting the TDS is increasing in other components, either organic or inorganic. More detailed breakdown of the data shows that the concentration of DOC does vary with season in surface waters for all basins (P < 0.001), but not in the hypolimnetic depth range (P = 0.295). For epilimnetic waters the general pattern is of highest [DOC] in June. The highest average concentration was in the south basin epilimnion (3.70 ± 0.19mg/L) although the hypolimnetic waters were of a similar magnitude. Like [TDS] the lowest values were recorded in March in the middle and north basins, although low concentrations were found in September in the south basin, unexpected as [TDS] was at its peak.

Total dissolved nitrogen (TDN) concentration was far lower than [DOC] and made up a far smaller percentage of the TDS pool. Values ranged from ~0.2 to 1.0 mg/L. Figure 27 shows the complex patterns of TDN distribution. Course analysis shows that only basin has a significant effect on [TDN] (P = 0.002). Both depth and season show no such significant relationship (P = 0.555 and P = 0.073 respectively). Figure 22 although shows for certain periods these bulk results are questionable, as at certain times of the year depth variation seems pronounced. Post-hoc analysis shows that March data is significantly different from June and September (P = 0.041 and 0.047), and although November is not significantly different from June or September, it is not highly insignificant for either (P = 0.079 and 0.090). [TDN] seems stable between November and March, in all basins but particularly north and middle. Variation becomes greater after March in all three basins, and the largest difference between depths is observed. This corresponds to lake stratification being observed. Between June and September, mean deep values in the middle basin rise sharply to surface water levels, while in the north basin epilimnion values decline sharply below that of hypolimnetic water.

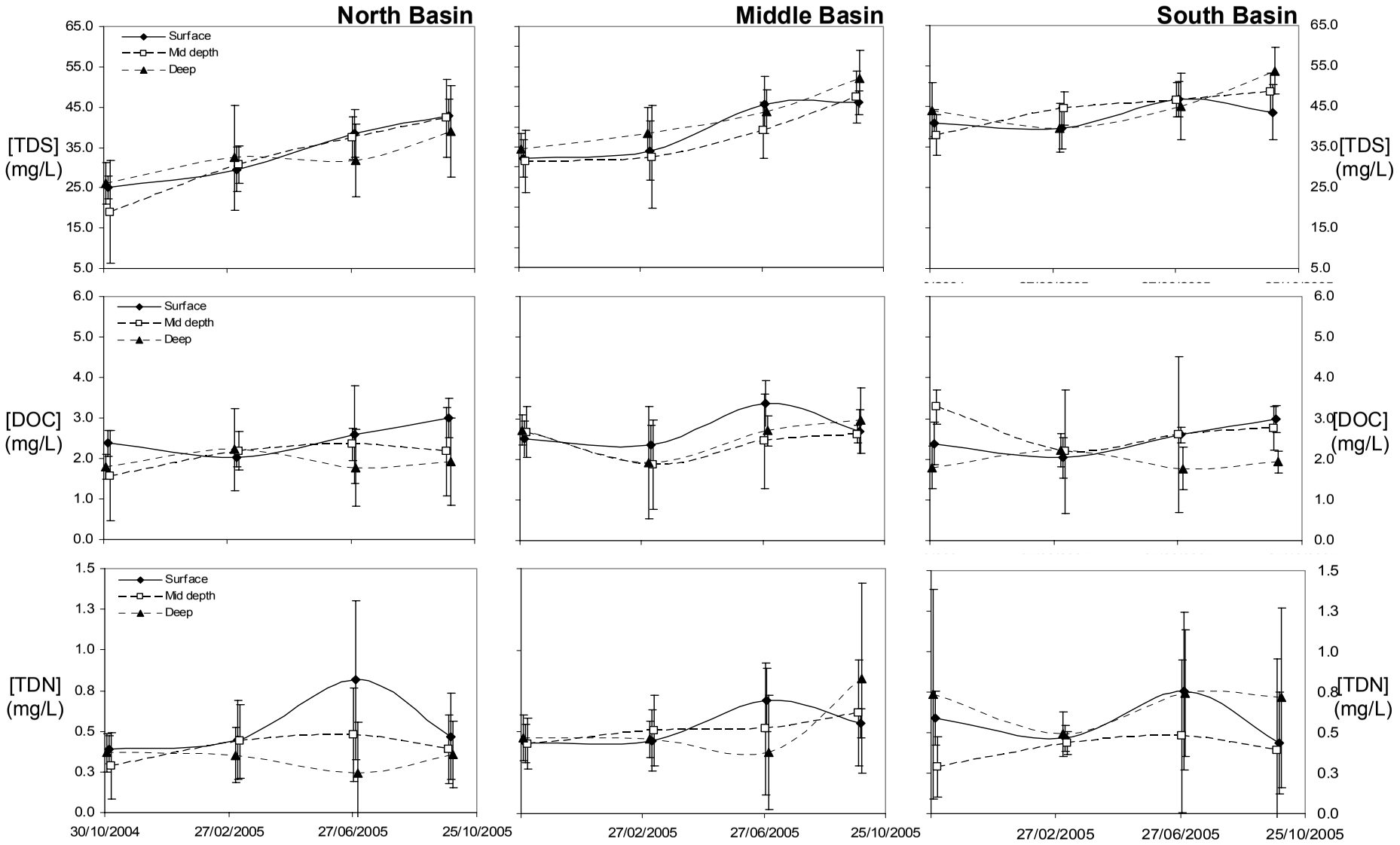

Fig 22: Seasonal variation in a) [TDS], b) [DOC] and c) [TDN]. Data divided into basin (south, middle, and north) and depth (Surface, middle, Deep). Each point represents the mean value for the whole basin. Data points plotted one day apart to allow resolution between error bars. Error bars represent ± 1 SD.

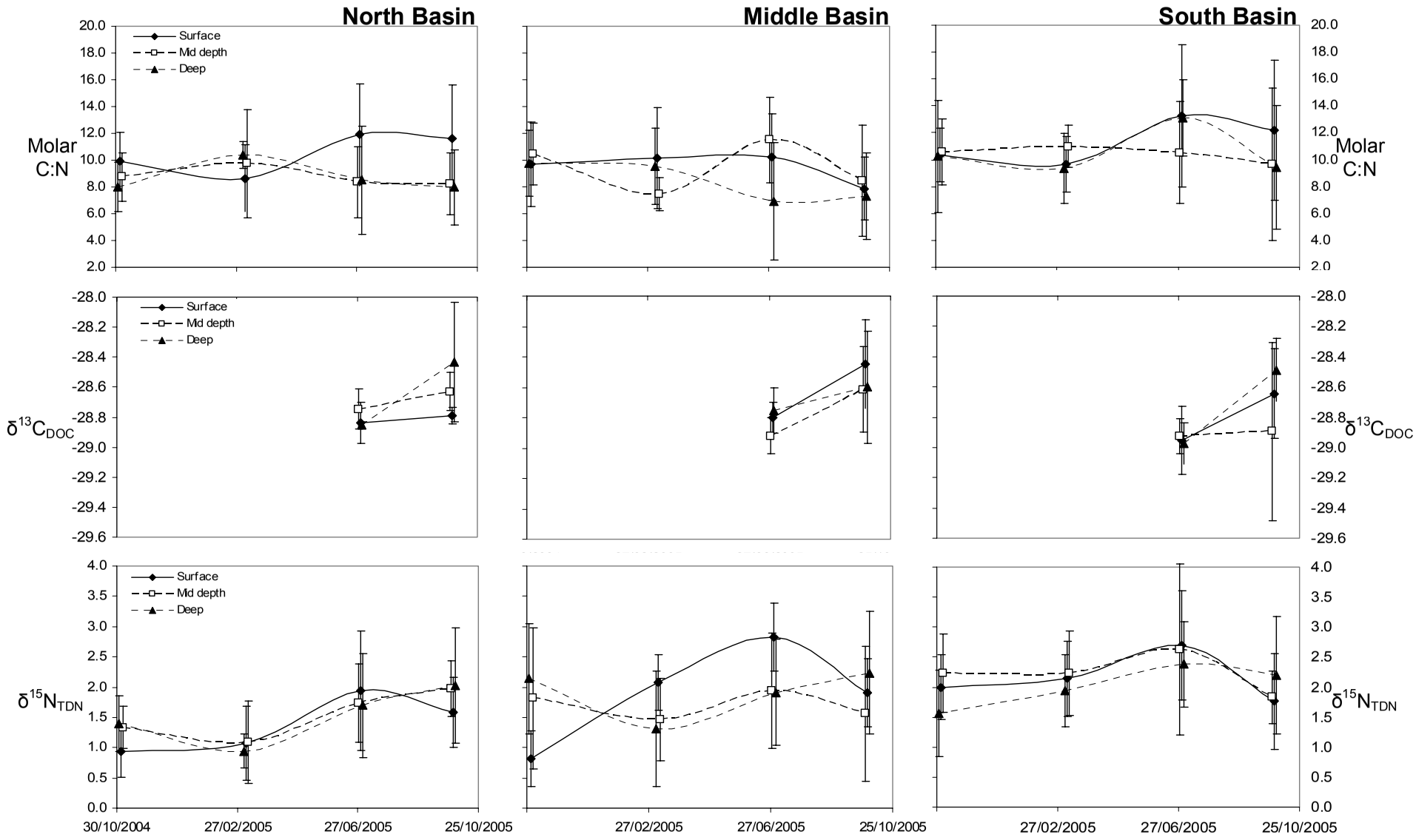

Fig 23: Seasonal variation in a) Molar C:N of DOM, b) $\delta^{13}C_{DOC}$ and c) $\delta^{15}N_{TDN}$. Data divided into basin (south, middle, and north) and depth (Surface, middle, Deep). Each point represents the mean values for the whole basin. Data points plotted one day apart to allow resolution between error bars. Error bars represent ± 1 SD.

Molar C:N of total dissolved solids showed variable seasonal patterns depending on depth and basin, although overall no significant effect caused by month or depth was observed (P = 0.152 and P = 0.050 respectively). However, Figure 23 shows that patterns are variable such that bulk statistical analysis is of little use. In the north and south basin epilimnion, molar C:N reaches a minimum in March '05 (8.64 and 9.66 respectively) and peaks in June (11.89 and 13.25), where values begin dropping again. North basin epilimnetic waters show a significantly different cycle from that of the deeper layers. Hypolimnion molar C:N values peak in March at ~10 with the rest of the year relatively constant at ~8. The south basin epilimnion too shows differences to mid depth water. Deep water in the south basin follows the same pattern as the epilimnion, but drops significantly more after the summer productivity high between June and September. The mid-depth water shows no significant difference over time (P = 0.211) although does appear to drop slightly between March and September. Mid-depth water in the middle basin follows the same seasonal cycle as surface water in north and south, peaking in June with a low in March. Surface water shows no such similarity, remaining constant at ~10 until dropping in September. The deep water drops earlier to ~7 in June.

$\delta^{13}C_{DOC}$ data is only available for June and September (Fig. 23) as November and March data was unable to be corrected in a similar manner to [DOC]. Variation is small, ranging from –29.0 ‰ to –28.4 ‰. $\delta^{13}C_{DOC}$ does not vary significantly with depth or basin between these two sampling times (P = 0.135 and P = 0.107) but varies significantly with season (P < 0.001). In all three basins there is a general trend towards more enriched $\delta^{13}C_{DOC}$ in September than in June, although how it changes at different depths appears to vary between basins. In the north basin deep water shows the largest increase from -28.9 ‰ to –28.4 ‰. It is epilimnetic water in the south too that shows the greatest enrichment (-29.0 ‰ to –28.5 ‰). However, in the middle basin both intermediate and surface waters show a greater enrichment over the same time period. Although not included in the graphical representation or the statistical analysis two samples from March were acidified also. One from surface waters and one from middle water, both in the north basin. The surface water value was –28.5‰, which is more enriched than June and September. The middle depth is even more enriched at –27.4‰. Both these results suggest that there is a possibility of more enriched $\delta^{13}C_{DOC}$ in March, but variation of 0.5 ‰ or more recorded in June and September suggests variability within the basin could be significant and using these two values as representative could be uncertain.

$\delta^{15}N_{TDN}$ (Fig. 23) shows seasonal variation ($P < 0.001$), with the most enriched signatures generally being recorded in June for all basins. The highest values are typically recorded in the epilimnion; even although on average depth has no significant interaction with $\delta^{15}N_{TDN}$. Variation with basin was also found ($P < 0.001$) with more enriched signatures occurring further south. Different basins also gave rise to variable seasonal patterns. For example, no significant difference between November and March was recorded at any depth in the north and south basins. However, contrary to patterns observed in these two basins, the middle basin surface water showed a significant increase between these months. The general pattern in the north and south basin is of depleted $\delta^{15}N_{TDN}$ in winter, becoming more enriched in the summer and the falling again in the autumn. Variation from this pattern is found in the north basin between June and September where enrichment continues in middle and deep water, and in the south basin deep water where no significant depletion occurs. The middle basin surface water had a different seasonal cycle, with signatures becoming more enriched all the way from November to June, and then falling in the autumn. Although bulk analysis revealed an insignificant effect of depth on $\delta^{15}N_{TDN}$, more detailed analysis showed this varied with the time of year (Month*Depth $P = 0.037$). For example, the middle basin surface and deep waters are different in November, March and June.

3.3.2) *Controls on [TDS], [DOC], [TDN] and molar C:N.*

During certain times of the year, temperature has a significant relationship with [TDS], [DOC] and to a lesser extent, [TDN], via its potential control on organic matter producing / consuming processes. Periods of higher temperature correspond with elevated [TDS], probably reflecting the different inflow regimes of the different basins, which have previously been shown to have different temperature characteristics (Chapter 2, Fig. 10), along with increased levels of autochthonous production. Only in September does temperature have no predictive power of [TDS] ($R^2 = 0.002$, $P = 0.706$). The concentration of TDS can be explained only partially by temperature range (R^2 from 0.199 to 0.203) between November and June, suggesting more than the described inflow variation between basins is responsible. Temperature variability can describe significant amounts of the observed [DOC] variation in November, March and June also ($R^2 = 0.337$, 0.318 and 0.548 respectively, all $P < 0.001$). However, [TDN] is only significantly correlated with temperature in June ($R^2 = 0.275$, $P < 0.001$). Temperature had no significant predicting power on molar C:N of TDS in any sampling month.

Dissolved inorganic carbon is added to aquatic systems, amongst other methods, by production during respiration. Bacteria utilise DOM and inorganic nutrients (mainly P and N) for heterotrophic breakdown so theoretically the concentrations of DOC / TDS and DIC may be linked. The concentration of DIC increases linearly with DOC (Fig. 24a) for November, March and September. November had the strongest correlation (R^2 = 0.541, P < 0.001) and the steepest increase of DOC. March and June showed similar slopes with a more gradual increase in DOC with DIC. September showed no correlation (P = 0.096).

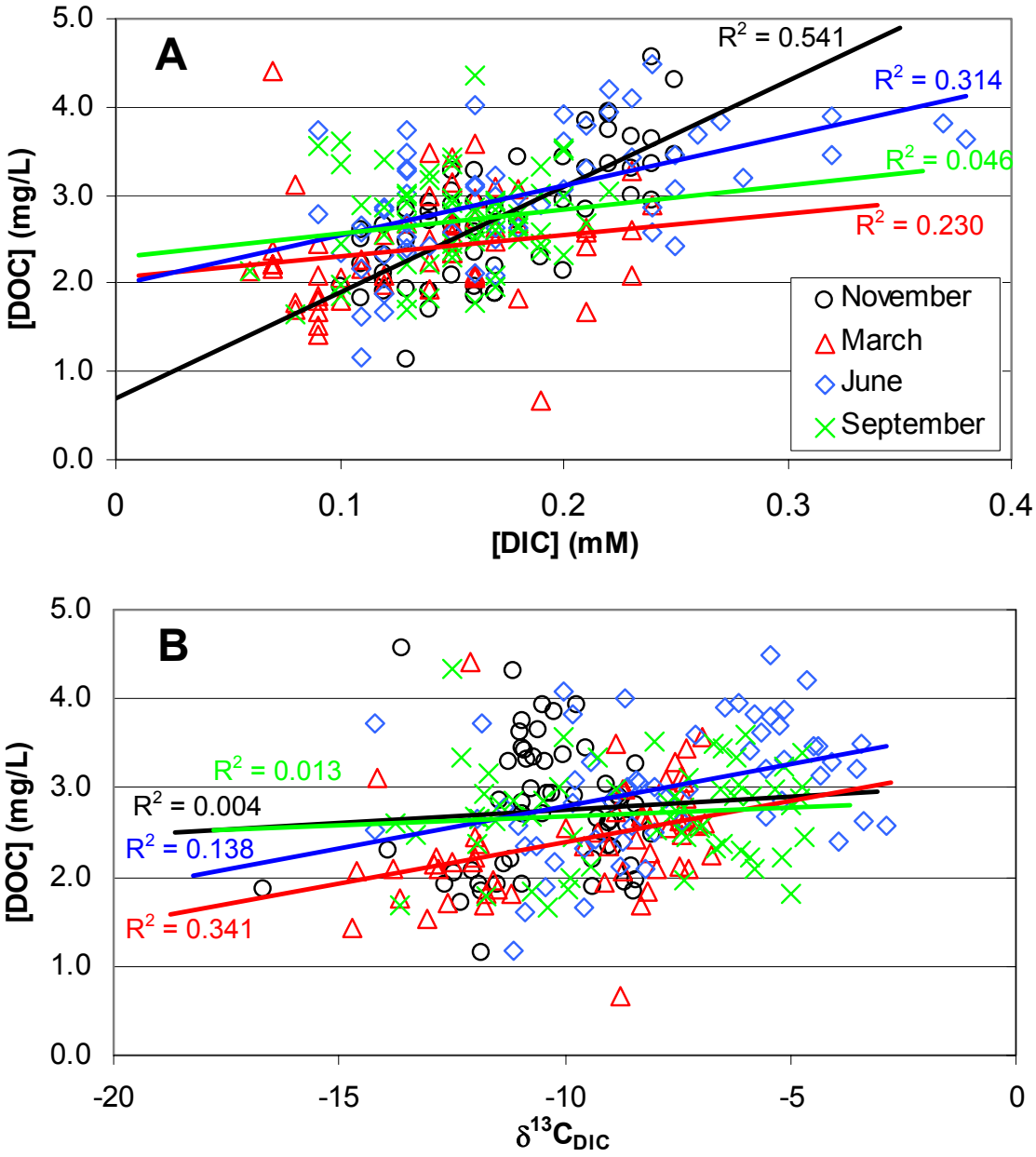

Figure 24: [DOC] against a) [DIC] and b) $\delta^{13}C_{DIC}$ for all sampling periods.

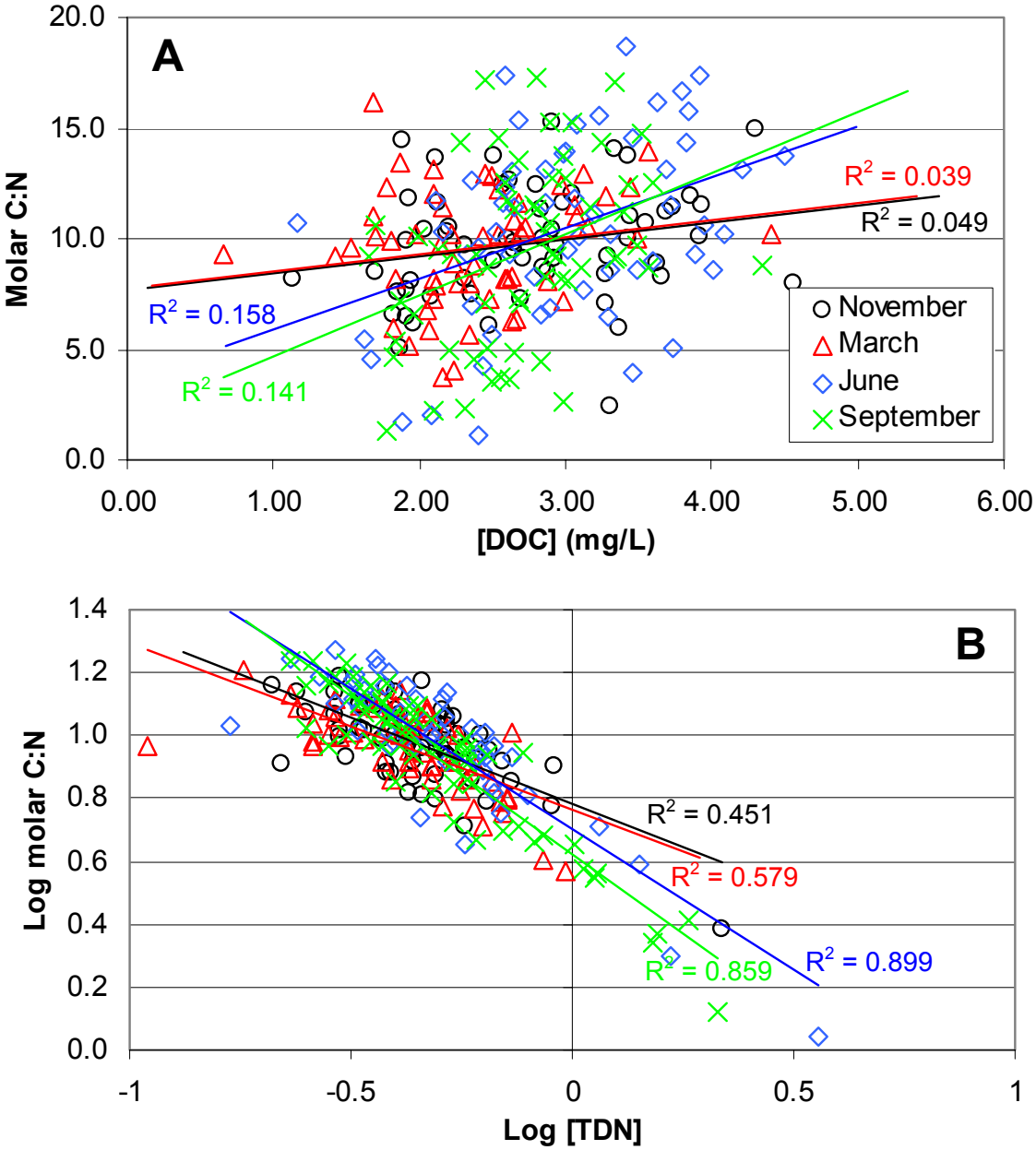

Figure 25: a) Molar C:N against a) [DOC] and b) Log C:N against log [TDN].

The concentration of DOC has a variable statistical relationship with $\delta^{13}C_{DIC}$ signature throughout the year (Fig. 24b). Both March and June showed a significant linear relationship between [DOC] and the $\delta^{13}C_{DIC}$ signature (Fig. 24b), with the $\delta^{13}C_{DIC}$ becoming more enriched with rising [DOC]. This relationship appears to only be present during the early spring to early summer months however as no significant relationship was found in November 04 or September 05 (R^2 = 0.004, P = 0.655 and R^2 = 0.013, P = 0.378 respectively).

Molar C:N is predicted to change seasonally as the balance between allochthonous and autochthonous organic matter sources vary. If molar C:N is related

to productivity peaks and there corresponding production of DOM / C, we can hypothesise that a rise in [DOM / C] may correspond to low molar C:N.

Molar C:N of dissolved organic matter showed no describable correlation with the concentration of TDS. The strongest correlation was found in September but this was still highly insignificant (R^2 = 0.026, P = 0.217). DOC proved to be a weak predictor of molar C:N in November and March (Fig. 25a), but during the spring / summer productivity peaks in June and September the relationship was more significant (R^2 = 0.158, P = 0.002 and R^2 = 0.141, P = 0.003 respectively). TDN was found to be the most reliable predictor of molar C:N in all months, showing highly significant logarithmic relationships (Fig. 25b). All sample periods show significant negative correlations, with increasing [TDN] leading to lower molar C:N (R^2 from 0.451 to 0.899, P < 0.001).

3.3.3) *Spatial Variation in epilimnetic [DOC], [TDN], $\delta^{13}C_{DOC}$, $\delta^{15}N_{TDN}$ and molar C:N.*

Figures 26 and 27 show spatial distribution of dissolved organic carbon and total dissolved nitrogen in the epilimnetic waters of Loch Lomond. The epilimnion has been defined as detailed in chapter 3. DOC and TDN concentrations were converted to g / m^3, and then multiplied by the epilimnion depth to provide integrated values.

Epilimnetic DOC concentration shows significant heterogeneity (Fig. 26), even in March when the lake is relatively well-mixed the far north can have less than half the [DOC] of the south west corner. In all four sampling periods there is a gradient of increasing epilimnetic DOC with distance south. November shows variation from north to south of over 60g / m^2. June has consistently the highest [DOC] throughout the lake, with the entire south basin [DOC] greater than 64g / m^2. During late summer/ early autumn the concentrations drop significantly in the south basin, but remain relatively stable in the north. The north and south basins show different seasonal patterns in terms of complexity. The north basin shows one peak in [DOC] in June, dropping in September. If it is assumed November ’05 (not sampled) will be similar to November it is likely the [DOC] then drops between September and November. The south basin however shows peaks in both June and to a lesser extent in November as stratification begins to breakdown. The middle basin may reflect the response observed in each of its neighbouring basins, with the north part behaving much like the north basin and the south part behaving like the south basin.

Total dissolved nitrogen shows a more homogenous distribution. Concentrations tend to stay around 1-7g / m^2 range for much of the year. Here the observed variation focuses on local areas of high concentrations. November, June and September all

show these 'hotspots' of [TDN] but location varies. In November elevated values are seen in the southwest corner. In June two 'hotspots' are obvious, one in the centre of the south basin, the other on the east coast of the middle basin, near the Cashell inflow. In September the far north at the mouth of the Falloch and the southwest corner near the Leven outflow each have higher values than the rest of the lake.

ARC GIS has also been used to plot spatial variability in C:N and $\delta^{15}N_{TDN}$ However, values have not been multiplied by depth as is unnecessary. Instead, any value that falls between 0-13 m is taken as an epilimnetic value, and anything below is considered the hypolimnion. If more than one point is present in the depth range, the average was taken. The lack of variability in the $\delta^{13}C_{DOC}$ signature means the distribution has not been plotted.

The epilimnetic distribution of $\delta^{15}N_{TDN}$ shows variable spatial patterns (Fig. 27). The seasonal cycle already described (Fig. 22) is again apparent with $\delta^{15}N_{TDN}$ becoming more enriched in the summer months, particularly June. Using the interpolated values, which include values for all extrapolated pixels, mean $\delta^{15}N_{TDN}$ for the epilimnion varies from a minimum of 1.74 ± 0.40‰ in March to 2.60 ± 0.47‰ in June.

Complex patterns of surface variation are seen in all four sampling periods (Fig. 28). In November, $\delta^{15}N_{TDN}$ ranged from 0.33‰ to 2.65‰, greater than the average seasonal difference. The most enriched area is found in the southwest corner by the River Leven outflow. March has a similar range in values (from 0.51‰ to 2.56‰) but the distribution is slightly different. In general the south and middle basins appear to have slightly more enriched signatures, but the 'hotspot' by the River Leven is not present. Instead the centre of the south basin shows an area of higher enrichment. As with November, $\delta^{15}N_{TDN}$ in March become more depleted in the north basin. June shows the most enriched values in general across the whole lake, and similarly to November and March, there is an area of depletion in the far north (~0.3‰), but few areas drop below ~1.5‰ in June. A latitudinal gradient is pronounced with the south basin showing enriched signatures consistently above 3‰. Peak enrichment occurs around M2 (See Introduction, section 1.8) near the Cashell Burn inflow, at the boundary between south and middle basins. Another area of high enrichment is found in the centre of the south basin; although curiously there is one site nearby where relatively depleted values occur, showing significant variability on a small spatial scale. However, in general the south and middle basins are consistently enriched in $\delta^{15}N_{TDN}$. In September the enriched values seen in June are maintained in the area between middle and north basins. However, the $\delta^{15}N_{TDN}$ signatures are

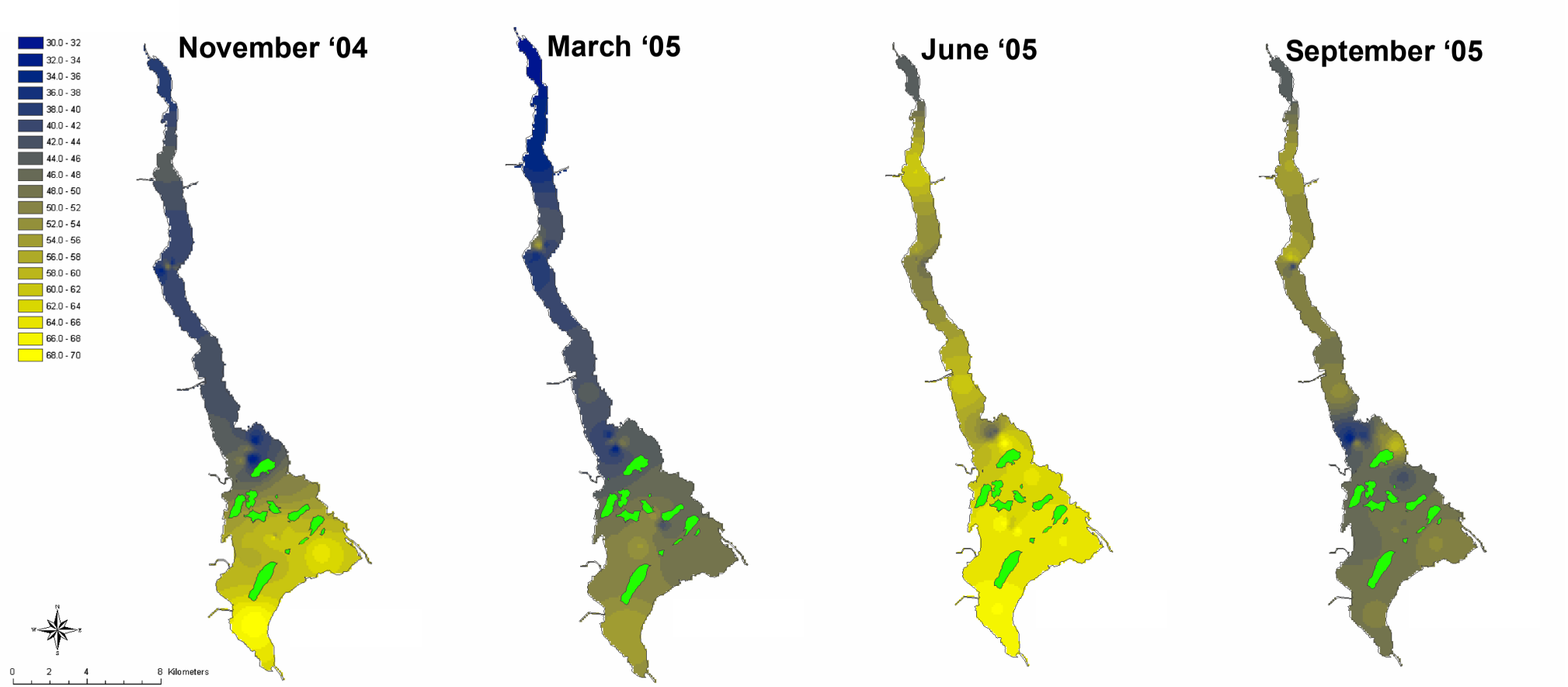

Figure 26: Epilimnetic distribution of dissolved organic carbon (DOC) in Loch Lomond for all sampled months. Islands are shown in green.

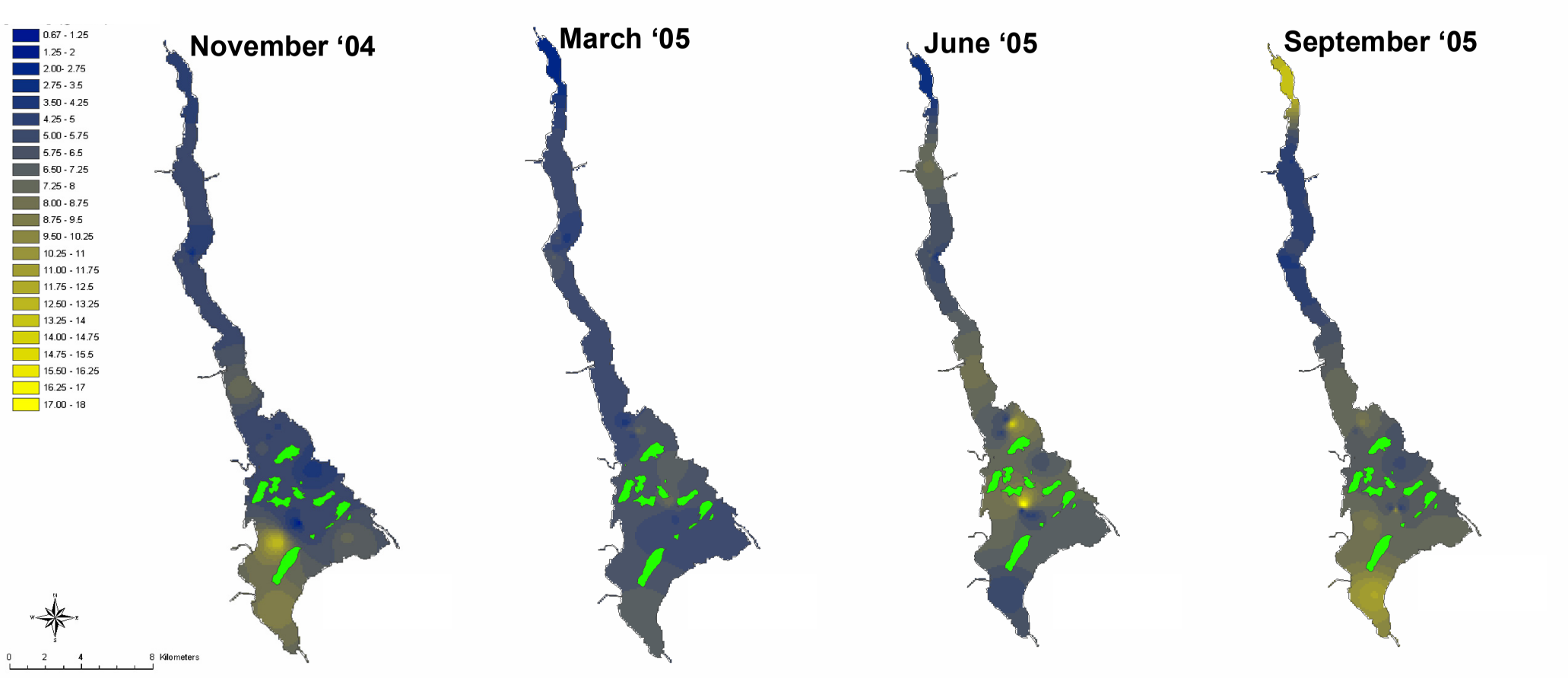

Figure 27: Epilimnetic distribution of total dissolved nitrogen in Loch Lomond for all sampled months. Islands are shown in green.

significantly more-depleted in the south basin. As with November there is an area of higher enrichment in the southwest corner.

The molar C:N shows the seasonal variability previously described in figure 22. Average values are lowest in March with a mean of 9.6 ± 0.6, with June having the highest (10.5 ± 3.7). The range of values changes between sampling trips also. The homogeneity of the water column in March is such that C:N ranges only from 7.8 to 11.4. In June, when the lake is stratified and showing varying productivity levels, this range increases to ~12.4, from 4.2 to 18.6. November and September are intermediary between these two extremes. As well as the seasonal variation, GIS mapped compositions reveal variable surface distributions of molar C:N

In November an area of higher C:N is seen near the Cashell inflow at the bottom of the middle basin. In March the values vary little across the lake. June and September each show significant horizontal variability. In June the highest values are seen across the south basin with values reaching a maximum in the middle of the south basin and in the southwest corner. However, as with $\delta^{15}N_{TDN}$ an area of particularly high C:N in the middle of the south basin is in close proximity to an area of low molar C:N. Similarly to the $\delta^{15}N_{TDN}$ distribution at the far north of the lake, near the River Falloch inflow, molar C:N differs significantly from the rest of the lake giving a relatively low value. The areas of high/low values change in September. The south basin has consistently lower values than in June and little heterogeneity. The highest molar C:N is observed in the lower parts of the north basin, with values above 9-10. Like June there is still the change close to the far north, with a low molar C:N recorded.

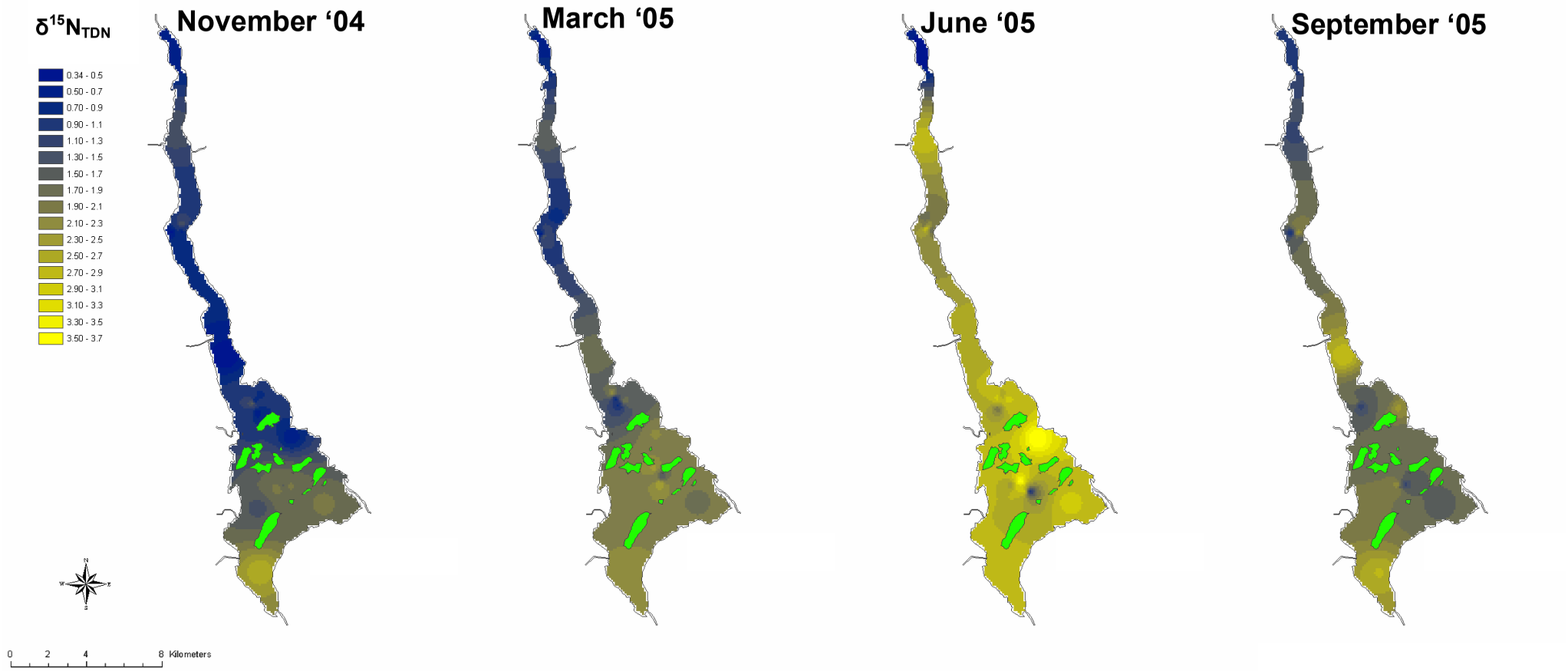

Figure 28: Epilimnetic distribution of $\delta^{15}N_{TDN}$ in Loch Lomond for all sampled months. Islands are shown in green.

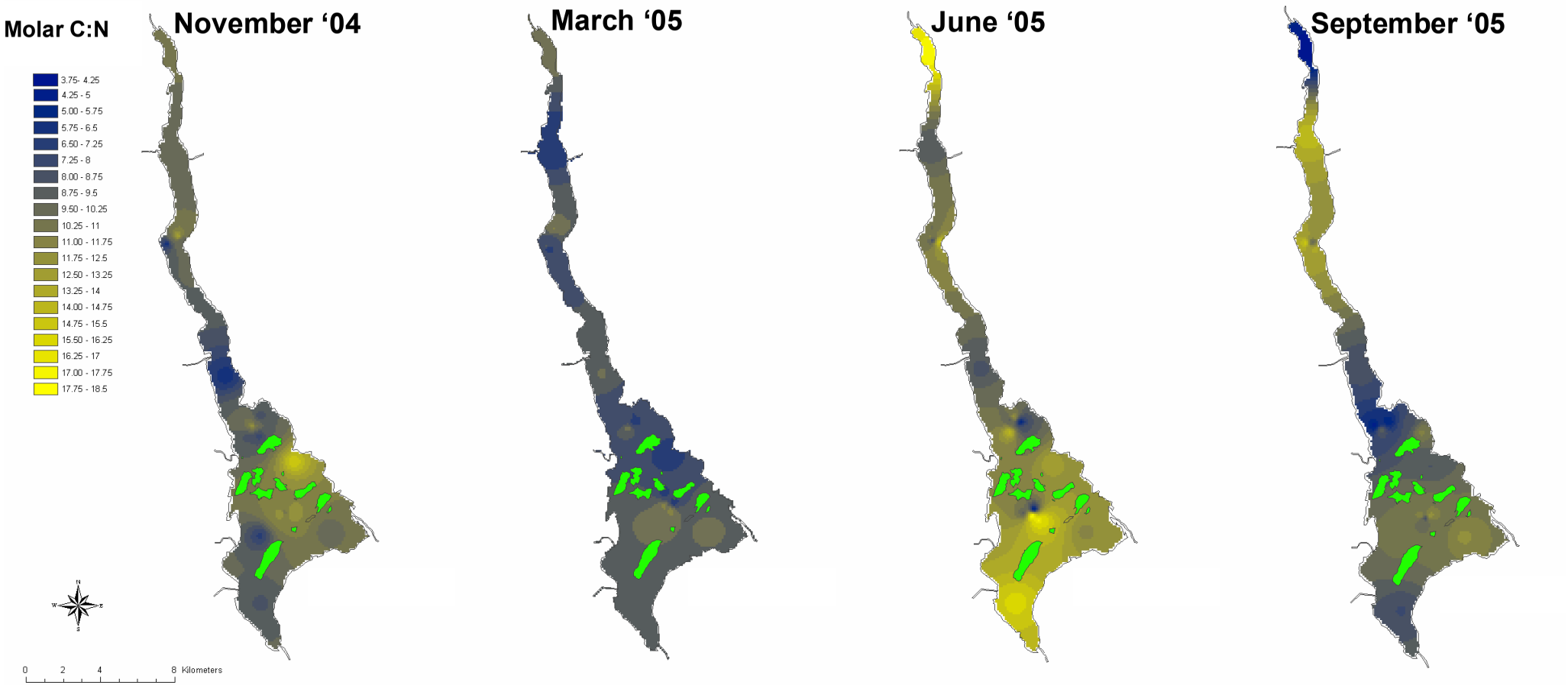

Figure 29: Epilimnetic molar C:N of TDS distribution in Loch Lomond for all sampled months. Islands are shown in green.

3.3.4) Generalised and localised flux of DOC

In the same way as for DIC (Chapter 2) total DOC quantities for different lake segments can be calculated (Table 4).

kg's DOC	November	March	June	September
Lake total	6510	6705	7592	6347
Epilimnion total	2792	*not stratified*	3327	2697
Hypolimnion total	3718	*not stratified*	4265	3651
South basin epi	1295.5	*not stratified*	1382.4	1036.1
South basin hyp	232.54	*not stratified*	237.1	187.3
South basin total	1538.1	1268.5	1619.5	1223.4
Middle basin epi	934.3	*not stratified*	1213.1	956.1
Middle basin hyp	1193.3	*not stratified*	1270.6	1222.1
Middle basin total	2127.6	2034.5	2483.7	2178.2
North basin epi	562.1	*not stratified*	731.6	704.6
North basin hyp	2292.5	*not stratified*	2756.9	2241.0
North basin total	2854.7	3402.2	3488.5	2945.7

Table 4: Mass in kilograms of DOC in different sections of Loch Lomond. Lake has been divided into basin and epilimnion/hypolimnion.

As with DIC quantities, the bulk of DOC is contained in the hypolimnion for all sampling periods when considering whole lake quantities. On average the hypolimnion contains 57 ± 0.01% of the lake DOC. Such generalisations do not consider spatial differences, where volume variability per basin and varying epilimnion / hypolimnion ratios are influential.

The percentage of DOC contained in the epilimnion compared to the hypolimnion follows a very similar pattern to that of DIC. In the south basin the epilimnion contains 84.9 ± 0.4% of the basins DOC. This proportion decreases in the middle basin to 45.6 ± 2.9% and again in the north to 21.5 ± 2.2%.

The total quantity of DOC in the epilimnetic water decreases from south to north in all sampling periods. The calculated difference was greatest in November when the epilimnion in the south contains over double the quantity of DOC in the north. The difference is less but still significant in both June and September. DOC quantity in the hypolimnion however shows the opposite pattern due to varying volumes between basins. The hypolimnion in the north basin in all sampling months contains over ten times the bulk quantity of DOC compared to the south basin and at least twice that found in the middle basin.

Using the data available on total concentrations of DOC in the lake, fluxes, both absolute and relative can be calculated (Table 5). The method and equation used, as

well as the underlying assumptions are the same as for DIC calculations and detailed in chapter 2, section 2.3.5.

Absolute (kg) and % change	Sept - Nov	Nov - Mar	Mar - June	June - Sept
total lake DOC	4.65 *(0.03)*	1.57 *(0.03)*	7.78 *(0.13)*	-13.53 *(-0.16)*
South basin epilimnion	7.41 *(0.25)*	-1.76 *(-0.17)*	2.67 *(0.28)*	-3.76 *(-0.25)*
Middle basin epilimnion	-8.22 *(-0.24)*	-0.06 *(-0.01)*	2.51 *(0.31)*	0.10 *(0.01)*
North basin epilimnion	-4.07 *(-0.20)*	1.37 *(0.30)*	-0.01 *(0.00)*	-0.29 *(-0.04)*
South basin hypolimnion	1.29 *(0.24)*	-0.33 *(-0.18)*	0.40 *(0.24)*	-0.54 *(-0.21)*
Middle basin hypolimnion	-0.83 *(-0.02)*	-0.69 *(-0.07)*	1.43 *(0.15)*	-0.53 *(-0.04)*
North basin hypolimnion	1.47 *(0.02)*	3.04 *(0.16)*	0.77 *(0.03)*	-5.61 *(-0.19)*

Table 5: Absolute and *percentage* change per day of DOC for the entire lake, as well as basin and depth specific values.

The rate of DOC change varies over time and between basins. Total lake DOC increases from September to June, although from November to March the increase is relatively small at only 0.03% per day. Between June and September is when most DOC is lost/utilised at a rate of 0.16% loss per day.

Considering individual lake segments more complicated fluxes are calculated. Between September and November for example, although total lake DOC is rising at a rate of 4.65 kg/day, both the middle and north basin epilimnion, along with the middle basin hypolimnion are loosing a significant proportion of their total DOC (8.22, 4.07 and 0.83 kg/day respectively. Similarly between November and March, only the north basin shows an increase in DOC content, but the lake still has a net gain in DOC. The time period between March and June is the only period that shows an almost unanimous increase in DOC content in all lake segments (the epilimnion in the north does lose DOC but at a rate less than 0.001% per day).

3.3.5) *Two-way mass balance to estimate the balance between allochthonous and autochthonous dissolved solids.*

The source of dissolved organic matter is of significance when considering its effects on lake-metabolism. High proportions of allochthonous material can be indicative of systems dominated by heterotrophic pathways, fuelled by imported organic material. Greater proportions of autochthonous material reveals the significance of within-lake production. Using observed variability in molar C:N of TDS the significance of these components was estimated.

Using the molar C:N of the TDS a simple 2-way mixing model was calculated to estimate the % allochthonous material made of the bulk material. $\delta^{13}C_{DOC}$ was unsuitable due to small seasonal and spatial variability, and finding suitable allochthonous end members using $\delta^{15}N_{TDN}$ was difficult. For these reasons a model based on molar C:N was used.

The model estimates the autochthonous end member values of molar C:N, and the allochthonous end members. Thus, using the assumption that the measured molar C:N is a reflection of a combination of these two end members, the following equation is derived.

$$\mathbf{C{:}N_T * M_T = (C{:}N_{auto} * M_{auto}) + (C{:}N_{allo} * M_{allo})} \quad \text{Eq. 8}$$

Where $C{:}N_T$ is the measured C:N ratio of TDS, M_T is fractional mass of total DOM, $C{:}N_{auto}$ is the estimated ratio of autochthonous material, M_{auto} is the fractional mass of autochthonous material, $C{:}N_{allo}$ is the estimated ratio of allochthonous material and M_{allo} the desired fractional allochthonous component of DOM.

Mass values for the equation are expressed as a fraction, meaning M_T will equal 1 with both M_{auto} and M_{allo} being <1. The estimated C:N ratio of 100% autochthonous production was taken as the Redfield ratio (6.625:1), which is the general ratio of carbon to nitrogen in aquatic phytoplankton and thus assumed to be a reasonable estimate for produced DOM / TDS. The molar C:N allochthonous end member was varied around estimated ranges from other studies (30:1 - 60:1) (e.g., Hutchinson 1956, Royer and Minshall 1997). The model has been run twice using these two extreme end members to obtain the range of possible values. Results are shown in Figure 30.

The amount of TDS of allochthonous origin changes on a seasonal basis much the same way as molar C:N, an artefact of the fact the model is based on molar C:N. As such the statistical analyses which held for molar C:N holds here also.

Mean % allochthonous TDS peaks in the south basin in June reaching 30% ± 19% when using the 30:1 model. The large deviation around the mean reflects significant spatial heterogeneity within the basin. E.g., the highest spot value is in the middle of the south basin surface waters where 31% allochthonous TDS was estimated. The middle basin and north basin both had times when the % allochthonous contribution approached zero, particularly in north basin deep/middle water during the summer months.

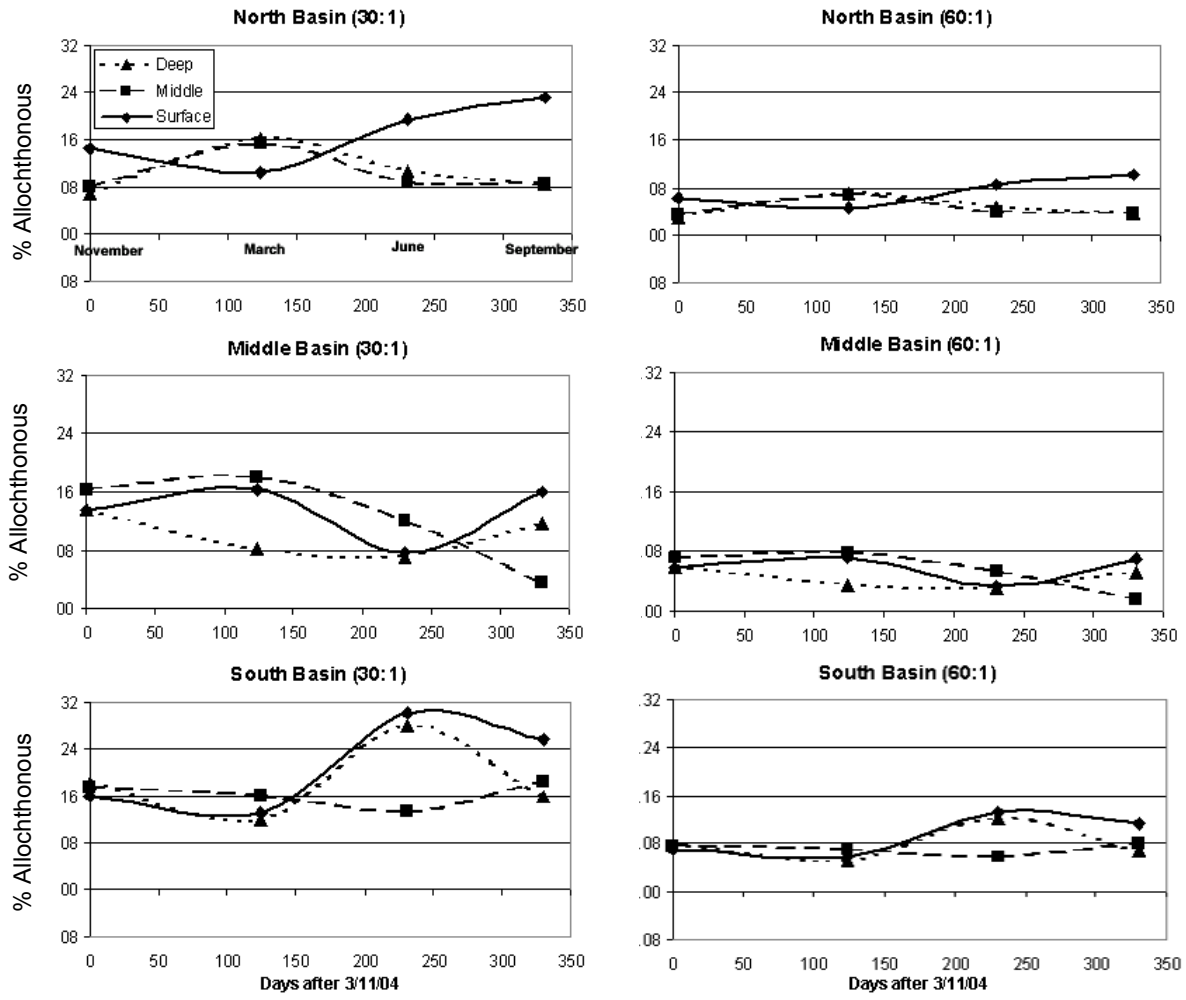

Figure 30: Percentage allochthonous material in bulk dissolved solids from molar C:N using estimated allochthonous end members of 30:1 and 60:1.

Although there is significant variability on a temporal and spatial scale, this model estimates that at most the % allochthonous material in DOM will be between 30-40% at times of high productivity and inflow volume. And at its lowest the contribution could be consistently less than 16%.

Our model estimates are complicated by the presence of inorganic nitrogen in the DOM samples. Although acidification to ~pH 4 ensures removal of the inorganic carbon the inorganic nitrogen (mainly nitrate) remains. Although not directly measured in this study, previous measurements (Habib *et al* 1997) indicate NO_3^- concentrations between 0.15 mg/l in spring and 0.12 mg/l in winter for the north basin. In the south basin winter nitrate concentration was approximately 0.25 mg/l, and ~0.17 mg/l in the spring. Thus in both basins inorganic nitrogen is a significant contributor to reported TDN values. The measured molar C:N will thus be lowered and show a greater proportion of the DOM to be of autochthonous origin. In chapter 5

and 6 direct measurements of phytoplanktonic and bacterial production have been used to assess the contribution of allochthonous material to total pelagic production and were used to validate or contradict the conclusions in this chapter.

3.4) *Discussion*

3.4.1) *Factors controlling temporal and spatial variation in [TDS], [DOC], and [TDN].*

Loch Lomond presents an interesting system in which to elucidate patterns in DOC and TDS dynamics. Due to the varying characteristics of the catchments, geological, hydrological and biological, the inputs of both allochthonous and autochthonous sources of DOM are variable in both quantity and quality. In brief, the north basin of Loch Lomond drains high altitude, base-poor catchments, with steep sides leading to poor water retention time and quick run off. The south basin drains a low-altitude, shallow-sloping base-rich catchment, with relatively extensive areas of farming, grazing and urban living. Such differences in catchment characteristics will now be considered as part of a temporal and spatial control of TDS, DOC and TDN concentrations.

The concentration of TDS, DOC and TDN will be influenced in two main ways (e.g., Kaplan and Bott 1982, McDowell and Likens 1988): i) by the creation and consumption by living organisms within the lake and; ii) by the varying balance between inputs and exports in the hydrological cycle. The concentration of dissolved solids in Loch Lomond varied on a seasonal and spatial scale. The north and middle basins show the largest seasonal change, with the south basin remaining relatively constant. In the north and middle basin there is a general increase in TDS concentration from November ‘04 to September ’05 (Fig. 22, page 76). There are two possible explanations for this trend. Previous studies have suggested the amount of precipitation to have an effect on the amount of DOM entering a lake (e.g., Meybeck 1988, Spitzy and Leenheer 1991). It is possible that the dissolved solids concentration increase is reflective of increasing precipitation levels in the periods preceding the June and September sampling, and subsequent additions from greater inflow volumes. Indeed, sampling trips in November and March were preceded by relatively dry periods, less so in June and September. The second possible explanation is that the input of autochthonous DOM / TDS increases as productivity in the lake increases. During phytoplankton production, and the food web that ensues,

DOM can be produced (and lost) in numerous ways. This could explain the increase seen in Loch Lomond. Neither control is mutually exclusive.

The concentration of TDS also shows significant spatial variability. The temporal variation described is only seen in the north and middle basins. The north basin in general has significantly lower TDS concentrations (33.5 ± 9.0 mg/L) than both the middle (39.8 ± 9.3 mg/L) and south basins (44.2 ± 6.7 mg/L). Spatial variability can be explained by the different catchment characteristics from north to south, but why the north basin shows a clear temporal change whereas the south does not is less clear. Rasmussen *et al* (1989) showed that the concentration of DOM was negatively correlated to increasing watershed slope, mean lake depth and lakes area. Houle *et al* (1995) found similar determining factors in 59 lakes in Quebec, Canada. Hence topographic and bathymetric controls could in part explain the lower [TDS] in the north basin. Coupled with differences in land use (more arable / forestry and urban areas in the south)

Further supporting the idea that [TDS] in Loch Lomond is mainly driven by inflow contribution is that river [TDS] in the main rivers entering the south basin is significantly higher than the north. Mean [TDS] for inflows entering the south basin is 82.3 ± 28.7 mg / L, compared to the north, which is 27.5 ± 10.1 mg / L. Also, the main catchments in the south drain significantly larger areas (Endrick and Fruin 264 and 161 km^2 respectively) compared to the north (Falloch and Inveruglas 113 and 158km^2 respectively), at lower altitudes and slope allowing more DOM / DIM collection on route to the lake. Residence time can also influence [TDS] (Curtis and Shindler 1997): the longer the residence time the more potential for microbial utilisation of the DOM pool and thus reduced concentrations. Average residence time in Loch Lomond is 1.9 years (Eurolakes D24), although this varied at different points in the lake. North basin deep water was shown to have residence times up to 4 years, with the south basin around 6 months (although residence times as short as a few days were modelled in some meteorological conditions). Longer residence times in the north and middle basin allow more processing time of DOM, particularly below the thermocline in deeper water which has generally longer residence times than the epilimnion. This implies DOM / TDS in the north should have a lower molecular weight, more enriched $\delta^{13}C$ and higher molar C:N

Within the same climatic area, the rainfall experienced in different catchments can be variable (Curran and Poodle 1992). In Loch Lomond the north sub-catchments can experience up to 3 times the precipitation of the south. Variation in the DOM / TDS pool could potentially be caused by the north basin experiencing a greater input of TDS from drainage basin run off in the summer months, whereas the precipitation

level in the south basin remains more constant. This is possible but unlikely to explain all the variation seen as more often than not precipitation levels are similar in the north and south catchments. It is likely that spatial variation between catchments is due to inorganic nutrient variability and its greater concentrations in the south.

The north basin may respond more dramatically to peaks in productivity (being an oligotrophic water body). During winter [TDS] is low, with an increase concomitant with productivity rises during the spring/summer. The middle basin also shows an increase in [TDS] during the summer months, but not to the same magnitude. The south basin however shows no seasonal variation. Maybe the supply of allochthonous DOM to the south basin is more constant than further north, and this concentration is high enough that inputs from an autochthonous source during summer do not make an observable difference in overall concentration? Or, that the addition of TDS, from autochthonous or allochthonous sources is balanced by its removal, by heterotrophic breakdown or drainage from the lake. As with observed variability in [DIC] (Chapter 2), lake volume variation was insufficient to explain the measured variation in TDS, DOC or TDN.

The concentrations of DOC and TDN showed a different response to temporal and spatial change than TDS (Fig. 22). Unlike the TDS pool, [DOC] showed no significant variation with season in hypolimnetic water in any basin. However, as with [TDS], [DOC] increased from north to south in surface waters, and likely for many of the same reasons as TDS. A conspicuous feature of lakes in other studies has been no change in the DOC concentration with depth and time (e.g., Wetzel *et al* 1972, Fukushima *et al* 1996). This absence of vertical stratification and seasonal variation is in contrast to other parameters (e.g., temperature, [DIC]) that do show such variation. This suggests that the bulk of DOC will be refractory and relatively recalcitrant to rapid bacterial decomposition, or generally that inflow / autochthonous production of DOC is matched by the rate of bacterial utilisation or export. Direct and concurrent measurements of bacterial production (Chapter 5) and [DOC] (Chapter 7) can help elucidate whether they are directly linked as predicted here. If variation in [DOC] is to occur it is generally in the epilimnetic waters (Wetzel 2001), and this was observed within Loch Lomond. This arises as, especially during stratified periods the autochthonous contribution of DOC is highest, and is processed so quickly (often < 48 hours (Wetzel 2001)) the impact on the hypolimnion bulk [DOC] appears insignificant. Additionally most allochthonous carbon enters the upper layers of a lake, thus variation is most likely to manifest in the surface waters.

It is possible that the DOC pool is changing more than elucidated via this study. As already stated, autochthonous production of DOC is readily utilised in days, so a sampling frequency of every three months will likely not reveal the changes. It is likely that this study reveals only surface detail of the dynamism of the DOC pool and change observed reflects the more stable and long lasting allochthonous component.

The temporal variation observed in surface water [DOC] follows a trend of increasing in the more productive periods. Strangely though, in September surface waters in the south are as low in DOC as in March (Fig.22), but [TDS] is at its highest. This may be caused by significant inflow of inorganic nutrients into the lake at the start of the autumnal period adding to the total dissolved solids, but not affecting the DOC pool.

The concentration of dissolved nitrogen shows interesting and variable changes both spatially and temporally (Fig. 22). The north basin surface water has the highest concentration of TDN in the summer months, although the increase seems to coincide with the observed rise in DOM. This suggests that unlike DOC, TDN concentrations are closely linked with DOM variation.

The concentration of TDS, DOC and TDN revealed spatial and temporal variability, supporting our first hypothesis that peak DOC / TDN concentrations will likely be recorded in the productive seasons and autumn, related to biomass production and increasing allochthonous input respectively. Evidence also suggests that the onset of high productivity periods and varying inflow dynamics could explain much of the temporal variability observed. Experiments presented in chapter 5 support these ideas as the observed periods of maximum dissolved organic material coincides with measured peaks in phytoplanktonic and bacterial production. Whether variability in $\delta^{13}C_{DOC}$ and $\delta^{15}N_{TDN}$ support these conclusions shall now be explored.

3.4.2) Factors controlling temporal and spatial variation in $\delta^{13}C_{DOC}$ and $\delta^{15}N_{TDN}$.

Variation in $\delta^{13}C_{DOC}$ and $\delta^{15}N_{TDN}$ signatures can help elucidate changes in various processes in lacustrine systems. The $\delta^{13}C_{DOC}$ of organic material has been used to examine changes in both primary productivity and the balance between pCO_2 (aq) and CO_2 (aq) versus HCO_3^- (e.g., Lehmann *et al* 2004, Hollander and McKenzie 1991, Ostrum *et al* 1997). Isotopic variation in dissolved nitrogen has also been linked to various biological reactions such as nitrogen uptake (e.g., Teranes and Bernasconi 2000), denitrification (e.g., Lehmann *et al* 2004 and references therein) and organic matter utilisation (e.g., Lehmann 2002).

$\delta^{13}C_{DOC}$ showed a small but significant enrichment from June to September, with hypolimnion samples generally showing the largest increase. C3 vegetation produces a characteristic isotope signature in its DOC pool, and whilst this changes somewhat as it is processed through the soil horizons and flow paths (Aitkenhead-Peterson *et al* 2003), has a narrow range, between –28‰ and –31‰ approximately. DOC isotope signatures in Loch Lomond appear well-constrained around the value of terrestrial organic matter, suggesting a mainly terriginous origin of the DOC analysed, and present a significant piece of evidence that allochthonous sources of DOC are important in this system. While there is some enrichment during the summer, the $\delta^{13}C$ never reaches beyond the threshold of allochthonous DOC. However, autochthonous organic carbon $\delta^{13}C$ can show significant variability over a temporal and spatial scale (e.g., Rosenfield & Roff, 1992, Zah et al., 2001, Rounick et al., 1982; Winterbourn et al., 1986; Boon & Bunn, 1994) and can range from -35‰ up to approximately -8‰. Variability in the autochthonous source could potentially provide insight into source, however the range observed in this study is typical of allochthonous DOC and as such no evidence of other sources can be gained from the $\delta^{13}C_{DOC}$ alone.

There is still a small but detectable enrichment in the summer months. The enrichment of the DOC pool between June and September can be explained by decreasing isotope fractionation during photosynthesis and the subsequent conversion of dissolved inorganic carbon to organic carbon, caused as the concentration of DIC drops (see chapter 2) in late summer. As DIC is utilised during photosynthesis, [DIC] drops and $^{13}C_{DIC}$ increases. Thus further photosynthesis utilises a $^{13}C_{DIC}$-enriched pool, which in turn is then reflected in the synthesised organic matter. Bacterial processing of DOC can lead to the same result. During heterotrophic breakdown of DOC and preferential utilisation of ^{12}C, the remaining DOC will become more enriched in ^{13}C. Both of these factors likely have an effect on overall $\delta^{13}C_{DOC}$ values. In eutrophic lakes DOC enrichment can be relatively large, from –34‰ to –24‰ (e.g., Lehmann *et al* 2004). In Loch Lomond the production of autotrophic carbon may not be large enough to significantly affect the overall $\delta^{13}C_{DOC}$. Drawing any conclusions over an annual scale is impossible due to lack of data in November and March, but $\delta^{13}C_{DOC}$ data has been collected as part of incubation experiments and will be further explored in chapter 7 (Fig. 51, page 178).

Nitrogen occurs in freshwater ecosystems in numerous forms and can undergo various different processing events (Wetzel 2001). Nitrogen is present as a number of organic compounds, such as amino acids and proteins, as well as refractory humic compounds. It is also present as ammonia, nitrite and nitrate. Loch Lomond has little

humic input so this should be minimal, particularly in the middle and north basins, though influx of fertiliser nitrogen may be of significance in the south. Nitrogen can be gained and lost in various ways. Gains include addition via precipitation, nitrogen fixation and inputs from groundwater drainage. Loses include outflow from the lake, reduction of nitrate to nitrogen gas by denitrification and loss to the sediments (Wetzel 2001).

Although much work and knowledge has been gained on carbon isotope fractionation during photosynthesis and respiration, and overall balances in aquatic systems, nitrogen cycling is less well understood (Goericke *et al* 1994).

$\delta^{15}N_{TDN}$ became more enriched in the summer months. Phytoplankton have been shown to preferentially incorporate ^{14}N during nitrate assimilation (Fogel and Cifuentes 1993) which would lead to more-depleted TDN produced, thus lowering $\delta^{15}N$. However, during the summer months the concentration of nitrate would be expected to decline as it is utilised, and as it does phytoplankton are forced into taking up more ^{15}N nitrate, which would thus raise the $\delta^{15}N_{TDN}$ signature. Such nitrogen cycling may be occurring in Loch Lomond, although lack of any specific nitrate measurements render confirmation difficult. However, SEPA work has shown that both the lake concentrations and inflow (Endrick and Falloch) DIN concentrations are highest in the autumn / winter at ~0.15 mg/l (north) and 0.25 mg/l south (SEPA report, Habib *et al* 1997), which may support the idea. That said, the concentration of dissolved nitrogen increases during the summer months, thus if nitrate levels are decreasing as reported in previous work (Habib *et al* 1997), the increase is being masked by a greater increase in other areas of the TDN pool such as amino acid or protein production, possible with high bacterial production for example (discussed further in chapter 5).

It has been known since the early forties that certain species of cyanobacteria can carry out nitrogen fixation (Burris *et al* 1943). However, the full significance of nitrogen fixation has been discovered and explored relatively recently. Nitrogen fixation is carried out by cyanobacteria and photosynthetic bacteria mainly, although heterotrophic N-fixation can be significant in lakes with high organic carbon (Hill 1992). The process is light-dependent in cyanobacteria and as such is likely to only have an appreciable impact on the surface waters, becoming very inefficient at night (<10% daytime production (Horne 1979, Livingston *et al* 1984)). I could find no records of nitrogen fixing cyanobacterial species in Loch Lomond. However, the root systems of alder trees support significant quantities of nitrogen fixing bacteria and are present on the shores of the lake. Thus any effect nitrogen fixation may have in Loch Lomond is likely from importing of material processed terrestrially. Variability in

external supply of inorganic nitrogen species and photosynthetic utilisation are likely far more significant factors controlling TDN concentration in Loch Lomond.

During nitrogen fixation, atmospheric nitrogen is converted into more reduced forms (e.g., N and NO_3^-). This will have the effect of raising the concentration of dissolved nitrogen in the epilimnion. Increased [TDN] is prevalent in both the middle and north basin epilimnion in the summer months, which may suggest an input of nitrogen fixation. The same increase is observed in the south basin in spring (between March and June) but concentrations drop in late summer. It's possible that even if nitrogen fixation was occurring in Loch Lomond, [TDN] increase would be counteracted by organic nitrogen uptake by phytoplankton. $\delta^{15}N_{TDN}$ may offer an insight into these patterns in some lakes.

Isotopic fractionation is less during nitrogen fixation than by photosynthesis (Wetzel 2001). Dissolved N_2 in water has a similar composition to that in the atmosphere, 0‰ (Benson and Parker 1961, Miyake and Wada 1967, Cline and Caplan 1975). As nitrogen fixation dominates then $\delta^{15}N$ values tend towards atmospheric values of 0‰. $\delta^{15}N_{TDN}$ in the south basin is the most ^{15}N-enriched suggesting that photosynthetic incorporation of inorganic nitrogen species by algae dominates over nitrogen fixation (assuming baseline levels are similar in each basin). The north basin shows more depleted values implying either N-fixation is occurring or, more probable, a reduction in photosynthetic incorporation levels in the south. Epilimnetic waters in the middle basin show unusual temporal patterns significantly different from south and north. The reason for this unusual pattern and very large variation is as yet un-explained. In oceanic studies of the Cyanobacteria, *Trichodesmium* sp, $\delta^{15}N$ values of between –2.1‰ and 0.05‰ were used to suggest nitrogen fixation was an important contribution to the overall nitrogen component of these bacteria (Wada 1980). Higher values in Loch Lomond, between ~1‰ to 2.5‰ suggests nitrogen fixation is likely not significant in these waters, and any influence from tree-root nitrogen fixation is minimal on pelagic communities.

If nitrogen-fixation is not, as likely, to be a significant driving force behind the low $\delta^{15}N_{TDN}$ in Loch Lomond, it can be assumed that enrichment via processing of inorganic nitrogen is a significant factor. However, in deep oligotrophic systems the preferential export of ^{15}N from the epilimnion (to deeper water) due to fractionation effects caused by zooplankton feeding and excretion (Altabet and Small 1990, Montoya *et al* 1992) may be contributing. This may be especially important in the north basin as algae in oligotrophic systems tend to use significant amounts of recycled nitrogen, and little of the sinking material is re-suspended in the epilimnion (Montoya *et al* 1992).

Unfortunately, a useful interpretation of natural abundance stable isotope values is only really possible with a detailed knowledge of source values (Robinson 2001), which is lacking from this work. The problems associated with this lack of data become apparent when the range of values found in other work are considered. Nitrogen fixation has been measured to lead to $\delta^{15}N$ values between –1.0‰ (Wada *et al* 1978) and +8‰, and inorganic nitrogen uptake from –9.7‰ to +23‰ depending on the nitrogen species involved (Wada and Hattori 1978). In reality it is possible that all the processes described (inorganic N uptake, N-fixation and export to the hypolimnion) may influence the $\delta^{15}N_{TDN}$ signature to varying degrees. For more information data on source $\delta^{15}N$ signatures is required, and while not an aim of this research would be valuable in the future for looking at balances between autochthonous and allochthonous DOM sources in the lake.

Although uncertainties remain in the conclusions drawn by stable isotope distribution, there is at least preliminary evidence that they are controlled by within lake production balances and the supply / degradation of terrestrial sources of organic material. Although the dynamics of the organic pool is likely more complex than represented by a survey of this resolution, the hypothesis predicting peaks in the summer / autumn periods may be correct. A more detailed temporal survey, coupled to a similarly comprehensive spatial survey would likely provide more insight.

Also apparent from the above discussions is the heterogeneity of the Loch Lomond water body, predicted in hypothesis 3 (page 72). As with DIC dynamics (chapter 2), DOM in Loch Lomond is rarely homogenous over small or large spatial scales. Reasons for this have been discussed previously in this section, and the conclusion is similar to that of chapter 2. When considering a water body of this size, and indeed any hydrological / morphological complexity, an understanding of this variability is essential in understanding whole lake nutrient cycles. One spot sampling is likely inaccurate for this lake, and while for others it may be, consideration of possible heterogeneity should be carried out.

3.4.3) *The molar C:N of TDS and the allochthonous / autochthonous balance.*

The molar C:N of TDS can give indications of both the quality and origin of dissolved organic material in a lake system. In its simplest interpretation the higher the molar C:N, the less nitrogen and thus, as nitrogen is often limiting in lakes, the poorer the quality of the DOM (Wetzel 2001). Low C:N ratios are indicative of TDS with a high proteinaceous content, preferable for microbial utilisation. Unfortunately various other factors affect the C:N ratio of organic matter and must be considered.

Other parameters measured, such as [DIC], [DOM], $\delta^{13}C_{DIC}$, $\delta^{18}O_{DO}$, etc all show variability possibly linked in part to the production to respiration ratio in the lake. The molar C:N is another tool to consider changes in the balance of primary/secondary production being a driving force behind nutrient concentrations and isotopic compositions. During times of high nutrient availability cells can become more protein-rich (Hama and Honjo 1987, Hama 1988) being able to synthesise proportionally high quantities of protein, whereas elevated light and thus UV exposure can lower the protein content (Goes *et al* 1995,1996) through damage to nucleic acids and DNA synthesising apparatus. Molar C:N in the surface waters of the north and south basin increases in the summer months. After the initial spring bloom period when nutrients are becoming exhausted, C:N ratio of cellular components and thus DOM produced would be high. As blooms progress nutrient availability becomes less and the quality decreases (Bertilisson and Jones 2003). This coupled with higher levels and longer periods of illumination could explain the elevated summer ratios observed in Loch Lomond surface waters.

The spatial variability in molar C:N of TDS (Fig. 29), likely reflects the balance between allochthonous and autochthonous material in the lake, as well as differing productivity levels and rates of processing. In all four sampling trips molar C:N has areas of high values in the south basin. This can be hypothesised to be due to increased productivity in the south basin and thus more complete utilisation of the available nitrogen, or low levels of inflowing inorganic nitrogen. In June there are areas of low molar C:N surrounded by otherwise high numbers. One low C:N area is next to the Cashell inflow, and is possibly showing the influence of run off from a nearby campsite. Another site of low C:N in the south basin is not near any measured inflows but still significantly different from the surrounding. This suggests variability on a both small and large spatial scales. Areas of higher productivity support a greater level of microbial processing, and microbial processing preferentially utilises nitrogen sources (Wetzel 2001). Indeed, incubation work discussed in chapter 5 revealed bacterial production, particularly in summer is significantly greater in the south basin. As such more nitrogenous compounds are likely utilised and the molar C:N was observed to be highest.

The isotope ratios of $\delta^{13}C_{DOC}$, $\delta^{15}N_{TDN}$ and molar C:N of DOM can all potentially provide information on the source of dissolved organic material in Loch Lomond. As $\delta^{13}C_{DOC}$ data was only available for June and September model calculations based on $\delta^{13}C_{DOC}$ variability have not been conducted. Instead a mixing model using molar C:N has been used based on assumptions of autochthonous and allochthonous end

member compositions. The estimated molar C:N of 100% autochthonous production was taken as the Redfield ratio (6.625: 1), and the C:N allochthonous end member was varied around estimated ranges from other studies (30:1 - 60:1) (eg., Hutchinson 1956, Royer and Minshall 1997).

All conclusions drawn from the allochthonous / autochthonous model used are likely subject to significant error and based on various assumptions, particularly that the contribution of inorganic nitrogen species will be insignificant. However, this model can be used as a baseline minimum estimated contribution of allochthonous material to pelagic water. The contribution of allochthonous material to bulk organic matter depends on numerous factors. These range from the amount of primary production, the depth of the lake, the residence time and the area of littoral zone (Wetzel 2001). Loch Lomond is deep, with a high residence time and low percentage littoral area. Catchment characteristics will also have an effect. It would be predicted that the steep-sloping, base-poor catchment in the north would bring in less allochthonous organic matter than the shallow gradient, base-rich catchment in the south. The generalised view of systems like this will be of a low relative contribution of allochthonous material to bulk organic matter. In general, it was believed only reservoir systems will be dominated by allochthonous organic material (e.g., Romaneko 1966).

TDS shows a variable seasonal pattern depending on both basin and depth. The estimated % allochthonous material is highest in the south basin, approaching 32% in the summer in the surface waters. Both south and north basins have peak allochthonous contributions in the summer months, in contrast to seston data (chapter 4). The middle basin has lower percentages in the summer months, possibly reflecting the lack of large inflow in this basin. The overall % contribution of allochthonous matter to dissolved organic matter is predicted, by a two-source mixing model to be low. Depending on which allochthonous end member is used the contribution can be < 1%, and never more than ~ 32%. This supports the idea that in unproductive lake systems autochthonous matter can dominate the overall organic pool, contradicting the terrestrial signature of $\delta^{13}C_{DOC}$ measured. However, the previously described presence of inorganic nitrogen species (mainly NO_3^-) is likely skewing the results towards the autochthonous end member, so values presented here represent a minimum allochthonous contribution.

The influence of inorganic nitrogen (and indeed zooplankton remains in seston, chapter 4) is confirmed in chapters five and six, where direct measurements of productivity reveal a far greater contribution of allochthonous carbon utilisation to pelagic production. Thus, although evidence in this chapter may contradict our

second hypothesis that DOM sources will be dominated by allochthonous material, known inaccuracies with the modelling procedure make a clear conclusion difficult. Instead we can assume that the minimum contribution of allochthonous DOM to lake metabolism is within the range reported, and that there is likely a temporal and spatial variability in the balance between autochthonous and allochthonous sources. Chapter 4 explores the concept with regard to particulate matter using similar modelling techniques to provide more insight into the metabolic / morphological / hydrological factors discussed in this chapter.

Chapter 4

What does the stoichiometry and stable isotope signatures of sinking particulate material (seston) tell us about nutrient cycling and carbon processing in Loch Lomond?

4.1) Introduction

Research elucidating the dynamics of seston in Loch Lomond was started as part of an honours project carried out by Scott Barclay and supervised by Dr Susan Waldron. Hence a significant amount of the analysis, sample processing and interpretation was carried out by them. However, additional analysis and interpretation has been carried out in this thesis to further the concepts first examined in the earlier work.

Sestonic material is that which falls through a water column (Tippett 1994). Whether this definition includes particulate material, buoyant in the water column, is ambiguous, but for this study was included. Owen *et al* (1999) used a small correction factor to eliminate suspended material in the water column on the day of collection. This was not done here and has been assumed to be constant throughout the year. This assumption was tested during monthly productivity incubations (see chapter 7) and although variation was detected the concentrations were generally low and unlikely to significantly influence the results shown here.

Particulate material is produced by various different processes in aquatic systems. Like DOM (Chapter 3), particulates can come from either autochthonous or allochthonous sources. Allochthonous particulate sources will include dust particles, leaves, branches, detritus and various other substances washed down rivers and into the lake itself. Autochthonous will include whole algal cells, the remains of algal cells following grazing/lysis, zooplankton remains, algal exudates, zooplankton/vertebrate defecations, etc. Using a combination of molar C:N, $\delta^{13}C_{seston}$ and $\delta^{15}N_{seston}$, by mass balance, the relative proportions of these inputs can be estimated and is discussed in detail later.

Loch Lomond is a monomictic system, i.e. there is one period per year of complete water turnover, with a time of thermal stratification in between. The annual sedimentation cycle of lakes in the size range of Loch Lomond is characterised by significant increases in deposition rate during the turnover period (Wetzel 2001). This increase is believed to be due to increased lakebed disturbance caused by increased

water turbulence, coupled with a general increase in imported particulate material accompanying wetter weather (Pennington 1974).

When considering sestonic material in Loch Lomond three hypotheses will be addressed.

i) Although less comprehensive in scale (fewer sampled sites) there will be a measurable difference in accumulation rate between basins in Loch Lomond. As with both DIC and DOM a more crude assessment of single point sampling strategies will be tested.

ii) There will be a range in the seasonal flux of seston to the lake bed and possible implications for both pelagic and benthic energy mobilisation.

iii) The proportion of autochthonous or allochthonous seston will change with season, reflecting variability in production and terrestrial input.

4.2) *Sample collection and processing*

Particulate material was collected in specifically designed traps (Fig. 31), designed and built by Stuart Wilson. One trap was deployed per basin. The traps consisted of three plastic tubes, fitted with rubber taps, suspended around a central column. Each trap had two different rosettes of traps each with three individual traps, one rosette at the middle depth and one at the deepest depth (deepest depths were selected to be ~5m from the lake bed. Thus depths were 9m and 17m in the south basin, 30m and 55m in the middle basin, and 80m and 170m in the north basin). The traps were designed to be in free rotation around the rope they were suspended by and to ensure the three replicates at each depth were comparable. The traps were designed with a height to diameter ratio of ~5:1, in accordance with optimal ratios (Hargrave and Burns 1979) that maintain a turbulent free zone at the base of the traps to limit loss after collection. During the field recovery traps were raised at a gentle speed to keep disruption of the material to a minimum, (it is unknown how much disruption occurred, but water in the trap was generally clear). Once above the surface of the loch the contents were released through the bottom taps and collected into acid washed 2L polyethylene

Fig 31: Seston traps used to collect particulate material. Each rosette has three replicate tubes.

bottles. The sample (lake water + accumulated particulates) was run through each trap three times to ensure maximum retrieval of material and keep sampling procedure consistent between replicate tubes. Post collection the traps were re-lowered and their GPS position recorded. Although care was taken to maintain the traps in the same location, boat drift and windy conditions often meant this was not possible. The filled bottles were refrigerated to await analysis.

Before processing the collected bottles were allowed to sit to let as much material settle as possible. The supernatant liquid was removed using an Eppendorf pipette. When disturbance of the settled material was a possibility, further concentration of the seston was achieved using repeat centrifugation, and decanting of the supernatant. This was repeated until all the particulate material could be contained in a small plastic beaker and frozen. Beakers were freeze-dried and weighed both before filling and after freeze-drying, to allow the mass of seston recovered to be quantified. This freeze dried seston was scraped to a glass vial, homogenised with a spatula and then prepped for stable isotope analysis. As with DOM preparation, ~2mg of material was weighed into 5x7mm tin capsules, which were then sealed and crushed. Stable isotope analysis was carried out on the same mass spectrometer and by the same method as DOM (chapter 3)

Seston traps were first deployed on the 13th of May 2005 with regular collections being carried out until early June 2006.

4.3) *Results*

4.3.1) *Influence of basin, depth and season on seston.*

Seston traps to collect particulate organic matter were deployed on the 13th of May 2005. They were subsequently emptied on 11 occasions between then and the 3rd of May 2006. The north basin traps were lost after the second collection for an unknown reason and new traps were built and deployed in April 2006, and collection of other traps was sometimes impossible due to weather. Exact sample dates are shown in table 6. Values of seston quantity are expressed in mg m ^{2} day $^{-1}$, calculated by scaling up the cross sectional area of each trap, to one square metre, and assuming accumulation occurred linearly in each time period.

Spatial analysis in the resolution obtained for DOM / C / N work is not possible with seston work as only one site per basin was considered. Each site was then divided into two depths, one midway through the water column and one 5 m above the bottom sediments. The deepest locations per basin were selected as considered

most likely to represent integrated processes. These three sites provided information on spatial and temporal variability on an intra-basin scale.

The mass of seston accumulated showed no significant difference with depth. Middle and deep arrays consistently gave statistically the same result ($P = 0.454$). Bulk seston accumulation did however vary with both basin and time of year (Fig. 32). Seston deposition was greater in the south basin and differed significantly from both the middle and north basins ($P < 0.001$); no significant difference between the middle and north basin was recorded ($P = 0.298$). Seston accumulation peaked in the deep waters (~18 m) of the south basin in early November (~day 140) with a mean accumulation rate of 4167 ± 349 mg m^2 day^{-1}. The shallower trap (~10 m) was less than this at 3026 ± 226 mg m^2 day^{-1}. A seasonal pattern is easy to distinguish in both the south and middle basins, with deposition rates reaching a maximum around November/December. Unfortunately, although seasonal variability is observed in the middle basin, data is not available when peaks were recorded in the south. Thus whether a similar peak would have been measured in the middle basin is unclear.

Basin	Collection date	Days after 13 / 05 / 05
North Basin	29 / 06 / 2005	46
	3 / 08 / 2005	82
	7 / 03 / 2006	298
	4 / 04 / 2006	326
	3 / 05 / 2006	355
Middle Basin	29 / 06 / 2005	46
	3 / 08 / 2005	82
	28 / 08 / 2005	107
	28 / 10 / 2005	168
	30 / 12 / 2005	231
	4 / 02 / 2006	267
	7 / 03 / 2006	298
	4 / 04 / 2006	326
	3 / 05 / 2006	355
South Basin	29 / 06 / 2005	46
	3 / 08 / 2005	82
	28 / 08 / 2005	107
	1 / 10 / 2005	141
	11 / 11 / 2005	182
	30 / 12 / 2005	231
	4 / 02 / 2006	267
	7 / 03 / 2006	298
	4 / 04 / 2006	326
	3 / 05 / 2006	355

Table 6: Dates each basin's seston traps were emptied, between 13/5/05 and 3/5/06.

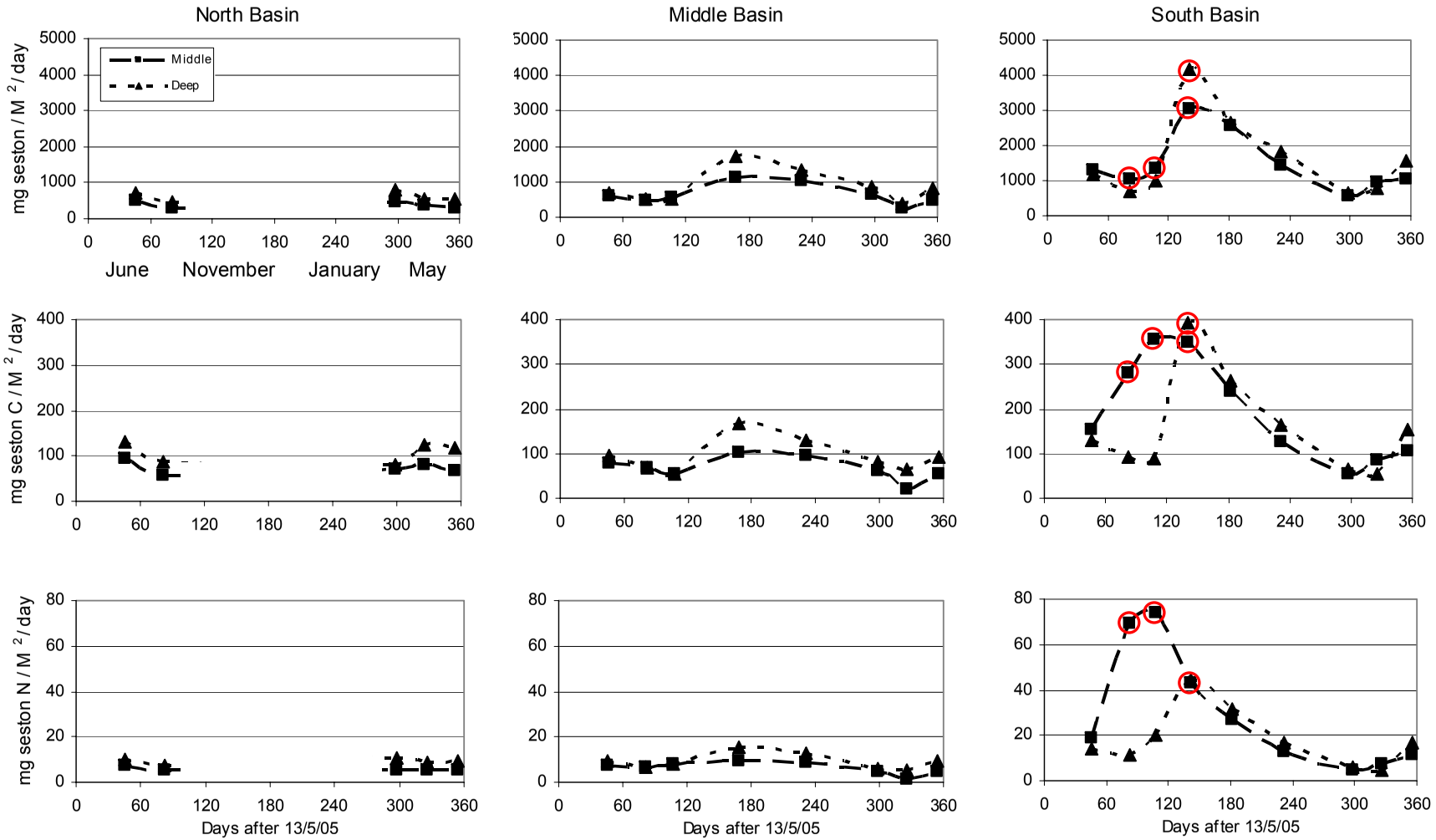

Fig 32: Seasonal variation in a) particulate organic matter concentration, b) particulate organic carbon concentration and c) total particulate nitrogen concentration. Data divided into basin (south, middle, and north) and depth (middle and Deep). Each data point is a mean of the three individual collection tubes. Highlighted samples contained copepods. Mid depths were 9m, 30m and 80m in the south, middle and north basin respectively. Deep depths were 17m, 55m and 170m.

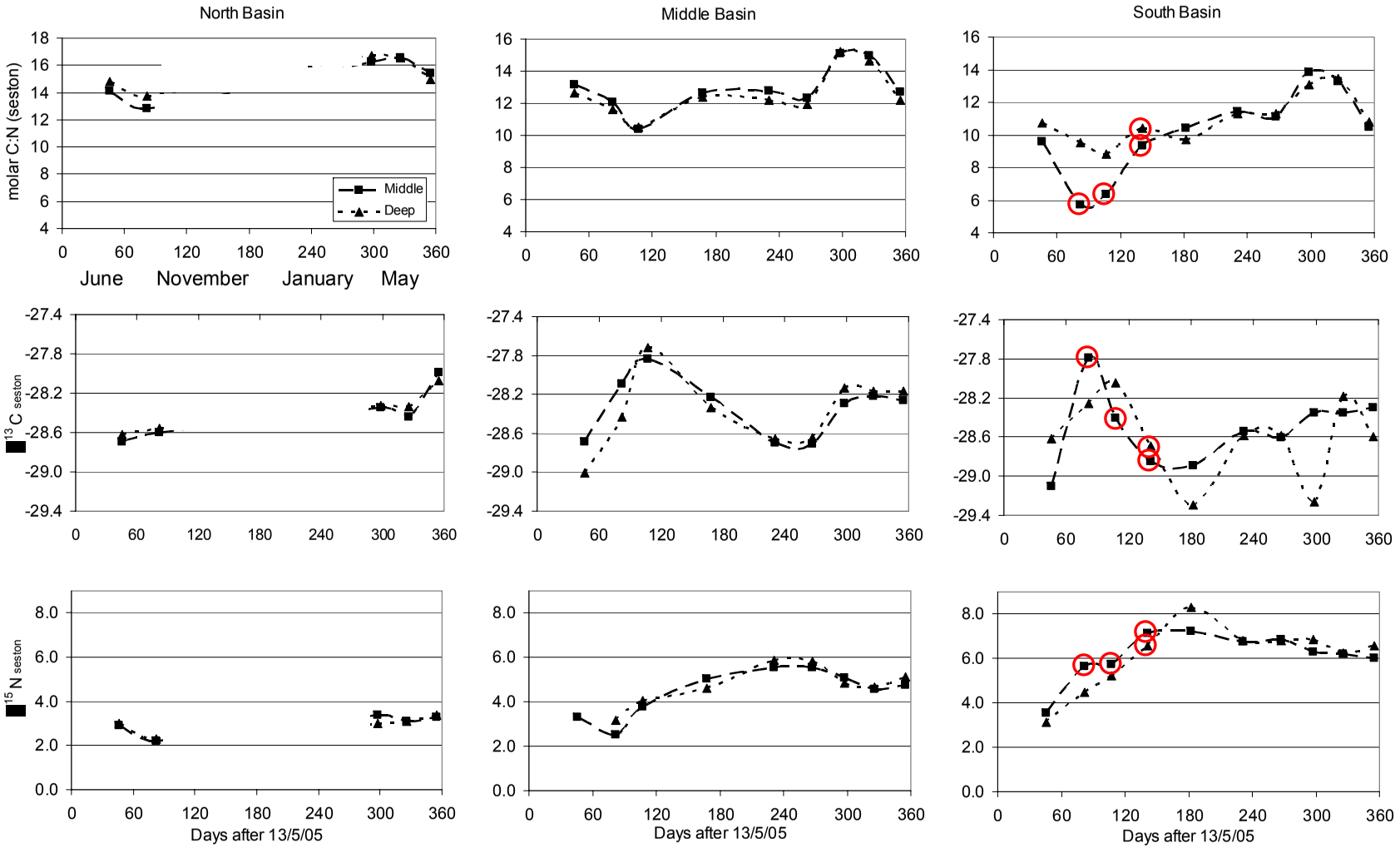

Fig 33: Seasonal variation in a) Molar C:N of seston, b) $\delta^{13}C_{Seston}$ and c) $\delta^{15}N_{Seston}$. Data divided into basin (south, middle, and north) and depth (middle and Deep). Each data point is a mean of the three individual collection tubes. Highlighted samples contained copepods. Mid depths were 9m, 30m and 80m in the south, middle and north basin respectively. Deep depths were 17m, 55m and 170m.

Thus the recorded peak in deposition rate in December (between 1100 and 1700 mg m^{2} day^{-1}) may not be the actual peak in the middle basin. As traps were open ended with no preservative, grazing is probable and as such accumulation rates likely represent a minimum value.

Both south and middle basins show the lowest deposition rates during the summer months. Even though production is higher, weather is generally far more stable and flow into the Loch from the watershed is reduced. In chapter 5, the implication that allochthonous production must be a significant supplier of both dissolved and particulate carbon, particularly in late summer was tested and validated. In either the south or middle basins no significant variation occurred in deposition rates of seston between June and late August 2005 (LSD post hoc P values from 0.100 and 0.928), or between April and June 2006 (LSD post hoc P values from 0.116 to 0.511). Deducing seasonal patterns in the north basin is not possible as data for the autumn/winter is not available. However, similar to the other two basins, deposition rates are low in the summer/spring months, reaching a peak of only 719 ± 380 mg m^{2} day^{-1} in April in the deep-water traps. No significant difference was found between any months sampled in the north basin, suggesting this basin too has stable bulk deposition rates from ~April to December at least.

From stoichiometric characterisation during stable isotope analysis and bulk seston deposition values the accumulation rates of sestonic carbon and sestonic nitrogen can be deduced. The deposition rates of sestonic carbon in deep water closely match the pattern of bulk seston (Fig. 32). However, differences are apparent in the summer months (2005) in the south basin. Here the shallow water traps record far more sestonic carbon than in the deeper traps, and the increase in sestonic carbon is not matched by an increase in bulk seston for the same period. This suggests that the proportional amount of carbon in the seston is increasing, not just bulk seston quantity. This is also the case for nitrogen content in the seston (Fig. 32) where the shallow depth traps record significantly higher deposition rates of sestonic nitrogen than if just a function of seston total mass. Highlighted points show where copepods were definitely present and correspond to these periods of increased weight percent carbon and nitrogen.

The deposition rate of sestonic carbon showed no significant variation with depth ($P = 0.922$) in all basins and all months. Seasonal patterns remained the same as bulk seston excluding the south basin in summer as described previous. But in general sestonic carbon varied significantly with both basin and season in the same way as bulk seston. No significant variation was found in the spring / early summer months, with only the late summer / early winter showing significant increases in

sestonic carbon deposition rates, corresponding to copepod presence / winter turnover periods respectively.

Sestonic nitrogen followed the same patterns described for carbon, although correlation between the two depths sampled is even greater than carbon / bulk seston in the middle and north basins.

Molar C:N of seston (Fig. 33) varies significantly with basin and season ($P < 0.001$) but not with depth ($P = 0.701$). Only in August '05 in the south basin does a difference in depth occur, where on two sampling occasions the shallower trap recorded more nitrogen rich material, again corresponding to observations of copepod biomass. The general pattern between basins is of an increase in molar C:N from south < middle < north, suggesting more nitrogenous compounds further south. The south and middle basins have similar seasonal patterns. The lowest molar C:N values are recorded at the start of the sampling cycle, around May/June. This should be at a time of relatively high productivity, both primary and secondary. The C:N then gradually rises until January. From January the molar C:N rises more steeply from ~11 in the south and 12 in the middle to ~13 and 15 respectively. This rise occurs until April, when the spring blooms likely commences and molar C:N falls again. Patterns in the north seem to closely match that of the middle basin for the periods sampled. Whether the same would have occurred for the rest of the year is unknown.

Seasonal cycles in the $\delta^{13}C_{Seston}$ and $\delta^{15}N_{Seston}$ signatures were not synchronous in the south and middle basins (Fig. 33), although bulk statistical analysis suggests no significant difference between the basins ($P = 0.219$). In both basins, $\delta^{13}C_{Seston}$ becomes more enriched in the summer months between June and late August. Shallow depth samples in the south become ^{13}C-depleted with the autumn/winter thermocline turnover and then steadily more ^{13}C-enriched from late November to June 06. Deeper water shows more variation in this period with two significant depletions recorded in late November and April. Shallow and deep water show close agreement in the middle basin. After the initial enrichment in June '05, $\delta^{13}C_{Seston}$ becomes more depleted until the spring (~day 260) where enrichment begins again, likely coinciding with start of the spring bloom events. The $\delta^{13}C_{Seston}$ shows greater variability than dissolved organic carbon, varying from ~-27.8 to –29.4‰.

In contrast to $\delta^{13}C_{Seston}$, the $\delta^{15}N_{Seston}$ (Fig. 33) shows significant variability with basin ($P < 0.001$) and month ($P < 0.001$). $\delta^{15}N_{Seston}$ tends to become more ^{15}N-depleted from the south basin to the north (Fig. 33). $\delta^{15}N_{seston}$ becomes more enriched from 3.5 ± 0.3‰ in June to 7.2 ± 0.2‰ in late November in south basin shallow water. This increase is mirrored in deeper water but reaches a slightly higher maximum (8.3 ± 0.2‰). Following this enrichment, signatures remain quite constant

between 6.0 and 7.0‰ although do appear to be gradually depleting. $\delta^{15}N_{seston}$ in the middle basin initially becomes more depleted between June and July, then gradually becoming more enriched over the summer months, reaching a plateau between December '05 and January '06. The measured maximum value in the middle basin was less than the south (5.9 ± 0.7‰ in the deeper water trap). The north basin again is difficult to usefully interpret due to lack of data, but the existing data exhibits the same initial depletion as the middle basin does, and also shows more depleted values in the spring/summer of 2006 (day 300 onward).

4.3.2) *Sestonic carbon/nitrogen isotope interaction.*

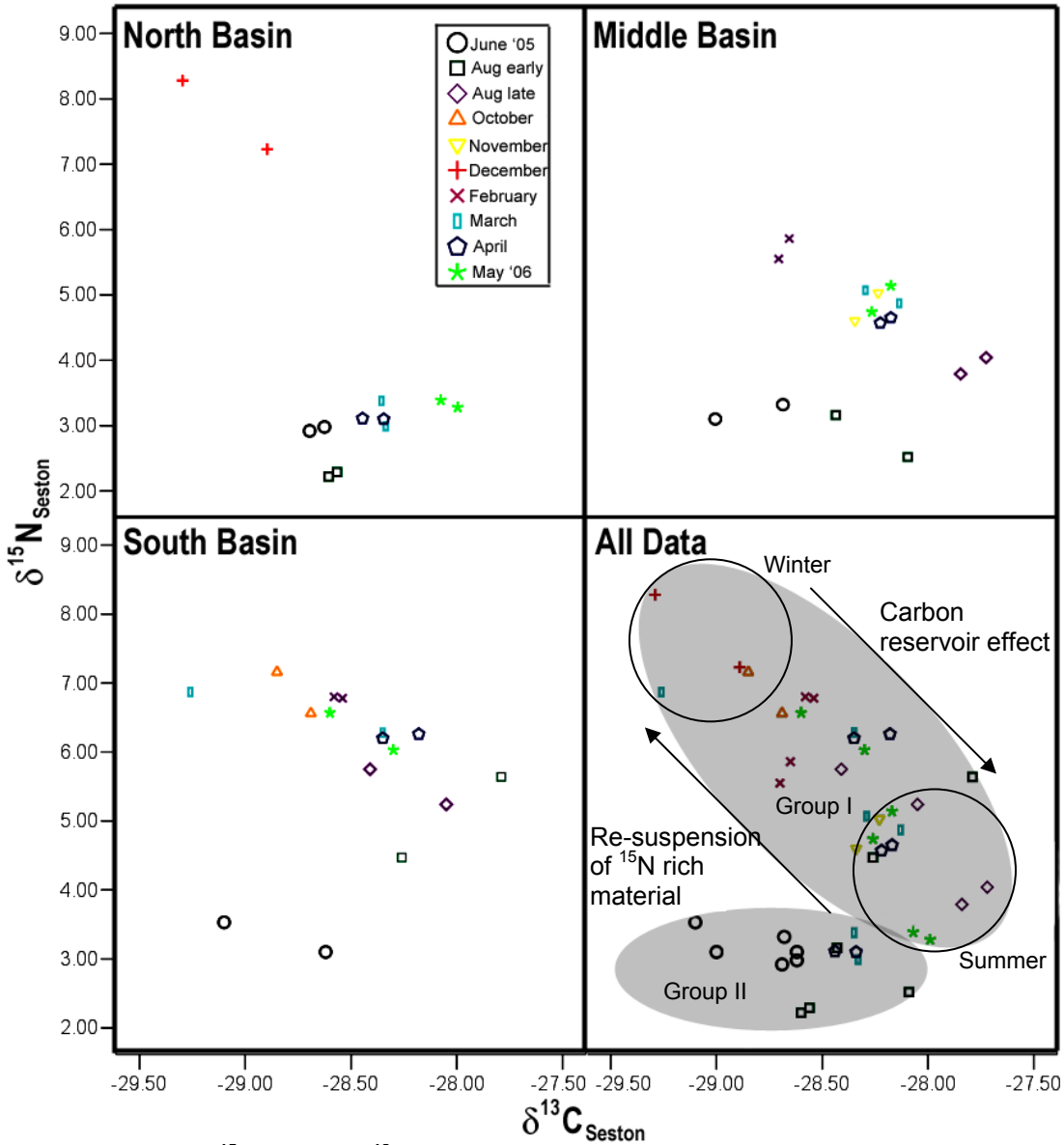

Figure 34: $\delta^{15}N_{Seston}$ / $\delta^{13}C_{Seston}$ cross-plots. Different symbols and colours represent different months sampled. For all data together no significant relationship was found ($R^2 = 0.071$, P = 0.081)

No significant relationship was found when plotting $\delta^{15}N_{seston}$ against $\delta^{13}C_{seston}$ for all the seston data. However, if data for June '05 and early August are not included there is a general trend of enrichment in the carbon pool coinciding with depletion in the nitrogen pool ($R^2 = 0.492$, $P < 0.001$), which is not driven by copepod presence. April '06 has northern basin values that deviate from the main trend and cluster with the data seen for the previous June and August. In June and early August the $\delta^{15}N_{seston}$ signature remains relatively stable although the $\delta^{13}C_{seston}$ tends to change noticeably. For the purposes of future discussion two groups have been defined in the cross plot (Fig. 34).

Group one makes up the majority of sampling points. A significant negative linear correlation exists in this group. It seems in general, winter seston during lake turn-over occupies the ^{15}N-enriched, ^{13}C-depleted zone, with the more productive months at the other end.

Group two contains fewer sample sites than group one. All June 05 samples are in this group, with the north basin traps being more depleted in $\delta^{13}C_{Seston}$. Early August middle and south basin traps, and north basin traps from April, May and June 06 are also in this section. Group two seems to represent mid to late summer seston signatures in all three basins.

4.3.3) Source of sestonic material

Using the molar C:N values of the seston a 2-way mixing model was calculated to estimate the % allochthonous material made of the bulk material. $\delta^{13}C_{seston}$ showed insufficient variability for significant temporal resolution to be obtained. Thus two models were calculated using molar C:N of seston and $\delta^{15}N_{seston}$.

A model was calculated according to equation 8 (page 91) using molar C:N of seston:

$$\mathbf{C{:}N_T * M_T = (C{:}N_{auto} * M_{auto}) + (C{:}N_{allo} * M_{allo})}$$

where $C{:}N_T$ is the measured molar C:N of seston, M_T is fractional mass of total seston, $C{:}N_{auto}$ is the estimated ratio of autochthonous material, M_{auto} is the fractional mass of autochthonous material, $C{:}N_{allo}$ is the estimated ratio of allochthonous material and M_{allo} the desired fractional allochthonous component of seston.

Mass values for the equation are expressed as a fraction; M_T will equal 1 with both M_{auto} and M_{allo} being < 1. The estimated C:N ratio of 100% autochthonous production was taken as the Redfield ratio (6.625:1), and the C:N allochthonous end member

was varied around estimated ranges from other studies (30:1-60:1) (e.g., Hutchinson 1956, Royer and Minshall 1997). The model has been run twice using these two end members to obtain the range of possible values. Results are shown in Table 7 and Figure 35.

By varying molar C:N allochthonous end members, the fractional contribution of allochthonous material to bulk seston showed a pronounced variability. When using the lowest estimated ratio for terrestrial material of 30:1, in general the percentage of allochthonous material was greater by up to 20%.

In general the percentage allochthonous component of the seston is lower than 40% and can be < 10% when using the 30:1 Model. Using molar C:N of 60:1, allochthonous seston never reaches more than 20% of the bulk sestonic material. The true contribution is likely between these two extremes. Seasonal and spatial trends are visible in the results. In general there is a decrease in the % allochthonous material in the late summer months (days 0 - 150). The southern basin at this time shows 100% autochthonous material, coinciding with periods of high primary productivity, and the recorded presence of zooplankton, which due to low molar C:N of the biomass, will have skewed the results significantly towards autochthonous seston. This initial low period is followed by a rise during the autumn/winter turnover events (~ December onward) with allochthonous contribution reaching a maximum around day 300, at the end of winter. Percentage contribution of allochthonous seston then begins to drop again as spring approaches.

Intra-basin variability is also evident (Tables 7, 8 and Fig. 35) with the fractional contribution of allochthonous material being greatest in the north basin, followed by the middle, followed by the south regardless of end member composition. South basin top traps give the lowest values in late spring/summer where no allochthonous material is shown. However, this is again due to methodological difficulty separating out a zooplankton component from the bulk material, skewing the estimates to unlikely values.

Due to uncertainty of molar C:N end members of allochthonous material, the percentage contribution of allochthonous material to seston was also investigated using the $\delta^{15}N_{Seston}$ instead of molar C:N. $\delta^{15}N$ of autochthonous and allochthonous sources tend to be quite variable, thus defining typical end member signatures is also difficult. For this model an end member for autochthonous sources was defined as 6‰ (Grey *et al* 2004) and allochthonous sources ranging from –2.7‰ (Admundson *et al* 2003) to –1.1‰ (Owens *et al* 1999). As with the C:N model each extreme value will be modelled to give a range of possible values. The model thus becomes:

$$\delta^{15}N_T * M_T = (\delta^{15}N_{auto} * M_{auto}) + (\delta^{15}N_{allo} * M_{allo}) \quad \text{Eq. 9}$$

30: 1	June '05	August early	August late	October	November	December	February	March	April	May '06
North surface	32%	26%						41%	43%	38%
North deep	35%	30%						43%	43%	35%
Middle surface	28%	23%	16%		26%		26%	36%	36%	26%
Middle deep	26%	21%	17%		25%		24%	34%	34%	24%
South surface	13%	0%	0%	12%			21%	28%	28%	16%
South deep	18%	13%	9%	16%			20%	29%	29%	18%
60: 1	June '05	August early	August late	October	November	December	February	March	April	May '06
North surface	14%	12%						18%	19%	16%
North deep	15%	13%						19%	19%	16%
Middle surface	12%	10%	7%		11%		12%	16%	16%	11%
Middle deep	11%	9%	7%		11%		10%	16%	15%	10%
South surface	6%	0%	0%	5%			9%	14%	12%	7%
South deep	8%	5%	4%	7%			9%	12%	13%	8%

Table 7: Percentage contribution of allochthonous organic material to bulk seston using mass balance model based on molar C:N, using two end allochthonous end member values of 30:1 and 60:1. Missing data represents periods when trap recovery could not take place.

-2.7‰	June '05	August early	August late	October	November	December	February	March	April	May '06
North surface	35%	43%						30%	33%	31%
North deep	35%	43%						35%	33%	30%
Middle surface	31%	40%	25%		11%		5%	11%	16%	14%
Middle deep	33%	33%	23%		16%		2%	13%	16%	10%
South surface	28%	4%	3%	0%			0%	0%	0%	0%
South deep	33%	18%	9%	0%			0%	0%	0%	0%
-1.1‰	June '05	August early	August late	October	November	December	February	March	April	May '06
North surface	43%	53%						37%	41%	38%
North deep	43%	52%						42%	41%	37%
Middle surface	38%	49%	31%		14%		6%	13%	20%	18%
Middle deep	41%	40%	28%		20%		2%	16%	19%	12%
South surface	35%	5%	4%	0%			0%	0%	0%	0%
South deep	41%	22%	11%	0%			0%	0%	0%	0%

Table 8: Fractional contribution of allochthonous organic material to bulk seston using mass balance model based on $\delta^{15}N_{Seston}$, using two end allochthonous end member values of -2.7‰ and -1.1‰.. Missing data represents periods when trap recovery could not take place.

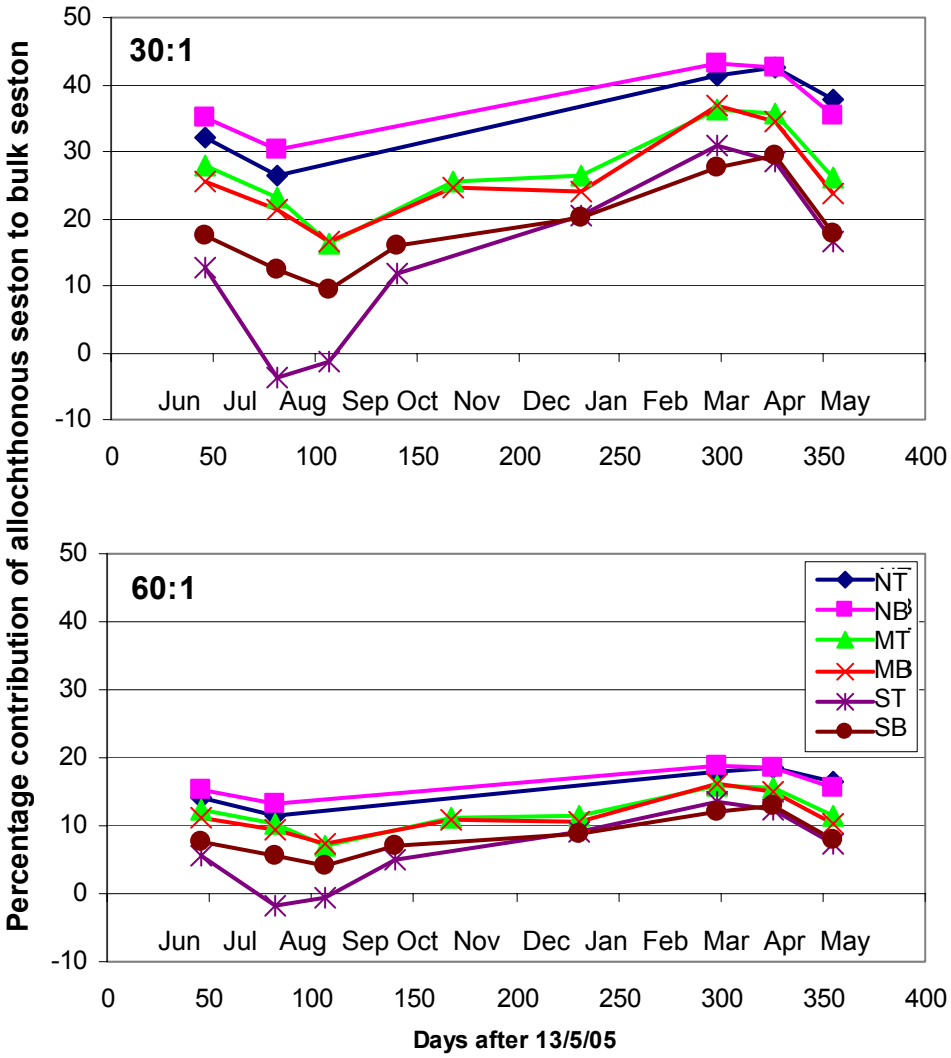

Figure 35: % allochthonous material in bulk seston from molar C:N, using 30:1 and 60:1 allochthonous end members. Data divided by basin (N = north, M = middle, S = south) and trap depth (T = top trap, B = bottom trap).

Values obtained from the second model show higher proportions of allochthonous material in late summer, over 50% in the –1.1‰ end member model. As opposed to an increase in the % allochthonous contribution to sestonic material during winter turnover, increase in the $\delta^{15}N$ model predicts the opposite. This is unlikely as winter is generally the period of highest allochthonous material.

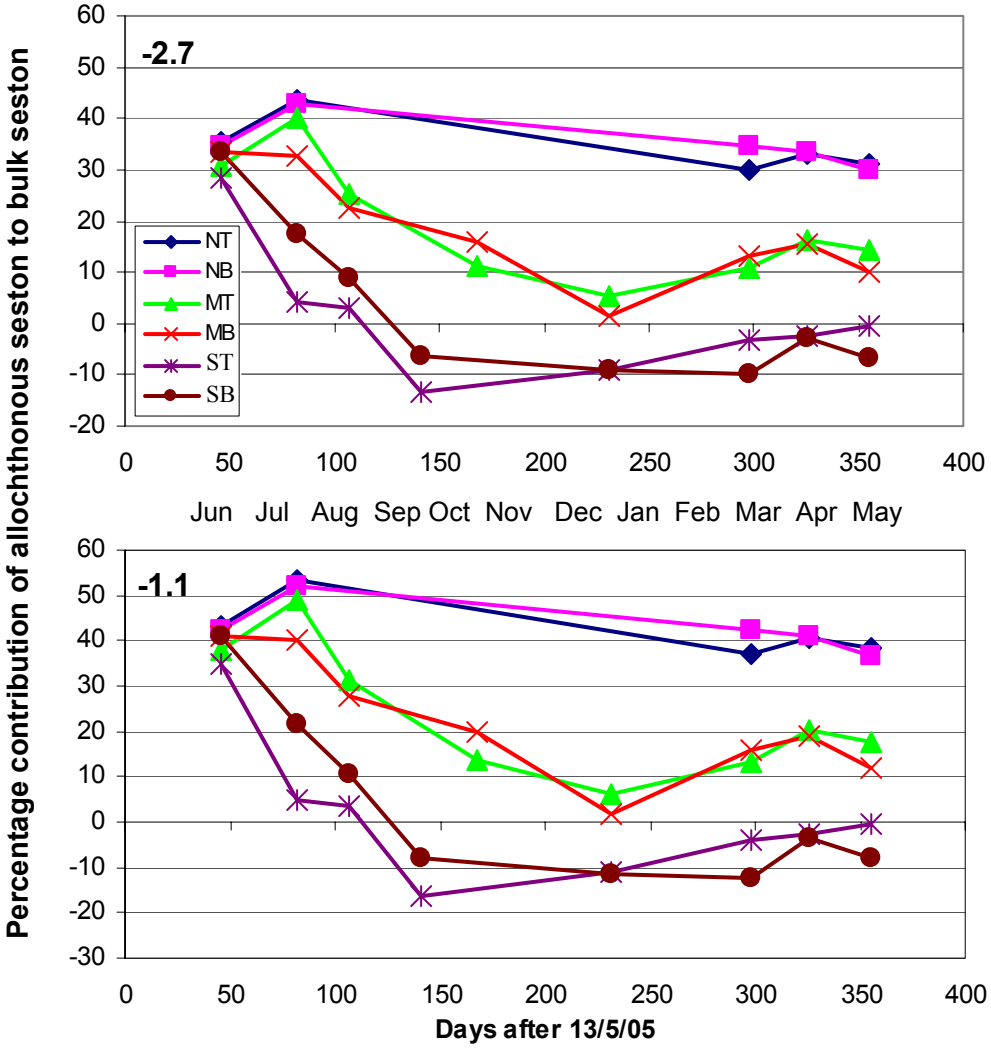

Figure 36: Percentage of allochthonous material in bulk seston calculated from $\delta^{15}N_{seston}$, using -2.7‰ and -1.1‰ allochthonous end members. Data divided by basin (N = north, M = middle, S = south) and trap depth (T = top trap, B = bottom trap).

4.4) Discussion

4.4.1) Temporal and spatial variation in seston accumulation.

Seston has been shown in other studies to attain peak deposition rates in the winter turnover period (Pennington 1974, Wetzel 2001). In Loch Lomond the seasonal cycle of sestonic deposition varies between basins. Due to lack of data for the north basin for most of the study period, this basin will be considered little from this point onwards.

In general the seston accumulation rates drop from south > middle > north (Fig. 32, page 108). Greater depths in the middle and north basin render disturbing of bottom sediments by rough weather more difficult, although it likely still occurs during winter turnover). Past productivity estimates (e.g., Maitland 1981, SEPA and EUROLAKE reports) point to a less productive north basin also, so less autochthonous material would be produced in surface waters to sink to the lake bed. Also, catchment characteristics such as the base-poor soils, low population and steep gradients mean less allochthonous material entering the basin. A combination of these factors likely accounts for the observed inter-basin variability in sestonic accumulation rates.

Peak deposition rates correspond to the predicted period of autumn / winter turnover, specifically between September and December (Fig. 32). This increase, observed also in other lakes (e.g., Pennington 1974, Habib *et al* 1997), is believed to result from re-suspension of bottom sediments as lake mixing occurs, coupled to increased allochthonous contributions during the winter period. The south basin shows both the highest average deposition rates and the highest turnover peak (~ 4000 mg m^2 day^{-1}). This is likely due to the shallowness of the basin and the consequence that bottom sediments are likely easier to disturb and re-suspend, due to the closer proximity of the more energetic surface waters. Along with the fact that higher productivity in the south leads to more settling material in general, of both phytoplankton and zooplankton origin (Habib *et al* 1997), and the presence of the largest inflows bringing large amounts of allochthonous particulate material into the lake.

It could be hypothesised that the deep traps would be more significantly affected by winter turnover than the shallower traps, particularly in the middle and north basins. Due to the depth of the water column it is unclear whether disturbed bottom sediments would be mobilised enough to reach the higher traps at shallower depths. However, it can also be suggested that falling allochthonous material brought to

surface waters in the winter will add proportionally more to shallower traps, gradually being degraded by microbial activity as it descends. Thus the two processes may balance each other out and explain why in all basins no significant variability between shallow and deep traps was observed. This is highly speculative and in need of further research to validate or deny.

Observed variation between traps has revealed that sestonic accumulation rates vary between basins in Loch Lomond, and on an annual cycle previously described in other water bodies. The effect this potentially has for basin production values is unclear. Much labile material will be processed before it sinks through the water column so nutritional values for sediment bacteria may be limited, although previous work has suggested particulate material is actually processed little while sinking (e.g., Ducklow *et al* 1982). Processing during descent may be of particular importance in the deeper middle and south basins, where sinking material has a greater time period to be processed, as well as significantly less source material to begin with.

Particulate material can provide a surface on which bacterial production can exceed free living bacterial cells (e.g., Friedrich *et al* 1999). Although in general attached bacteria make up a smaller component of the total bacterial community in the water column, if there are significant differences in the concentration of material the effect on production may be significant. Higher recorded values of sestonic material in the south basin for example may be a factor in possible bacterial production differences between basins, examined further in chapter 5.

4.4.2) *Temporal and spatial variation in $\delta^{13}C$, $\delta^{15}N$ and C:N of seston.*

The measured range in the $\delta^{13}C$ of seston, ~ 1.6‰, exceeds that observed for DOC, ~ 0.4 - 0.6‰ but is still relatively small. In both the south and middle basins there is a small enrichment in the summer months followed by a depletion over the autumn / winter turnover period, followed again by subsequent enrichment as spring approaches (Fig. 33). $\delta^{13}C_{Seston}$ enrichment likely reflects decreasing isotopic fractionation between input inorganic and output organic carbon as the summer progresses, a phenomenon termed the “reservoir effect” by Lehmann *et al* (2004). As phytoplankton fix DIC, they preferentially incorporate ^{12}C, leaving the remaining pool enriched in ^{13}C. Thus as inorganic carbon becomes less available during times of high productivity, and when the water column is stable and undergoes little mixing, phytoplankton use more of the remaining ^{13}C-enriched DIC, and their biomass becomes part of the more enriched particulate pool. This reservoir effect is supported

by the enrichment of the $\delta^{13}C_{DIC}$ pool seen in the summer of 2005 (Chapter 2). During the autumn / winter turnover events, the mixing of benthic material and an increased inflow of inorganic and organic nutrients from the watershed bring more $^{12}C_{DIC}$ (inflow $\delta^{13}C_{DIC}$ average most depleted in September = -11.7‰), fractionation again increases lowering the $\delta^{13}C_{Seston}$ signature.

Seston $\delta^{13}C$ in Loch Lomond is similar to that found in two Adirondack lakes where values between – 28 and – 30‰ were recorded (Owen *et al* 1999). Most enriched values were recorded in the summer months. Further evidence that increasing utilisation of DIC by algae is responsible for observed seasonal rises in $\delta^{13}C_{POC}$ is supported by Bernasconi (1997) where summer values in a eutrophic lake reached –22‰.

As well as decreased photosynthetic rates, heterotrophic production rates may increase at the end of the summer period, utilising the remaining organic material from the bloom events of spring and summer, along with the increasing supply of allochthonous material. During this heterotrophic breakdown, ^{12}C is preferentially incorporated leading to ^{13}C-depleted DIC produced (e.g., Rau 1978). This gradual decline was observed in the middle basin until the spring bloom period where a sharp enrichment was observed (Fig. 33). This contrasts with the south basin where following an initially significant depletion in November $\delta^{13}C_{Seston}$ becomes steadily more enriched from late November to June in the shallow traps. The mechanism for such enrichment is unclear, but could possibly be linked to bacterial breakdown of seston becoming proportionally more prolific in the winter. i.e., the labile / refractory balance of organic material is a continuous scale and not two discrete forms. During times of little labile supply, bacteria may process material usually considered refractory. Thus during the winter more sestonic material may be continually processed, and via the selective incorporation of ^{12}C by bacteria, leave the remaining seston isotopically heavier. $\delta^{15}N_{seston}$ was most depleted in the late summer / early autumn months which supports this hypothesis, reflecting selective incorporation of ^{14}N by phytoplankton. Fresh supply of inorganic nitrogen from the catchment and overturn of lake sediments likely cause the enrichment then observed in autumn / winter.

The deep traps in all three basins show the same general enrichment in $\delta^{13}C_{seston}$, but in the south basin an additional period of depletion was observed in March. The observed periods of depletion may be caused by rough weather around that time, stirring up more of the bottom sediments which could potentially bring more depleted material into the lower traps, although the amount of bulk seston did not differ between depths at this time (Fig. 32). Or an increased supply of C3 plant material

washed into the lake during a period of high precipitation. More information would be required to elucidate with less doubt the cause of this measured depletion.

As well as the balances between photosynthesis, respiration and possibly nitrogen fixation, other factors from higher trophic levels may be influencing the observed change in $\delta^{13}C$ of seston. Although copepod species were removed where possible if any did remain, along with other known zooplankton species, their isotope signature may be reflected. Removed copepods had an isotope signature of ~-27.6‰, which is more enriched than found in other studies (e.g., Mathews *et al* 2003) possibly due to an isotopically heavier diet. Their isotope signature implies that if they or other zooplankton were present in the seston analysed during the summer they may have contributed to the observed enrichment. When examining the data without any dates recording copepods, the patterns do remain but seasonal changes are less pronounced.

Along with analysing extracted copepod remains, a leaf found in June was also analysed. Though C3 plant $\delta^{13}C$ can vary by ~10‰, the leaf $\delta^{13}C$ of – 28‰ is considered typical of surrounding C3 vegetation. Although one leaf does not equate to all allochthonous material, it is an indication at least of the $\delta^{13}C$ of particulate material transported to the water column.

The nitrogen isotope signature of seston can offer further insight into the metabolic processes occurring in the lake and acting on sestonic material. The link between phytoplankton and thus seston $\delta^{15}N$ and utilisation of inorganic nitrogen has been explored in the marine (Wada and Hattori 1976, Altabet 1989) and freshwater (Owen *et al* 1999) environments. Here the lack of differentiation between the inorganic and organic nitrogen pools complicates a definitive interpretation.

There are two main patterns to be considered; the observed rise in $\delta^{15}N_{Seston}$ during the winter months and the observed decline in $\delta^{15}N_{seston}$ values from south to middle to north. $\delta^{15}N_{seston}$ values can very significantly from lake to lake depending on trophic status and other parameters, which make inter-lake comparison difficult, but patterns can be elucidated.

The observed increase in $\delta^{15}N_{seston}$ in the south basin (Fig. 33) during the late autumn / winter months appears to concur with other studies (e.g., Hodell and Shelske 1998) where isotope signatures were lowest in the summer and increased during winter. It is possible that autumnal overturn had started in the south basin around August although (~day 80) and the rise is due to that. Lehmann *et al* (2004) observed the same pattern in Lake Lugano, with minimum ^{15}N values in summer and maximum in winter. Both these studies and Owen *et al* (1999) recorded enrichment in

the $\delta^{15}N_{Seston}$ with a concurrent depletion in the $\delta^{13}C_{Seston}$. This pattern is seen in Loch Lomond also in both the south and middle basins (Fig. 33).

Possible explanations for the low summer $\delta^{15}N_{seston}$ are that that during summer inorganic nitrogen (mainly NO_3^-) is readily utilised by algae. While inorganic nitrogen sources are not limited there is a selective incorporation of ^{14}N by phytoplankton and a subsequent depletion in $\delta^{15}N$ of the sestonic material produced (Hodell and Shelske 1998). During the winter months, seston is likely dominated by material broken down via heterotrophic activity and detrital sources, which tend to be more isotopically enriched (Hodell and Shelske 1998), coupled to the fact more ^{15}N-enriched nitrate is continually added from the water shed and not utilised. This interpretation is supported by other studies that have found a negative relationship between nitrate concentration and $\delta^{15}N$ of phytoplankton (e.g., Wada and Hattori 1976, Altabet 1989, Altabet and Francois 1994, Owen *et al* 1999). Photosynthetic activity and thus inorganic nitrogen incorporation to biomass is reduced during the winter so the observed enrichment decreases. Both the middle and north basins show an initial autumnal decrease in the $\delta^{15}N_{seston}$, suggesting the thermocline may not yet have broken down and decreasing signatures are observed in response to mineralization of inorganic nitrogen.

More enriched $\delta^{15}N_{seston}$ in the winter months may be caused by the contribution of heavily degraded organic matter making up more of the bulk material. As the bulk seston is degraded remaining material becomes enriched in ^{15}N. Material in the lake sediments and hypolimnetic water column is likely more enriched in ^{15}N as bacterial degradation will have preferentially utilised the ^{14}N fraction. Input of this enriched particulate material from below the thermocline during winter turnover may also be a significant factor in the observed elevated $\delta^{15}N$.

Cross plotting $\delta^{13}C_{POC}$ and $\delta^{15}N_{TPN}$ revealed two main groups defined on Figure 34. Previous work by Bernasconi *et al* (1997) on Lake Lugano, a deep oligotrophic lake in Switzerland, showed two distinct groups in their study also, but with different temporal patterns to this study. In Lake Lugano, depleted $\delta^{13}C$ corresponded to enriched $\delta^{15}N$ in winter, and vice versa in the summer, consistent with the previously discussed patterns of increasing $\delta^{13}C_{seston}$ and falling $\delta^{15}N_{seston}$ in the summer.

Loch Lomond group I sample response (Fig. 34) is relatively consistent with the conclusions drawn by Bernasconi *et al* (1997). There is a negative linear relationship. Seasonal dependence would predict summer values to cluster at one end of the relationship, and winter the other. In group one this is observed, but is less distinctive than in Lake Lugano. Early November, December and January occupy the

end of highest $\delta^{15}N$ and lowest $\delta^{13}C$, with April, May, June and August at the other end, consistent with Lake Lugano. It has already been shown that $\delta^{15}N$ particularly varies across the lake, implying that spatial variability may be adding observed noise to the linear negative relationship that seems to be seasonally controlled.

Group II shows areas where the $\delta^{15}N$ signatures are relatively stable, but the $\delta^{13}C$ is variable. This group is occupied by all June 05 samples, middle and south basin early August samples and north basin traps in April, May and June also. This group may represent the lower limit of $\delta^{15}N$, dependent on the source inorganic nitrogen composition. North basin traps in December 2005 occupy a section away from group II where all other North sample points are, being depleted inc $\delta^{13}C$ and enriched in $\delta^{15}N$.

The molar C:N of seston in Loch Lomond is generally quite low (Fig. 33), between 6 and 14. These ratios reflect the quality of sestonic material and thus possible availability to other organisms for metabolic utilisation. In some studies molar C:N has been observed to increase with lake depth, presumably as a response to higher rates of secondary processing in shallow waters and preferential processing of nitrogenous compounds (Pennington 1974). This pattern was only observed in Loch Lomond in the summer months in the south basin. A lack of variation with depth in the middle basin suggests that particulate material produced in the epilimnion may not be significantly processed while sinking to the hypolimnion. Lack of data for the north basin makes interpretation difficult, but the five available data points show no molar C:N variation with depth.

During the spring / summer months there were copepods present in the sestonic material. Although care was taken to remove them as thoroughly as possible, it is likely parts of them remained. Zooplankton is high in protein and would thus lower the molar C:N of the bulk seston. This may also contribute to the observed decline in molar C:N during the spring / summer sampling periods.

Molar C:N increases from south basin, > middle basin, > north basin. This supports previous conclusions that productivity is significantly less in the northern parts of the lake, and as such low C:N compounds, indicative of within lake production and high productivity are less common. In both the south and middle basins there is an initial drop in the molar C:N during the summer, likely associated with the higher productivity and associated biomass increase. After this initial drop the molar C:N gradually rises to peak at the end of winter. This rise is likely due to both the secondary processing of organic matter by bacteria, preferentially incorporating nitrogen, and the increased input of allochthonous material from the watershed, which generally has a higher C:N ratio than autochthonous production

(LaZerte 1983, Hecky *et al* 1993). Both basins then show the beginnings of a drop in the molar C:N as spring begins, likely accompanying the first algal blooms of the year.

Conclusions drawn here were tested during the incubation experiments also (chapter 5), which revealed a significant rise in molar C:N (maximum of 17.9 in December) in the winter months of the north basin, dropping with the onset of spring and reaching a minimum in late summer (minimum of 6.1 in September). The south basin failed to show a similar magnitude winter peak, but still revealed a noticeable drop in molar C:N in spring / summer reaching 5.6 in April. This data further supports the conclusions of this chapter that molar C:N is influenced significantly by the annual production cycles and the likely balance between autochthonous and allochthonous sources of material (further explored in section 4.4.3). Copepods were not observed in any incubation experiment, although likely some less apparent zooplankton remains may have been present at times of high productivity. The observed spring / summer molar C:N minimum values are likely caused by a combination of increased autochthonous production, discussed above and presence of protein rich zooplankton remains.

4.4.3) *Source of sestonic material*

The isotope ratios of carbon and nitrogen and molar C:N were studied to help understand the source (autochthonous or allochthonous) of sestonic organic material in Loch Lomond. $\delta^{13}C_{Seston}$ varied little, so was not useful in resolving the source. Various other difficulties exist in applying $\delta^{13}C$ values to elucidate organic material origin (see France 1996 and references within), and although similar difficulties likely exist with $\delta^{15}N$ and molar C:N, clearer differences in end member values mean estimates using these have been produced. Both $\delta^{15}N$ and C:N were used to estimate allochthonous contribution to seston, however patterns varied between models. $\delta^{15}N$ use yielded a high proportion of values below 0%, likely due to the presence of zooplankton remains, which have significantly higher $\delta^{15}N$ being one trophic level higher than phytoplankton or detritus. As such the $\delta^{15}N$ model is believed to be the less accurate of the two, so the model based on molar C:N will be used for further discussion.

As discussed in chapter 3, the proportion of autochthonous material to allochthonous material in limnetic systems is dependent on several different factors, ranging from the catchment characteristics, to within system production levels. Seston showed a generally low contribution of allochthonous material to bulk organic

matter ranging from 0% to ~42%. There was significant variability both temporally and spatially. The north basin showed the greatest proportion of allochthonous material, followed by the middle basin and then the south. The north basin has a steep, fast flowing catchment draining base poor soils, which would suggest less allochthonous material will enter the lake here compared to the shallow gradient, base-rich soils of the south basin. However, higher productivities in the south basin also add a greater amount of autochthonous material via phytoplankton and zooplankton, and could thus mask the increased quantity of allochthonous material from the catchment yielding the higher proportions of allochthonous material estimated in the north basin. The higher residency times in the north basin also mean that organic material has greater opportunity for microbial utilisation, which would likely utilise autochthonous material preferentially and increase the observed proportion of allochthonous organic material.

As the model used is dependent on changes in the molar C:N, seasonal patterns follow the same pattern shown in Figure 33. The contribution of allochthonous organic material to seston is least in the summer months in all three basins. A minimum value is estimated in the south basin where 100% of the organic material is predicted to be autochthonous. Although it is entirely plausible that autochthonous organic material will be proportionally more significant in the spring / summer than winter, the likely presence of zooplankton remains in these samples mean 100% is likely an overestimate of autochthonous contribution to seston. Allochthonous organic material would be less prevalent in the productive periods as phytoplankton and zooplankton bloom, releasing significant quantities of autochthonous material. With the onset of winter this productivity declines and coupled with generally higher levels of precipitation and thus inflow into the lake, the proportion of allochthonous material increases in the seston.

Observed variability in the dynamics of sestonic source has provided further information on possible changing roles and dominance between within lake and terrestrial sources of energy. The presented model is certainly skewed in favour of autochthonous production via presence of zooplankton biomass but seasonal trends may still be of significance. The increased prevalence of allochthonous seston during the winter months is suggestive of a system dominated at that time by processing of material from the watershed, imported into the lake, with the opposite being true in the spring / summer. The re-mobilisation of sestonic material in the winter months, be it from the lake-bed or the catchment raises the possibility of increased substrate for

bacterial processing and potential heterotrophic dominance. The conclusions and implications of this chapter are further examined in chapters 5 and 6.

Chapter 5

The autotrophic / heterotrophic production balance in Loch Lomond

5.1) Introduction

Classical views of aquatic ecosystems were of food chains dependent on phytoplankton production and the potential consumption and utilisation of this production by higher trophic levels. The significance of heterotrophic bacteria in the recycling of dissolved organic matter (DOM) has been known for some time (e.g., Kuznetsov 1970, Simon 1987), but their subsequent utilisation by heterotrophic nanoplankton and re-incorporation into trophic transfer via microbial loop pathways was elucidated later (Azam *et al* 1983).

More recent observations have shown that the balance between autotrophic and heterotrophic production is variable, and in oligotrophic aquatic environments both net heterotrophy (e.g., Dortch and Packard 1989; Gasol *et al* 1997, del Giorgio *et al* 1997, Biddanda *et al* 2001) and net autotrophy (Carignan *et al* 2000) have been measured. The question of which method of production, phytoplanktonic or bacterial, dominates a system has fundamental consequences to the overall carbon processing of each system, as does the coupling or de-coupling between the two. Net autotrophic systems, dominated by photosynthetic pathways can be net sinks for CO_2, whereas net heterotrophy can lead to supersaturation of CO_2 and subsequent evolution of CO_2 to the atmosphere (Cole *et al* 1994) making these systems possible net sources of carbon.

Lakes present an interesting scenario as many receive large subsidies of DOM from terrestrial (allochthonous) sources, suggested as a substrate for bacterial utilisation, and thus potentially increasing the importance of heterotrophic processes. Net heterotrophy has also been observed in river systems which also receive large terrestrial subsidies to the within-system (autochthonous) produced organic material (Maranger *et al* 2004), resulting in evasion of CO_2 to the atmosphere and higher DIC concentrations downstream (e.g., Raymond *et al* 1997, Raymond and Bauer 2001).

The apparently contradictory conclusions of oligotrophic systems being net heterotrophic (e.g., del Giorgio *et al* 1997) and net autotrophic (e.g., Carignan *et al* 2000) has more recently been explored with respect to concentrations of dissolved organic carbon (Prairie *et al* 2002). It has been suggested that far from the previous studies giving contradictory results, each study was examining systems on different

extremes of a DOC concentration gradient. Prairie *et al* (2002) proposed a threshold value in DOC below which a system will be autotrophic and above which will be heterotrophic. This concentration of DOC suggested was between 4-6 mg/L.

Much of the boreal and temperate zones are known as net sinks of CO_2 from the atmosphere (e.g., Apps *et al* 1993), however many estimates do not include the flux, in or out, of CO_2 from lakes, dictated by the relative balance between autotrophic carbon fixation (consuming CO_2) and heterotrophic carbon utilisation (producing CO_2). For more accurate modelling of these systems, essential in times of increasing interest in global carbon dynamics, data on relative productivities is required. By using concurrent measures of primary and secondary productivity over space and time the net flux of carbon through the system can be elucidated.

Here I present data considering the temporal and spatial metabolic balance in Loch Lomond. From previous survey work (chapter 3) [DOC] is known to vary over time and space due to the varying trophic, morphometric and catchment characteristics (Fig.22 and 26, chapter 3), but are generally below the autotrophic / heterotrophic boundary defined by Prairie *et* al (2002). Thus, Loch Lomond should be net autotrophic. By elucidating how such variations in [DOC] relate to metabolic balance in the lake, the validity of this [DOC] boundary to pelagic production in Loch Lomond can be tested, and potentially complete models of carbon transfer through the metabolic pathways can be elucidated over time and space for Loch Lomond. This lake has the potential to show varying ratios between phytoplankton production (PP) and bacterial production (BP) over both time and space due to varying trophic levels between basins and heterogeneous distribution of both inorganic (chapter 2) and organic matter (chapter 7). Hence the question of its net heterotrophic / autotrophic state is not straightforward. Previous work has examined variation between PP and BP in variable systems of similar trophic states (e.g., Biddanda *et al* 2001, Jansson *et al* 2003), and at different timescales. By conducting a year long survey, that accommodates Loch Lomond's spatial trophic / physico-chemical heterogeneity, both temporal and spatial variability on the balance between autotrophic / heterotrophic pathways and its relationship to various different parameters was considered.

Bacterial production has been known to be of significance in aquatic systems for some time. However, due to uncertainty and variability in bacterial respiration to bacterial production ratios their use in elucidating robust models of carbon pathways

has been limited (e.g., Brock 1987, Pomeroy and Weibe 1993). Bacterial production is a combination of bacterial biomass production (BBP) and bacterial respiration (BR), and only when considered together can more accurate assessment of their role in aquatic carbon cycling be examined (Jahnke and Craven 1995). Respiration in lakes and other aquatic systems is a growing area of research (e.g., Schwaerter *et al* 1988, del Giorgio *et al* 2005) as its significance is revealed. In this chapter PP, BBP have been measured, BR and total BP (BBP + BR) have been estimated to shed more light on lake carbon cycling and the relative contributions of phytoplankton and bacteria to overall lake metabolism.

In this chapter I set out to examine 3 hypotheses

i) The south basin of Loch Lomond will have higher fluxes of carbon through both the phytoplanktonic and bacterial metabolic pathways than the north basin due to its slightly higher trophic state.
ii) Despite being a generally oligotrophic system, in the epilimnion of Loch Lomond algal autotrophic production will exceed bacterial heterotrophic production.
iii) Due to large areas of the lake that are below the photosynthetically active epilimnion, Loch Lomond will be, in total, a heterotrophic system.

5.2) *Methodology*

To directly measure primary and secondary productivity an isotope tracer method was used. The method detailed relies on selective uptake of a ^{13}C-labelled DIC source (bicarbonate) for photosynthetic measurement, and a ^{13}C-labelled DOC source (leucine) for bacterial productivity and respiration. Productivity is calculated by measuring the rate of tracer uptake in each case. Details on spike preparation can be found in section 5.2.3.

Samples were taken at approximately one month intervals for a year between August 2006 and July 2007. Using data obtained on variability of $\delta^{13}C_{DIC}$, $\delta^{18}O_{DO}$ and $\delta^{13}C_{DOC}$ across the Loch (see chapter 2 and 3) and its interpreted relationship to productivity, two sites were chosen in the north and south basin, considered to be representative of the basin as a whole. Selected sites are shown in chapter 1, Figure 8.

5.2.1) *Method Development*

Incubation experiments were first planned to individually assess phytoplankton and bacterial production independently of each other, using filtration separation techniques, adapted from Gurung *et al* (2002). At each site 5 L of lake water was collected using a Van Dorn water sampler and subsequently stored in aspirators. Water was collected from two depths, one just below the surface and one from approximately mid-depth.

The sample water was returned to the Scottish Centre for Ecology and the Natural Environment (SCENE) as soon as possible after collection. In the lab, sufficient sample to fill each incubation (~600 ml), was filtered through 3 µm cellulose nitrate membrane filters to separate bacteria and phytoplankton. In tests carried out to validate the procedure (see Appendix 2), culturing different filtrates on agar plates, 51% – 65% of bacteria passed through the 3 µm filter and no phytoplankton was recorded in the filtrate. Filtration was carried out at no more than 20 mg of Hg to maintain cell integrity (Gurung and Urabe 1999, Gurung *et al* 2002).

Post-filtration, phytoplankton and other particulate material on the 3 µm membrane filter were re-suspended into lake water filtered through a 0.2 µm silver filter. At 0.2 µm all biological material is removed and only the water and nutrient content remains. This enabled the re-suspended material to be added to natural lake water with no other organisms present. Once completed the re-suspended sample bottles were spiked with sufficient 98% ^{13}C-labelled sodium hydrogen carbonate ($NaHCO_3$) for a final concentration ~5 % ambient. Approximate ambient concentrations were obtained from survey work carried out between 2004 and 2005, with the closest date to the sampling time used. Filtered samples containing bacteria were spiked with 99% ^{13}C-labelled leucine for a final concentration of 20 nM. Two methods of leucine spiking are regularly used; one is to closely match the background concentration, believed to be close to 20 nM in natural waters (e.g., Preston *et al* 1996, Sommerville and Preston 2001), the other to saturate the water with excess leucine. Matching ambient concentration was chosen to limit the risk of forcing leucine into phytoplankton cells during the incubation, although incubations in July 2007 were carried out with both methods to examine the differences.

Samples were incubated in 500 ml Nalgene polycarbonate bottles for 24 hours. For consistency, 24 hours was chosen for both bacterial and phytoplankton incubations. This time period is greater than in some other experiments which use smaller volumes (e.g., Biddanda *et al* 1994, Berglund *et al* 2007) but less than other experiments using comparable volume (e.g., Gurung and Urabe 1999, 165 ml bottles

for 2 days), and as such bottle effects in this volume and length of time will be minimal. Bottles were incubated in surface water in a bay next to the SCENE facility at approximately 0.5 m depth. Temperature in the surface waters of the incubation site was only significantly different from deep water in the north basin in the summer. Experiments to examine how this temperature change affected uptake, and how to correct for the difference were carried out (discussed later). Water collected from surface waters was incubated in transparent bottles to simulate light conditions in the epilimnion, water from depth in opaque bottles to simulate the light-limited conditions in the hypolimnion.

Results from the first incubations showed little to no uptake of spike by either bacteria or phytoplankton. This was due to miscalculation of the leucine spike and no viability of re-suspended phytoplankton cells. For the latter reason the procedure was changed, now removing the separation step and relying on selectivity of the tracers to the production process being measured.

5.2.2) *Incubation experiments, final method.*

A diagram of the updated method is shown in Figure 37. Sample sites remain as previously stated. On the boat water stored in aspirators was first passed through a 250 µm zooplankton mesh to remove large zooplankton and other particulates. Aspirators were returned to shore. Before any processing, natural abundance DIC samples were taken from each aspirator via the method described in chapter 2. A dissolved oxygen reading and temperature were recorded using an YSI 550 DO probe. The temperature reading was used to compare sample sites to incubation sites for subsequent correction, and dissolved oxygen to calculate community respiration.

The contents of each aspirator was divided into two, 2L glass flasks. Approximately 2 L was gravimetrically added to each flask. This was carried out for light and dark samples, two reservoirs per depth, giving a total of four 2L reservoirs, two for surface samples and two for deep. To one reservoir representative of each depth, the bicarbonate spike was added to render a field sample with a pre-determined [DIC]. To the remaining two reservoirs, leucine spike was added. All spikes were added using an Eppendorf Pippeter Mulitipette Plus system. The master sample was spiked instead of individual bottles to reduce variability between replicates. Water from each reservoir was then added to incubation bottles, the exact volume recorded by weighing the sample. Incubation bottles were filled to maximum

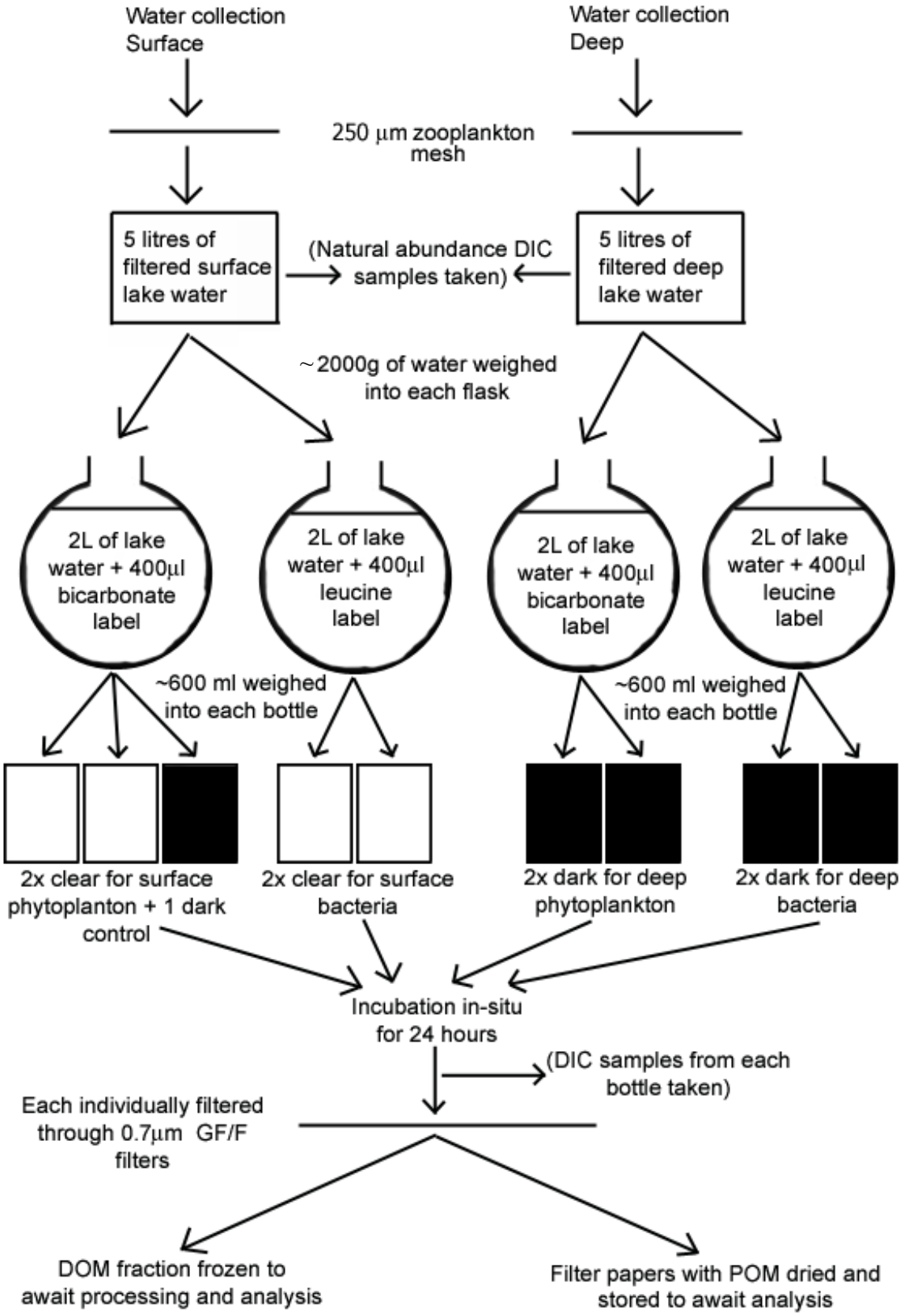

Figure 37: Flow chart of incubation preparation procedure. Diagram represents one sample site only so procedure would be replicated for the second site.

volume to minimise any possible headspace in the sample, and limit gas ingression or egression between air and water.

A total of five bottles were used in surface-sample incubations (Fig. 37): two clear bottles with bicarbonate spike to assess phytoplankton incorporation; two clear bottles with leucine spike for bacterial incorporation; one dark bottle with bicarbonate spike as a control to assess dark photosynthesis. Reservoirs for deep samples were treated the same but all bottles were opaque as no light is assumed to naturally penetrate to these samples in-situ, and no phytoplankton control was required.

Once weighed all bottles were attached to a thin wire frame and deployed in the lake for 24 hours at ~0.5m depth. During this time the remaining water from each aspirator was filtered through pre-combusted 0.7 µm GF/F filters, via a 25 mm Whatman glass frit membrane holder at a pressure of ~20 mg/Hg, used to prevent lysis of algal and other cells (as this may affect the natural abundance DOM and POM stable isotope measurements). Filter papers were dried at 60 ^{o}C for at least four hours in preparation for generation of a natural abundance POM measurement. Natural abundance of $\delta^{13}C_{POM}$ was required in calculations of ^{13}C uptake. Filtrate was frozen to await DOM analysis as detailed in section 3.2.3 chapter 3.

After 24 hours, sample bottles were returned to the lab. Dissolved oxygen and temperature were again measured from the unspiked bottles. All other samples were sub-sampled for DIC analysis and then stored in a refrigerator.

Samples were filtered the same way as the natural abundance samples described previously (chapter 3). Four samples could be filtered consecutively attached a vacuum rig connected to a Buchi V-500 vacuum pump. Reservoirs on the glass frit filter units were 100 ml and as such required continual topping up. Towards the end of each filtration the 25 mm radius filter circle can readily become clogged. If filtration reached a suitably slow pace a small spatula, first rinsed with acetone and air-dried, was used to gently agitate the surface of the filter paper. This action was taken above using multiple filter papers to load as much POM on to the surface area as possible and thus aid in isotope analysis. All filtration start times were recorded to account for any discrepancies in incubation duration.

Upon filtration completion, filter papers were removed and dried. Filtrate was added back to the original incubation bottles, which had been rinsed thoroughly in distilled water to remove any remaining particulates, and then frozen to await DOM analysis. D / POM isotopic analysis was undertaken on a different IRMS than described in chapter 3. Here samples were analysed on a Europa Scientific 20: 20 IRMS, interfaced with a Roboprep CN biological sample converter. Samples were

calibrated against ammonium sulphate and sucrose laboratory working standards that had been calibrated against IAEA secondary standards.

The described procedure was carried out for both north and south sample sites. The logistics of lab processing meant processing two sample sites simultaneously was impossible. The south basin site was sampled on day one and the north basin site the following day.

5.2.3) *Spike preparation procedure*

Bicarbonate and leucine spikes were produced in batches to ensure little variation between field campaigns. The aim was to have a dry powder of identical mass in each tube, which could be diluted with a know amount of distilled water in the field and then added to samples to achieve the desired concentrations. Storage of the spikes dry was preferred as it extends the time before spike composition changes, particularly of importance with bicarbonate (Preston per. comm). Logistically pre-weighed dry spikes are more practical as no weighing of small amounts of spike in the field is required.

5.2.3.1) *Bicarbonate*

Bicarbonate spikes were made up using 98% ^{13}C-labelled sodium hydrogen carbonate ($NaHCO_3$). The target spike concentration for each incubation was approximately 5% ambient background concentration for that time of year.

Spike preparation comprised three main steps:

1) ~0.648 g of bicarbonate was weighed into a 25 ml standard volume flask. 25 g of distilled water was added gravimetrically. The solution was mixed well until complete dissolution of the substrate.
2) 2 ml aliquots (Gilson pipette calibrated gravimetrically, accurate to 0.1 ml) were pipetted into individual vials. 12 vials containing 2 ml were prepared at once. The vials were freeze-dried, while rapidly rotating so that the dry spike collected in the vial tip. The spike was then stored with cap on and in a desiccator until use in the field.
3) In the field to re-dissolve the spike, ~10 ml of distilled water was gravimetrically added to the vial. From this solution the volume needed to reach the desired concentration was pipetted into each master incubation solution.

5.2.3.2) *Leucine*

Leucine spikes were made up using 99% ^{13}C-labelled leucine, where C-1 of six carbon atoms was labelled with ^{13}C. Spikes here were made up to achieve a final concentration of 20 nM in a 500 ml sample.

Spike preparation comprised three main steps:

1) ~0.39 mg of Leucine was weighed into an acid washed glass bucket and added to a 250 ml standard volume flask. Approximately 250 ml of distilled water was then gravimetrically added to the flask. The solution was mixed well to ensure the leucine had dissolved.
2) 2 ml aliquots were pipetted into individual vials. With the greater volume available, 24 vials were filled (as opposed to 12 for bicarbonate). The vials were freeze-dried, while rapidly rotating so that the dry spike collected in the vial tip. The spike was then stored with cap on and in a desiccator until use in the field.
3) In the field ~10 ml of distilled water was gravimetrically added to the vial. From this solution the desired volume was pipetted into each master incubation.

5.2.4) *Data extrapolation*

5.2.4.1) *Phytoplankton production*

Phytoplankton production was measured by the incorporation of ^{13}C from labelled bicarbonate. ^{13}C composition was measured in atom %. Fractional synthetic rate (% increase in carbon per day (FSR)) was expressed by the following equation:

$$FSR = \left(\frac{\Delta atm\%\,{}^{13}C_{excess(POC)}}{atm\%\,{}^{13}C_{excess(DIC)}} \right) \times 100 \qquad \text{Eq. 10}$$

where $atm\%_{excess(POC)}$ is the change in atm% ^{13}C measured by subtracting the final recorded value of each incubations POC from a starting natural abundance value prior to incubation. $Atm\%_{excess(DIC)}$ is the calculated atm% value of the incubation water at the start combining natural abundance DIC signatures and the bicarbonate spike.

FSR fails to take into account concentration of carbon in each incubation system; as such absolute synthetic rate (ASR) is calculated by the following equation and expressed in units of µg C / L / day:

$$ASR = \frac{([POC] \times FSR)}{100} \quad \text{Eq. 11}$$

where [POC] is the concentration of particulate carbon measured on each filter paper (µg C / L) and FSR as calculated in Eq. 10.

5.2.4.2) *Bacterial production, respiration and growth efficiency*

Bacterial biomass production (BBP) expresses the amount of carbon that is utilised by bacteria for the formation of new bacterial tissue. Bacterial respiration represents the carbon that is taken up by the bacteria and processed in the respiratory pathways and evolved as inorganic carbon. BBP was calculated in the same manner as photosynthetic production (see overleaf) by assessing uptake of the leucine spike. Direct measures of bacterial respiration are difficult to obtain without suitable separation of size fractions, not undertaken in this work. As such, for estimates of bacterial respiration, the following equation derived by Rivkin and Legendre (2007) that relates bacterial respiration to bacterial production and temperature was used:

$$BR = \left(\frac{BP}{0.374} - 0.0104 * T \right) - BP \quad \text{Eq. 12}$$

where BR is bacterial respiration (µg C / L / day), BP is bacterial production (µg C / L / day), 0.0104 is a constant and T is in situ water temperature (^{o}C). Where temperature measurements were not available (see results section) BR has been estimated by taking the mean proportion BR made up of total BP from samples with temperature data available. Mean BR / BP for deep south basin water was 1.60 ± 0.03 (n=10), and 1.61 ± 0.05 (n=11) for surface water. Mean BR / BP for deep, north basin water was 1.59 ± 0.11 (n=11), and 1.57 ± 0.13 (n=12) for deep and surface water respectively.

Fractional and absolute synthetic rates for BBP was calculated as for primary production, but were subsequently corrected for the fraction of the leucine labelled

(one in six carbon atoms) and the percentage of bacterial carbon made up of leucine (assumed to be 10%, Preston *et al* 1996).

Bacterial growth efficiency (BGE) expresses the proportion of gross bacterial production utilised for biomass production, and not for maintenance respiration (del Giorgio and Cole 1998). It is expressed as:

$$BGE = \frac{(BBP)}{(BBP + BR)} \qquad \text{Eq. 13}$$

Where BBP and BR are expressed in µg C/ L / day.

5.2.5) *Temperature correcting bacterial productivity*

Temperature has been shown to have an effect on both photosynthetic and respiratory rates in aquatic systems (e.g., Robarts and Zohary 1987, Scavia and Laird 1987, Tibbles 1997). In general increased temperatures lead to higher production rates as in general enzymatic reactions in each process proceed quicker at higher temperatures. In this study, water was removed from the hypolimnion in both the north and south basin and incubated in shallower water near the SCENE facility. However, only deep water from the north basin showed significant temperature differences from the incubation site.

Experiments were carried out in July 2007 whereby water from the north basin hypolimnion was collected and incubated at a series of different temperatures. From these incubations a calibration line was obtained that showed the relative increase in production related to temperature (Fig. 38). This correction was then applied to any month's data with a significant temperature difference. Bacterial production values from the deep-water north basin have been corrected when incubation temperature varied significantly from collection temperature. Phytoplankton production values were not corrected as production at this depth is minimal.

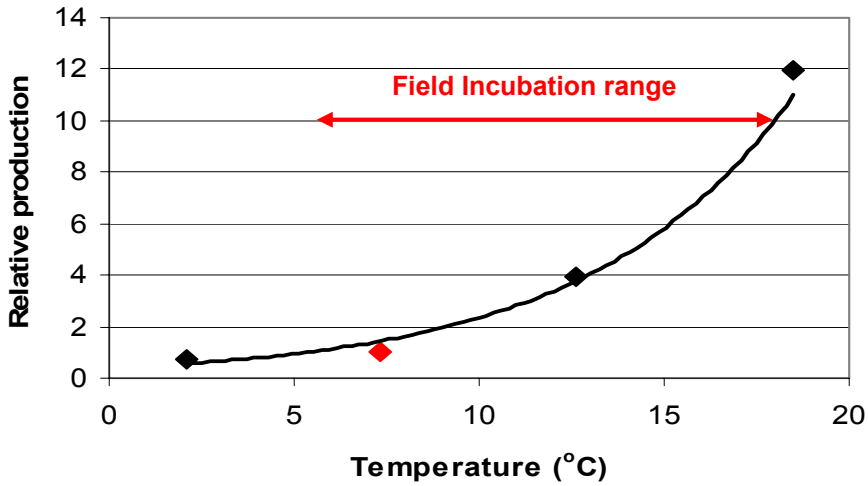

Figure 38: The effect of temperature on bacterial production rate. In-situ temperature is marked in red and Y values represent the multiplication factor needed to either increase or decrease the BP. Each value is an average of 3 replicates. The equation used for subsequent corrections was: *Relative production = 0.3796 e* $^{0.1819 * Temperature}$. Temperature range measured in the field was 6°C to 17.7°C.

5.2.6) *Depth integrated primary and secondary production estimates*

Light is extinguished through the water column (e.g., Raven and Falkowski 1997, Wetzel 2001) and it has been observed that this decline in irradiance has a negative effect on phytoplanktonic photosynthetic rate (e.g., Irwin *et al* 1975, Platt and Jassby 1976, Mallin and Paerl 1992, Wetzel 2001). This attenuation of light and thus photosynthesis follows an exponential decline to the base of the epilimnion (~1% of surface irradiance). However (due to photoinhibition in the shallowest areas) in most cases the maximum photosynthetic rate is below the surface (Falkowski and Raven 1997). In this work there is no data on sub-surface chlorophyll maxima, so for general calculations an exponential decline has been used to calculate an extinction co-efficient (e.g., Fig. 39) between the epilimnion value and the measured hypolimnion value. Production below 13 m (chosen as the maximum possible limit of the epilimnion) is assumed to be light independent so no correction is applied for depths below this. Extinction curves calculated for each sampling trip at each site were used to calculate primary production reduction at 1 m intervals from 0 m to 13 m, so yielding integrated production values in the epilimnion in μg C m^{-2} day^{-1}.

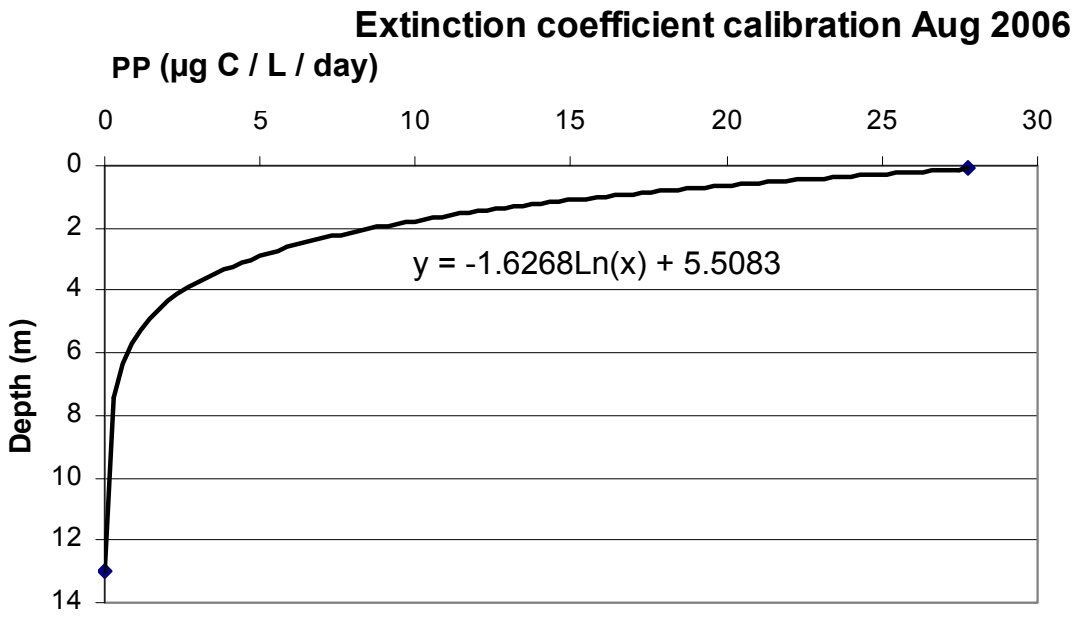

Figure 39: Example calibration line for primary production extinction co-efficient using measured values at the surface, and the hypolimnion for base values at the top and bottom of the epilimnion respectively.

It has been assumed that below the epilimnion irradiance will have no effect on productivity, so as such any integrated production values are the sum of production at > 13m, multiplied by the depth.

Bacterioplankton show varying responses to depth, not being directly limited by light availability. If limited by autochthonous nutrient supply from phytoplankton it can be hypothesised that bacteria will respond to depth in a similar way to phytoplankton (e.g., Cole *et al* 1988), and this has been observed in numerous situations for some time (e.g., Overbeck 1968). However, bacterial variability with depth has been observed to be far more complex and dependent on numerous other factors, and as such assuming it follows the same decline as phytoplankton is uncertain. Bacteria have been shown to respond to various other parameters such as changes in precipitation, allochthonous inputs, nutrient variability temperature, mixing, parasitism and grazing (e.g., Lane 1977, Goulder 1980, Coveney and Wetzel 1992, 1995, Wetzel 2001). For the reasons outlined above two extremes were calculated for bacterial water column production; the first assumes that bacterioplankton are closely coupled to primary production and as such follow the same exponential decline; the second is that bacterioplankton operate independently. As I have no data on relative peaks or drops through the water column a linear relationship is assumed between the measured epilimnion value, and the measured hypolimnion value (which is then assumed to represent everything below 13 m).

	Date	Water temp (°C)	[DOC]	[POC]	[DIC]	$\delta^{13}C_{DOC}$	$\delta^{13}C_{POC}$	$\delta^{13}C_{DIC}$	PP	BBP	BR	BP	BGE
North Basin	Aug-06		3532.83	453.06	1778.90	-24.8	-27.0	-4.8	29.14 ± 0.43	4.50 ± 0.02	7.06 ± 0.02	11.56 ± 0.03	0.39
Epilimnion	Sep-06	15.3	3651.48	279.40	1898.85	-27.7	-27.7	-9.5	12.83 ± 0.63	3.45	5.62	9.07	0.38
	Nov-06		4891.28	283.08	790.60	-27.7	-27.7	-8.5	0.09	1.48 ± 0.52	2.32 ± 0.81	3.79 ± 1.32	0.39
	Jan-07		4755.54	528.83	696.26	-27.9	-31.2	-9.9	1.62 ± 0.68	4.63	3.63	5.95	0.39
	Feb-07	6.6	3578.73	627.67	1364.78	-28.2	-32.3	-9.0	0.65 ± 0.02	1.41 ± 0.95	2.30 ± 1.59	3.71 ± 2.53	0.38
	Mar-07	7.5	3302.59	236.97	735.67	-28.1	-29.3	-10.0	0.02 ± 0.01	0.30 ± 0.20	0.42 ± 0.33	0.73 ± 0.53	0.43
	Apr-07	12.3	3997.99	557.81	2106.89	-26.8	-28.6	-4.4	11.63 ± 0.35	0.99 ± 0.41	1.52 ± 0.69	2.51 ± 1.11	0.40
	Jun-07	13.3	965.80	317.20	1206.89	-30.5	-31.6	-5.1	17.97 ± 2.20	7.88 ± 0.14	13.03 ± 0.23	20.89 ± 0.37	0.38
	Jul-07	15.6	2149.34	426.04	1525.21	-29.6	-30.3	-5.0	40.53 ± 2.61	5.43 ± 0.52	8.93	14.36	0.38
North basin	Aug-06		3649.92	377.61	1538.06	-26.6	-25.8	-10.7	0.91 ± 0.19	0.74 ± 0.13	1.17 ± 0.21	1.90 ± 0.34	0.39
Hypolimnion	Sep-06	11.4	3495.44	153.78	1414.62	-27.7	-27.3	-11.9	0.23 ± 0.03	1.07 ± 0.06	1.67 ± 0.11	2.74 ± 0.17	0.39
	Nov-06		2412.54	107.06	1070.28	-27.9	-28.1	-13.2	0.00 ± 0.00	0.03 ± 0.05	0.05 ± 0.07	0.03 ± 0.04	0.39
	Jan-07		3690.81	628.33	703.27	-28.1	-29.3	-10.0	1.21 ± 0.19	2.61 ± 0.09	4.13 ± 0.15	6.74 ± 0.24	0.39
	Feb-07	6.4	3006.30	632.51	1340.21	-28.1	-25.9	-8.7	1.29 ± 0.17	2.36 ± 0.38	3.88 ± 0.63	6.24 ± 1.01	0.38
	Mar-07	6.0	2535.16	241.76	778.27	-28.8	-28.5	-9.9	0.00 ± 0.00	0.03 ± 0.44	0.11 ± 0.74	0.18 ± 0.74	0.38
	Apr-07	6.5	3129.34	182.09	1911.32	-27.7	-30.0	-3.9	0.10 ± 0.01	0.59 ± 0.03	0.92 ± 0.04	1.51 ± 0.07	0.39
	Jun-07	9.4	1159.41	245.95	1094.07	-30.1	-29.8	-10.6	0.14 ± 0.01	0.57 ± 0.16	0.86 ± 0.26	1.43 ± 0.42	0.40
	Jul-07	10.9	1499.95	191.87	1071.42	-29.2	-30.8	-10.3	0.27 ± 0.08	0.86	1.32	4.46	0.38

Table 9: Data collected from north basin site including natural abundance concentrations (μg/L) and $\delta^{13}C$ (‰) of DOC, POC and DIC along with corresponding production values. Primary production (PP), bacterial biomass production (BBP), bacterial respiration (BR) and total bacterial production (BP) are expressed as μg C / L / day. Production values are means (n=2) ± 1 standard deviation. Values with no standard deviation represent one replicate only.

	Date	Water temp (°C)	[DOC]	[POC]	[DIC]	$\delta^{13}C_{DOC}$	$\delta^{13}C_{POC}$	$\delta^{13}C_{DIC}$	PP	BBP	BR	BP	BGE
South Basin	Aug-06		4535.93	755.27	1908.24	-27.2	-24.0	-4.1	27.79 ± 3.18	3.24 ± 0.45	5.18 ± 0.71	8.42 ± 1.16	0.38
Epilimnion	Sep-06	17.7	4729.83	320.55	2166.09	-26.8	-28.2	-9.2	9.83 ± 0.08	14.77 ± 0.85	24.54 ± 1.42	39.31 ± 2.26	0.38
	Nov-06		4446.42	315.41	1760.33	-26.2	-29.2	-8.4	0.88 ± 0.11	4.34 ± 0.13	6.93 ± 0.21	11.26 ± 0.35	0.38
	Jan-07		5284.58	304.78	1388.08	-26.6	-20.0	-7.8	1.33 ± 0.63	3.67 ± 0.03	5.86 ± 0.04	9.53 ± 0.07	0.38
	Feb-07	6.4	3737.79	313.00	1840.21	-27.5	-21.8	-6.8	1.63	1.42	1.12	1.83	0.39
	Mar-07	6.0	3382.23	294.15	1417.91	-27.6	-29.8	-5.4	0.16	0.55 ± 0.06	0.86 ± 0.10	1.41 ± 0.16	0.39
	Apr-07	14.3	4616.01	431.00	1766.22		-27.6	-3.6	21.82 ± 0.04	1.42 ± 0.77	2.22 ± 1.29	3.64 ± 2.06	0.39
	Jun-07	13.5	1360.58	359.39	1646.09	-29.7	-32.9	-4.4	20.09 ± 2.11	6.67 ± 0.82	11.02 ± 1.38	17.69 ± 2.19	0.38
	Jul-07	16.5	2151.49	426.04	1447.01	-28.4	-30.3	-5.0	41.51 ± 2.51	3.67 ± 0.54	5.96	9.63	0.38
South basin	Aug-06		3278.20	699.84	2165.60		-22.4	-5.2	0.92 ± 0.15	0.09	0.68	0.55 ± 0.78	0.38
Hypolimnion	Sep-06	16.7	6855.06	379.47	1477.13	-28.2	-27.1	-6.7	0.76 ± 0.12	2.12 ± 0.14	3.37 ± 0.23	5.48 ± 0.37	0.39
	Nov-06		5367.59	310.58	1825.09	-27.0	-29.3	-8.2	0.12 ± 0.03	4.47 ± 0.71	7.14 ± 1.13	11.61 ± 1.83	0.38
	Jan-07		3278.01	343.23	1513.19	-27.0	-23.0	-7.5	1.79 ± 1.48	4.01 ± 0.45	6.40 ± 0.72	10.41 ± 1.17	0.39
	Feb-07	6.0	4142.01	340.91	2212.92	-26.8	-22.2	-6.4	1.38 ± 0.20	0.66	0.49 ± 0.78	0.82	0.39
	Mar-07	6.0	5971.60	310.34	1481.81	-27.0	-30.0	-6.5	0.02 ± 0.00	0.97 ± 0.16	1.55 ± 0.27	2.52 ± 0.43	0.38
	Apr-07	11.2	3950.46	451.23	1744.68	-25.7	-28.5	-6.0	0.67 ± 0.19	0.37 ± 0.51	0.50 ± 0.85	0.87 ± 1.35	0.40
	Jun-07	12.9	2065.01	428.08	1689.46	-31.2	-31.4	-6.8	0.41 ± 0.41	2.44 ± 0.62	3.96 ± 1.03	6.40 ± 1.65	0.38
	Jul-07	16.1	2387.21	359.29	1664.07	-29.9	-31.5	-5.2	1.20 ± 0.73	3.64 ± 1.37	5.93 ± 2.29	9.58 ± 3.66	0.38

Table 10: Data collected from south basin site including natural abundance concentrations (μg/L) and $\delta^{13}C$ (‰) of DOC, POC and DIC along with corresponding production values. Primary production (PP), bacterial biomass production (BBP), bacterial respiration (BR) and total bacterial production (BP) are expressed as μg C / L / day. Production values are means (n=2) ± 1 standard deviation. Values with no standard deviation represent one replicate only.

5.3.1) *Algal: Bacterial production balance*

All data from this point, that required temperature correction has been carried out. The balance between PP and gross BP is shown in figures 40 and 41 for the north and south basin respectively. Bacterial production has been calculated by adding measured bacterial biomass production to estimated bacterial respiration. PP shows significant seasonal changes ($P < 0.001$) in the epilimnetic waters of both basins (Fig. 40a and 41a), peaking in the summer months between April and August. The magnitude of the bloom periods is similar between basins reaching similar peaks in both July and August. However, the epilimnion of the south basin appears to respond to the spring bloom conditions quicker, showing approximately double the productivity recorded in the north basin in April. PP between November and march

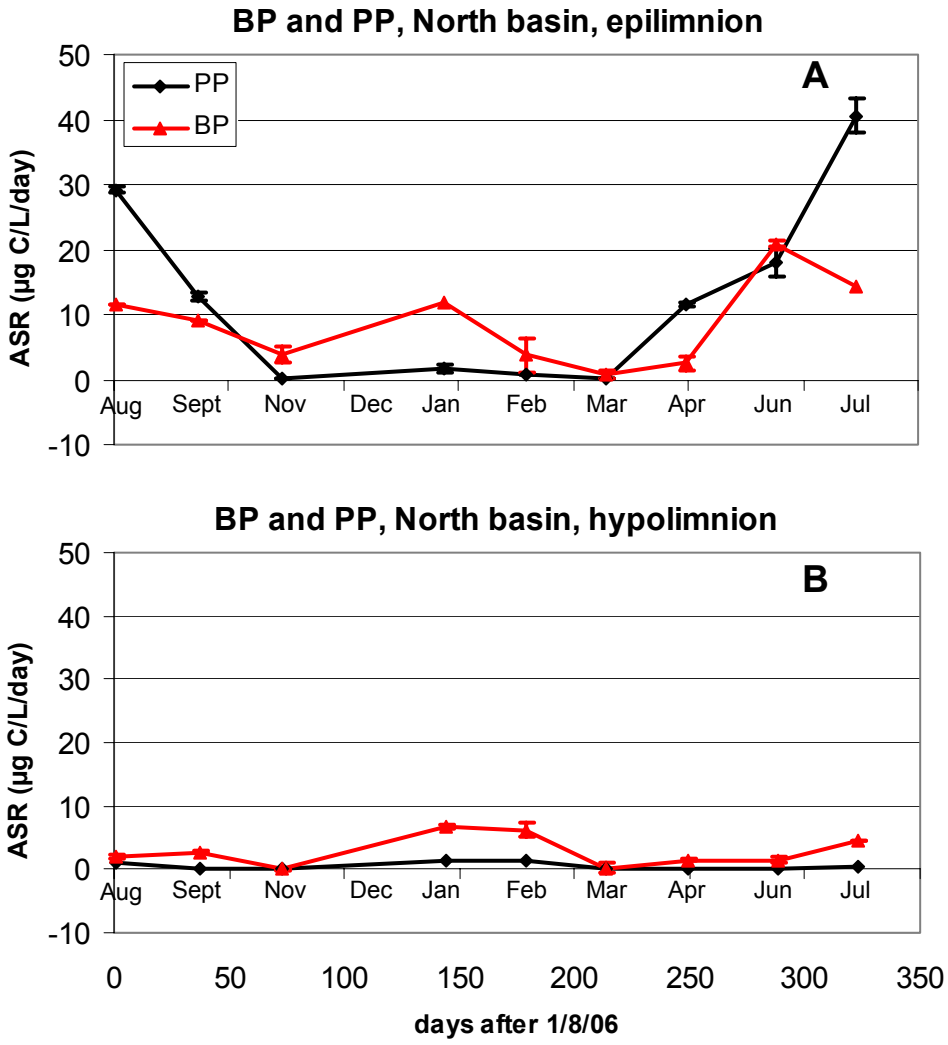

Figure 40: Bacterial production (BP) and primary production (PP) throughout an annual cycle in the a) epilimnion and b) hypolimnion of the north basin. Error bars represent standard deviations. ASR = Absolute Synthetic Rate.

in the epilimnion of each basin is not significantly different from zero. In each basin there is no significant variation in PP in the winter months, i.e., November does not significantly differ from January, February or March in the north (P = 0.231, 0.653 and 0.951 respectively) and in the south (P = 0.771, 0.852 and 0.464 respectively).

BP in the epilimnion of each basin has similar seasonal patterns with one significant difference; the south basin shows a significant peak in BP in September 2006 (P < 0.001). This peak is not shown in the north basin. Excluding this peak each basin behaves similarly with respect to BP, with highest levels similarly in the summer months, although generally BP does not exceed PP in this period. However, BP is significantly greater than PP throughout the winter months with productivities

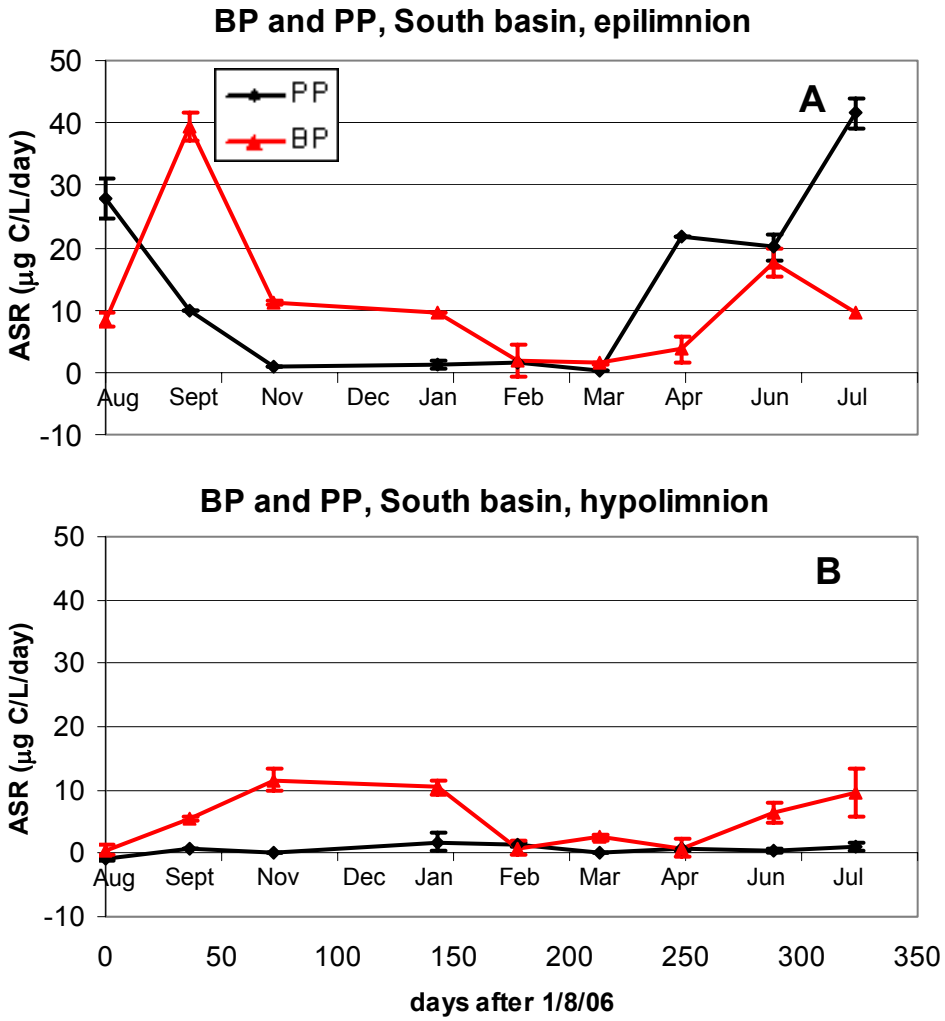

Figure 41: Bacterial production (BP) and primary production (PP) throughout an annual cycle in the a) epilimnion and b) hypolimnion of the south basin. Error bars represent standard deviations. ASR = Absolute Synthetic Rate.

up to 11.89 µg C/L/day in January in the north basin, and 9.53 ± 0.07µg C/L/day in the south.

PP never rises significantly above zero in the hypolimnion of the north or south basin (Figs. 40b and 41b). Hypolimnetic BP was never measured to reach the same level as in the epilimnion of each basin, but still shows significantly higher values than PP at certain times of year. The south basin hypolimnion showed peak BP between November and January (Fig. 41b), which may have continued to rise in December. The highest recorded hypolimnion BP value was 11.61 ± 1.83 µg C/L/day in the south, comparable to the winter maxima in the epilimnion. The north basin hypolimnion showed peak bacterial productivities in January and February. Both north and south basin hypolimnia show a rising BP in summer 2007, between June and August (this assumes August 2006 would be similar in 2007). An increase in BP in the north basin hypolimnion was observed one month after the south basin, and showed approximately half the productivity.

5.3.2) *Epilimnetic depth integrated production estimates*

Figure 42 shows the integrated production values in the north and south basin for the epilimnion only. As data on bacterioplankton vertical distribution was not available, two estimates of bacterial production have been given that span an estimated high and low distribution. The epilimnion has been assumed to be from 1-13 metres for both basins.

Epilimnetic primary production in the north basin reaches a peak in July 2007 at 133.34 mg C m^{-2} day^{-1}, and a minimum in March when only 0.07 mg C m^{-2} day^{-1} was produced, (likely not significantly different from zero). In only three months sampled did primary production in the epilimnion exceed bacterial production, both low and high estimates. August showed the largest difference where PP exceeded BP by between 8.9 and 45.8 mg C m^{-2} day^{-1}. April and July 2007 were the other two dates where the epilimnion was net autotrophic. For the rest of the year the north basin epilimnion is net heterotrophic with BP exceeding PP. The highest estimated difference is in January where, BP utilises between 92.5 and 112.4 mg C m^{-2} day^{-1}. by integrating production throughout the year the epilimnion in the north basin is heterotrophic with a PP: BP ratio of 0.56:1 to 0.80:1.

PP in the epilimnion of the south basin reaches its highest value at the same time as the north basin with a comparable rate of 145.4 mg C m^{-2} day^{-1}. Likewise the lowest rates occurred in January and March. Like the north basin, PP in the south basin exceeds BP in April, July and August. The basin is also just net autotrophic in

February, by up to 5.1 mg C m^{-2} day^{-1}. BP exceeds PP by larger amounts in the south basin than in the north basin, particularly from November to January, where at the least, BP processes a minimum of 117.6 mg C m^{-2} day^{-1} more than PP. This offset rises to as much as 275.3 mg C m^{-2} day^{-1} more BP than PP in November. The epilimnion in the south basin is more strongly heterotrophic than the north basin with a PP: BP ratio of between 0.44:1 and 0.55:1.

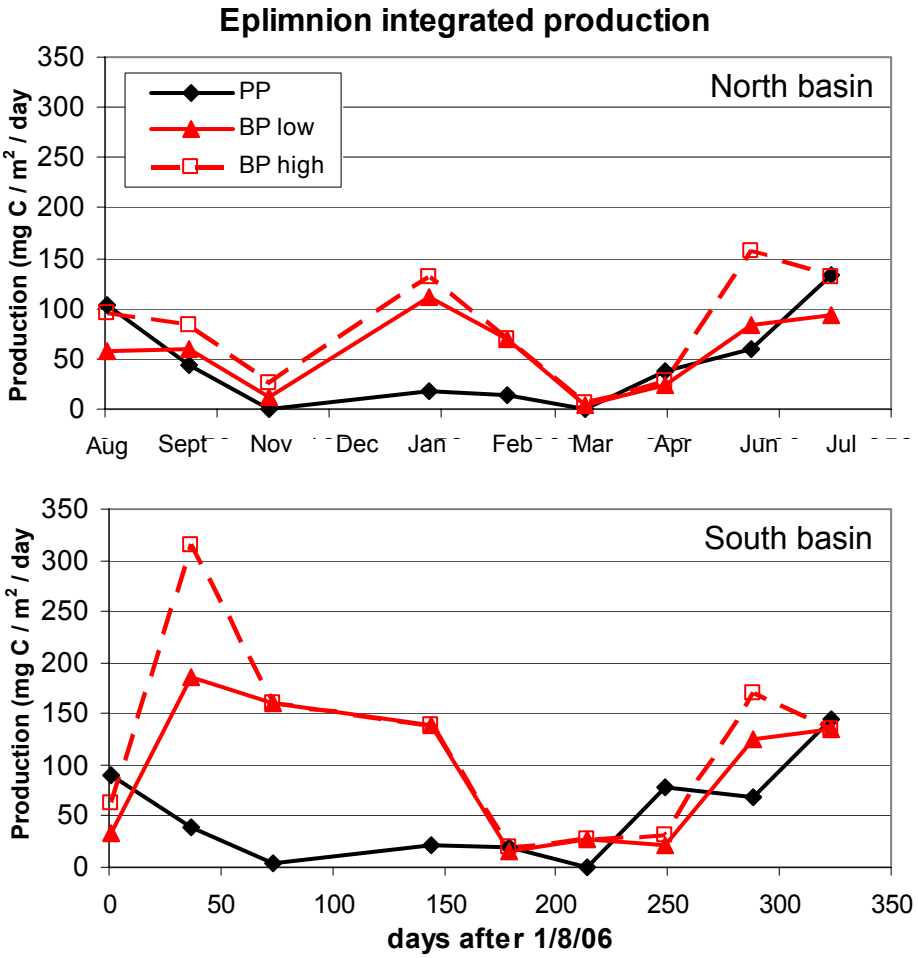

Figure 42: Depth integrated algal and bacterial production estimates for each sampling period. Dashed red lines represent high bacterial production estimates obtained by linear regression, with solid red lines representing low bacterial production estimates using an exponential decline from the surface to 13 m.

At this juncture the assumptions used for these estimates should be clarified. PP through the epilimnion has been calculated using an exponential decline in productivity between 0 and 13 m depth. In reality this pattern is likely to be inaccurate. Although irradiance attenuation through a water column does follow an exponential decline, PP can often increase in the top few metres before dropping with

depth, due to photoinhibition in near surface waters (Wetzel 2001). The magnitude of this sub-surface maximum is generally lower in less productive waters (Wetzel 2001), but integrated PP estimates are likely lower than actual values. However, as no data on depth variability is known an exponential decline has been chosen.

Likely more errors exist in estimates of integrated BP with depth. Bacterial production, although influenced significantly by temperature, is not directly affected by irradiance. Other factors such as organic matter availability, parasitism, grazing, etc all can have significant effects (Wetzel 2001). As such vertical distribution of bacterioplankton can be variable and unpredictable (e.g., Saunders 1971). For this reason it was decided to estimate a theoretical upper limit and lower limit, based on the assumption that BP will closely follow PP with depth, thus an exponential decline was used. However, evidence suggesting BP may be partly independent of PP is already accumulating in this research, thus production values presented are likely to be a significant underestimate. The second estimate is a simple linear decline from surface to hypolimnion. In truth populations may shrink or swell through the water column but lack of information means this cannot be further explored in this work.

5.3.3) Total and net lake production

Month	South basin PP	Mid basin PP	North basin PP	South basin BP	Mid basin BP	North basin BP	Total lake production
Aug	2514	2739	2404	934 - 1780	1366 - 2284	1515 - 2125	**1465 - 3839**
Sept	1131	1249	895	5248 - 8886	3686 - 5789	1786 - 2186	**13587 - 7445**
Nov	119	74	5	4572	2842 - 3040	201 - 440	**7855 - 7417**
Jan	645	877	1233	3985	4142 - 4413	3826 - 4152	**9797 - 9199**
Feb	589	751	1211	435 - 540	1484 - 1536	2952	**2321 - 2478**
Mar	21	13	1	788	539 - 567	69 - 104	**1362 - 1426**
Apr	2185	1670	716	598 - 896	726 - 923	855 - 918	**1833 - 2391**
Jun	1948	1817	1086	3578 - 4793	3179 - 4790	1799 - 3027	**3707 - 7761**
Jul	4102	3961	2408	3832 - 3838	3614 - 4135	2209 - 2834	**336 - 815**

Table 11: Complete basin and lake production estimates for Loch Lomond. All numbers in kg C production day^{-1}. Range in BP represents high and low estimates for depth integrated production. Total lake production shows the estimated maximum and minimum production values where RED numbers represent net bacterial production and BLACK numbers net algal production.

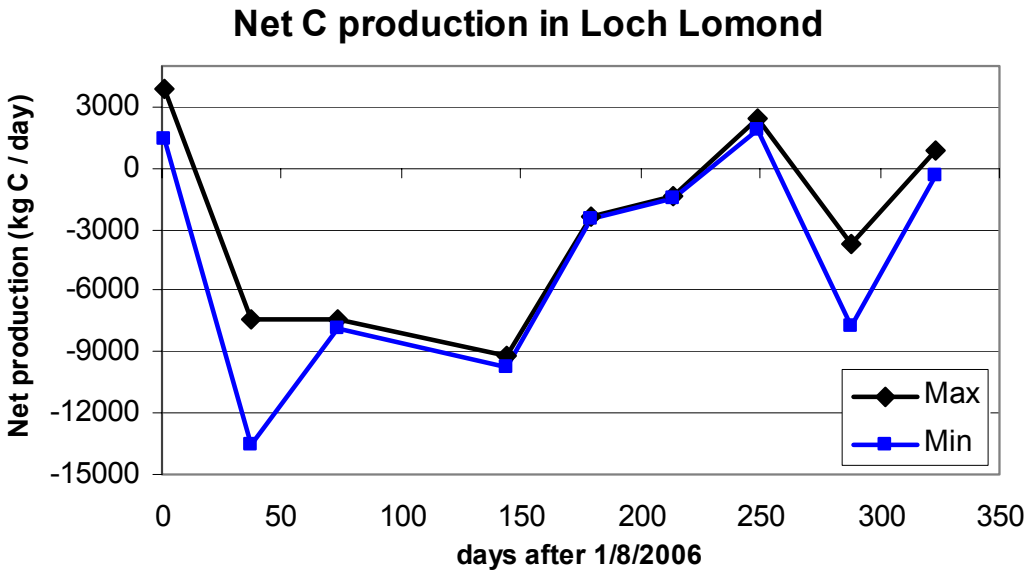

Figure 43: Seasonal change in net productivity in Loch Lomond. Black lines show values using minimum bacterial production estimates and blue lines maximum. Values above 0 represent net autotrophic production, below 0 net heterotrophic.

The total quantity of carbon processed by both the algal and bacterial pathways was estimated for each sample month as follows. Integrated epilimnion production values have been multiplied by the surface area of each basin obtained from GIS analysis. As hypolimnion production values are believed not to be affected

significantly by depth and irradiance decline, etc, production values in mg C m^{-3} have been multiplied by the volume of the respective basin. Volumes were again obtained via GIS analysis provided by Dr Jane Drummond. Total basin production was then calculated by combining hypolimnion production and epilimnion production. Two estimates of bacterial production, using exponential decay with depth, and a linear change have been used to estimate upper and lower limits of total basin / lake production. Middle basin production estimates have been obtained by using the midpoint value between south basin and north basin values, assuming the middle basin to be intermediary between the two.

Estimates of whole lake productivity reveal Loch Lomond is a net heterotrophic system (Table. 11 and Fig. 43). With the exception of April, August and possibly July, bacterial production exceeds primary production. The magnitude of net heterotrophy varies throughout the year with a maximum possible value estimated in September 2006, where net bacterial production was 13,587 kg C day^{-1}. The lowest estimated difference between primary and secondary productivity was in July '07 where the lake could either be net autotrophic, with production of 815 kg C day^{-1}, to net heterotrophic, with production of 336 kg C day^{-1}, depending on the bacterial production estimate used.

Variability in the estimated bacterial production arising, from different approaches to depth integrated production estimation, changes the magnitude of net heterotrophic / autotrophic balance, but with the exception of July does not change the overall direction of production (Fig. 43). These tracer experiments indicate that primary production rarely exceeds bacterial production, thus there must be an external source of organic carbon for bacterial utilisation.

By integrating values below total productivity graphs (not shown) the total annual production via the phytoplanktonic and bacterial pathways has been estimated. Phytoplanktonic production for the lake was estimated at 970.8 tonnes of carbon processed per year. Bacterial production was estimated twice for high and low values, depending on the method of depth integration previously detailed. The lowest estimate yielded a bacterial production of 2283.5 tonnes of carbon per year, and the highest 2794.4 tonnes carbon per year. These estimates suggest that Loch Lomond is net heterotrophic to a value between 1312 – 1823 tonnes of carbon processed per annum. Using these values the PP: BP for the entire lake ranges from 0.35: 1 to 0.43: 1.

Several sources of error need to be considered for whole lake estimates of carbon production. Firstly, when calculating epilimnetic production in the south basin, production per m^2 was multiplied by the surface area of the basin. This leads to an

overestimation of epilimnion production as some areas of basin are shallower than the 13 m assumed in the calculations. However, this is likely not a large source of error in the north or middle basin as they are generally steep sided and nearly always deeper than 13 m. Secondly, the middle basin has been shown in chapters 2 and 3 to potentially function as a significantly different lake segment to the south and north, so assuming it to be an intermediate in production terms is an uncertain assumption. However, in the absence of any data to confirm or deny this, an intermediate value seemed the most sensible estimate. Thirdly, the thermocline in Loch Lomond is dynamic in distribution and whilst 13 m has been used to cover all possible ranges, for times when the thermocline may have been shallower, epilimnion production may have been overestimated.

5.4) *Discussion:*

This chapter set out to test three main hypotheses, each of which will now be considered in turn.

5.4.1) *The South basin of Loch Lomond will have higher fluxes of carbon through both the phytoplanktonic and bacterial metabolic pathways than the north basin due to its higher trophic state and nutrient availability.*

Primary production patterns in Loch Lomond followed predictable seasonal changes (Fig. 40a and 41a) and are comparable with work on lakes of similar nutrient states and location (e.g., Smith 1979, Taipale *et al* 2007, Karlsson 2007). Seasonal patterns in primary production have been extensively studied and described in the past and as such only a brief discussion will follow. In temperate zone lakes early winter is dominated by cold temperatures, little incident radiation and short day-lengths. Also, surrounding catchments are often frozen limiting the supply of nutrients into the lake. In mid-winter in high latitude, but moderate climate lakes, phytoplankton production remains very low. Water is continually well mixed below the minimal depth critical to net photosynthesis, which along with the low light and temperature conditions limit any primary productivity (e.g., Sommer 1985).

As spring approaches water temperatures are generally still low, but both the duration of illumination and the quantity of incident radiation increases. With a build up of inorganic nutrients, transported up from deep water over the winter mixing period, and a more stable water column as stratification occurs, phytoplankton populations start to grow rapidly. This has been observed here in Loch Lomond (Fig. 40 and 41) in April and has been measured in work previously undertaken on this lake at approximately the same time (e.g., Maulood and Boney 1980, Habib *et al* 1997). This spring bloom period is likely the point of highest algal biomass, although measured production continues to rise past this point. As summer approaches nutrients become more limiting and algal biomass will start to decline. This is also likely due to the stability of the water column, which allows the dominant diatom species (*Staurodesmus, Scenedesmus* and *Tabellaria* in Loch Lomond (Eurolakes report D5)) to sink through the water column, and possibly silica limitation (e.g., Lund *et al* 1963, Neale *et al* 1991). Also, by this time zooplankton communities have responded fully to the algal bloom and grazing will be at its maximum rate. During late summer and early autumn *Asterionella* and *Melosira* can dominate the algal flora but don't reach the biomass of the spring bloom forming diatoms. However,

cyanobacteria blooms also occur which can increase productivity substantially (e.g., Ganf and Oliver 1982).

Autumn sees productivity levels begin to decline (from August onward), due to decreasing temperatures, lower irradiance times and quantity, and increasing mixing below critical photosynthetic depths. In Loch Lomond significant algal productivity had finished by November in both north and south basins.

The north and south basin epilimnia follow the described seasonal pattern in PP with little variation between the two. Although the south basin shows higher productivities in April than the north there is generally no significant difference between epilimnetic PP between basins ($P = 0.594$), despite the differing trophic states of the basins. Hypolimnetic PP would be expected to be minimal in both basins and as predicted, neither basin differs significantly from 0 and show no difference to each other ($P = 0.805$).

Bacterial production follows varying seasonal cycles between basins and depth ranges. The epilimnion of the north basin shows a seasonal pattern with similarities to PP. BP is initially higher in late summer (although not to the same extent as PP) and then drops through autumn. However, unlike PP there is a winter peak in BP in January of the same magnitude as August and is significantly higher than the other winter months (November, February and March, all $P < 0.001$). BP responds slower to spring bloom conditions not rising significantly from March to April ($P = 0.153$), but then rises above PP in June. BP estimates in the south basin epilimnion follow a similar pattern to the north basin, with one significant outlier in September. Here BP increases to double any value seen throughout the year and is ~ four times greater than concurrent PP at the time. However, in general, BP in the epilimnion of the north and south do not significantly differ ($P = 0.273$). That north and south epilimnetic BP seems to mimic to some extent the PP seasonal cycle, suggests some dependence of bacteria on organic carbon produced during PP. However, significantly higher values of BP than PP in the winter months, and particularly September in the south basin, show PP cannot be fuelling BP on its own, another external supply of energy must be being utilised. This concept is further examined in chapter 6.

BP in the hypolimnion of the north basin is generally low, even during the summer months of peak epilimnetic productivity, although significantly higher values are seen in January and February. A hypolimnetic minima in BP has been described in numerous other water bodies (e.g., Overbeck *et al* 1969, Chrost and Rai 1994, Simon *et al* 1998, Gurung and Urabe 1999) at least until the lower hypolimnion is reached and sediment productivity is detected. In the south basin hypolimnion BP was consistently higher than in the hypolimnion of the north ($P = 0.01$). This difference is

likely a reflection of the different depth hypolimnion water was sampled from. In each basin water was taken from ~5m above the lake bed, which were ~22m in the south and 55m in the north. Thus distance from the productive epilimnion to the hypolimnion sample point is over double in the north basin, and as such any transport down of organic material will take longer allowing opportunity for utilisation by the time it reached deep water in the north.

Bacterial seasonal and spatial distribution can be less well constrained than that of phytoplankton, as many short-term process, along with longer term trends in temperature, light, etc can influence population size and structure (e.g., Saunders *et al* 1980, Pomeroy and Weibe 2001, Ducklow *et al* 2002), for example, phytoplankton condition and hence rate of exudation, external organic carbon supply and community loss via grazing or viral lysis.

Therefore, it is concluded that there is no detectable difference in PP between the mesotrophic south basin and the oligotrophic north basin in Loch Lomond, with one exception being the spring bloom period in April. However, algal blooms can be very short-lived events, lasting less than weeks (e.g., Blomqvist *et al* 1994). As such there is a high probability that events, which may have included blooms of greater magnitude than recorded in this work, have been missed in-between sampling campaigns. With greater temporal resolution, significant differences between basins may have been observed, the difference in chlorophyll levels between basins in particular is well documented (Maitland 1981). However, in this study no difference was detected in either epilimnion or hypolimnion water bodies, and seasonal fluctuations are both similar and predictable.

BP shows no statistically significant variation between the north and south basin in the epilimnetic water masses, with the notable exception of September (A replicated measurement so likely not an experimental artefact or analytical error). The reason for such a large discrepancy at this point is unclear, but possible explanations could be varying influxes of organic matter into each basin. September corresponds to high levels of foliage deposition and thus run off of organic material in the watersheds. In the lower altitude, relatively base-rich and slow-flowing catchments located in the south, allochthonous organic carbon could be fuelling bacterioplankton at this point. Indeed, PP is insufficient to support BP of this magnitude at this time of year, implying an external source. Whereas this likely applies to the north basin also, the high altitude, fast-flowing, base-poor catchment suggests it may transport less organic material in this manner.

Another possibility is breakdown of the thermocline and re-suspension of organic material. In the south basin stratification is often readily broken down with rough weather, whereas the north is generally more stable until late autumn. September's peak in BP in the south could possibly be due to an influx of organic material stored in the hypolimnion and sediments, mobilised by rough weather. Prior to the September sampling date the south basin may have had a significant summer bloom, which was in the process of decline during sampling. A combination of algal senescence following death, exudation, etc could possibly have supplied the south basin with significant quantities of labile organic material for bacterial utilisation. The reason for this peak is unknown. More detailed temporal sampling would be required to elucidate the cause in future work.

BP in the hypolimnion was the only bulk data set which showed significant variation between the north and south basin, with the south basin consistently more productive. This is likely a reflection of varying concentrations of DOC available, and the quality of DOC (C:N north hypolimnion = 7.3 ± 3.4, south basin hypolimnion = 6.3 ± 2.4) in the different water bodies. The hypolimnion in the south basin in all but one month showed significantly higher [DOC] than in the north, either due to transport from the productive epilimnion being easier due to proximity in the south, or due to regular breakdown of stratification and subsequent water mixing in the south, which could include re-suspension of organic material in the sediments.

The conclusion that PP does not differ between basins, but that BP does in the hypolimnion, and at certain times in the epilimnion, supports the idea that PP and BP are not always tightly coupled. Hence factors other than autochthonous supply of organic carbon to bacteria must be of significance in Loch Lomond at certain times and places. Our hypothesis that productivities will significantly differ between the two basins has been shown in-correct for all but the north basin hypolimnion.

5.4.2) *Despite being a generally oligotrophic system, in the epilimnion of Loch Lomond algal autotrophic production will exceed bacterial heterotrophic production.*

In recent times the significance of bacteria in limnetic systems has become apparent, particularly the possibility of significantly more heterotrophic breakdown of allochthonous organic carbon sources than PP, leading to net heterotrophic water-bodies (e.g., Cole *et al* 1994, del Giorgio and Peters 1994, Kritzberg *et al* 2004). The concept of allochthonous DOC utilisation in Loch Lomond is explored more comprehensively in chapter 6, but examining measured differences in autotrophic

and heterotrophic production, hypotheses relevant to the potential significance of autotrophic Vs heterotrophic production can be considered.

When estimating depth integrated production in the epilimnion, BP is found to exceed PP in nearly all sampling periods (Fig. 42, page 146). Depending on which estimate is used for BP the offset in rates changes, but a general trend of BP > PP remains. PP in the north basin epilimnion peaks at 133.34 mg C/m^2/day, which is generally higher than BP throughout the year. However, BP is significantly greater in the winter months and in early summer. The south basin shows even larger differences, with BP exceeding PP from September to February, and rate in the order of magnitude greater.

Thus the second hypothesis is disproved, rather the epilimnion of Loch Lomond appears to function as a heterotrophic environment for much of the year and over much of its extent.

Although data from sources already listed suggests many limnetic systems to be heterotrophic (particularly oligotrophic water bodies), and as such sources of carbon to other ecosystems, other evidence (also presented) suggests the opposite, and that oligotrophic systems in particular are likely to be dominated by autotrophic production (Carignan *et al* 2000). The suggestion by Prairie *et al* (2002) that [DOC] was of critical importance in determining which of these hypothesis applied to a particular lake, and that a threshold exists at 4 - 6 mg/L DOC above which a lake's epilimnion was net heterotrophic, is interesting, for in Loch Lomond [DOC] is lower than or between these two numbers consistently.

Thus the data colleted supports the idea of unproductive lake systems being net heterotrophic. The north basin shows clearly higher levels of BP in the epilimnion than PP. This is consistent with previous hypotheses by Cole *et al* (1994) who used supersaturation in surface water CO_2 to imply similarly, net heterotrophy. Other recent studies also support the idea that BP exceeds PP in unproductive systems and thus the carbon cycle is supported by external sources of organic carbon (e.g., del Giorgio and Peters 1994, Jansson *et al* 1999, Kritzberg *et al* 2004).

However, this is contrary to hypothesis by Prairie *et al* (2002) that [DOC] is a control, as [DOC] in the north basin is regularly lower than 4 mg/L (Fig. 26, chapter 3). There is no general correlation with [DOC] and the magnitude of bacterial production in Loch Lomond (see Chapter 7), which may be indicative of generally small amounts of variation in the [DOC], that lacks the magnitude to cause a noticeable effect on BP rates. Or productivities are being limited by N or P availability and not DOC. More replicates would be needed to examine any correlation more fully.

The relationship between [DOC] and lake heterotrophy is supported however, by results from the south basin. Here [DOC] is consistently higher than the north basin in the epilimnion and BP is significantly greater than PP for large portions of the year. The peak BP estimate for the epilimnion does in fact correspond with the highest recorded [DOC] and only value above 6 mg / L measured.

BP is likely controlled by different factors at different times in Loch Lomond. High BP values in the south basin epilimnion in winter suggest a de-coupling from PP, and potential use of terrestrial organic carbon supplies. In the summer months BP is more closely matched to PP, suggesting more interdependence between the processes. This could be due to less organic carbon entering the lake in the summer months, that PP is meeting the majority of the energy supply of the bacterial community, or that grazing pressure during the spring summer productive period is limiting the bacterial population and thus production.

With the available evidence it can be concluded that the epilimnion of Loch Lomond varied from an autotrophic system at some points, to a heterotrophic one at others. However, generally the epilimnion of Loch Lomond is net heterotrophic, with PP to BP ratios of 0.80 - 0.56 in the north, and 0.44 - 0.56 in the south, so hypothesis 2 can be rejected. The hypothesis was based on the assumption [DOC] would be of direct influence on BP, which is suggested to not be the case. Explanations for the lack of correlation are currently unclear, but it is possible the range in [DOC] in Loch Lomond is not great enough to have a detectable impact above other controlling factors (e.g., temperature).

The conclusion that hypothesis 2 is incorrect, and that heterotrophy dominates the epilimnetic waters in Loch Lomond, the validity of hypothesis 3 is implied as a result. Hypolimnetic areas will likely always be dominated by bacterial production and add to the magnitude of net heterotrophy in this lake.

5.4.3) *Due to large areas of the lake that are below the photosynthetically active epilimnion, Loch Lomond will be, in total, a heterotrophic system.*

Loch Lomond is a heterotrophic lake that is dominated by bacterial utilisation of organic matter sources additional to those generated via autotrophic production. To add further evidence to the above hypothesis, I estimated complete lake carbon production and utilisation, and from this determined how much extra carbon is processed by bacteria each year and therefore how much is potentially exported from the Lomond waters.

The hypolimnion of a lake is characterised by a lack of photosynthetic activity (Wetzel 2001), below the level of 1% surface irradiance. In Loch Lomond a large proportion of the middle and north basins in particular are below epilimnion. In these areas it would be expected that bacterial production would exceed primary production if there is another source of organic carbon for utilisation. In general all our data shows that BP exceeds PP in the hypolimnion. The only exceptions to this were observed in the south basin in February and April. The magnitude of the difference was small however and could be due to complete circulation in the shallow waters of this basin.

All three basins were net heterotrophic over an annual cycle. Each basin however was responsible for varying levels of production and the south basin showed the largest net level of production. This would be expected in-spite of the earlier findings that there was no significant difference in PP between basins. BP was significantly higher in the south. Although the considerably larger volume of hypolimnion in the north reduced the overall difference, BP in this reservoir was not high enough to surpass the south or middle basins in net productivity.

Loch Lomond is a heterotrophic system based on sampling times in this work. Due to often short durations of algal and cyanobacterial blooms it is possible period of significantly higher PP have been missed however. More detailed temporal surveying, particularly during the spring / summer would be needed to explore this possibility. The fact that BP is consistently greater than PP suggests that allochthonous carbon must be a significant energy source for production in Loch Lomond. This has consequences on the ecosystem scale and will be further explored in chapter 6.

Chapter 6

The contribution bacterial processing of allochthonous carbon makes to pelagic production in Loch Lomond.

6.1) *Introduction*

Bacteria have been known for some time to be responsible for processing organic matter produced by phytoplankton (e.g., Azam *et al* 1983) and to subsequently allow the reintroduction of this organic material into aquatic food webs via the microbial loop. In recent times evidence has accumulated that bacteria can also utilise external (allochthonous) sources of organic carbon which was largely believed recalcitrant to bacterial attack, and contribute to pelagic production independently of phytoplanktonic photosynthesis (e.g., Tranvik 1988, Jones 1992, Cole *et al* 1994, del Giorgio and Peters 1994, Jansson *et al* 1999, Cole *et al* 2000, Kritzberg *et al* 2004). Although it has been shown that brown water lakes with high humic content are dominated by bacterial breakdown of allochthonous organic carbon (e.g., Hessen 1992), there is now increasing evidence that bacterial processing of terrestrial carbon may support the bulk of bacterial production in other, even clear-water systems and net heterotrophy can result (Tranvik 1998, Moran and Hodson 1994, Cole *et al* 2000).

The realisation that secondary production may be of significance in many limnetic systems has profound consequences for ecosystem carbon cycling as a whole. Lakes have long been considered sinks for carbon, and while measured pelagic heterotrophy doesn't discount this, as it takes no account of sediment accumulation, it does present the possibility the magnitude of such sinks could be inaccurate. In the extreme many systems thought to be sinks for carbon and atmospheric CO_2 may in fact be sources (Cole *et al* 1994).

Changing proportions of heterotrophy in pelagic systems also has consequences for food webs and efficiency of energy transfer (e.g., Jansson 2003). It has been suggested that food webs based on heterotrophic breakdown of allochthonous DOC have lower energy mobilisation efficiency and thus support significantly less productive subsequent trophic steps. This phenomenon has long been known in brown-water lakes, which have been observed to have a high proportion of bacterial production, but support unproductive food webs (e.g., Thienemann 1925) and were given the title dystrophic. However, lakes not strongly coloured have been shown to

have similar energy mobilisation patterns (Jansson 2003), many of which were clear-water, oligotrophic systems.

Molar C:N mass balance modelling (Chapters 3 and 4) initially suggested that organic carbon (dissolved and sestonic) was likely mainly autochthonous in origin. However, uncertainties in organic C:N caused by the presence of inorganic nitrogen species, complicates interpretation such that estimates are minimum contributions of allochthonous organic carbon (between 8% and 40%). Direct measures of algal and bacterial production (Chapter 5) has presented several pieces of evidence that suggest Loch Lomond is a heterotrophic system dominated for the majority of the time by bacterial utilisation of carbon additional to that supplied from algae.

In this chapter I set out to calculate approximate proportions of the total pelagic production that is fuelled by allochthonous organic carbon, with an overall aim of estimating how much allochthonous and autochthonous carbon is utilised by pelagic bacteria in Loch Lomond.

6.2) Methods

Primary production and bacterial production were estimated as described in detail in chapter 5. Using this data methods and equations used by Jansson (2003) have been used to estimate the allochthonous contribution to total pelagic production, as follows.

The amount of PP that is made available to bacteria varies between aquatic systems. Here it is assumed an average value of 30% of PP lost via exudation can subsequently fuel bacterial production (Arvola *et al* 1996). The fraction of PP released in this way can vary from < 10% to > 50% (Jordon and Likens 1980, Sondergaard *et al* 1985, Rieman and Sondergaard 1986) although most estimates are between 15 - 30% (Sensitivity of the model was tested by using upper and lower limits of 20% and 40%). Also DOC produced by feeding of zooplankton on algal cells is estimated to be 10% of gross PP (Lampert *et al* 1978 and references therein). Therefore, primary production utilisable by bacteria (PP_{bac}) is expressed as;

$$PP_{BAC} = (0.3 \times PP) + 0.1 \times \left(PP - (0.3 \times PP)\right) \qquad \text{Eq. 14}$$

From this equation, the extent to which measured BP is supported by the available carbon from PP can be estimated. By subtracting the amount of BP supported by

autochthonous sources from total bacterial production, bacterial production supported by allochthonous carbon can be estimated:

$$BP_{ALLO} = BBP - (PP_{BAC} \times BGE) \qquad \text{Eq. 15}$$

In the estimates of bacterial production presented in chapter 5 a statistical relationship (Eq. 12) was used to estimate bacterial respiration from bacterial production and temperature. Using these estimates yielded bacterial growth efficiencies (BGE) of ~39%. Average BGE for freshwater ecosystems has been estimated at 26% (del Giorgio and Cole 1998) with an upper limit of ~37%. This means that likely our previous estimates of bacterial respiration have been underestimated and for this reason in this model I will use bacterial biomass production values from chapter 5, which were directly measured, and use various BGE reported in the literature. Values from 20% to 37% have been taken from del Giorgio and Cole (1998). Total pelagic production was calculated by adding gross PP to BP_{ALLO}, and the subsequent proportion BP_{ALLO} makes to pelagic production estimated from the following;

$$TBP_{ALLO} = \frac{BP_{ALLO}}{TPP} \qquad \text{Eq. 16}$$

Where TBP_{ALLO} is the total contribution of allochthonous carbon to bacterial production and TPP is the total pelagic production (PP + BP_{ALLO}).

Integrated BP estimates for epilimnetic values were calculated assuming linear relationships between surface and 13 m depth.

6.3) *Results*

The contribution of bacterial production fuelled by allochthonous inputs of organic carbon varies between the epilimnion and hypolimnion in the north basin (Table. 11). Over an annual cycle, 70 ± 21% of hypolimnion bacterial production is modelled to be fuelled by allochthonous organic carbon. The contribution to the epilimnion is on average less but is considerably more variable throughout the year (41 ± 39%). Contribution of BP_{ALLO} to pelagic production peaks in the hypolimnion in March prior to the spring bloom period, where 100% of the carbon utilised by bacteria is of allochthonous origin. Lowest values were estimated in July (39%) and August (42%), but values never reach as low as in the epilimnion. The epilimnetic contribution of allochthonous carbon is below the detection limits of this model during the spring bloom start in April, and is regularly below 10% from April to August. Highest modelled allochthonous carbon contribution to pelagic production correspond to periods of low productivity e.g., 94% (November), 63% (February) and 93% (March). The epilimnion of the north basin reveals an annual cycle where at times BP is 100% supported by allochthonous carbon, and at others 100% supported by autochthonous.

	Date	BBP	PP	PP_{BAC}	BP_{ALLO}	Total pelagic production	Contribution of BP_{ALLO} to PelP (%)
North basin Hypolimnion	August	0.74	0.91	0.34	0.65	1.56	42
	September	1.07	0.23	0.08	1.05	1.27	82
	November	0.00	0.00	0.00	0.00		
	January	2.61	1.21	0.45	2.49	3.70	67
	February	2.36	1.29	0.48	2.23	3.52	63
	March	0.14	0.00	0.00	0.28	0.28	100
	April	0.59	0.10	0.04	0.58	0.69	85
	June	0.57	0.14	0.05	0.56	0.70	79
	July	0.86	0.27	0.10	0.83	1.10	39
North basin Epilimnion	August	4.50	29.14	10.78	1.70	30.83	6
	September	3.45	12.83	4.75	2.22	15.05	15
	November	1.48	0.09	0.03	1.47	1.56	94
	January	4.63	2.10	0.60	4.43	6.53	68
	February	1.41	0.65	0.24	1.35	2.00	63
	March	0.30	0.02	0.01	0.30	0.32	93
	April	0.99	11.63	4.30	0.18	11.57	2
	June	7.87	17.97	6.65	6.14	24.11	26
	July	5.43	40.53	15.00	1.53	42.06	4

Table 12: The contribution of allochthonous carbon to pelagic production in the north basin showing bacterial biomass production (BBP), primary production (PP), estimated PP available to bacteria (PP_{BAC}), the estimated amount of BP fuelled by allochthonous carbon (BP_{ALLO}) and estimated total pelagic production (PelP), (all in µg C/L/day). The final column is the estimated percentage allochthonous fuelled BP makes up of total pelagic production.

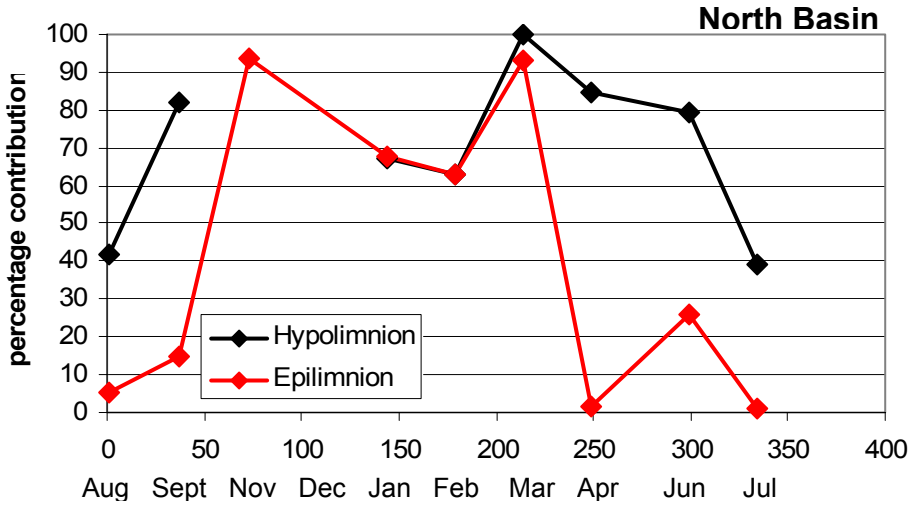

Figure 44: Seasonal change in the percentage contribution of allochthonous fuelled bacterial production in the north basin. Missing data point represents period when both PP and BP were below detection limits.

As in the north the hypolimnion of the south basin is modelled to have consistently higher proportions of pelagic production fuelled by allochthonous organic carbon. The annual average is 73 ± 28% in the hypolimnion, which is not significantly greater than in the north. The range in percentage contribution of BP_{allo} to PeIP in the south basin epilimnion is greater than in the north basin epilimnion gets both higher (August, 100%) and lower (February, 10%), the hypolimnetic water in the south is generally fuelled by allochthonous carbon for most of the year, with only two months with significantly low values (February and April). A large supply of autochthonous carbon in February seems unlikely, and if these two points are removed from the data mean allochthonous C contribution increases to 87 ± 13% for the whole year. However, no valid reason exists to exclude the points from the data set, and indeed these points may represent a re-suspension of autochthonous material on the lake bed during rough weather.

The epilimnion in the south basin has a similar annual mean value to the north basin epilimnion with 39 ± 35%. As with the north the epilimnion in the south shows a wide range of values from 0% (April and July) to 83% (November). The highest contributions of allochthonous carbon are observed in the late autumn and winter months from September to March.

	Date	BBP	PP	PP_{BAC}	BP_{ALLO}	Total pelagic production	Contribution of BP_{ALLO} to PelP
South basin Hypolimnion	August	0.42	0.00	0.00	0.21	0.21	100
	September	2.12	0.76	0.28	2.04	2.80	72
	November	4.47	0.12	0.05	4.46	4.58	97
	January	4.01	1.79	0.66	3.83	5.63	70
	February	0.66	1.38	0.51	0.54	1.78	30
	March	0.97	0.02	0.01	0.97	0.99	97
	April	0.37	0.67	0.25	0.30	0.97	23
	June	2.44	0.41	0.08	2.42	2.63	93
	July	3.64	1.10	0.41	3.54	4.64	77
South basin Epilimnion	August	3.24	27.79	10.28	0.57	28.35	1
	September	14.77	9.83	3.64	13.83	23.66	58
	November	4.34	0.88	0.32	4.25	5.13	83
	January	3.67	1.33	0.49	3.54	4.87	73
	February	1.42	1.63	0.30	0.63	1.45	44
	March	0.55	0.16	0.06	0.54	0.69	77
	April	1.42	21.82	8.07	-0.68	21.14	0
	June	6.67	20.09	7.43	4.74	24.83	19
	July	3.67	41.51	15.36	-0.33	41.18	0

Table 13: The contribution of allochthonous carbon to pelagic production in the south basin showing bacterial biomass production (BBP), primary production (PP), estimated PP available to bacteria (PP_{BAC}), the estimated amount of BP fuelled by allochthonous carbon (BP_{ALLO}) and estimated total pelagic production (PelP), (all in µg C/L/day). The final column is the estimated percentage allochthonous fuelled BP makes up of total pelagic production.

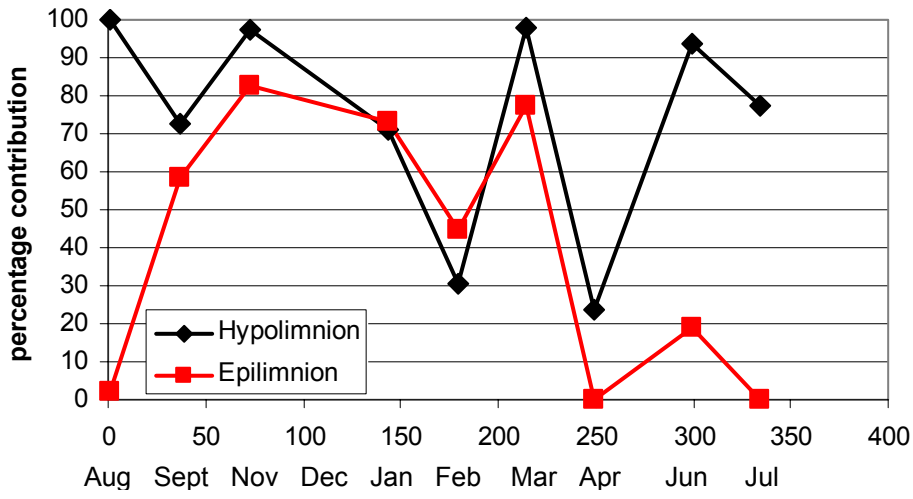

Figure 45: Seasonal change in the fractional contribution of allochthonous fuelled bacterial production in the south basin. Missing data point represents period when both PP and BP were below detection limits.

A consequence of the equation 14 is that the bacterial production fuelled by autochthonous carbon (BP_{auto}) can be estimated from measured primary production using bicarbonate incorporation. This relationship is shown in Figure 46 and reveals estimated BP_{auto} to generally be one third of total PP in the water column of Loch Lomond, though significant uncertainty is apparent at higher productivities.

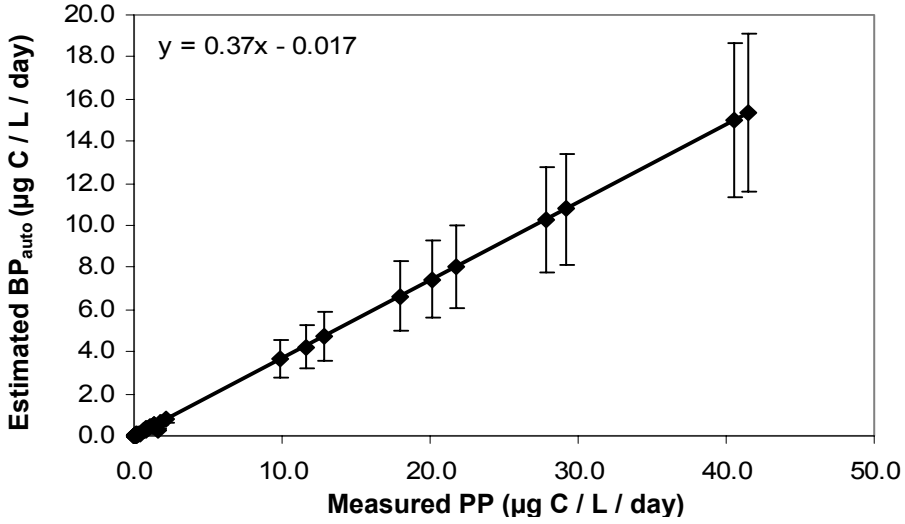

Figure 46: Estimated bacterial production fuelled by autochthonous carbon (BP_{auto}) based on measured photosynthetic production.

Epilimnetic integrated production values were calculated assuming linear decreases for bacterial biomass production (as detailed in chapter 5, section 5.2.6). Integrated primary production was taken directly from estimates in chapter 5, and these values have been used in equation 1, 2 and 3 to estimate the % contribution of bacterial utilisation of allochthonous carbon, to total epilimnetic production in the top 13 m of the north and south basins. Absolute quantities of allochthonous carbon utilised by bacterial production are also shown as a first stage in elucidating total amounts of allochthonous subsidies to Loch Lomond.

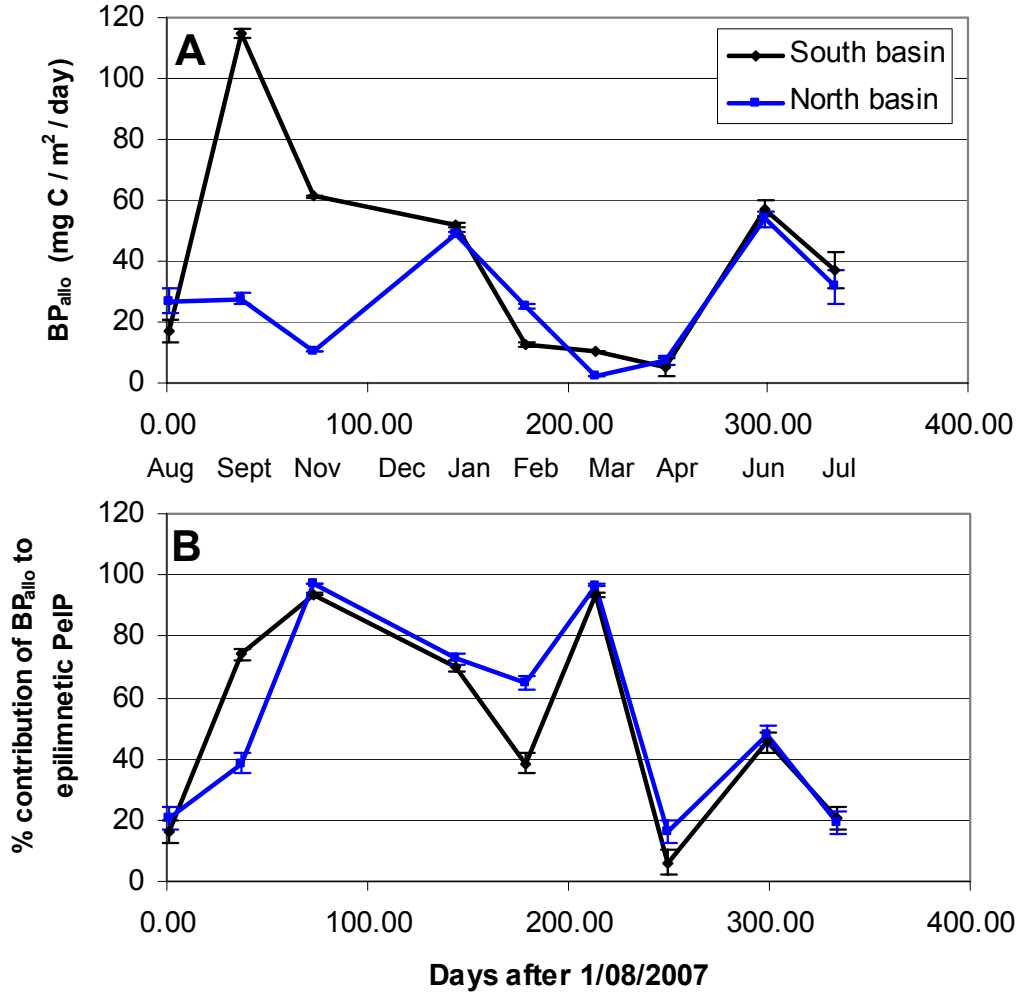

Figure 47: Absolute (A) and fractional (B) contribution of allochthonous carbon to total pelagic production in the upper 13 m of the south and north basin. Error bars represent errors associated with varying estimates of BGE and PP_{BAC} in earlier calculations.

North and south basins show similar seasonal patterns in epilimnetic utilisation of allochthonous organic carbon during winter and onto spring / summer (Fig. 47a). In January allochthonous carbon is utilised by bacteria at a rate of ~ 50 mg C / m^2 / day for both basins. This drops during February and March, beginning to rise again in April. From this point both basins show significant growth in allochthonous carbon processing, reaching ~ 60 mg C / m^2 / day in June, and then beginning to drop in July.

Between August and January significant differences in the quantity of allochthonous carbon processed are observed between north and south basins (Fig. 47a). The north basin shows relatively low production values of between 15 and 30 mg C / m^2 / day, contrasting with the south basin where ~ 120 mg C / m^2 / day of allochthonous carbon is processed by bacteria in September.

The disparity in quantity of allochthonous carbon processed observed between north and south basins in September, is reflected in the fractional contribution bacterial processing of allochthonous carbon makes to total pelagic production at this time (Fig. 47b). In the south basin over 70% of the total production is fuelled by allochthonous carbon, compared to ~40% in the north. All other months show good agreement between north and south suggesting that overall quantities of allochthonous carbon in the epilimnion of each basin are similar, as is the proportion this carbon makes up of the total available in the water column.

The total amount of allochthonous carbon utilised by bacteria on a basin wide and whole lake scale can be estimated (Fig. 48). Epilimnion integrated values (upper 13 m of water column) have been multiplied by basin surface areas obtained from GIS data. The hypolimnion values (in units / m^3) have been multiplied by the hypolimnion volume obtained again from a GIS source. As with whole lake production estimates in chapter 5, the middle basin has been assumed to be an intermediary between the measured north and south basins. Numbers for the middle basin are thus averages of the other two.

The highest absolute quantities of allochthonous carbon utilised by bacteria were observed in the autumn and winter months in the middle and south basins (Fig. 48). The south basin shows the largest individual recorded flux of allochthonous carbon in September where 3264 kg C day^{-1} is estimated to be used by bacteria. All three basins show a drop during November, particularly in the North where only 171 kg C day^{-1} was estimated to be being processed. A substantial increase in the north basin was observed in January, peaking at 2716 kg C day^{-1} utilised. This significant rise was not observed in the middle or south basins.

All basins show a drop in the quantity of allochthonous carbon processed during the late winter and early spring months, with minimum values being recorded in March and April, likely corresponding to the onset of the algal bloom season. A rise was recorded in all three basins after April as the summer months of June and July are reached.

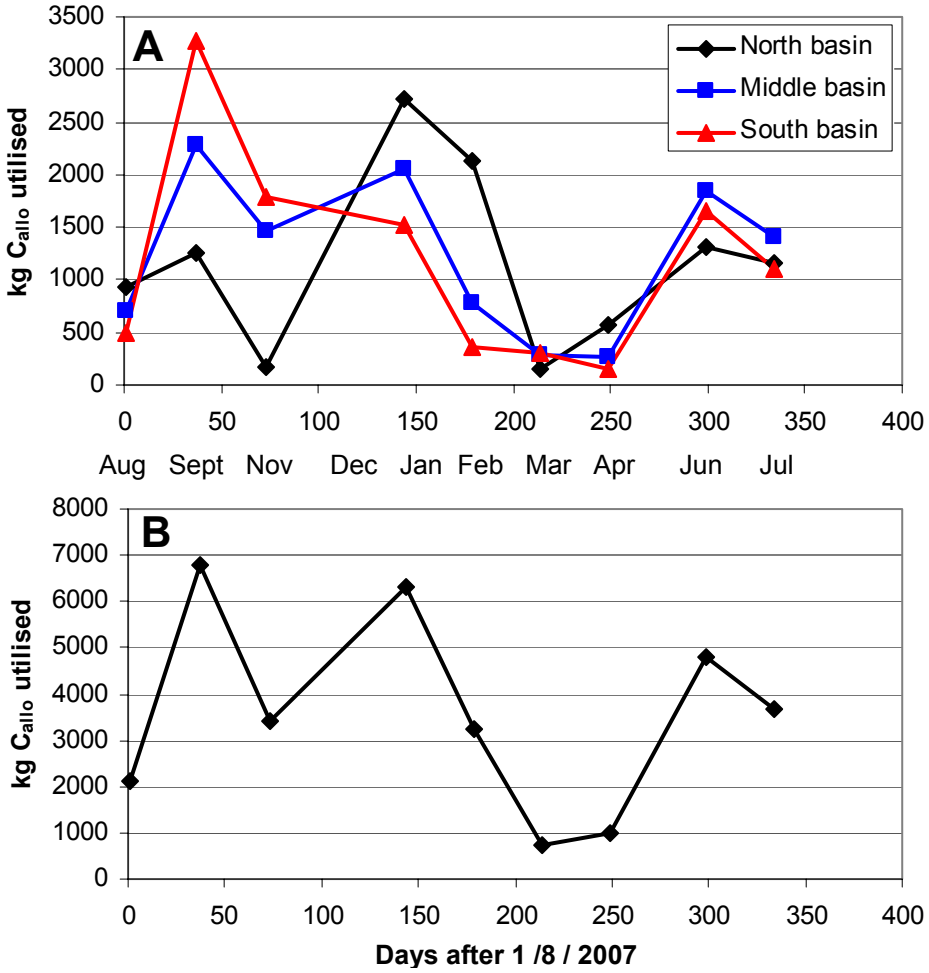

Fig 48: Estimated absolute amounts of allochthonous carbon processed via bacterial utilisation in a) each basin and b) the whole lake over an annual cycle.

The whole lake shows similar patterns to the basin specific results. Highest values were observed in September and January where 6805 and 6296 kg C day^{-1} respectively was estimated to be processed. The high in the autumn / winter months was again followed by a significant drop at the start of spring, reaching a minimum in March and April, and rising during the summer.

There was significant variability between the processing areas of the majority of allochthonous carbon, epilimnion or hypolimnion. In the north basin the percentage of total allochthonous carbon processed in the hypolimnion ranged from 14.5% in June to 61.2% in February. This was significantly higher than in the south basin where at most the hypolimnion accounted for 2.1% (February and March) of total allochthonous carbon processing. The middle basin was intermediate between the

two other basins, but in general most of the allochthonous carbon is processed in the top 13 m of Loch Lomond.

6.4) *Discussion*

Phytoplankton and bacteria are the most important producers of particulate organic material in the water column of lakes (Berglund *et al* 2007) and, in chapter 5 we have shown that in Loch Lomond bacterial production exceeds primary production by phytoplankton by a significant margin. This outcome supports the growing consensus that most unproductive water bodies are dominated by bacterial utilisation of organic carbon compounds and thus are heterotrophic systems, with primary production the dominant process only in productive systems (e.g., Gasol *et al* 1997). Although the southern basin's greater overall bacterial production levels are greater than the north basin's, contradicting the previous conclusion, this is likely a reflection of significantly different loading of organic material from the watersheds. The difference in loading quantities may be masking any difference trophic level may be having.

Organic carbon for bacterial utilisation can originate from two sources as previously discussed (see Chapter 5). Exudation from live phytoplankton cells, release from dead phytoplankton cells and release from metazoans and higher trophic levels constitute the autochthonous supply. Any bacterial productivity that cannot be accounted for by the sum of the previous processes must originate from the catchment and a terrestrial source. In the first 4 chapters, I present evidence to suggest allochthonous carbon may be of significance, and direct measures (chapter 5) of greater bacterial production compared to primary production have supported this idea. Finally modelled estimates of allochthonous carbon utilisation in this chapter have suggested a large proportion of pelagic bacterial production is fuelled by allochthonous sources.

Loch Lomond shows a strong seasonal influence on the absolute quantity of allochthonous carbon utilised and the relative fraction of pelagic production that was fuelled by allochthonous carbon (Fig. 44 and 45). In the south and middle basin, the majority of allochthonous carbon was processed in the epilimnion, hence discussion will focus there. The trend towards the highest contribution of allochthonous carbon to pelagic production during the winter months, is likely due to a lack of primary production at this time of year, caused by low temperatures and low irradiance. Hence, even although overall production may be lower in the winter, the majority of bacterial carbon must be supplied from terrestrial sources. In some lakes in winter

there is a drop in the quantity of allochthonous carbon available as soil mobilisation is limited by freezing. This is not the case in the Loch Lomond catchment where winter temperatures rarely drop below freezing. Indeed during the winter of 2006/07 temperatures were generally relatively warm and rainfall, particularly in December / January was high. November was when allochthonous carbon fuelled the highest proportion of pelagic production in the south basin, reaching over 80%. This implies however that there is still a source of autochthonous carbon driving some energy flow, although lowest lake productivities were recorded (along with March) at this time.

As spring approaches the days become longer, temperatures rise and phytoplankton begin to bloom. During blooms the quantity of autochthonous carbon increases compared to allochthonous. Such temporal variability in the supply of algal produced autochthonous organic carbon has been observed for some time (Coveney 1982, Brock and Clyne 1984, Vadstein *et al* 1989). Autochthonous DOC is more readily utilised by bacteria (Pomeroy 1974; Azam and Cho 1987) as allochthonous DOC has undergone several degradation steps en route to the lake (Yano *et al* 2000) and is more recalcitrant to bacterial utilisation. Thus as autochthonous supply increases, the maximum bacterial production (limited by temperature, grazing and viral lysis) may be completely met by this source. In the epilimnion of the south basin 100% autochthonous supply to pelagic production was estimated in April, July and August (Page 163). April likely corresponds to the primary phytoplankton bloom dominated by diatoms, whereas July and August were more likely to coincide with summer cyanobacteria blooms (Eurolakes). Lack of information on species composition during sampling trips mean these assumptions have not been clarified. During spring and summer blooms, primary production can often be limited by exhaustion of the nutrient pool, during which time production, although high, is not as prolific as during bloom periods. This is one possible reason for 20% of pelagic production being fuelled by allochthonous carbon in June in the south basin epilimnion, as primary production may not quite be reaching the required level to support all bacterial production. A greater input of less refractory carbon from the watershed is another possibility.

The epilimnion of the north basin showed a similar seasonal cycle to the south basin. However, during the summer months algal primary production does not reach the level that it supports 100% pelagic production (Fig. 44). In April it comes closest, supporting 98%. This may imply that either primary production isn't quantitatively as important as in the south, or that there is a greater supply of more readily useable allochthonous carbon. Each of these is a likely scenario and chances are each

contributes to the observed difference. The north basin is known to be oligotrophic year round, compared to the south which is regularly mesotrophic during the summer months. Although no significant difference in annual primary production between basins was observed, differences are still likely. Blooms are often short-lived and events may have been missed in either basin. If the south basin for example had a bloom just before data collection, which ended before our measurements, peak productivity may not be recorded, but its influence on the autochthonous carbon pool could still be present. The same bloom may not have occurred in the north and as such allochthonous carbon is still being utilised to some extent. The north basin also drains a far steeper catchment than the south, so although it has less opportunity to pick up organic carbon en route, and the organic carbon it transports will likely have less chance to be degraded and be thus be less refractory upon utilisation by pelagic bacteria.

The hypolimnion in the north shows a similarly high contribution of allochthonous carbon to pelagic production in the winter months, with the lack of primary production in the epilimnion again the most likely reason. However, the hypolimnion also shows elevated contributions during the summer months when the epilimnion is almost entirely supported by autochthonous carbon. The north basin stratifies from approximately May to November, during which time export of particulate and dissolved organic matter from the epilimnion to hypolimnion is generally little. Hence, during the summer months, in spite of the productivity in the surface waters, the hypolimnion may be completely cut off from this supply and thus processing the allochthonous carbon that remains (Sondergaard *et al* 1985, Vadstein 1989).

Although the absolute quantities of allochthonous carbon processed in the epilimnia of the south and north basin show significant differences at certain times of the year (Fig. 47a), the fractional contribution to both is similar (Fig. 47b). Relative contributions are never as low as in values previously discussed which dealt with epilimnetic and hypolimnetic waters in m^3, as they include an exponential decline in primary productivity with depth. Thus, while bacterial productivity often stays relatively constant through the water column the amount of autochthonous carbon available to it declines. However, in April both the north and south epilimnetic water show low contributions of allochthonous carbon. Each basin also has low values in July and August, with the previously discussed increase in June. Due to lack of primary production due to temperature and light limitation, etc, the contribution of allochthonous carbon in winter is high in both basins, approaching 100% in November and March.

Estimations of allochthony in other systems support these findings. For example, Kritzberg *et al* (2004) found that the bacterial community in two un-productive, slightly acidic lakes comprised between 35-70% allochthonous carbon. Although estimates presented in this work vary, and there is likely uncertainty in the exact figures caused by various errors, allochthonous carbon is certainly a significant component of total lake carbon cycling in Loch Lomond. These conclusions support a growing consensus that the importance of allochthonous carbon in limnetic systems increases significantly with increasing latitude (Alin and Johnson 2007), and is proportionally the most important source of organic matter in this temperate latitude lake.

Temporal differences in the dependence of pelagic production on allochthonous sources of organic carbon has been observed, along with differences in source carbon flow patterns between basins. The question now arises: what are the implications for energy cycling in Loch Lomond?

The primary source of production in a water column, be it phytoplanktonic or bacterial, affects the subsequent transfer of that production through the food web. It has previously been observed in marine systems that oligotrophic and strongly eutrophic systems have lower energy transfer efficiency than moderately nutrient rich areas (Sommer *et al* 2002). In oligotrophic pelagic environments this is due to domination of the plankton by pico-plankton (< 2-3 μm) which are too small to be directly ingested by zooplankton. The same scenario occurs in oligotrophic lake systems, dominated by bacterial processing of allochthonous organic carbon. Although there is undeniably a significant extra quantity of organic carbon processed, the amount that can flow to the higher trophic levels could be relatively little (e.g., Fenchel 1988). Approximately 90% of energy fixed at each trophic level is lost when transferred to the next level. This implies more energy will pass up a classical food chain to primary / secondary consumers, than similar quantities flowing through a microbial food web. Microbial food webs will on average require two extra trophic steps before carbon originally fixed by bacteria for example would be available to the metazoan community. Recent work has shown that bacterial based food webs are considerably less efficient than phytoplankton based ones (e.g., Jansson 2003, Bergland *et al* 2007).

This research has implied that there may be a considerable source of extra carbon / energy made available in Loch Lomond additional to algal production and the classic pelagic food chain. However, with the additional trophic steps required to transfer this energy to higher levels, the effect it has outside the microbial community is uncertain. It is known that energy mobilisation is significantly less in microbial

dominated communities, but the large quantities of extra production may counter balance the in-efficiency. Further work should address the question of how much of the observed extra production is actually of influence to higher trophic levels in Loch Lomond, as well as constraining the likely destinations of allochthonous carbon utilised by bacteria. i.e., how much supports subsequent biomass at higher trophic levels? How much is directly respired and lost as CO_2 to the atmosphere? How much is sequestered in the sediments and removed from the ecosystem for a prolonged period? These questions are essential in elucidating the role of lakes such as Loch Lomond to ecosystem carbon dynamics, and the possible consequences of varying inputs / outputs of allochthonous carbon in the future.

Chapter 7

Predicting primary and secondary production in Loch Lomond from natural abundance stable isotopes and various physico-chemical factors.

7.1) Introduction

The previous chapters have presented the natural abundance isotopes of DOC and DIC, their respective concentrations and various other physical parameters (temperature, pH, etc). In chapters five and six, productivity measurements quantified by isotope labelling, were presented and discussed. During isotope labelling experiments all the parameters measured in chapters 2 and 3 were measured concurrently.

The goal of this chapter is thus to explore the full data set obtained during incubation procedures for relationships between natural abundance isotopes, concentrations and other physico-chemical parameters, and measured productivities, (both secondary and primary). i.e., effectively linking the natural abundance survey work (chapters 2 and 3) with productivity incubations.

Examples of such linkage have been documented, e.g., Previous work by others has related the rate of primary production to the concentration and $\delta^{13}C$ of dissolved inorganic carbon (e.g., Juday 1935, Schindler and Fee 1973, Quay *et al*. 1986; Keough *et al*. 1996, Bade *et al* 2004). Low [DIC] and enriched $\delta^{13}C_{DIC}$ are considered indicative of high photosynthetic activity and vice versa. In chapter 2 these assumptions were utilised to imply variability in photosynthetic activity on a temporal and spatial scale in Loch Lomond. These assumptions can be assessed in this chapter by direct comparisons between natural abundance [DIC] and $\delta^{13}C_{DIC}$ with measured rates of primary productivity. These parameters have also been used to draw conclusions about rates of community respiration, as respiration yields the opposite result to photosynthesis, raising the [DIC] and depleting the $\delta^{13}C_{DIC}$.

Although the $\delta^{13}C_{DOC}$ signature varies little in Loch Lomond, the relative changes in concentration can also be used to make assumptions about rates of bacterial processing and organic matter loading, either autochthonous or allochthonous (see chapter 3). In this chapter direct relationships will be tested for to elucidate any dependence / relationship [DOC] may have with bacterial or photosynthetic productivity.

The aims of this chapter are as follows

i) Elucidate seasonal trends in the various physico-chemical parameters measured during the incubation field programme.

ii) Elucidate any possible relationships between parameters measured in chapters two and three and use concurrent productivity measures to support or contradict the conclusions made in those chapters, and examine the predictive power of different parameters on algal / bacterial productivity.

iii) To assess inter-annual variability by comparing annual time-series data to assess how representative survey work in chapters two and three is on more detailed timescales.

7.2) Methods

$\delta^{13}C_{DIC}$, $\delta^{13}C_{DOC}$, [DOC], [DIC], pH, temperature and [DO] were all recorded along side stable isotope tracer incubations to assess lake productivity. The method for these incubations is described in detail in chapter five.

Natural abundance samples for $\delta^{13}C_{DIC}$ / [DIC] were taken before lake water was separated for isotope spiking (Chapter five, Fig. 36). After spiking was carried out and incubation bottle suspended in-situ, remaining water was filtered via the method described in chapter five, and the filtrate was frozen and prepared for DOC analysis via the method described in chapter three. pH was measured during DOC preparation before acidification, and temperature / [DO] were measured before incubations using a YSI 550 DO probe.

Data has been explored for relationships using step-wise linear regressions, step-wise non linear regressions and standard regression analysis.

7.3) Results

7.3.1) Seasonal trends in 2006/2007

Natural abundance time series were recorded throughout the incubation experiments. Although the spatial resolution is far less than the survey work of 2004 / 2005, they constitute a more detailed time series data set. Section 7.3.1 presents these new time series for purposes of examining inter-annual variability.

During the year long incubation programme all basins and depths of Loch Lomond showed significant temperature variation (Fig. 49). All measured sites show a drop in temperature between August and March, followed by an increase coinciding with the onset of spring, continuing to rise into late summer.

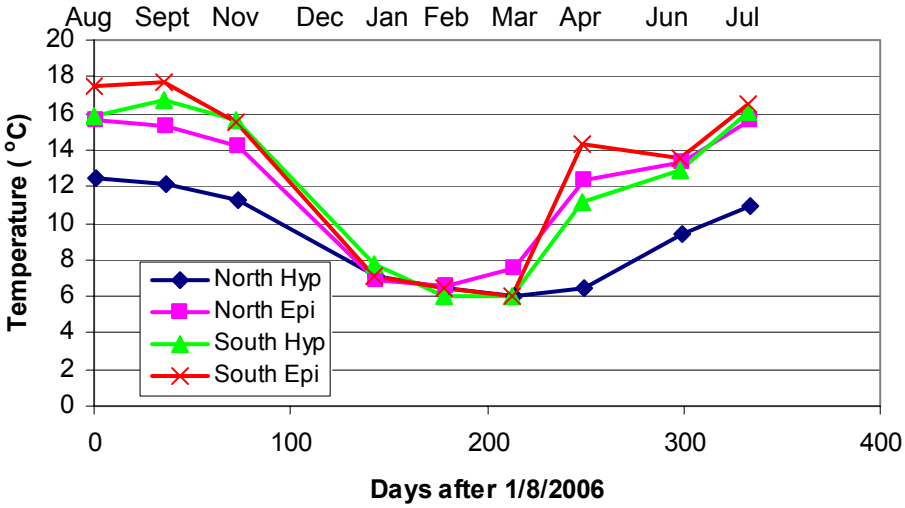

Figure 49: Seasonal temperature variation for the four measured areas of Loch Lomond between August 2006 and July 2007.

Hypolimnetic water in the north basin shows the smallest annual range with a minimum of 6.0°C in March and a maximum of 12.5°C in August. North basin epilimnetic water and all south basin water show changes of similar magnitude with the south basin epilimnion having the greatest annual range from 6.0°C in March to 17.7°C in September.

Figure 50 shows concentrations changes in DOC, DIC and POC over the sample year for each lake segment. DOC makes up the majority of the carbon pool in the lake (mean 67.8 ± 7.0% all data), followed by DIC (mean 25.8 ± 6.7% all data) and then POC (mean 6.6 ± 2.9% all data).

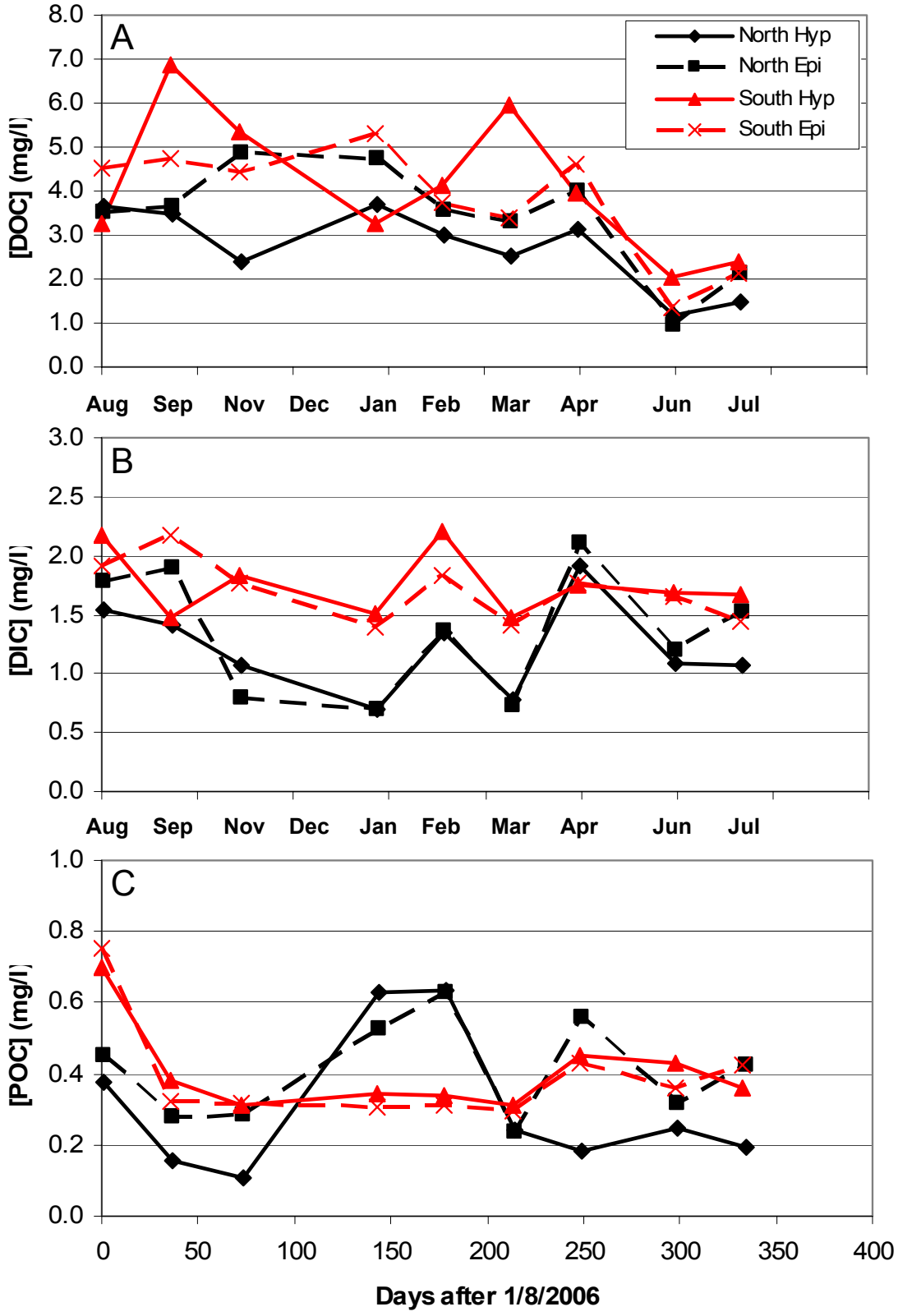

Figure 50: Seasonal change in concentrations of a) DOC, b) DIC and c) POC. Data divided into basins (north and south) and depths (epilimnion and hypolimnion).

[DOC] showed varying seasonal patterns (Fig. 50a) depending on the sample site and the depth. Hypolimnetic water in the south basin showed the largest range from 3.28 mg / L in August and January to a maximum of 6.9 mg / L in September. The north basin epilimnion and south basin epilimnion follow similar seasonal cycles, having relatively constant concentrations between August and January, followed by a drop from February to April. Lowest [DOC] were observed in the hypolimnion of the north basin, with 2.4 mg / L in November, the same time the north basin epilimnion was at its peak (4.9 mg / L).

Surface water in the south and north show similar patterns in [DOC] concentration variation. Concentrations are highest in the winter months between November and January at around 5 mg / L. This winter peak is followed by a fall with the onset of spring and the bloom season. The [DOC] in the south and north basin epilimnion rises again from June onwards. Hypolimnetic water in the north has a generally lower [DOC] than any other measured lake segment, reaching a minimum of 2.4 mg / L in November 2006, and showing an annual mean of just 3.1 ± 0.5 mg / L. Hypolimnetic water in the south basin shows the largest range in [DOC] with a minimum of 3.3 mg / L in August and January, and a peak of 6.9 mg / L in September. The hypolimnion in the south shows a different annual cycle than other measured water masses, showing two distinct peaks in [DOC] in September and March, with significant drops in between.

Concentration changes in DIC follow similar annual cycles in each basin between the epilimnion and hypolimnion (Fig. 50b), but more dissimilar patterns between basins. The south basin has a more steady concentration throughout the year, ranging only between ~1.5 and 2.0 mg / L, compared to the north basin which ranged from ~0.7 to 2.0 mg / L. Variability in the south basin is underlined by two key features, a peak in [DIC] in August, and a second in February. [DIC] in the north basin shows similar peaks in these months, although contains an even greater rise in April 2007.

[POC] in the south basin follows similar seasonal patterns in both the epilimnion and hypolimnion (Fig. 50c). Concentrations drop between autumn and winter, remaining relatively constant until spring bloom time when concentrations rise again. The north basin shows a different pattern with the highest concentrations being recorded in January and February, when the south basin was showing minimum concentrations. The high concentrations were repeated in both the epilimnion and hypolimnion in the north basin.

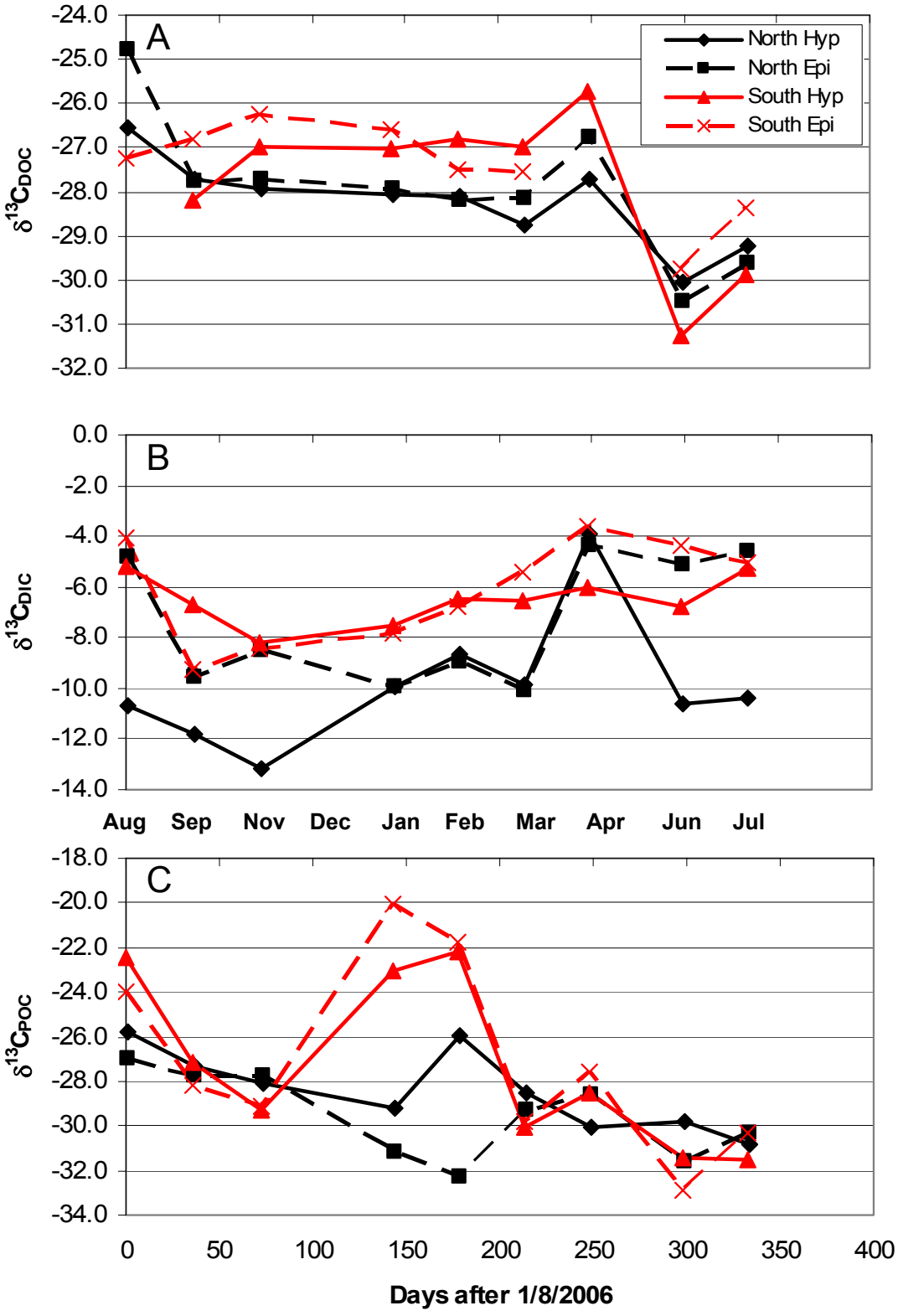

Fig 51: Seasonal variation in a) $\delta^{13}C_{DOC}$, b) $\delta^{13}C_{DIC}$ and c) $\delta^{13}C_{POC}$. Data divided into basins (north and south) and depths (epilimnion and hypolimnion).

$\delta^{13}C_{DOC}$ is consistently more ^{13}C-enriched in the south basin than in the north (Fig. 51a). The epilimnion in the south is more ^{13}C-enriched than the hypolimnion in the early winter months (November - January) becoming more depleted as spring begins. $\delta^{13}C_{DOC}$ in the south hypolimnion, north epilimnion and hypolimnion are all stable through the late summer and winter months (approximately -27 ‰, -28 ‰ and -28 ‰ respectively) and all three become more enriched from April and the start of the spring bloom period. Data for the epilimnion in the south is not available for April as the incubation bottles detached from the support frame during incubation, but the consistency between all other lake segments suggests it is likely enrichment occurred also. The north basin epilimnion and hypolimnion show enriched values in August at the end of the spring/summer productive season. Surface water in the north showed the highest value at this time of year reaching -24.7 ‰. In general $\delta^{13}C_{DOC}$ values are all well constrained in a signature consistent with allochthonous DOC produced by C3 vegetation.

$\delta^{13}C_{DIC}$ (Fig. 51b) in the south basin epilimnion and hypolimnion follows similar seasonal patterns. $\delta^{13}C_{DIC}$ reaches a minimum in late autumn / early winter (September / November), and the pool steadily became more enriched through winter and into spring, before becoming more depleted again between April and July. The epilimnion shows the largest range in the south basin with $\delta^{13}C_{DIC}$ at - 9.3 ‰ in September, and - 3.6 in April. $\delta^{13}C_{DIC}$ variability in the north basin shows different seasonal patterns in the epilimnion compared to the hypolimnion. Epilimnetic water follows a similar pattern to south basin $\delta^{13}C_{DIC}$, falling at the start of winter and then rising again in the spring, and values remain enriched (~ - 5 ‰) until the next winter. The hypolimnion $\delta^{13}C_{DIC}$ has the most depleted values in the lake in autumn / winter. Minimum measured $\delta^{13}C_{DIC}$ was - 13.2 ‰ in November, with values continually < - 10.0 ‰ from June through to November. Values rise between January and June, reaching enriched values comparable to the south basin of - 4.0 ‰ in April. In both the south and north basin epilimnion and hypolimnion $\delta^{13}C_{DIC}$ matches closely, separating approximately when the lake stratifies in spring.

7.3.2) *Controls on primary productivity.*

Primary production was significantly correlated with temperature in the epilimnion (Fig. 52). Hypolimnion temperatures vary a small amount throughout the year and PP is generally low in these waters so have been excluded from the regression analysis. PP showed an exponential relationship with increasing temperature in epilimnetic

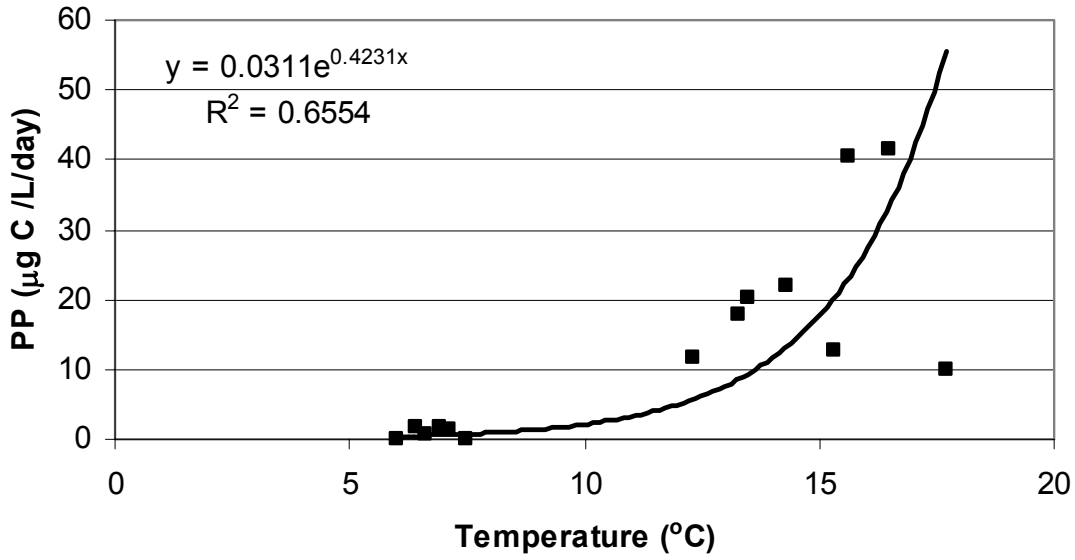

Figure 52: Relationship between primary production and temperature (°C) in epilimnetic waters (north and south basin combined). Exponential relationship is significant at the 0.05 level (P = 0.001) (n = 16).

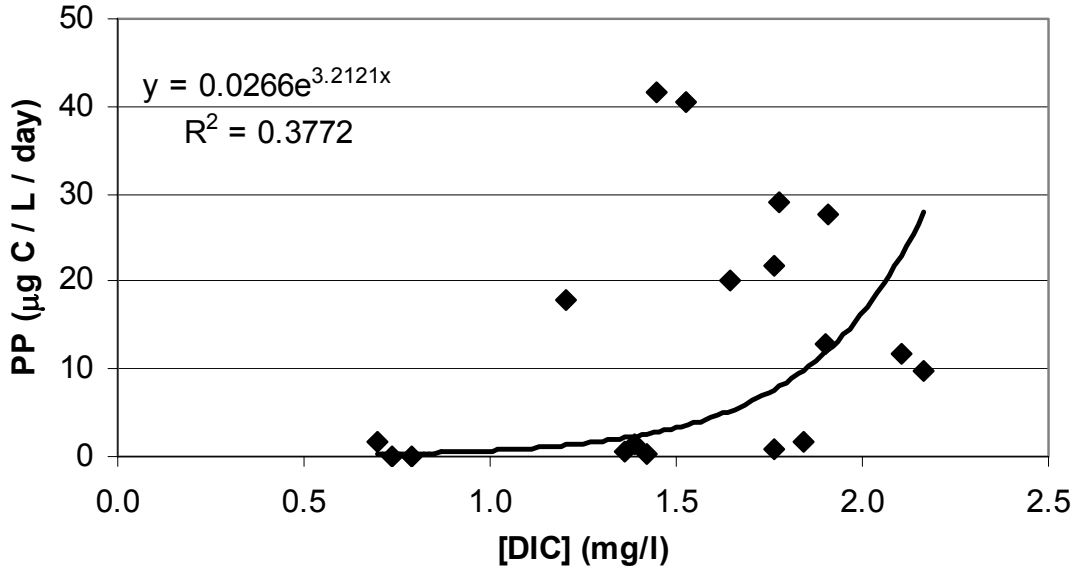

Figure 53: Relationship between DIC concentration and primary production in epilimnetic waters (north and south basin combined). Exponential relationship is significant at the 0.05 level (P = 0.007) (n = 18).

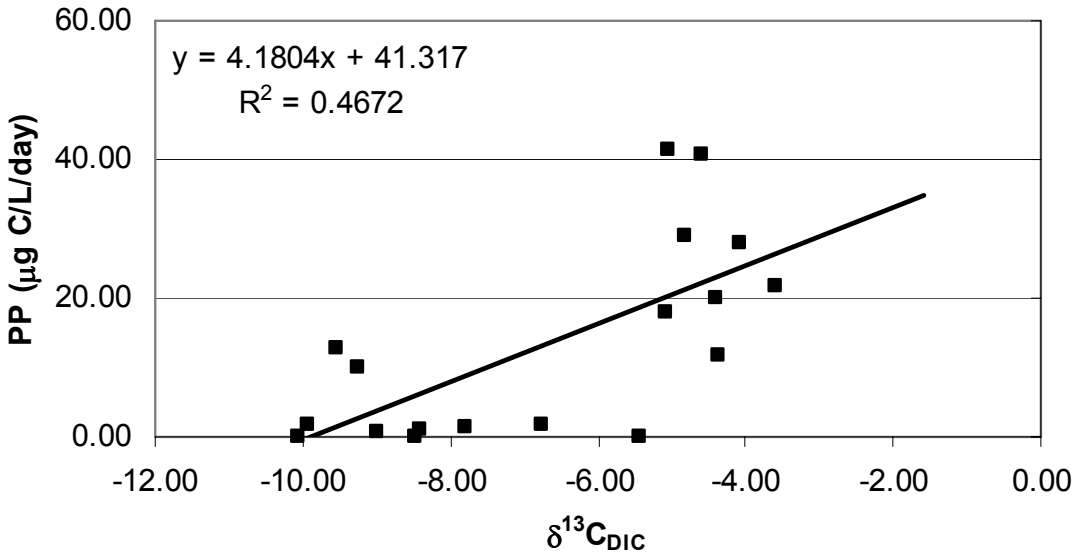

Figure 54: Relationship between $\delta^{13}C_{DIC}$ and primary production in epilimnetic waters (north and south basin combined). Linear relationship is significant at the 0.05 level (P = 0.002) (n = 18). An exponential relationship (not shown) was significant also, but explained less of the observed variation (R^2 = 0.40).

waters. Temperature could account for 68% of the variation seen in PP in the epilimnion of the north and south basins combined (n = 12, R^2 = 0.683, P = 0.001).

DIC concentration showed a significant (n = 18, P = 0.007) exponential relationship with PP in epilimnetic waters, although the regression explained less of the observed variability than temperature (R^2 = 0.377). As with temperature variation hypolimnion values were excluded as little PP variation above zero was observed.

$\delta^{13}C_{DIC}$ could explain more of the variability in PP than [DIC] showing a significant positive linear relationship (n = 18, P = 0.002, R^2 = 0.467). The most depleted $\delta^{13}C_{DIC}$ (~ -10‰) was measured at times of low PP, and the most enriched (~ -4‰) at times of low PP, although there is noticeable scatter of the data around this trend.

7.3.3) *Controls on bacterial productivity.*

Figure 55 shows bacterial production plotted against temperature for all sampled values including epilimnion and hypolimnion. Hypolimnion values have not been excluded because theoretically bacterial production is unaffected by the low light conditions. Bacterial production showed a significant exponential relationship with temperature when looking at all data (n = 36, P < 0.001, R^2 = 0.538). More variability is explained when just considering the epilimnion (R^2 = 0.619, data not shown), which

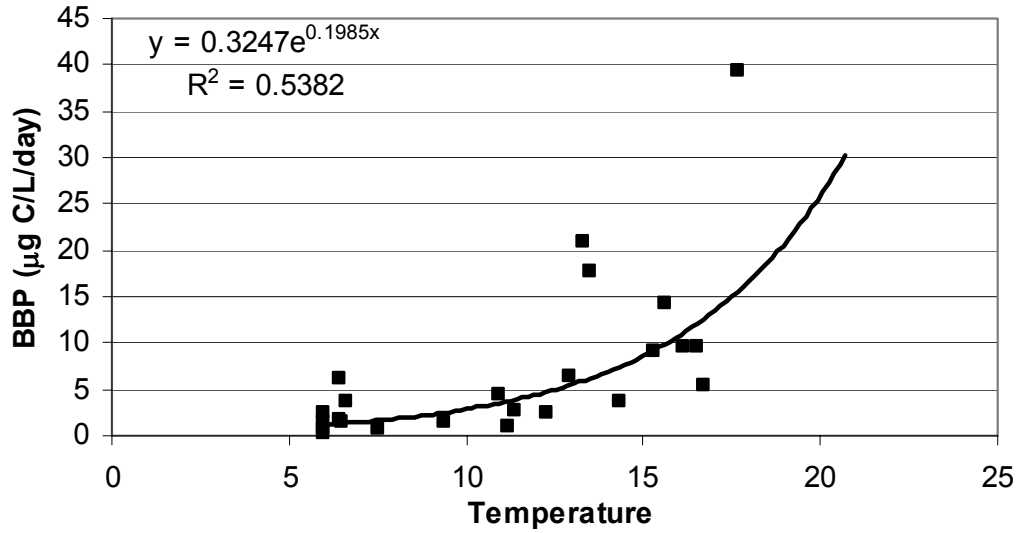

Figure 55: Relationship between bacterial production and temperature (°C) in epilimnetic and hypolimnetic waters (north and south basin combined). Exponential relationship is significant at the 0.05 level (P < 0.001) (n = 24).

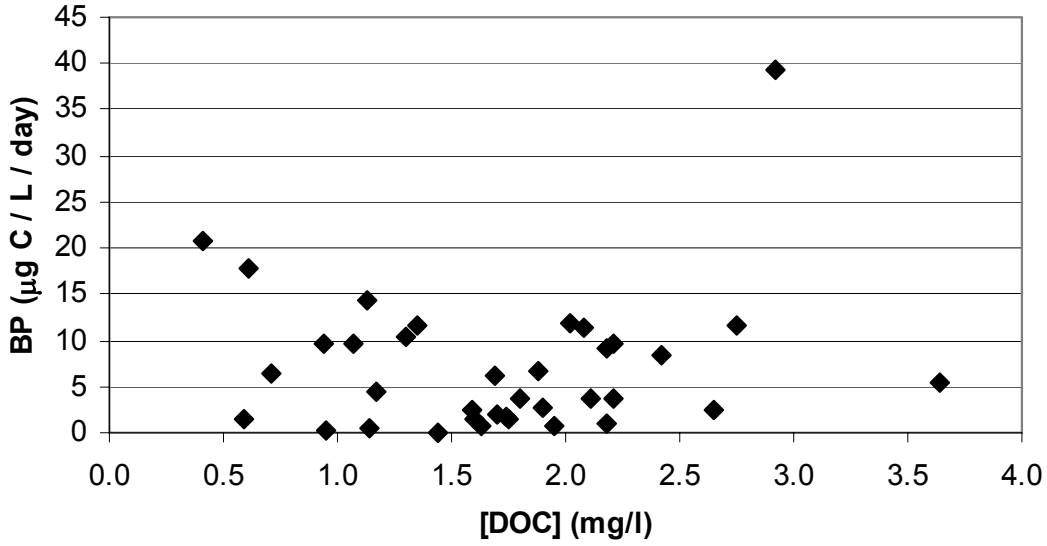

Figure 56: Relationship between bacterial production and DOC concentration in epilimnetic and hypolimnetic waters (north and south basin combined). No significant relationship was observed.

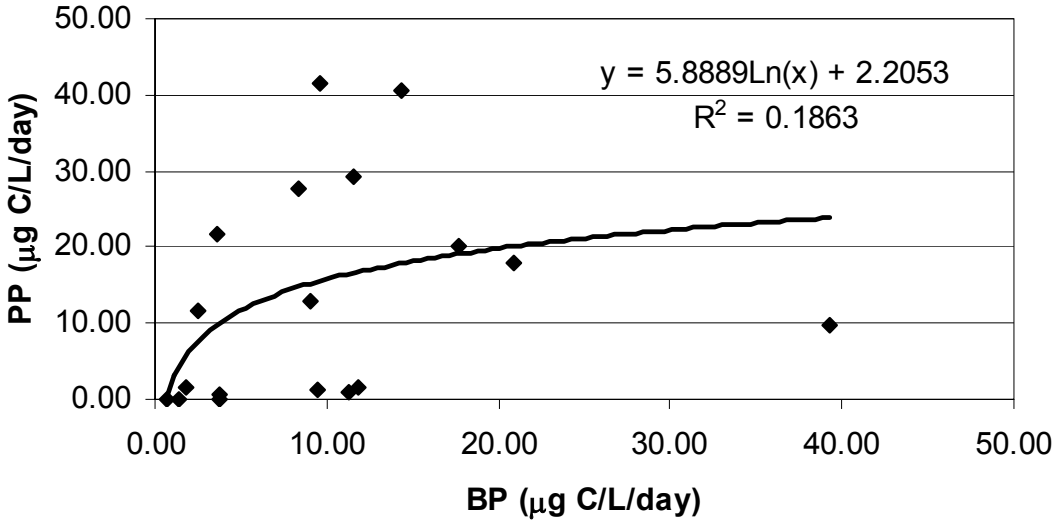

Figure 57: Relationship between phytoplanktonic and bacterial production in epilimnetic waters. A logarithmic relationship explained the most variation (R^2 = 0.186) but was still insignificant at the 0.05 level (P = 0.067).

could either be a function of reduced sample size, or as hypolimnion bacterial production shows greater dependence on non-temperature parameters.

Neither DOC concentration (Fig. 56) or $\delta^{13}C_{DOC}$ (data not shown) had any predictive power on bacterial production. This applied to bulk data and when examining each lake segment specific data. Primary production also had no significant effect on bacterial production although was only just insignificant (P = 0.067). [DIC] and $\delta^{13}C_{DIC}$ each showed no significant relationship with bacterial production. Only temperature was observed to have a significant influence on bacterial production.

7.3.4) *Detailed annual productivity estimations.*

Temperature data is collected at the SCENE research facility daily throughout the year in one point of the south basin. Using the described relationships between temperature and productivity, algal (Fig. 52) and bacterial (Fig. 56), a detailed time series of production can be estimated (Fig. 58). Water temperatures are only recorded in the south basin, epilimnetic water so these patterns are only relevant to this lake segment.

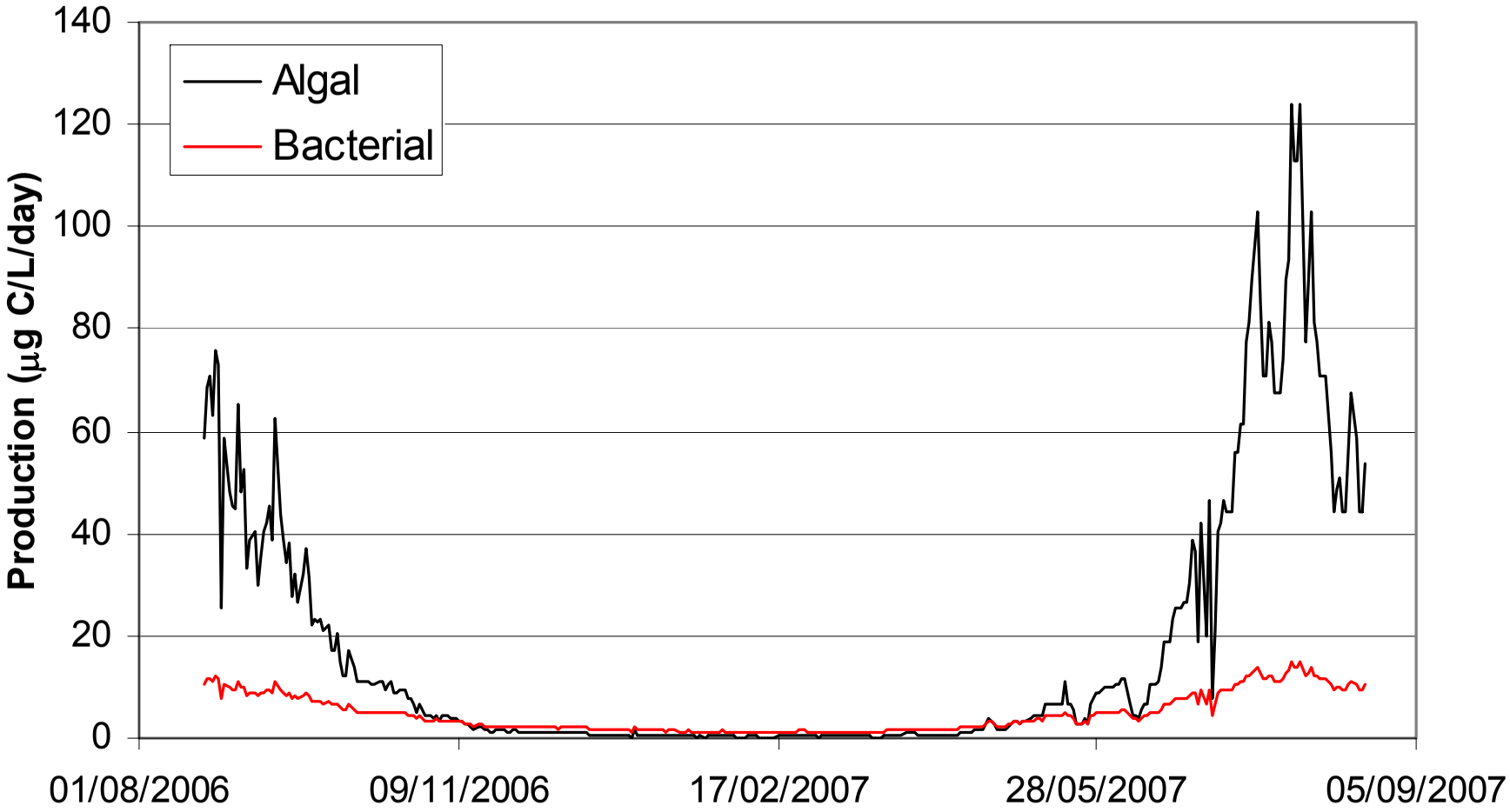

Figure 58: Estimated phytoplanktonic and bacterial production over an annual cycle in the south basin epilimnion using described temperature / productivity relationships.

Primary production in the south basin shows clear seasonal fluctuations (Fig. 58). There is little productivity during the winter months when the temperature is below approximately 8^{o}C. Productivity increases in the spring just after April. Primary productivity rises rapidly during this period but the rise is not continuous. On at least two occasions a drop in water temperature causes the estimated productivity to drop. At the end of the summer productivity begins to fall again and is less than 1 µg C / L / day by November / December.

Bacterial productivity in the south basin epilimnion shows a seasonal trend also, but lacks the dramatic peaks seen in primary production. Bacterial production follows primary production increasing during the spring/summer and dropping in the winter. Bacterial production exceeds primary production throughout the winter months in the south basin epilimnion.

7.3.5) *Inter-annual variability in [DIC], $\delta^{13}C_{DIC}$ and [DOM].*

More detailed time series data was collected between August 2006 and July 2007 than in the preliminary survey work presented in chapters two, three and four. Although the spatial resolution is not of the same detail, by comparing seasonal patterns observed in 2004 / 2005 with those of 2006 / 2007 deductions can be made about how representative these initial surveys were of a more comprehensively recorded annual cycle.

Figure 59, 60 and 61 show seasonal trends from each sample period plotted on the same time scale. Samples sites in ‘06 / ‘07 have been plotted against the closest corresponding site from ‘04 / ‘05. [DIC] in the south basin showed different trends in ‘04 / ‘05 compared to ‘06 / ’07 (Fig. 60c and d). Throughout the year [DIC] was consistently lower in ‘06 / ‘07, particularly approaching spring when in ‘04 / ‘05 the highest values were recorded approaching 0.4 mM. No corresponding peak was observed in ‘06 / ‘07 and [DIC] in the south basin remained relatively constant between 0.10 mM and 0.18 mM in both the epilimnion and hypolimnion.

More detailed resolution for the ‘06 / ‘07 time series reveals a fluctuating [DIC] in the north basin, both in the epilimnion and hypolimnion (Fig. 59a and b). Between February and April there are noticeable rises and falls in [DIC], which are not shown with the less detailed time series of ‘04 / ‘05 where [DIC] shows little seasonal variation in this location. However, [DIC] in the north basin was generally comparable between years in both the epilimnion and hypolimnion.

$\delta^{13}C_{DIC}$ in the epilimnion of the north basin (Fig. 60a and b) showed depletion from -6‰ to -12‰ between September and March 04 / 05, followed by an enrichment

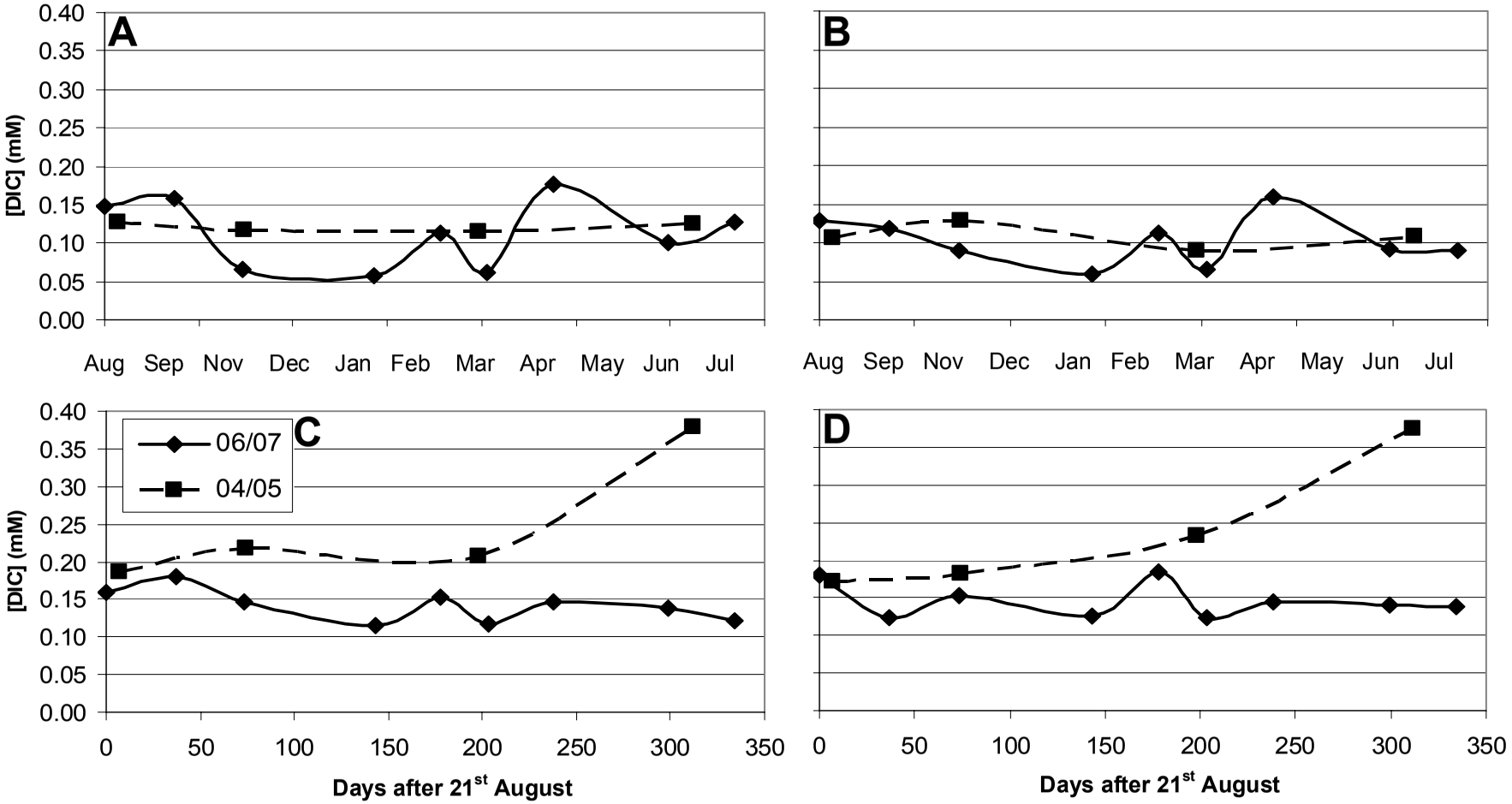

Figure 59: DIC annual concentration variation in 2004 / 2005 and 2006 / 2007 in a) north basin epilimnion, b) north basin hypolimnion, c) south basin epilimnion and d) south basin hypolimnion.

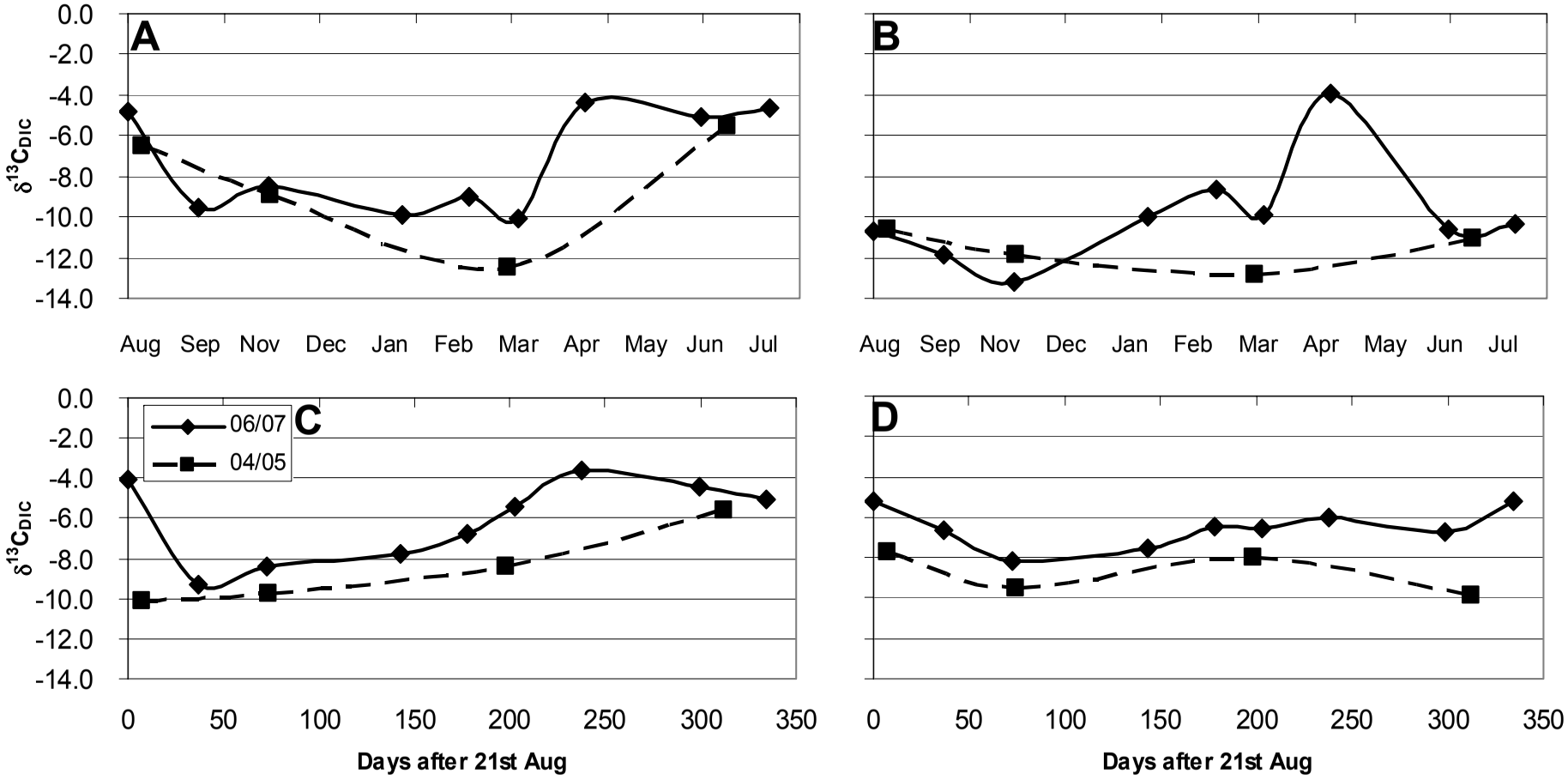

Figure 60: $\delta^{13}C_{DIC}$ annual variation in 2004 / 2005 and 2006 / 2007 in a) north basin epilimnion, b) north basin hypolimnion, c) south basin epilimnion and d) south basin hypolimnion.

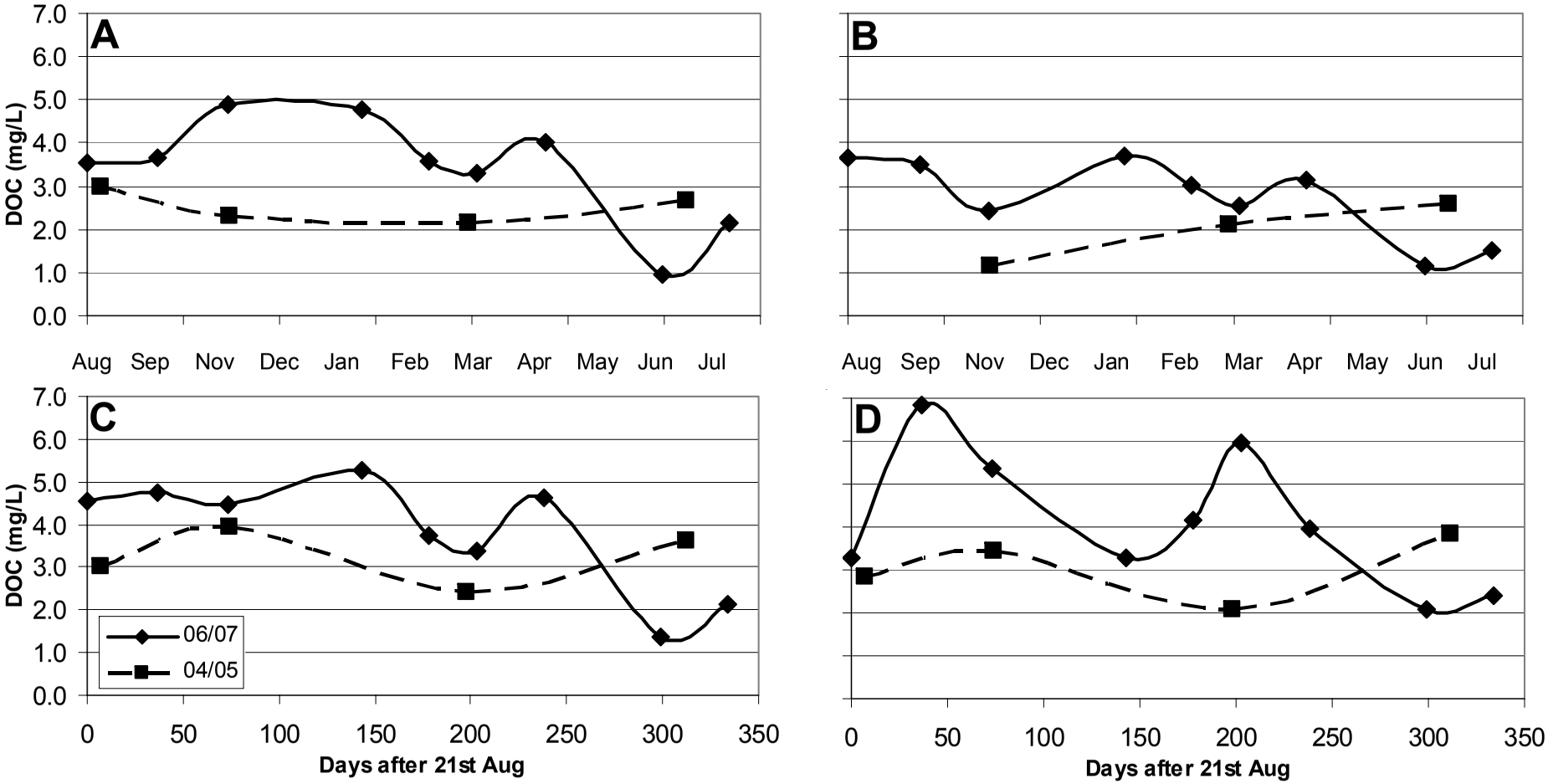

Figure 61: DOC annual concentration variation in 2004 / 2005 and 2006 / 2007 in a) north basin epilimnion, b) north basin hypolimnion, c) south basin epilimnion and d) south basin hypolimnion.

between March and June. The same general pattern was observed during the incubation experimental period with an overall decline between August '06 and March '07, but as with [DIC] various peaks and troughs occur in this period. Between August and September $\delta^{13}C_{DIC}$ dropped from ~-4.8 ‰ to ~-9.5 ‰, which was the most significant depletion in this time period. Between September and March $\delta^{13}C_{DIC}$ varied only between -8.5 ‰ and -10.0 ‰. The measured enrichment in $\delta^{13}C_{DIC}$ observed between March and June in '04 / '05 is also present in the '06 / '07 data, but this data set reveals the increase to be far more rapid, with the majority of the enrichment occurring between March and April, corresponding to the spring algal blooms. $\delta^{13}C_{DIC}$ remained relatively constant between April and July.

The hypolimnion in the north basin showed no significant seasonal variability in '04 / '05, but showed significant spring summer enrichment in '06 / '07. No sample site from '04 / '05 was an exact match for the '06 / '07 site, and the closest was significantly deeper (>100 m compared to 55 m), which may influence the comparison. Changes in $\delta^{13}C_{DIC}$ in the hypolimnion of the north are relatively rapid compared to the sampling frequency of '04 / '05, and as such corresponding enrichment peaks (e.g., between March and April) may have been missed during '04 / '05. $\delta^{13}C_{DIC}$ becomes rapidly depleted after April in '06 / '07, not retaining the enriched state observed in the epilimnion in June and July, expected due to differences in dominant metabolic processes between depth ranges (see chapter 2).

The south basin epilimnion in '04 / '05 shows enriching $\delta^{13}C_{DIC}$ from September through to June. The more detailed temporal information of '06 / '07 follows a similar trend of enrichment over this period, but like in the north basin, shows a peak in April. The enrichment is more gradual than in the north basin, but peak $\delta^{13}C_{DIC}$ of -3.6 ‰ is reached in April. A second peak is also observed in the epilimnion in the south in late summer, a corresponding peak in the '04 / '05 season was not recorded. The hypolimnetic water in the south basin shows similar seasonal patterns in both the '04 / '05 and the '06 / '07 season. In general $\delta^{13}C_{DIC}$ in '06 / '07 was 1 – 2 ‰ more enriched in the '06 / '07 season reaching a maximum of -5.2‰ in July and August.

The concentration of DOC showed significant temporal heterogeneity in '06 / '07 (Fig. 61) in all measured lake segments. The north basin epilimnion (Fig. 61a) had greater variability than the hypolimnion (Fig. 61b) peaking in November and January (4.9 and 4.8 mg / L respectively), with minimum concentrations in June 07 (1.0 mg / L). [DOC] in the '04 / '05 season never exceeded ~ 3.0 mg / L in the north basin epilimnion. The north basin hypolimnion had a narrow range in [DOC] compared to the epilimnion (1.2 – 3.7 mg / L) but still showed peaks (September, January and April) and troughs (December, March and June) throughout the '06 / '07 season. An

increase between November and June was observed in '04 / '05 but [DOC] was consistently lower than in '06 / '07.

[DOC] in the south basin epilimnion (Fig. 61c) was relatively stable in '04 / '05 (mean [DOC] = 3.3 ± 0.7 mg / L), with a minimum concentration measured in March (2.4 mg / L). '06 / '07 generally had higher [DOC] (mean 3.8 ± 1.3 mg / L) and greater temporal heterogeneity revealed by the increased sampling frequency. Peaks in DOC were observed in September (4.7 mg / L), January (5.3 mg / L) and April (4.62 mg / L). Deep water in the south had the greatest [DOC] of any measured lake segment in '04 / '05 and '06 / '07, although as with the other sections [DOC] was generally greater in '06 / '07. [DOC] in '04 / '05 was observed to fall from September to March, rising again from March to June. [DOC] in '06 / '07 followed a similar pattern, with an initial rise between August and September being followed by a decline between September and January. This preceded an increase between January and July.

7.4) *Discussion*

This chapter set out three aims which will now be considered.

7.4.1) *Elucidate seasonal trends in various physico-chemical parameters during the incubation field programme.*

Temperature varied predictably for a monomictic system with a single annual period of stratification. All lake segments reached minimum temperatures in the winter months rising through the spring and summer with increasing day length, air temperatures and quantity of incident radiation, falling again in the autumn as day length reduces, air temperature drops and incident radiation decreases. Only the north basin hypolimnion varied significantly from the other lake segments, never reaching more than 11^{o}C, whereas all other areas peaked between 15.5 and 17.5^{o}C.

The concentration of DOC varied between all four lake segments, as well as seasonally (Fig. 50), with the greatest range observed in the south basin hypolimnion. The highest concentrations measured occurred in autumn. The observed peak in [DOC] is likely due to increased loading from the watershed as rainfall increases coupled to foliage losses from terrestrial vegetation during the autumn months (e.g., Kaplan and Bott 1982, McDowell and Likens 1988). Chapter 3 examines variability in [DOC] in more detail. The time-series of 2006 - 2007 measured a significant drop in both [DOC] and the molar C:N of the DOM in all four lake segments in April. The concentration of bulk DOM did not decline in the same manner (data not shown)

suggesting this represents a period of increased supply of nitrogenous compounds, possibly linked to the end of bloom events where phytoplankton / zooplankton death and decay can add significant quantities of nitrogen rich DOM to the system. Alternatively / additionally, nutrient stress is causing the remaining phytoplankton to exude a higher proportion of their photosynthate (e.g., Lancelot 1983, Baines and Pace 1991) and thus raising the quality of the available DOM.

[DIC] had the most seasonal variability in the north basin (Fig. 50). Concentrations were lowest during the winter months from November through March. This supports seasonal findings reported in chapter 3, but once again is contradictory to results found in other lake systems (e.g., Hanson *et al* 2006) where highest concentrations were observed in the winter. Hanson *et al* (2006) implied low photosynthetic utilisation of DIC in this time allowed accumulation and there is no reason to assume this would not be the case in the winter months in Loch Lomond. Therefore, another driving force e.g., increased inflow of DIC or low winter respiratory rates must account for the low [DIC]. Bacterial production during the winter has been shown to be low in Loch Lomond (Chapter 5), so little DIC will be being added via this pathway, which implies low concentrations in the inflowing waters may be responsible for low concentrations in the north basin.

The south basin has a less variable seasonal pattern in [DIC]. The epilimnion has two distinct peaks in August and February. The August peak is likely a response to measured increases in bacterial production and respiration during this period (see chapter 5) linked to the end of the summer productive period. Primary productivity in February is negligible so cannot explain the observed peak in [DIC], instead it may possibly be due to increased run-off bringing in more DIC, or re-suspension of lake-bed sediments by rough weather allowing a temporary increase in heterotrophic activity. Although measured BP was not high in February, more elevated levels were observed between November and January so the DIC produced may still be present in the system in February.

As with DIC, the concentration variation of POC measured to be greatest in the north basin. The north basin hypolimnion generally had the lowest concentrations year round, likely reflecting a greater proportion of the POC being processed as it sinks through the deeper water column, along with little primary production of biomass in the deep waters. Little POC produced in the epilimnion during stratification will reach below the thermocline also for two reasons; one that little exchange takes place between layers during stratification and two; any labile organic material that is produced is rapidly broken down in the epilimnion as has an insignificant effect on hypolimnetic concentrations (Wetzel 2001). However, both the

epilimnion and hypolimnion show peaks in POC during January / February. Primary and secondary production were both low during this period so increased contribution from this source can be ruled out. Likely winter peaks in POC are associated with lake turnover and subsequent re-suspension of bottom sediments, as well as greater quantities of allochthonous particulate material imported from the watershed. Chapter 4 examined seasonality in sinking particulates and recorded similar patterns. The epilimnion in the north basin had another rise in [POC] in April, likely corresponding to the spring bloom and increased phytoplankton / zooplankton biomass.

No winter peak in [POC] was observed in the south basin, although peaks corresponding to the spring and summer productive periods were. The reason no winter peak was observed is not clear as each basin is exposed to similar climatic conditions and if anything the south basin should receive greater organic matter subsidies from the catchment. Further investigation would be required to elucidate possible explanations. The epilimnion and hypolimnion in the south show similar seasonal trends and magnitude, illustrating the shallow depths and often comprehensive water column mixing in this basin. Highest concentrations of POC were measured in August 2006, likely higher than the April spring bloom peak due to an increased supply of allochthonous POC at the start of autumn.

$\delta^{13}C_{DOC}$ showed relatively constant signatures between September '06 and March '07, ranging between ~ -26.5 ‰ and ~ -28 ‰, typical of DOC of a terrestrial origin. During this period there is an approximately 1‰ difference between south basin water (~ -27 ‰) and north basin water (~ -28 ‰), likely a reflection of varying catchment DOC sources. The range observed in $\delta^{13}C_{DOC}$ is consistent with other aquatic systems with strong terrestrial connection (e.g., Schiff *et al* 1997, Palmer *et al* 2001).

All lake segments showed an enrichment of approximately 1 ‰ between March and April, followed by a significant depletion in June. More depleted $\delta^{13}C_{DOC}$ is possibly due to the increased algal biomass and production in this period. A depletion at the same time in the $\delta^{13}C_{POC}$ signature along with low molar C:N of both DOC and POC suggest autochthonous supply of organic material (e.g., Ziegler and Brisco 2004). Between June and August $\delta^{13}C_{DOC}$ became significantly more positive in each basin, from a minimum of -31‰ to -25‰. The more enriched values are consistent with DOC that has been derived from diagenetically altered organic matter, specifically microbially re-worked POC. Low molar C:N (POC) in the autumnal period (approximately August – November), when phytoplankton biomass is usually low is

likely caused by a greater microbial presence, supporting the hypothesis that enriched $\delta^{13}C_{DOC}$ is due to bacterial breakdown of POM.

Most depleted signatures of DOC were observed to correspond with the lowest DOC concentrations. There is a possibility the depleted values are a result of the autochthonous DOC signature being more visible when not flooded by a more enriched allochthonous one. The summer dry period is likely to reduce the supply of allochthonous DOC to the lake, and as such the proportion it makes of the total DOC signature reduces. Therefore the depleted $\delta^{13}C_{DOC}$ in the summer months could be due to increased primary productivity (supported by $\delta^{13}C_{DOC}$, $\delta^{13}C_{POC}$ and molar C:N), or concentration of the autochthonous carbon pool (supported by [DOC], and earlier models of autochthonous / allochthonous DOC balance (see chapter 3)). These two hypotheses are not mutually exclusive.

$\delta^{13}C_{DIC}$ was observed to rise steadily in the south basin peaking in April during the phytoplanktonic bloom season. As discussed in chapter 2, enriched $\delta^{13}C_{DIC}$ is a likely result of algal processing of the inorganic carbon pool (e.g., Myrbo and Shapley 2006). In the south basin from April to July, and again in September epilimnion and hypolimnion signatures diverge. During stratification, as primary production is more prevalent in the surface waters, hypolimnion $\delta^{13}C_{DIC}$ will tend to be more depleted than the surface waters (e.g., Quay *et al*. 1986; Keough *et al*. 199). Signatures are approximately equal in July and August which is demonstrative of the unpredictable stratification patterns in the south basin, likely corresponding to a period of rough weather and water column mixing. The north basin has a more stable period of stratification which is demonstrated by the significant divergence in epilimnetic and hypolimnetic $\delta^{13}C_{DIC}$ from August to November, and again between April and July. The pattern is as already described with depleted values in the hypolimnion, where primary production is limited and bacterial respiration dominates metabolic processing (Chapter 5). The north basin DIC signature peaks in April in the epilimnion and hypolimnion. Similarity of the $\delta^{13}C_{DIC}$ signatures suggest the water column is still well mixed, and the enriched signature suggests significant primary production at this time.

7.4.2) *Elucidate any possible relationships between parameters measured in chapters two and three and use concurrent productivity measures to support or contradict the conclusions made in those chapters.*

Both phytoplanktonic and bacterial production was observed to be closely linked to water temperature (Fig. 52 and 55). The dependence of photosynthesis (e.g., Hew

et al 1969) and bacterial production / respiration (e.g., Rivkin and Legendre 2001) on temperature has been well documented and is as expected. The direct effect of temperature is an increase in the specific activity of enzymatic processes involved in metabolism. However, increasing temperature is concurrent with increasing irradiance leading to higher photosynthetic rates. Whereas bacterial production is not directly effected by irradiance, the bloom in phytoplankton and associated labile organic matter allow bacterial production rates to increase.

Temperature explained the most variation seen in both phytoplanktonic and bacterial production with an exponential increase in production rate (R^2 = 0.680 and 0.538 respectively). For this reason temperature was used to estimate detailed time series of production using water temperature data collected daily in the south basin epilimnetic waters (Fig. 58). Patterns in production follow the predicted pattern for primary production, peaking during the spring and summer before falling during the autumn and winter. Occasional drops in PP during the periods of a general rise are likely caused by fluxes of colder rainwater entering the lake. Whether this temperature change would actually affect the PP level is unclear, and would need to be investigated further were this relationship to be used to predict PP. From bacterial production estimates calculated previously (Chapter 5, Fig. 41b) using temperature seems to give underestimates of production. This is likely as temperature is one of several factors that can limit bacterial production and respiration rates, such as nutrient availability, concentration and chemical composition of DOC (e.g., Findlay and Watling 1997, White and Findlay 1988). No data on nutrient availability was available during this work so cannot be commented on, and the influence of DOC is discussed shortly. However, bacterial production, although clearly co-dependent on temperature, cannot be reliably predicted via simple temperature relationships. More work would be required to refine and test these relationships and their predictive power.

In chapter 2 and this chapter, $\delta^{13}C_{DIC}$ variability has been used to suggest changes in metabolic balance in Loch Lomond. It has been shown conclusively that the photosynthetic pathways are selective for isotopically light DIC (see chapter 3 and references therein), resulting in isotopically heavier DIC in the water column. Thus, in general higher primary productivity leads to enriched $\delta^{13}C_{DIC}$, and vice versa. Respiration acts in the opposite direction. A general pattern in lakes is thus more enriched $\delta^{13}C_{DIC}$ in the spring / summer than the winter, and more depleted values in the hypolimnion than the epilimnion. However, as well as biological controls $\delta^{13}C_{DIC}$ is driven by the acid base system. Base line $\delta^{13}C_{DIC}$ will be dictated by the physical and

chemical properties of the catchment, so if they change the resulting lake signature can also alter.

Primary production was observed to be significantly correlated with $\delta^{13}C_{DIC}$, implying that the variability recorded is at least in part, biologically controlled. A linear relationship explained 46% of the variability seen in PP. PP also increased with increasing [DIC], contrary to what may be predicted. This may be explained by higher bacterial production values recorded at times of high PP raising the [DIC] faster than it is utilised by phytoplankton on photosynthesis. Indeed, chapter 5 reported bacterial production greatly exceeding PP, making it feasible that overall [DIC] could increase in the productive periods.

Although from the evidence presented $\delta^{13}C_{DIC}$ is likely in part biologically mediated, the relationship is not as strong as with temperature. However, evidence from the incubation season has supported the conclusions of chapter 2 that variability over both time and space in $\delta^{13}C_{DIC}$ is dictated in part by metabolic variability. A problem arises in that other factors can influence baseline $\delta^{13}C_{DIC}$ (e.g., productivity range in the catchment, pH and the resulting carbonate equilibrium, ingression or egression of CO_2). Thus using previously measured signatures to predict productivity could be inaccurate. By examining inter-annual variability in $\delta^{13}C_{DIC}$ their use as a predictor can be assessed (see section 7.3.5). If inter-annual variability is significant $\delta^{13}C_{DIC}$ would be of little use in predicting PP.

Bacterial production is dependent on temperature for reasons already discussed. The relationship however is not as strong as PP suggesting other controlling factors. It can be hypothesised that DOC may be related to BP as it will constitute the main source of organic carbon for bacterial utilisation, and indeed other studies have shown the dependence (e.g., Warren *et al* 1964, Bott *et al* 1984, Kaplan and Bott 1983). However, in this work no relationship between [DOC] and BP was observed. Possible reasons are simply a lack of data not allowing any discernable patterns to be deduced, or that the pool of allochthonous DOC is so large compared to potential bacterial demand (set by nutrient availability, temperature, etc) that variability caused by BP is not detectable. More research would be needed to elucidate any possible relationships. This could include collection of more data points to be sure of any relationships or lack there of, or artificial addition of labile DOC at a range of concentrations to elucidate any effect on BP. Also, measurements of both nitrogen and phosphorus levels would be required, as any possible dependence on DOC by bacteria could be masked if under nitrogen or phosphorus limitation.

DOM / C concentration is generally dictated by two processes, biological addition and removal, and the balance between hydrological import and export (e.g., Kaplan and Bott 1982, McDowell and Likens 1988). Lack of any describable relationship between [DOC] and bacterial production suggests BP is not an important factor in controlling dissolved organic material in Loch Lomond, or vice versa. However, more information would be needed to rule out any contribution (see previous paragraph). It is likely that biological activity does have a role in regulating organic matter dynamics in Loch Lomond, but that its contribution is relatively minor compared to variability in inflow / outflow balance and hydrodynamic processes in the lake. This could potentially be achieved by culturing populations of lake bacteria and incubating in controlled environments with set concentrations of inorganic and organic nutrients and assessing each factors influence individually.

As both primary production and bacterial production seem dependent to some degree on temperature, the effects of predicted climate change can be explored at this juncture. Recent projections from the Hadley Centre computer models (http://www.metoffice.gov.uk/research/hadleycentre/) forecasts a doubling of atmospheric CO_2 concentrations and a 2 - 3^{o}C rise in sea level surface air temperatures in the next century (to approximately the year 2100). Both primary production and bacterial production were observed to have an exponentially increasing relationship with temperature, although PP shows a more rapid increase. For example, taking average epilimnion temperatures in July, PP was estimated to increase from 22 to 58 µg / L / day in the south basin assuming a 2^{o}C rise in temperature. BP showed a far more modest increase from 7 to 11 µg / L / day. 2-3^{O}C is possibly a conservative estimate and as such this effect could be greater than estimated here.

From the observed relationships I can thus hypothesise algal production will respond more significantly to an increase in average temperatures. In turn the autochthonous supply of organic carbon will be greater, thus fuelling a greater proportion of the bacterial population. The predicted response by bacteria is less, thus the relative proportion of the total production that is fuelled by allochthonous sources may be reduced if most of their carbon demand is met through autochthonous subsidies. Potentially lakes of this type may become significantly less heterotrophic, and maybe even autotrophic in some cases? This in-turn may lower the level of dissolved CO_2 in the lake water and lower egression rates to the atmosphere. However, an increase in precipitation rates is expected by approximately 1 mm / day over the same period (Hadley models), which could

increase the amount of DOC transported from the watershed. Although this research failed to detect a bacterial production dependence on [DOC], if one does exist an increased allochthonous supply may have an effect on metabolic balance.

Such speculation is also based on the assumption that both PP and BP are not nutrient limited in Loch Lomond. This is known not to be the case, particularly in the north basin. Thus any increase possible due to rising air temperatures may be capped at a maximum level dictated by nutrient availability. Research assessing both PP and BP to controlled temperature rises could help elucidate theoretical maximum achievable production rates based on a nutrient limitation cap.

7.4.3) *To assess inter-annual variability by comparing annual time-series data to see how representative survey work in chapters two and three is on more detailed timescales.*

In chapters 2 and 3 fluxes of DIC and DOC were estimated using four sampling times between November and the following September. In estimating fluxes between points concentrations were assumed to change an even amount each day. Figures 59 - 61 show that annual fluxes are more complex and variable than these simple assumptions.

[DIC] in the north basin was of a similar magnitude both years, but infrequent temporal sampling was shown to miss significant peaks and troughs associated with productivity blooms and declines. The south basin too illustrated the limitations of the first seasons sampling frequency, particularly in summer when [DIC] was measured to be considerably higher in the 2004 / 2005 season. This variability between years suggests predicting concentration changes and magnitudes from year to year is impossible and likely depends on various factors from prevailing climatic conditions, to the onset of bloom conditions. These conclusions hold with $\delta^{13}C_{DIC}$ (Fig. 60) and DOM (Fig. 61) also. Infrequent sampling in '04 / '05 may possibly have missed pronounced peaks, particularly in the north basin during spring / summer productive periods. In the south basin $\delta^{13}C_{DIC}$ seasonal variability was relatively consistent between years, but was generally 2 ‰ more enriched in '06 / '07 than '04 / '05, again illustrating the problems with using one season's values to predict on other timescales.

Fluxes and standing stocks estimated in chapters 2 and 3 are likely only vague estimates of actual numbers. Biological controls on [DIC], [DOC] and $\delta^{13}C_{DIC}$ can be rapid as blooms can manifest rapidly and be short lived, and potentially be missed altogether. For this reason, sampling on as frequent a timescale as possible is

recommended when elucidating seasonal changes in both chemical parameters and productivity estimates.

Chapter 8

Concluding remarks

Loch Lomond is a morphometrically, hydrologically and biologically complex system. In this research such complexities have been reflected in various different parameters. Variability in [DIC], $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$ have been elucidated on a temporal and spatial scale, likely related to biological variability on seasonal scales coupled to changing inflow characteristics.

[DIC] did not reach maximum values in the winter like in various other studies (e.g., Hanson *et al* 2006) suggesting that it is not only rates of PP that dictate concentration. More likely is an increasing contribution of respiratory CO_2 during the summer / autumn coupled to high concentrations imported from inflowing waters. Evidence throughout the work has suggested heterotrophic processes to be of greater significance than autotrophic so [DIC] is more likely to reflect changes in bacterial activity rather than algal. The spatial variability observed, notably higher [DIC] in the south basin reflects higher input from the surrounding watershed. [DIC] also reveals the first piece of evidence that Loch Lomond is a heterotrophic system as surface waters are generally saturated beyond atmospheric equilibrium in CO_2.

The isotopic signatures of both DIC and DO varied significantly over time and space. By combining the two measurements I have concluded that metabolism is the driving factor behind these isotope distributions in Loch Lomond. Photosynthetic discrimination of ^{12}C leads to an enrichment of the remaining inorganic carbon pool, explaining enriched values observed in spring / summer consistent with previous work (Herczeg 1987, Hollander and McKenzie 1991, Wang and Veizer 2000). $\delta^{18}O_{DO}$ reaches maximum enrichment after the $\delta^{13}C_{DIC}$ peak and corresponds to late summer early autumn when bacterial production rates are highest. Changes observed with depth support further the idea of metabolic control on stable isotopes in Loch Lomond. Depletion in $\delta^{13}C_{DIC}$ coupled to enrichment in $\delta^{18}O_{DO}$ with increasing depth suggest a shift in dominance between phytoplanktonic and bacterial production (e.g., Myrbo and Shapley 2006), later proven to be the case with direct production measurement.

The assumption of $\delta^{13}C_{DIC}$ variability being related to changes in the production balance, particularly PP was tested during incubation experiments and a clear relationship between the two was observed. Although no corresponding data was available to examine the $\delta^{18}O_{DO}$ conclusions, accumulating evidence suggests these

two isotopic signatures are driven by the varying metabolic processes in Loch Lomond.

GIS spatial analysis showed significant spatial variability in both $\delta^{13}C_{DIC}$ and $\delta^{18}O_{DO}$. The complex hydrological patterns, varying inflow characteristics, light availability, etc likely all contribute to this variation. The large range in $\delta^{13}C_{DIC}$ observed in Loch Lomond (~ 11‰) compared to work by others (e.g., Quay *et al* 1986, ~3.6‰) suggests not all lakes will exhibit the same degree of spatial variability. However, the ubiquitous nature of this variability in all sample periods implies this should be considered, particularly in large lake systems. To my knowledge this is the first comprehensive spatial review of isotopic distribution in a lake of this size and complexity.

Chapter 3 revealed that the dynamics of TDS too are complex both spatially and temporally in Loch Lomond. [TDS] changed significantly with season in the north and middle basin but remained relatively constant in the south. Highest concentrations were measured in late summer / early autumn and likely reflect both increased loads of allochthonous TDS in inflowing water, and a greater quantity of inflowing water caused by increased precipitation levels (e.g., Meybeck 1988, Spitzy and Leenheer 1991). Increased supply of autochthonous TDS, corresponding to summer productive periods likely also effects the over all [TDSM] and contributes to elevated levels.

Spatial variability can be complex, but a simple pattern of [TDS] being least in the north, and increasing from the middle to the south basin was recorded. This supports conclusions in other studies linking watershed slope, lake depth and lake area to [DOM] (Rasmussen *et al* 1989, Houle *et al* 1994). Significantly greater concentrations of TDS in south basin inflows support the idea that at least in part [TDS] is controlled by the catchment characteristics and flow regimes. This, coupled to greater potential processing time of TDS in the north basin (due to greater residence times) potentially explains the general latitudinal trends seen in [TDS].

Prevalence of seasonality in the north and middle basin [TDS] is likely caused by a combination of factors. Being less productive than the south basin, the north basin my respond more dramatically to brief bloom periods in the spring / summer. It may also be that the allochthonous [TDS] in the south basin is so much greater than the autochthonous supply that seasonal variability caused by production variation is not resolvable, which may not be the case in the north / middle basins where overall [TDS] is lower.

As with $\delta^{13}C_{DIC}$, the isotope ratios of carbon and nitrogen in DOM can be used to elucidate possible metabolic functioning in Loch Lomond. $\delta^{13}C_{DOC}$ has limited

functionality in delineating metabolic functioning as signatures from autochthonous and allochthonous sources vary over the same ranges, and as such observed variation may come from several different and indistinguishable sources (e.g., Rosenfield & Roff, 1992, Zah et al., 2001, Rounick et al., 1982; Winterbourn et al., 1986; Boon & Bunn, 1994). However, the observed enrichment from June to September could be due to the corresponding enrichment in $\delta^{13}C_{DIC}$. Phytoplanktonic production of biomass utilises enriched inorganic carbon and thus produces more enriched DOM and POM. Bacterial respiration of DOM during the same period would lead to enrichment also. Positive conclusions on the cause of $\delta^{13}C_{DOC}$ variability at this point are not clear.

Measured variability in the $\delta^{15}N_{TDN}$ is believed to reflect a combination of varying rates of inorganic nitrogen utilisation by phytoplankton and nitrogen fixation by cyanobacteria. More depleted $\delta^{15}N_{TDN}$ in the summer months is believed to reflect preferential incorporation of $^{14}N_{DIN}$ by phytoplankton during primary production into biomass, and thus subsequently produced TDN and has been observed in other systems. In Loch Lomond $\delta^{15}N_{TDN}$ became more enriched during the summer months however, likely a result of the concentration of DIN declining during the spring and summer (SEPA data) leading to forced incorporation of more ^{15}N-enriched DIN remaining by phytoplankton and thus production of enriched TDN. No specific evidence on the significance of nitrogen fixation in the lake was gathered, but nitrogen fixing species of cyanobacteria are present and as such could be affecting the isotope signature, particularly in the summer months. Preferential export of $^{14}N_{TDN}$ from the epilimnion via zooplankton feeding, excretion, etc may also add to the enrichment effect seen in the spring / summer months. This is particularly significant in deep oligotrophic systems like the north basin, where algae rely on significant amounts of recycled nitrogen and little sinking material is re-distributed to the epilimnion (Montoya *et al* 1992).

Molar C:N of DOM was used to support conclusions drawn on metabolic process variations, deduced by the above mentioned parameters. The principle used was that high C:N is indicative of nitrogen poor, refractory organic matter of little nutritional value to microbial utilisation, and low molar C:N is indicative of nitrogen rich, labile organic matter produced during productive periods. Further, low C:N is used to indicate an autochthonous source of organic matter and vice versa.

Molar C:N of DOM was lowest in the spring bloom periods when nutrients are readily available allowing phytoplankton cells to synthesis proportionally high quantities of protein (e.g., Hama and Honjo 1987, Hama 1988). Molar C:N declines in the summer, likely linked to exhaustion of nutrients post bloom (Bertilisson and Jones

2003) and increased UV exposure (Goes *et al* 1995, 1996). Therefore, variability in molar C:N of DOM has been used to imply varying productivities at different times and locations and thus the balance between autochthonous and allochthonous DOM. This idea was taken further in a mass balance model.

Mixing models detailed in chapter 3 suggested that autochthonous DOM was the dominant source of organic matter in Loch Lomond. However, inorganic nitrogen was not removed from DOM samples during any sampling trip. Therefore molar C:N values, upon which the model is based, will be pulled down by this presence and as such pull down the predicted proportional contribution of allochthonous DOM to total dissolved organic matter. Indeed, future models based on concurrent productivity measurements suggest this model is in-effective at predicting the source of organic material.

Chapters 2 and 3 each included detailed spatial sampling of various different measurements and showed clearly the heterogeneity of the lake. Using GIS interpolation techniques allowed contour mapping to be conducted and an idea of whole lake patterns to be elucidated. Small scale changes were observed at certain times and locations, suggesting that there will inevitably be inaccuracies in the interpolated values produced. However, the scale and ubiquity of that heterogeneity has shown that this method of interpolation is certainly preferable to single point sampling, often used in other limnetic studies. This work has concluded that spatial variability in [DIC], [TDS], $\delta^{13}C_{DIC}$, $\delta^{18}O_{DO}$, $\delta^{13}C_{DO}C$ and $\delta^{15}N_{TDN}$ is regularly significant and such variability should be considered in future studies of Loch Lomond. Whether similar conclusions will be found in other lake systems is unknown, but this work strongly suggests consideration of the possibility.

Sestonic accumulation seasonal patterns followed trends previously described in other water bodies (e.g., Pennington 1974, Habib *et al* 1997, Wetzel 2001). Peak deposition rates occurred in winter, which corresponds to turnover of the thermocline and subsequent mobilisation of bottom sediments into the water column. Increased quantity of allochthonous material linked to autumnal leaf loss, etc is also another reason for the observed winter maxima. Accumulation rates vary with basin and reflect varying quantities of organic material transported in each catchment and the depth of and thus ease of which each basins bottom sediments are disturbed. These factors lead the north basin having the lowest accumulation rates, and the south the highest.

$\delta^{13}C_{seston}$ and $\delta^{15}N_{seston}$ reflect metabolic balance in the lake and the previously mentioned cycle of summer stratification and winter turnover. Enrichment in $\delta^{13}C_{seston}$ in the summer months likely reflects enrichment in the DIC pool, and subsequent proliferation into the particulate pool via photosynthetic incorporation and biomass production. Depletion in $\delta^{13}C_{seston}$ in the winter indicates reduced photosynthetic activity, coupled to likely increased supply of $^{12}C_{DIC}$ from the watershed and bottom sediments. Possibly significant rates of heterotrophic breakdown at this time may also reduce $\delta^{13}C_{seston}$ as ^{12}C is preferentially processed giving ^{13}C depleted biomass. This may be significant in autumn / winter as heterotrophic processes become relatively more important. $\delta^{13}C_{seston}$ in Loch Lomond was consistent with that recorded in other studies (e.g., Owen *et al* 1999).

$\delta^{15}N_{seston}$ was most depleted in the summer months and most enriched in the winter. Selective incorporation of $^{14}N_{DIN}$ by phytoplankton likely explains the depleted signature of seston in the spring / summer. Dissolved inorganic nitrogen is utilised heavily during the spring and summer, and as such any further productivity has to incorporate the more ^{15}N-enriched DIN remaining, which along with higher contributions of heavily degraded organic matter from the watershed and the bottom sediments result in the observed enrichment in the autumn / winter.

Like TDS before, the molar C:N of seston was used in mixing models to estimate the source composition of falling particulate matter. The contribution of allochthonous material to bulk seston was estimated to generally be low, from 0 to ~40%. However, as well as the problems associated with presence of inorganic nitrogen, remains of zooplankton known to be present in the spring / summer months further confound the results, likely giving over-estimates of the autochthonous contribution.

Chapter 5 presented direct measurements of both phytoplanktonic and bacterial production, with the aim of elucidating varying balances in the auto – heterotrophic ratio. This information was used in conjunction with chapter 6 to further decipher this balance and the contribution of terrestrially derived organic matter utilisation to pelagic production.

No measurable variation existed in PP between the north and south basins, and each followed predictable seasonal cycles based on illumination, day length and nutrient annual cycles. Measurements of BP revealed similar patterns to PP, suggesting that the two processes may depend on one another to some extent. However, in the opposite way there are periods in both basins where BP exceeds PP, which implies allochthonous carbon utilisation is of importance at certain times of the year. BP in the south basin epilimnion in September was the most obvious example,

where BP exceeded PP by double. These initial finding provided the first evidence of heterotrophy in Loch Lomond, and to further explore this possibility more detailed assessment of water column, basin and whole lake production values was examined.

Integrated values for epilimnetic water column production were calculated to examine the contradictory conclusions by others that oligotrophic water bodies are a both a source (e.g., Cole *et al* 1994, del Giorgio *et al* 1997) and a sink (e.g., Carignan *et al* 2000) of carbon. Using integrated estimates of PP and BP in the epilimnion of each basin it was concluded that heterotrophic processes dominate over autotrophic. Although the highest rates of PP exceed the highest recorded BP, in general and for much of the year BP exceeds PP and the net balance is in favour of the heterotrophic pathways. As PP is expected to be effectively non-existent in the hypolimnion of a water body, the epilimnion is where, if anywhere, net autotrophy will result. The fact that this does not occur in Loch Lomond is strong evidence for a heterotrophic system and this lake as a potential source of carbon to other ecosystems, adding to a growing number of similar conclusions in other temperate and boreal systems (e.g., del Giorgio and Peters 1994, Cole *et al* 1994, Jansson *et al* 1999, Kritzberg *et al* 2004). In general for epilimnetic water the PP: BP ratio ranged from 0.6 - 0.8 in the north basin, and 0.4 - 0.6 in the south. It is believed the counter intuitive finding that PP exceeds BP to a greater degree in the oligotrophic north basin, compared to the mesotrophic south basin, is due to significantly greater quantities of allochthonous organic carbon available in the south.

Scaling these epilimnetic estimates up to whole basin values, and combining them with hypolimnetic production estimates show conclusively that in this system bacterial production significantly exceeds phytoplanktonic. With this in mind chapter 6 examined the quantities of carbon utilised from outside the lake.

From the work presented in chapters 2 to 5 it was concluded that bacterial processing of allochthonous organic material is likely a significant contributor to pelagic production in Loch Lomond. Chapter 6 elucidated estimates of the relative contributions of BPallo to pelagic production for different seasons and lake segments, leading to estimates of the annual utilisation of allochthonous carbon via bacterial processing.

Like both phytoplanktonic and bacterial production, the seasonal contribution of allochthonous carbon to pelagic production covered a wide range. Allochthonous sources of carbon were significantly more important to pelagic production during the winter months when autochthonous supply (from phytoplankton / zooplankton / vertebrates, etc) is low. In the south basin the contribution of BPallo to pelagic

production peaked in November, where it accounted for ~ 80% of the total. Between April and August the contribution was minimal and BP_{allo} was estimated to have no influence on total pelagic production in August, April and July. During this period primary productivity is relatively high and the supply of labile autochthonous carbon is sufficient to support the majority of bacterial production. In the south basin hypolimnion seasonal patterns were similar with BP_{allo} in winter contributing a high proportion to total pelagic production. In general however, the contribution of autochthonous carbon to the hypolimnion is less as most is likely utilised at the site of production (usually the epilimnion) and not transported to deeper water.

North basin epilimnetic water was estimated to have the same seasonal cycle as the south basin. However, autochthonous supply was never estimated to quite meet 100% of the bacterial production demand. August, April and July had the lowest predicted contribution of BP_{allo} to pelagic production at 6%, 2% and 4% respectively. Reasons for this are discussed previously.

The implication of the significant contribution allochthonous carbon makes to energy mobilisation in Loch Lomond is not clear. While it has been estimated a substantial quantity of carbon is processed from the water shed, and potentially available to higher trophic levels, due to energy loss at each trophic step its significance is not known. In order to assess this more fully experimental procedures would need to be undertaken that quantify the transfer of energy via the classical and microbial food web. Mesocosm experiments, inducing either a phytoplankton dominated food chain (using addition of N and P) or bacterial dominated food chain (addition of C, N and P), tracking an isotope tracer through each trophic level could elucidate the possible differences and efficiencies of each pathway. Thus while it has been suggested in this work, that hundreds of tonnes of carbon in addition to autochthonous supply are available to lake food webs per annum, the impact this has on the biota requires more investigation.

Estimated bacterial utilisation of terrestrial carbon for the whole lake is between 2.0 and 1.8 tonnes C / km^2 catchment / year. This varied between the north and south basins reflecting the varying catchment sizes. The south basin catchments are significantly larger (~475 km^2) than the north (~271 km^2). The respective quantities of terrestrial carbon processed are between 1.55 and 1.65 tonnes / km^2 / year in the south and 2.39 to 2.57 tonnes / km^2 / year in the north. As previously stated in this and other work (e.g., Apps *et al* 1993), the contribution of inland waters to terrestrial carbon balances has been under-explored, though is now being further clarified (e.g.,

Cole *et al* 2007). The production values derived in this work can be used to estimate the potential significance of lake bacterial carbon processing to the terrestrial budget.

Estimates of terrestrial production can be highly variable, and can regularly exceed 100 tonnes C / km^2 / year in rapidly growing temperate forests (e.g., Hollinger *et al* 2004, Cole *et al* 2007, Sasai *et al* 2007). In these systems the 1.55 to 2.57 tonnes terrestrial C / km^2 / year processed in this inland lake would be relatively insignificant, contributing at most 1-2% of the total biome carbon utilisation. However, when considered over broader spatial scales and over longer time periods, terrestrial production rates are generally far lower - indeed forestry makes up a small fraction of the Loch Lomond catchment land use. Boreal peatlands for example have been observed to be consistently one of the most significant terrestrial carbon production zones (Post *et al* 1982, Smith *et al* 2004) varying between 2 – 7 tonnes C / km^2 / year during the Holocene. If we thus use our extreme end member values for lake and terrestrial peatland production, lake bacterial carbon processing could be anywhere from 22% of terrestrial production, to matching or exceeding it in magnitude. This is in agreement with other work carried out (e.g., Dean and Gorham 1998, Stallard 1998) which estimated lake carbon burial rates to be between 4.5 and 14 g C / m^2 / year, compared to terrestrial rates of approximately 1.2 g C / m / year. Though this represents burial rate and not specifically allochthonous carbon utilisation by bacteria, if we assume the majority of lakes to be dependent on a significant fraction of allochthonous carbon, evidence here supports the conclusion that a significant percentage of total terrestrial carbon production is carried out in limnetic systems.

These estimates are crude as terrestrial production values are based on variable sources, over various spatial scales and locations; direct measurements of production in the Loch Lomond catchment would be required for more accurate estimation. However, as little of the Lomond catchment is rapidly-growing forest we can assume production rates closer to the lower end specified and as such lake processing of terrestrial carbon is likely a significant extra component of terrestrial carbon production budgets. Thus more recent attention to its significance is justified and future models would be unwise to ignore this contribution.

Using the derived productivity estimates we can expand the concept to global values. Though these calculations are filled with assumptions and uncertainties they can provide some insight. The total estimated volume of lakes on the planet is approximately 91,000 km^3 (Gleick 1996). Using this value and multiplying it by the upper and lower allochthonous carbon utilisation values between 0.041 Pg C $year^{-1}$ and 0.044 Pg C $year^{-1}$ allochthonous carbon utilised in lakes worldwide are estimated

(NB: 1 Pg = 1 billion metric tonnes). Estimates of annual CO_2 evasion from lakes combined with sediment storage are in the range of 0.1 to 0.22 Pg C $year^{-1}$ (Cole *et al* 2007), so our estimate seems feasible and suggests bacterial processing of allochthonous carbon contributes only a small fraction of total carbon flux in lakes. This estimate fails to account for numerous factors, for example, our numbers are valid for a system with a particular trophic state, particular depths, particular DOC concentrations, etc. The assumption that all lakes on the planet are sufficiently similar to process similar magnitudes of allochthonous carbon is likely too broad, and likely significant geographical variation exists.

Given our projected changing climate, the linking of the terrestrial carbon reservoirs with the atmospheric carbon cycle through limnetic allochthonous processing is of scientific importance and thus offers exciting challenges in refining such broad assumptions.

This study has formally demonstrated the complexity of carbon cycling dynamics in Loch Lomond, Scotland. The techniques utilised, though relatively complex are readily available to the scientific community, for realisation of the benefits ^{13}C tracer approaches offer. Such approach have the potential to allow wide scale monitoring of numerous lakes across the globe, quantifying carbon flow through algal and bacterial food chains, the extent to which allochthonous carbon is processed and potentially egressed as CO_2, and the potential availability of this carbon to subsequent trophic levels. Further development of the procedures could yield yet more information, e.g., tracking of the allochthonous carbon through the food web, efficiency of energy transfer via algal versus bacterial food chains, sedimentation versus utilisation of allochthonous carbon and atmospheric egression.

By utilising these techniques in various lakes of different sizes, depths, trophic levels and latitudes the techniques utilised inn this work could add further to a growing knowledge base of the role of lacustrine systems in the global carbon cycle. Additionally, this thesis provides benchmark level, for comparison with future studies to elucidate changes in algal / bacterial production, allochthonous / autochthonous carbon utilisation and metabolic balance as a consequence of predicted global climate change.

November, south basin	date collected	Time collected	Latitude	Longitude	Depth (m)	[DIC] (mM)	$\delta^{13}C_{DIC}$	[DOC] (mg/L)	$\delta^{13}C_{DOC}$	C:N DOM	$\delta^{18}O_{DO}$	$\delta^{18}O_{H2O}$	Temp	pH
L1.B	03/11/2004	09:30	-4.5939	56.0717	20	0.18	-9.50	3.43		13.77		-8.08	10	
L1.M	03/11/2004	09:55	-4.5939	56.0717	10			3.68		11.21		-8.18	10	
L1.T	03/11/2004	09:42	-4.5939	56.0717	0	0.22	-9.73	3.94		11.54		-8.18	10	
L2.B	03/11/2004	11:39	-4.6075	56.0289	17	0.21	-10.24	3.85		11.95		-7.46	10	
L2.M	03/11/2004	11:52	-4.6075	56.0289	7	0.24	-10.03	3.36		5.98		-7.57	10	
L2.T	03/11/2004	11:39	-4.6075	56.0289	0	0.24	-13.58	4.56		8.01			10	
L3.B	03/11/2004	12:06	-4.5528	56.0647	14	0.23	-10.59	3.65		8.29		-7.11	10	
L3.M	03/11/2004		-4.5528	56.0647	7	0.25	-10.92	3.45		10.96		-8.11	10	
L3.T	03/11/2004	12:06	-4.5528	56.0647	0	0.22	-10.48	3.93		10.12		-7.66	10	
LP1.B	03/11/2004	10:07	-4.5925	56.0728	20	0.21	-10.92	2.82		11.29		-8.03	10	
LP1.M	03/11/2004	10:20	-4.5925	56.0728	10	0.23	-10.74	2.98		11.61		-7.47	10	
LP1.T	03/11/2004	10:07	-4.5925	56.0728	0	0.22	-10.69	3.34		14.08		-7.34	10	
LP2.B	03/11/2004	10:28	-4.5911	56.0731	20	0.24	-10.40	2.93		9.11		-8.22	10	
LP2.M	03/11/2004	10:39	-4.5911	56.0731	10	0.22	-10.91	3.74		11.38		-7.88	10	
LP2.T	03/11/2004	10:28	-4.5911	56.0731	0	0.20	-10.88	3.43		9.97		-8.17	10	
LP3.B	03/11/2004	10:47	-4.5842	56.0733	20	0.25	-11.14	4.30		14.93		-7.78	10	
LP3.M	03/11/2004	11:55	-4.5842	56.0733	10			2.51		13.76		-7.54	10	
LP3.T	03/11/2004	10:47	-4.5842	56.0733	0	0.24	-11.00	3.62		8.90		-7.66	10	
LP4.B	03/11/2004	11:12	-4.6131	56.0589	15	0.23	-10.85	3.31		2.41		-7.34	10	
LP4.M	03/11/2004	11:31	-4.6131	56.0589	7	0.21	-10.43	3.29		9.18		-7.41	10	
LP4.T	03/11/2004	11:12	-4.6131	56.0589	0	0.20	-10.28	2.94		9.65		-7.18	10	

Table 14: 2004 / 2005 survey data, where L = Lower (South) basin, M = Middle basin, U = Upper (north) basin, T = Top (surface), M (after full stop) = Middle depth, B = Bottom (deep) depth and P = patchiness site

November, middle basin	date collected	Time collected	Latitude	Longitude	Depth (m)	[DIC] (mM)	$\delta^{13}C_{DIC}$	[DOC] (mg/L)	$\delta^{13}C_{DOC}$	C:N DOM	$\delta^{18}O_{DO}$	$\delta^{18}O_{H2O}$	Temp	pH
M1.B	02/11/2004	15:56	-4.6458	56.1378	50	0.16	-9.03	2.35		7.48		-7.97	7.50	
M1.M	02/11/2004	16:15	-4.6458	56.1378	25	0.17	-11.17	2.19		10.30		-7.90	9.00	
M1.T	02/11/2004	15:56	-4.6458	56.1378	0	0.13	-8.06	2.48		6.10		-7.51	10.00	
M2.B	02/11/2004	16:51	-4.5839	56.0978	35	0.15	-9.07	3.04		12.06		-7.83	8.00	
M2.M	02/11/2004	17:08	-4.5839	56.0978	20	0.17	-9.39	1.89		14.40		-7.40	10.00	
M2.T	02/11/2004	16:51	-4.5839	56.0978	0	0.14	-8.83	2.90		15.30		-7.37	10.00	
M3.B	03/11/2004	07:33	-4.6153	56.1192	50	0.16	-11.22	3.28		7.07		-7.93	8.00	
M3.M	03/11/2004	07:54	-4.6153	56.1192	25	0.18	-10.95	2.70		7.24		-7.57	10.00	
M3.T	03/11/2004	07:33	-4.6153	56.1192	0	0.16	-8.66	1.95		8.09		-7.28	10.00	
MP1.B	03/11/2004	08:03	-4.6186	56.1161	55	0.17	-11.42	2.85		8.68		-7.78	8.00	
MP1.M	03/11/2004	08:14	-4.6186	56.1161	30	0.20	-11.34	2.13		11.60		-7.40	9.00	
MP1.T	03/11/2004	08:03	-4.6186	56.1161	0	0.15	-8.29	2.64		9.79		-7.30	10.00	
MP2.B	03/11/2004	08:26	-4.6214	56.1147	55	0.19	-13.89	2.31		8.20		-7.27	7.50	
MP2.M	03/11/2004	08:38	-4.6214	56.1147	30	0.16	-9.76	2.91		10.45		-7.86	9.50	
MP2.T	03/11/2004	08:26	-4.6214	56.1147	0	0.14	-8.39	2.81		12.38		-8.05	10.00	
MP3.B	03/11/2004	08:49	-4.6683	56.1183	30	0.16	-8.98	2.62		12.60		-7.59	10.00	
MP3.M	03/11/2004	09:05	-4.6683	56.1183	15	0.15	-8.41	3.27		8.38		-7.43	10.00	
MP3.T	03/11/2004	08:49	-4.6683	56.1183	0	0.16	-8.48	1.85		7.55		-7.42	10.00	
MP4.B	03/11/2004	09:13	-4.6053	56.1189	20	0.16	-8.63	2.58		12.44		-7.99	10.00	
MP4.M	03/11/2004	09:27	-4.6053	56.1189	10			3.55		10.72		-7.29	10.00	
MP4.T	03/11/2004	09:13	-4.6053	56.1189	0	0.15	-8.76	2.88		8.51		-7.72	10.00	

Table 15: 2004 / 2005 survey data, where L = Lower (South) basin, M = Middle basin, U = Upper (north) basin, T = Top (surface), M (after full stop) = Middle depth, B = Bottom (deep) depth and P = patchiness site

November, north basin	date collected	Time collected	Latitude	Longitude	Depth (m)	[DIC] (mM)	$\delta^{13}C_{DIC}$	[DOC] (mg/L)	$\delta^{13}C_{DOC}$	C:N DOM	$\delta^{18}O_{DO}$	$\delta^{18}O_{H2O}$	Temp	pH
U1.B	02/11/2004	11:21	-4.7042	56.2942	50	0.17	-16.66	1.87		5.10		-7.68	8.50	
U1.M	02/11/2004	11:08	-4.7042	56.2942	25	0.14	-10.47	2.71		9.09		-8.14	9.50	
U1.T	02/11/2004	10:58	-4.7042	56.2942	0	0.11	-9.39	2.21		10.47		-7.60	9.50	
U2.B	02/11/2004	12:28	-4.7164	56.2528	100	0.14	-12.29	1.71		8.51		-7.73	6.50	
U2.M	02/11/2004	12:17	-4.7164	56.2528	50	0.12	-11.91	1.91		7.66		-7.94	9.50	
U2.T	02/11/2004	11:49	-4.7164	56.2528	0	0.11	-9.04	2.60		10.00		-7.66	9.50	
U3.B	02/11/2004	13:25	-4.6897	56.2014	100	0.13	-11.83	1.14		8.15		-7.97	6.00	
U3.M	02/11/2004	13:20	-4.6897	56.2014	50	0.14	-11.47	1.91		6.44		-8.08	6.50	
U3.T	02/11/2004	13:10	-4.6897	56.2014	0	0.12	-8.90	2.31		9.72		-8.11	9.50	
UP1.B	02/11/2004	13:54	-4.6894	56.2031	100	0.14	-12.67	1.91		9.90		-7.73	6.00	
UP1.M	02/11/2004	13:54	-4.6894	56.2031	50	0.11	-11.67					-7.52	7.00	
UP1.T	02/11/2004	13:49	-4.6894	56.2031	0	0.12	-9.28	2.64		9.48		-7.33	9.00	
UP2.B	02/11/2004	14:20	-4.6844	56.2050	100	0.12	-12.46	2.04		10.40		-7.91	6.50	
UP2.M	02/11/2004	14:30	-4.6844	56.2050	50	0.13	-10.93	1.92		11.80		-7.82	6.00	
UP2.T	02/11/2004	14:14	-4.6844	56.2050	0	0.12	-8.51	2.11		13.63		-7.35	9.00	
UP3.B	02/11/2004	14:49	-4.7008	56.2014	45	0.11	-11.84	1.83		6.55		-7.75	8.00	
UP3.M	02/11/2004	14:59	-4.7008	56.2014	20	0.11	-13.96			8.46		-8.05	9.50	
UP3.T	02/11/2004	14:59	-4.7008	56.2014	0	0.10	-8.43	1.96		6.20			9.50	
UP4.B	02/11/2004	15:15	-4.6869	56.2022	40	0.15	-12.04	2.08		7.34		-7.84	9.00	
UP4.M	02/11/2004	15:35	-4.6869	56.2022	20	0.11	-9.16	2.51		8.95		-7.72	9.00	
UP4.T	02/11/2004	15:15	-4.6869	56.2022	0	0.13	-8.90	2.84		9.96		-7.55	9.50	

Table 16: 2004 / 2005 survey data, where L = Lower (South) basin, M = Middle basin, U = Upper (north) basin, T = Top (surface), M (after full stop) = Middle depth, B = Bottom (deep) depth and P = patchiness site

March, south basin	date collected	Time collected	Latitude	Longitude	Depth (m)	[DIC] (mM)	$\delta^{13}C_{DIC}$	[DOC] (mg/L)	$\delta^{13}C_{DOC}$	C:N DOM	$\delta^{18}O_{DO}$	$\delta^{18}O_{H2O}$	Temp	pH
L1.B	07/03/2005	09:30	-4.5844	56.0739	20	0.23	-7.98	2.09		13.10	22.76	-5.97	4.50	7.26
L1.M	07/03/2005	09:56	-4.5844	56.0739	10	0.17	-7.75	3.08		11.06	23.19	-6.07	4.50	
L1.T	07/03/2005	09:42	-4.5844	56.0739	0	0.21	-8.40	2.43		10.12	22.97	-6.12	4.50	7.30
L2.B	07/03/2005	12:15	-4.6072	56.0267	13	0.17	-7.46	2.63		8.29	22.77	-5.62	4.50	7.33
L2.M	07/03/2005	12:28	-4.6072	56.0267	7	0.15	-7.28	3.44		12.32	22.71	-5.97	4.50	
L2.T	07/03/2005	12:15	-4.6072	56.0267	0	0.24	-7.32	2.88		8.04	22.84	-5.61	4.50	7.26
L3.B	07/03/2005	12:47	-4.5597	56.0661	10	0.15	-7.31	2.65		10.82	22.94	-7.72	4.50	7.33
L3.M	07/03/2005	13:03	-4.5597	56.0661	5	0.17	-7.36				22.86	-6.40	4.50	
L3.T	07/03/2005	12:47	-4.5597	56.0661	0	0.15	-7.69	2.54		10.19	22.68	-7.90	4.50	7.44
LP1.B	07/03/2005	10:06	-4.5897	56.0761	20	0.17	-7.42	2.48		7.25	22.88	-5.86	4.50	
LP1.M	07/03/2005	10:22	-4.5897	56.0761	10	0.17	-7.25			12.38	22.74	-7.58	4.50	
LP1.T	07/03/2005	10:06	-4.5897	56.0761	0	0.16	-6.95	3.57		13.96	22.57	-7.90	4.50	
LP2.B	07/03/2005	10:34	-4.5950	56.0839	20	0.16	-7.47	2.12		7.84	22.79	-7.88	4.50	7.33
LP2.M	07/03/2005	10:50	-4.5950	56.0839	10	0.21	-7.05	2.59		8.20	23.21	-6.06	4.50	
LP2.T	07/03/2005	10:34	-4.5950	56.0839	0	0.16	-7.27	2.10		8.20	22.69	-5.85	4.50	7.42
LP3.B	07/03/2005	10:06	-4.5897	56.0822	16	0.21	-7.36	2.63		6.28	23.27	-5.79	4.50	7.37
LP3.M	07/03/2005	11:30	-4.5897	56.0822	8	0.15	-7.39	3.15		10.50	22.76	-7.71	4.50	
LP3.T	07/03/2005	11:06	-4.5897	56.0822	0	0.23	-6.89	2.60		8.19	22.43	-7.63	4.50	7.36
LP4.B	07/03/2005	11:40	-4.6003	56.0675	20	0.23	-7.57	3.28		11.93	22.82	-7.65	4.50	7.21
LP4.M	07/03/2005	11:56	-4.6003	56.0675	10	0.18	-7.35	3.07		11.53	22.99	-7.94	4.50	
LP4.T	07/03/2005	11:40	-4.6003	56.0675	0	0.14	-6.75	2.24		8.90	22.81	-7.69	4.50	7.34

Table 17: 2004 / 2005 survey data, where L = Lower (South) basin, M = Middle basin, U = Upper (north) basin, T = Top (surface), M (after full stop) = Middle depth, B = Bottom (deep) depth and P = patchiness site

March, middle basin	date collected	Time collected	Latitude	Longitude	Depth (m)	[DIC] (mM)	$\delta^{13}C_{DIC}$	[DOC] (mg/L)	$\delta^{13}C_{DOC}$	C:N DOM	$\delta^{18}O_{DO}$	$\delta^{18}O_{H2O}$	Temp	pH
M1.B	08/03/2005	17:27	-4.6528	56.1375	55	0.14	-9.26	2.49		12.80	23.58	-5.96	5.00	7.06
M1.M	08/03/2005	17:42	-4.6528	56.1375	27	0.16	-8.72	2.07		5.90	23.43	-7.74	5.00	
M1.T	08/03/2005	17:27	-4.6528	56.1375	0	0.12	-9.95	2.54		12.20	24.01	-6.10	5.00	7.14
M2.B	07/03/2005	13:31	-4.5756	56.0775	30	0.16	-8.86	2.73		10.55	23.95	-5.95	5.00	7.09
M2.M	07/03/2005	13:42	-4.5756	56.0775	15	0.15	-9.03	2.34		8.00	23.61	-5.43	5.00	
M2.T	07/03/2005	13:31	-4.5756	56.0775	0	0.15	-9.55	2.34		5.65	23.66	-5.80	5.00	7.29
M3.B	07/03/2005	14:01	-4.6117	56.1175	25	0.14	-9.13	1.93		5.12	23.69	-7.66	5.00	7.19
M3.M	07/03/2005	14:22	-4.6117	56.1175	12	0.19	-8.42				23.76	-5.65	5.00	
M3.T	07/03/2005	14:01	-4.6117	56.1175	0	0.21	-8.29	1.68		16.14	23.41	-6.13	5.00	7.25
MP1.B	07/03/2005	14:30	-4.6086	56.1258	32	0.14	-8.86	3.49		10.05	23.25	-5.52	5.00	
MP1.M	07/03/2005	14:45	-4.6086	56.1258	16	0.11	-8.09	2.27		7.97	24.33	-7.48	5.00	
MP1.T	07/03/2005	14:30	-4.6086	56.1258	0	0.18	-8.17	1.83		8.19	23.18	-5.90	5.00	
MP2.B	07/03/2005	15:03	-4.6217	56.1228	42	0.13	-8.31	2.69		11.60	25.95	-5.82	5.00	
MP2.M	07/03/2005	15:21	-4.6217	56.1228	21	0.19	-8.76	0.66		9.26	24.75	-6.76	5.00	
MP2.T	07/03/2005	15:03	-4.6217	56.1228	0	0.13	-7.78			6.13	24.27	-5.84	5.00	7.26
MP3.B	07/03/2005	15:35	-4.6025	56.1197	43	0.13	-8.53				24.22	-7.96	5.00	
MP3.M	07/03/2005	16:04	-4.6025	56.1197	22	0.15	-8.25	2.67		6.33	24.06	-5.95	5.00	
MP3.T	07/03/2005	15:35	-4.6025	56.1197	0	0.18	-8.12	2.69		10.30	24.87	-7.67	5.00	
MP4.B	08/03/2005	17:59	-4.6197	56.1217	26	0.11	-8.91			7.21	24.19	-6.12	5.00	
MP4.M	08/03/2005	18:15	-4.6197	56.1217	13	0.14	-8.70	2.99		7.15		-6.74	5.00	
MP4.T	08/03/2005	17:59	-4.6197	56.1217	0	0.16	-8.64	2.97		12.42	24.40	-5.88	5.00	

Table 18: 2004 / 2005 survey data, where L = Lower (South) basin, M = Middle basin, U = Upper (north) basin, T = Top (surface), M (after full stop) = Middle depth, B = Bottom (deep) depth and P = patchiness site

March, north basin	date collected	Time collected	Latitude	Longitude	Depth (m)	[DIC] (mM)	$\delta^{13}C_{DIC}$	[DOC] (mg/L)	$\delta^{13}C_{DOC}$	C:N DOM	$\delta^{18}O_{DO}$	$\delta^{18}O_{H2O}$	Temp	pH
U1.B	08/03/2005	10:15	-4.7069	56.2936	53	0.09	-14.73	1.42		9.19	23.48	-7.63	5.00	6.95
U1.M	08/03/2005	10:45	-4.7069	56.2936	30	0.08	-13.64	1.77		12.28	23.18	-6.22	5.00	
U1.T	08/03/2005	10:15	-4.7069	56.2936	0	0.09	-11.78	1.69		10.98		-7.71	5.50	7.21
U2.B	08/03/2005	13:10	-4.7106	56.2503	80	0.09	-13.05	1.53		9.56	23.29	-5.87	5.50	7.00
U2.M	08/03/2005	14:00	-4.7106	56.2503	40	0.10	-14.60	2.06		6.72	23.43	-5.83	5.50	
U2.T	08/03/2005	13:10	-4.7106	56.2503	0	0.12	-13.79	2.09		7.23	23.30	-6.08	5.50	7.01
U3.B	08/03/2005	14:28	-4.7011	56.2000	100	0.09	-12.80	2.10		12.00	23.36	-8.03	5.50	6.54
U3.M	08/03/2005	14:56	-4.7011	56.2000	50	0.09	-11.50	1.86		13.40	23.57	-5.91	5.50	
U3.T	08/03/2005	14:28	-4.7011	56.2000	0	0.11	-12.50	2.16		3.70	23.29	-7.69	5.50	7.08
UP1.B	08/03/2005	15:04	-4.7019	56.2100	65	0.07	-12.09	4.41		10.20	23.29	-7.85	5.50	
UP1.M	08/03/2005	15:44	-4.7019	56.2100	30	0.08	-14.17	3.12		12.95	23.43	-5.90	5.50	
UP1.T	08/03/2005	15:04	-4.7019	56.2100	0	0.10	-11.17	1.80		9.90	23.28	-7.85	5.50	
UP2.B	08/03/2005	15:49	-4.6944	56.2100	45	0.08	-12.60	1.70		10.10	23.64	-8.08	5.50	
UP2.M	08/03/2005	16:10	-4.6944	56.2100	22	0.09	-12.01	2.45		12.97	23.30	-7.90	5.50	
UP2.T	08/03/2005	15:49	-4.6944	56.2100	0	0.07	-11.90	2.37		8.80	23.16	-7.91	5.50	
UP3.B	08/03/2005	16:17	-4.6967	56.2031	80	0.07	-12.02	2.16		11.40	23.14	-5.99	5.50	6.98
UP3.M	08/03/2005	16:42	-4.6967	56.2031	40	0.09	-11.72	1.81		5.93	23.12	-5.87	5.50	
UP3.T	08/03/2005	16:17	-4.6967	56.2031	0	0.06	-12.88	2.14		9.66	23.34	-6.20	5.50	7.08
UP4.B	08/03/2005	16:43	-4.6992	56.1992	80	0.07	-12.01	2.22		10.20	23.05	-7.59	5.50	6.98
UP4.M	08/03/2005	17:09	-4.6992	56.1992	40	0.07	-12.85	2.23	-27.67	4.00	23.42	-7.84	5.50	
UP4.T	08/03/2005	16:43	-4.6992	56.1992	0	0.12	-11.61	1.98		10.18	23.24	-7.79	5.50	6.98

Table 19: 2004 / 2005 survey data, where L = Lower (South) basin, M = Middle basin, U = Upper (north) basin, T = Top (surface), M (after full stop) = Middle depth, B = Bottom (deep) depth and P = patchiness site

June, south basin	date collected	Time collected	Latitude	Longitude	Depth (m)	[DIC] (mM)	$\delta^{13}C_{DIC}$	[DOC] (mg/L)	$\delta^{13}C_{DOC}$	C:N DOM	$\delta^{18}O_{DO}$	$\delta^{18}O_{H2O}$	Temp	pH
L1.B	29/06/2005	09:22	-4.5903	56.0703	20	0.37	-9.84	3.82	-28.98	14.34	26.06	-6.47	12.50	6.10
L1.M	29/06/2005	09:46	-4.5903	56.0703	10	0.28	-5.98				23.23	-6.61	15.50	
L1.T	29/06/2005	09:22	-4.5903	56.0703	0	0.38	-5.63	3.63		16.16	23.61	-6.19	17.50	6.20
L2.B	29/06/2005	10:04	-4.6106	56.0297	17	0.18	-7.23	2.98		13.83	25.71	-6.36	12.50	6.10
L2.M	29/06/2005	10:26	-4.6106	56.0297	8	0.28	-5.53	3.21	-29.01	11.06	23.43	-6.25	16.00	
L2.T	29/06/2005	10:04	-4.6106	56.0297	0	0.27	-5.77	3.84	-29.13	15.81	23.44	-6.38	17.00	6.20
L3.B	29/06/2005	10:46	-4.5569	56.0606	12	0.18	-7.42	2.63	-29.00	13.04	24.98	-6.44	14.00	5.85
L3.M	29/06/2005	10:59	-4.5569	56.0606	6	0.25	-9.34	2.43		4.21	25.67	-6.65	15.00	
L3.T	29/06/2005	10:46	-4.5569	56.0606	0	0.13	-14.23	3.72	-29.03	11.52	24.57	-6.42	15.00	6.20
LP1.B	29/06/2005	12:25	-4.5953	56.0697	20	0.21	-9.40	3.30	-28.71	8.57	25.35	-6.57	13.00	6.20
LP1.M	29/06/2005	12:45	-4.5953	56.0697	10	0.22	-6.15	3.95	-28.83	10.59	22.78	-6.23	16.50	
LP1.T	29/06/2005	12:25	-4.5953	56.0697	0	0.20	-6.45	3.91	-28.98	17.36	23.39	-6.27	17.00	6.30
LP2.B	29/06/2005	12:55	-4.5997	56.0722	20	0.25	-9.77	3.08	-29.01	15.25	26.18	-6.25	12.00	6.20
LP2.M	29/06/2005	13:05	-4.5997	56.0722	10	0.22	-4.62	4.20	-29.02	13.14	24.29	-6.53	17.50	
LP2.T	29/06/2005	12:55	-4.5997	56.0722	0	0.23	-5.88	3.42	-28.51	18.69	24.16	-6.46	17.50	6.40
LP3.B	29/06/2005	13:19	-4.6075	56.0731	6	0.21	-5.42	3.80	-29.06	16.67	23.17	-6.40	17.50	
LP3.M	29/06/2005	13:30	-4.6075	56.0731	3	0.24	-5.44	4.49	-28.99	13.66	23.50	-6.32	17.50	
LP3.T	29/06/2005	13:19	-4.6075	56.0731	0	0.32	-5.13	3.88	-28.96	9.29	24.03	-6.49	17.50	
LP4.B	29/06/2005	13:44	-4.5992	56.0750	18	0.23	-10.03	4.09	-29.08	10.22	26.52	-6.82	12.50	
LP4.M	29/06/2005	14:02	-4.5992	56.0750	9	0.17	-4.53		-28.77		24.19	-6.17	17.00	
LP4.T	29/06/2005	13:44	-4.5992	56.0750	0	0.32	-4.47	3.46	-29.09	3.90	23.52	-6.39	17.50	

Table 20: 2004 / 2005 survey data, where L = Lower (South) basin, M = Middle basin, U = Upper (north) basin, T = Top (surface), M (after full stop) = Middle depth, B = Bottom (deep) depth and P = patchiness site

June, middle basin	date collected	Time collected	Latitude	Longitude	Depth (m)	[DIC] (mM)	$\delta^{13}C_{DIC}$	[DOC] (mg/L)	$\delta^{13}C_{DOC}$	C:N DOM	$\delta^{18}O_{DO}$	$\delta^{18}O_{H2O}$	Temp	pH
M1.B	28/06/2005	16:47	-4.6439	56.1381	55	0.17	-8.23	2.08	-28.86	1.96	25.60	-7.47	8.00	6.30
M1.M	28/06/2005	17:15	-4.6439	56.1381	25	0.13	-8.72	2.41	-28.72	9.63	24.93	-7.25	9.00	
M1.T	28/06/2005	16:47	-4.6439	56.1381	0	0.13	-3.41	3.48	-28.85	8.60	23.83	-6.30	16.00	6.30
M2.B	29/06/2005	14:15	-4.5869	56.0978	40	0.17	-8.02	2.50	-28.91	5.69	25.59	-6.57	8.50	6.20
M2.M	29/06/2005	14:38	-4.5869	56.0978	20	0.16	-8.79	2.11		11.68	25.53	-6.50	11.00	
M2.T	29/06/2005	14:15	-4.5869	56.0978	0	0.26	-5.22	3.69	-28.61	13.10	23.67	-7.61	17.00	6.20
M3.B	28/06/2005	17:42	-4.6114	56.1181	50	0.13	-8.61	3.02		9.55	25.57	-7.36	8.50	6.10
M3.M	28/06/2005	17:51	-4.6114	56.1181	25	0.13	-4.08	3.28	-28.95	6.53	24.37	-6.48	10.50	
M3.T	28/06/2005	17:42	-4.6114	56.1181	0	0.09	-11.84	3.73	-28.84	5.09	21.64	-7.24	19.00	6.40
MP1.B	29/06/2005	15:05	-4.6056	56.1153	18	0.19	-7.74	2.89	-28.69	6.93	25.54	-6.35	11.50	6.40
MP1.M	29/06/2005	15:23	-4.6056	56.1153	9	0.14	-3.52				23.86	-6.34	15.00	
MP1.T	29/06/2005	15:05	-4.6056	56.1153	0	0.16	-8.65	4.01	-28.70	8.59	24.78	-7.67	17.00	6.60
MP2.B	29/06/2005	15:38	-4.6167	56.1142	55	0.16	-8.42	3.10	-28.85	10.13	25.68	-7.94	8.00	6.30
MP2.M	29/06/2005	15:51	-4.6167	56.1142	25	0.17	-3.53	3.23	-28.96	15.61	23.91	-6.19	9.00	
MP2.T	29/06/2005	15:38	-4.6167	56.1142	0	0.25	-4.37	3.47	-28.90	14.48	24.20	-7.54	16.50	6.40
MP3.B	29/06/2005	16:05	-4.6194	56.1186	50	0.14	-3.91	2.41	-28.52	1.05	23.97	-6.63	7.00	
MP3.M	29/06/2005	16:25	-4.6194	56.1186	25	0.20	-8.35	3.07	-28.95	11.79	25.53	-6.65	10.00	
MP3.T	29/06/2005	16:05	-4.6194	56.1186	0	0.17	-3.38	2.62	-28.89	9.34	24.37	-6.22	17.00	
MP4.B	29/06/2005	16:44	-4.6164	56.1197	40	0.24	-8.95	2.86	-28.67	13.09	25.72	-6.76		
MP4.M	29/06/2005	17:00	-4.6164	56.1197	20	0.17	-8.00	3.00	-29.02	13.86	25.72	-6.88		
MP4.T	29/06/2005	16:44	-4.6164	56.1197	0	0.15	-2.87	2.59	-28.83	12.27	24.06	-6.54		

Table 21: 2004 / 2005 survey data, where L = Lower (South) basin, M = Middle basin, U = Upper (north) basin, T = Top (surface), M (after full stop) = Middle depth, B = Bottom (deep) depth and P = patchiness site

June, north basin	date collected	Time collected	Latitude	Longitude	Depth (m)	[DIC] (mM)	$\delta^{13}C_{DIC}$	[DOC] (mg/L)	$\delta^{13}C_{DOC}$	C:N DOM	$\delta^{18}O_{DO}$	$\delta^{18}O_{H2O}$	Temp	pH
U1.B	28/06/2005	10:28	-4.7033	56.2925	55	0.11	-11.92	2.65		11.35	25.30	-8.24	7.00	5.95
U1.M	28/06/2005	11:13	-4.7033	56.2925	25	0.10	-10.86	2.35		7.00	25.43	-6.47	10.00	
U1.T	28/06/2005	10:28	-4.7033	56.2925	0	0.24	-8.49	2.58	-29.08	17.35	25.45	-7.85	14.00	5.80
U2.B	28/06/2005	11:31	-4.6958	56.2517	100	0.11	-10.88	1.62	-28.83	5.55	25.20	-7.31	6.00	5.95
U2.M	28/06/2005	11:54	-4.6958	56.2517	50	0.12	-9.56	1.67	-28.62	4.51	25.62	-7.67	6.50	
U2.T	28/06/2005	11:31	-4.6958	56.2517	0	0.20	-7.12	3.60	-28.71	8.97	24.30	-7.68	15.00	6.50
U3.B	28/06/2005	14:06	-4.6892	56.2022	100	0.11	-11.04	2.57		11.64	25.93	-7.28	6.00	5.90
U3.M	28/06/2005	14:23	-4.6892	56.2022	50	0.13	-10.65	2.35	-28.57	12.57	25.25	-6.09	6.50	
U3.T	28/06/2005	14:06	-4.6892	56.2022	0	0.13	-5.52	2.68	-28.97	15.45	23.87	-7.28	16.00	6.20
UP1.B	28/06/2005	14:39	-4.6953	56.2025	100	0.13	-9.96				25.18	-8.46	6.50	5.90
UP1.M	28/06/2005	15:12	-4.6953	56.2025	50	0.09	-11.31	2.79	-28.82	8.27	25.31	-6.12	6.50	
UP1.T	28/06/2005	14:39	-4.6953	56.2025	0	0.16	-4.34	3.13	-28.78	7.74	23.72	-6.77	16.50	6.20
UP2.B	28/06/2005	15:19	-4.6333	56.2044	55	0.13	-14.22	2.52	-28.88	10.27	25.77	-7.61	6.50	5.95
UP2.M	28/06/2005	15:39	-4.6333	56.2044	25	0.11	-10.22	2.16	-28.86	10.25	25.24	-7.65	7.00	
UP2.T	28/06/2005	15:19	-4.6333	56.2044	0	0.19	-4.53		-28.71		23.96	-7.30	16.00	6.30
UP3.B	28/06/2005	15:49	-4.6964	56.2039	85	0.11	-11.13	1.17	-28.84	10.70	26.63	-7.72	6.50	
UP3.M	28/06/2005	15:58	-4.6964	56.2039	45	0.12	-9.80	2.83	-28.71	6.56	25.25	-6.54	7.00	
UP3.T	28/06/2005	15:49	-4.6964	56.2039	0	0.12	-5.17	2.86	-28.77	11.61	24.64	-7.17	17.00	
UP4.B	28/06/2005	16:12	-4.6989	56.2114	70	0.12	-10.43	1.88		1.65	25.23	-6.59	6.50	
UP4.M	28/06/2005	16:30	-4.6989	56.2114	35	0.12	-9.56	2.32	-28.90	9.33	25.04	-6.55	7.50	
UP4.T	28/06/2005	16:12	-4.6989	56.2114	0	0.13	-5.12	3.31	-28.85	10.19	24.45	-6.10	17.00	

Table 22: 2004 / 2005 survey data, where L = Lower (South) basin, M = Middle basin, U = Upper (north) basin, T = Top (surface), M (after full stop) = Middle depth, B = Bottom (deep) depth and P = patchiness site

September, south basin	date collected	Time collected	Latitude	Longitude	Depth (m)	[DIC] (mM)	$\delta^{13}C_{DIC}$	[DOC] (mg/L)	$\delta^{13}C_{DOC}$	C:N DOM	$\delta^{18}O_{DO}$	$\delta^{18}O_{H2O}$	Temp	pH
M1.B	02/09/2005	09:37	-4.5908	56.0708	20	0.17	-7.71	2.84	-28.35	4.48	25.23	-6.23	16.00	7.05
M1.M	02/09/2005	09:48	-4.5908	56.0708	10	0.18	-7.35	2.79	-28.90	11.51	24.58	-6.03	16.00	
M1.T	02/09/2005	09:37	-4.5908	56.0708	0	0.19	-10.14	3.00	-28.80	12.77	24.37	-6.04	16.50	
M2.B	02/09/2005	12:06	-4.6058	56.0281	17	0.20	-8.85	2.99	-28.78	2.58	24.84	-6.09	15.50	6.90
M2.M	02/09/2005	12:23	-4.6058	56.0281	8	0.20	-8.01	3.53	-28.81	14.71	25.56	-6.19	15.50	
M2.T	02/09/2005	12:06	-4.6058	56.0281	0	0.17	-7.37	1.96	-28.93	6.55	24.33	-6.03	15.50	7.00
M3.B	02/09/2005	12:33	-4.5503	56.0450	10	0.22	-6.15	3.05	-28.40	15.27	25.04	-6.17	15.50	7.00
M3.M	02/09/2005	12:44	-4.5503	56.0450	5	0.19	-6.18	3.33	-28.86	17.03	24.75	-6.05	15.50	
M3.T	02/09/2005	12:33	-4.5503	56.0450	0	0.18	-7.39	2.50	-28.32	3.55	24.06	-5.91	15.50	7.10
MP1.B	02/09/2005	10:02	-4.5925	56.0733	20	0.20	-6.70	3.49	-28.79	9.72	24.61	-6.30	16.00	7.10
MP1.M	02/09/2005	10:19	-4.5925	56.0733	10	0.19	-6.52	2.38	-28.40	9.28	24.46	-5.95	16.00	
MP1.T	02/09/2005	10:02	-4.5925	56.0733	0	0.19	-7.30	2.45	-28.86	17.13	24.30	-6.33	16.00	7.00
MP2.B	02/09/2005	10:28	-4.5889	56.0725	20	0.18	-7.26	3.11	-28.31	8.68	24.66	-6.15	15.50	7.00
MP2.M	02/09/2005	10:39	-4.5889	56.0725	10	0.20	-6.64	2.31	-28.79	2.36	24.37	-5.38	15.50	
MP2.T	02/09/2005	10:28	-4.5889	56.0725	0	0.21	-7.66	2.69	-28.48	13.49	24.61	-5.89	16.00	7.10
MP3.B	02/09/2005	11:22	-4.5850	56.0736	20	0.19	-6.97	2.58	-28.45	12.57	24.66	-6.39	15.50	
MP3.M	02/09/2005	11:38	-4.5850	56.0736	10	0.17	-6.23	2.90	-30.13	10.71	24.76	-5.88	15.50	
MP3.T	02/09/2005	11:22	-4.5850	56.0736	0	0.16	-7.96	2.54	-28.23	14.59	23.94	-6.18	16.00	
MP4.B	02/09/2005	11:42	-4.6114	56.0653	20	0.15	-7.55	2.91	-28.35	12.70	24.83	-6.18	15.50	
MP4.M	02/09/2005	11:55	-4.6114	56.0653	10	0.17	-5.77	2.09	-28.37	2.20	25.10	-6.20	16.00	
MP4.T	02/09/2005	11:42	-4.6114	56.0653	0	0.16	-5.00	2.81	-28.91	17.24	24.64	-6.09	16.00	

Table 23: 2004 / 2005 survey data, where L = Lower (South) basin, M = Middle basin, U = Upper (north) basin, T = Top (surface), M (after full stop) = Middle depth, B = Bottom (deep) depth and P = patchiness site

September, middle basin	date collected	Time collected	Latitude	Longitude	Depth (m)	[DIC] (mM)	$\delta^{13}C_{DIC}$	[DOC] (mg/L)	$\delta^{13}C_{DOC}$	C:N DOM	$\delta^{18}O_{DO}$	$\delta^{18}O_{H2O}$	Temp	pH
M1.B	01/09/2005	15:38	-4.6458	56.1383	50	0.16	-11.14	2.86	-28.31	8.32	26.60	-6.54	10.00	6.65
M1.M	01/09/2005	15:32	-4.6458	56.1383	25	0.15	-11.44	2.80	-28.79	11.31	26.60	-6.44	12.50	
M1.T	01/09/2005	15:38	-4.6458	56.1383	0	0.13	-4.75	3.02	-28.78	8.12	24.24	-6.15	16.00	6.90
M2.B	02/09/2005	13:00	-4.5864	56.0969	35	0.16	-12.50	4.34	-28.90	8.81	26.31	-6.66	9.00	6.70
M2.M	02/09/2005	13:20	-4.5864	56.0969	17	0.17	-11.78	2.93	-28.30	9.16	27.30	-6.30	10.00	
M2.T	02/09/2005	13:00	-4.5864	56.0969	0	0.15	-4.70	2.46	-28.71	8.73	23.67	-6.18	16.00	7.45
M3.B			-4.6342	56.1153	55	0.14	-11.70	3.17	-28.96	11.37	26.01	-6.27	8.00	6.60
M3.M			-4.6342	56.1153	25	0.16	-11.54	2.62	-28.85	3.62	26.66	-6.46	11.00	
M3.T			-4.6342	56.1153	0	0.14	-5.00	1.82	-28.36	4.67	24.44	-6.11	15.00	7.05
MP1.B	01/09/2005	17:08	-4.6283	56.1131	45	0.16	-11.76	1.78	-28.87	1.32	26.20	-6.59	9.00	6.70
MP1.M	01/09/2005	17:25	-4.6283	56.1131	20	0.18	-11.89	2.64	-28.84	11.29	26.84	-6.29	13.50	
MP1.T	01/09/2005	17:13	-4.6283	56.1131	0	0.13	-5.86	2.90	-28.30	9.26	24.32	-6.18	15.50	7.10
MP2.B	02/09/2005	13:30	-4.5944	56.1128	43	0.15	-12.27	3.34	-28.64	9.05	26.25	-6.76	9.00	6.70
MP2.M	02/09/2005	13:43	-4.5944	56.1128	22	0.18	-11.91	2.65	-28.88	4.84	27.44	-6.56	10.00	
MP2.T	02/09/2005	13:30	-4.5944	56.1128	0	0.12	-4.73	3.40	-28.71	11.21	24.82	-6.30	15.50	7.10
MP3.B	02/09/2005	13:54	-4.6067	56.1169	25	0.16	-13.28	2.47	-28.58	5.10	26.83	-6.71	10.00	
MP3.M	02/09/2005	14:03	-4.6067	56.1169	12	0.15	-6.11	2.29	-28.33	14.38	24.60	-5.84	15.50	
MP3.T	02/09/2005	13:54	-4.6067	56.1169	0	0.15	-5.34	2.98	-27.97	8.19	24.61	-6.33	16.00	
MP4.B	02/09/2005	14:13	-4.6231	56.1172	60	0.17	-12.01	2.68	-27.94	7.23	26.36	-6.66	7.50	
MP4.M	02/09/2005	14:28	-4.6231	56.1172	30	0.15	-11.96	2.37	-28.31	4.56	27.36	-6.76	11.00	
MP4.T	02/09/2005	14:13	-4.6231	56.1172	0	0.14	-5.21	2.21	-28.32	4.99	24.31	-5.86	15.50	

Table 24: 2004 / 2005 survey data, where L = Lower (South) basin, M = Middle basin, U = Upper (north) basin, T = Top (surface), M (after full stop) = Middle depth, B = Bottom (deep) depth and P = patchiness site

September, north basin	date collected	Time collected	Latitude	Longitude	Depth (m)	[DIC] (mM)	$\delta^{13}C_{DIC}$	[DOC] (mg/L)	$\delta^{13}C_{DOC}$	C:N DOM	$\delta^{18}O_{DO}$	$\delta^{18}O_{H2O}$	Temp	pH
U1.B	01/09/2005	10:38	-4.7017	56.2928	52	0.17	-13.77	2.59	-28.71	11.74	26.75	-6.51	8.00	6.45
U1.M	01/09/2005	10:59	-4.7017	56.2928	25	0.13	-13.66	1.70	-28.73	10.58	26.28	-6.40	16.00	
U1.T	01/09/2005	10:38	-4.7017	56.2928	0	0.12	-7.00	2.55	-28.85	3.74	25.10	-6.31	16.00	6.60
U2.B	01/09/2005	11:16	-4.6958	56.2511	100	0.10	-9.93	1.87	-27.59	7.17	25.91	-6.79	6.00	
U2.M	01/09/2005	11:39	-4.6958	56.2511	50	0.13	-10.75	2.69	-28.81	7.06	26.17	-6.62	7.50	
U2.T	01/09/2005	11:16	-4.6958	56.2511	0	0.14	-5.15	3.24	-28.83	14.38	25.05	-6.28	15.00	6.90
U3.B	01/09/2005	12:31	-4.6886	56.1986	100	0.11	-10.64		-28.49	3.59	26.21	-6.78	6.50	6.65
U3.M	01/09/2005	12:42	-4.6886	56.1986	50	0.06	-9.43	2.13	-28.75	9.50	25.79	-6.62	8.00	
U3.T	01/09/2005	12:31	-4.6886	56.1986	0	0.13	-6.53	2.98	-28.71	13.73	24.69	-6.30	15.00	6.80
UP1.B	01/09/2005	13:01	-4.6869	56.2042	100	0.10	-9.87	1.99	-28.70	10.06	25.73	-6.50	6.50	6.10
UP1.M	01/09/2005	13:16	-4.6869	56.2042	50	0.10	-9.24		-28.56	4.02	26.13	-6.42	7.50	
UP1.T	01/09/2005	13:01	-4.6869	56.2042	0	0.15	-6.48	3.43	-28.72	12.42	24.57	-6.34	15.00	6.80
UP2.B	01/09/2005	13:36	-4.6931	56.2050	100	0.09	-10.03	3.57	-28.54	8.65	25.47	-6.54	7.00	6.60
UP2.M	01/09/2005	13:50	-4.6931	56.2050	50	0.10	-9.84	2.45	-28.49	7.06	26.12	-6.43	9.00	
UP2.T	01/09/2005	13:56	-4.6931	56.2050	0	0.10	-6.00	3.60	-28.80	12.52	25.47	-6.08	15.00	6.90
UP3.B	01/09/2005	14:15	-4.6986	56.2006	80	0.08	-10.36	1.66	-28.31	9.22	25.81	-6.55	7.00	
UP3.M	01/09/2005	14:30	-4.6986	56.2006	40	0.10	-9.27	3.35	-28.54	9.20	25.86	-6.63	12.00	
UP3.T	01/09/2005	14:15	-4.6986	56.2006	0	0.11	-4.81	2.89	-28.80	15.25	25.11	-6.42	14.50	
UP4.B	01/09/2005	14:40	-4.6878	56.2008	60	0.13	-10.72	1.84	-28.67	5.32	26.11	-6.72	7.50	
UP4.M	01/09/2005	15:03	-4.6878	56.2008	30	0.12	-10.46	2.86	-28.53	10.26	26.48	-6.41	10.50	
UP4.T	01/09/2005	14:40	-4.6878	56.2008	0	0.13	-5.87	2.22	-28.81	9.28	25.14		15.00	

Table 25: 2004 / 2005 survey data, where L = Lower (South) basin, M = Middle basin, U = Upper (north) basin, T = Top (surface), M (after full stop) = Middle depth, B = Bottom (deep) depth and P = patchiness site

Appendix 2:

Bacterial filtration Efficiency Experiments

Introduction.

In preparation for incubation experiments Loch Lomond water was filtered through a 3µm cellulose nitrate filter. The efficiency of this pore size was assessed with the following method.

Method.

500ml of Loch water was filtered through a 100µm zooplankton mesh and 500ml of loch water was filtered through 3µm cellulose nitrate filter. Four treatments were then examined for bacterial numbers.

1) Unfiltered loch water
2) Filtered through a 100µm zooplankton mesh
3) Filtered through a 100µm zooplankton mesh and a 3µm cellulose nitrate membrane filter.
4) Filtered through a 0.2µm silver filter.

And there were three replicates of each treatment. Samples were refrigerated before being cultured.

Bacteria were cultured on Agar plates prepared the day before and kept in sterile conditions. Three different volumes of loch water was plated out, 20µl, 100µl and 200µl to obtain suitable numbers. Serial dilution methods were originally carried out but gave to few bacteria for statistical analysis. All plating was done under sterile conditions. When plating was complete, agar plates were stored upside down in an incubation oven at 28˚C for 6 days. After this time colonies were recorded.

Results.

Treatment	Colonies (20µl)	Colonies (100µl)	Colonies (200µl)
1 (1)	6,4,5	18,19	23,20
1 (2)	4,4,5	16,15,15	20,19,22
1 (3)	4,6,5	18,18,16	26,22,24
2 (1)	3,5,4	13,15,16	21,21,20
2 (2)	4,4,3	12,14,11	17,21,21
2 (3)	4,5,6	13,16,10	21,22,23
3 (1)	2,3,3	7,9,12	12,12,9
3 (2)	3,2,5	8,11,13	10,11,10
3 (3)	2,4,4	13,8,10	12,13,12
4 (1)	0,0,0	0,1,0	0,0,0
4 (2)	0,0	0,0,0	0,0,1
4 (3)	0,0,0	0,0,0	0,0,0

Mean unfiltered = 4.78, 17 and 22
Mean 3µm filtered = 3.11, 10.11 and 11.22

% Bacteria which pass through 3µm filter = **51.01% – 65.12%.**

Appendix 3:

Incubation experiments first run. June 2006.

Introduction:

Original plans to assess phytoplanktonic and bacterial production in Loch Lomond were to physically separate the two components via filtration methodology.

Materials and methods:

Samples were collected from one site in the south basin (day 1) and one site in the north basin (day 2). What follows is a description of the method for one site; the same would have been carried out the following day.

Water was collected in a Van Dorn water sampler. In the south basin samples were taken at the surface and at ~20 m depth. In the north a surface sample and one from ~45 m. After collection water was filtered through a course (100 μm) zooplankton mesh while being decanted into two 5 L aspirators.

Once ashore, two procedures could be carried out, I shall describe each in turn.

Phytoplankton pre incubation:

600 ml of sample water was filtered through a 3 μm cellulose nitrate membrane filter. This pore size was selected to allow the majority of bacteria through and retain all phytoplankton. Filtration pressure never exceeded 20 cm/Hg to retain cell integrity. At the same time 600 ml of loch water was filtered through 0.2 μm membrane filters, giving loch water with no particulate organic material present. Once both filtrations had been completed the phytoplankton on the filter paper was gently agitated off the filter paper into the 0.2 μm filtered lake water and shaken.

The sample was then spiked with labelled bicarbonate to a concentration of 10% ambient. This equated to 100 μl of spike. The incubation bottle was then sealed, shaken and deployed as soon as possible after spiking (never more than 30 minutes). Phytoplankton incubations were run in duplicate for surface and deep water. Deep water incubations were in bottles covered in aluminium foil and black insulation tape to mimic light exclusion. Incubations lasted for 24 hours.

Phytoplankton post incubation:

Immediately upon collection three DIC replicates were taken (see method described in Waldron and Scott 2006). Samples were then filtered onto 2.7 µm glass fibre filter papers to collect particulate material. Filter papers were then covered in aluminium foil and dried for a minimum of 6 hours at 60^{o}C. The filtrate was frozen after filtration to await DOM analysis. Samples were condensed by rotary evaporation after being filtered through 0.7 µm glass fibre filters, frozen and freeze dried for later analysis.

Bacteria pre incubation:

600 ml of sample water was filtered through a 3 µm nitrose cellulite membrane filter. This pore size was selected to allow the majority of bacteria through and retain all phytoplankton. Filtration pressure never exceeded 20 cm/Hg to retain cell integrity. Water which passed through the filter is assumed to only contain free living bacteria. The proportion of bacteria which passed through the filter was assessed in separate experiments described in a separate report. The overall efficiency of bacterial throughput was 51.01% – 65.12%. No phytoplankton passed through the filters.

The sample was then spiked with 100µl of labelled Leucine for a final concentration of 20 nmols/L. The incubation bottle was then sealed, shaken and deployed as soon as possible after spiking (never more than 30 minutes). Phytoplankton incubations were run in duplicate for surface and deep water. Deep water incubations were in bottles covered in aluminium foil and black insulation tape to mimic light exclusion. Incubations lasted for 24 hours.

Bacteria post incubation:

Immediately upon collection three DIC replicates were taken (see method described in Waldron and Scott 2006). The remaining sample was then filtered onto 0.7 µm glass fibre filters to collect particulate material (assumed to be bacteria) and the filtrate was frozen to await DOM analysis.

Along with the phytoplankton and bacterial incubations a community sample was also deployed for each site that was unfiltered. This sample was measured for dissolved oxygen before and after the incubation to give a community respiration measure.

Results.

Data obtained from the incubations is shown in table 1. Here $\delta^{13}C$ values are shown for DIC, DOC, POC (on 2.7 µm filters) and POC (on 0.7 µm filters) after incubation completion.

Sample name	$\delta^{13}C_{DIC}$	$\delta^{13}C_{DOC}$	$\delta^{13}C_{POC}$ (2.7um)	$\delta^{13}C_{POC}$ (0.7um)
PL1 S	338.6		-26.4	-29.6
PL2 S	337.6	-26.2	-24.8	
PD1 S	-8.6	-31.8	-28.0	-30.6
PD2 S	-9.3	-27.7	-29.5	-31
BL1 S	-5.4	-27.9	-27.8	
BL2 S	-6.0	-26.5	-30.5	
BD1 S	-9.0	-29.0	-28.5	
BD2 S	-9.1	-31.2	-31.1	
PL1 N	267.9	-30.9	-26.0	-29.9
PL2 N	284.0	-24.2	-25.5	-27.6
PD1 N	-12.4	-30.7	-26.6	
PD2 N	-11.8	-29.0	-29.1	-26.9
BL1 N	-6.3	-26.9	-29.4	
BL2 N	-6.9	-27.3	-28.0	
BD1 N	-11.4	-29.3	-29.7	
BD2 N	-11.4	-29.2	-27.1	

Table 26: $\delta^{13}C$ Results for DIC, DOC and POC for incubation test run.

Discussion:

POC values should be a reflection of uptake of the tracer we have added to the system. For phytoplankton labelled bicarbonate will be taken up as a dissolved inorganic source of carbon during photosynthesis which will subsequently be utilised in tissue production. The same is true of bacteria, which will utilise the Leucine as a dissolved organic source of carbon and use it in respiration. If the incubations have worked then the tracer will show up in the respective tissues of bacteria and phytoplankton. The results show that no such enrichment in the POC pool for either fraction has been recorded.

The DIC spike is certainly present in the incubations, as shown by its presence in the DIC pool (was used in the phytoplankton light samples). Although the absolute delta value is not what we would expect (possibly an analytical problem to be resolved) the tracer is there in high enough amounts to register enrichment in the phytoplankton pool. This experiment gave POC enrichments of 0.002 to 0.005 atm % excess ^{13}C, which suggests almost no metabolic activity at all.

It is possible that the proposed re-suspension of phytoplankton cells has actually resulted in killing them or reducing cell fitness, and as such no/little photosynthesis is occurring in the incubation bottles. This suggests that the separation of individual fractions may not be possible, although whether it is necessary is debatable in hindsight. In order to test this hypothesis the next set of incubations will be done with no pre-incubation filtration step, relying instead on the specificity of the individual labels.

During the bacterial filtration efficiency tests it was shown that at least 51.01% of bacteria passed through the membrane filters in a suitable condition to continue to grow and metabolise. For this reason we can not explain the negative uptake result in the same way as for the phytoplankton. However, going back to the original calculations it has been shown a mistake was made and the amount of Leucine to be added to obtain a final concentration of 10nmol/L was underestimated by a factor of 1000. So even if all the tracer was used the uptake would have been too small to detect.

Conclusions and changes to future incubations:

- Separation of the phytoplankton/bacteria seems difficult if not impossible. Phytoplankton cells are almost certainly being killed in the process.
- Future incubations will not separate but instead use the fact bacteria will use leucine above phytoplankton and phytoplankton will use bicarbonate above bacteria. By not separating we also eliminate the problem with assessing attached bacterial production as we will no longer be removing the particulate material pre-incubation.
- The DIC spike is present so no change will be made to the concentration.
- The leucine spike will be remade with the concentration being increased by a factor of 1000.
- The experiment needs to be controlled more tightly. A natural abundance measure of DIC, DOC, and POC needs to be made for each site and at each depth.
- The phytoplankton control sample (not shown in results) needs to be spiked with bicarbonate, not leucine, as do the dark phytoplankton samples. They were spiked with leucine this time around.
- The community respiration samples need to be in opaque bottles, not clear. Respiration will be overshadowed by photosynthesis if in the light, particularly in the summer months.

Appendix 4:

The relative proportions of DIC, DOC and POC were plotted during the incubation campaign. This data was used to obtain the average proportion DIC contributed to organic matter, the percentage of which was then used to correct non-acidified DOM data from the spatial / temporal survey work.

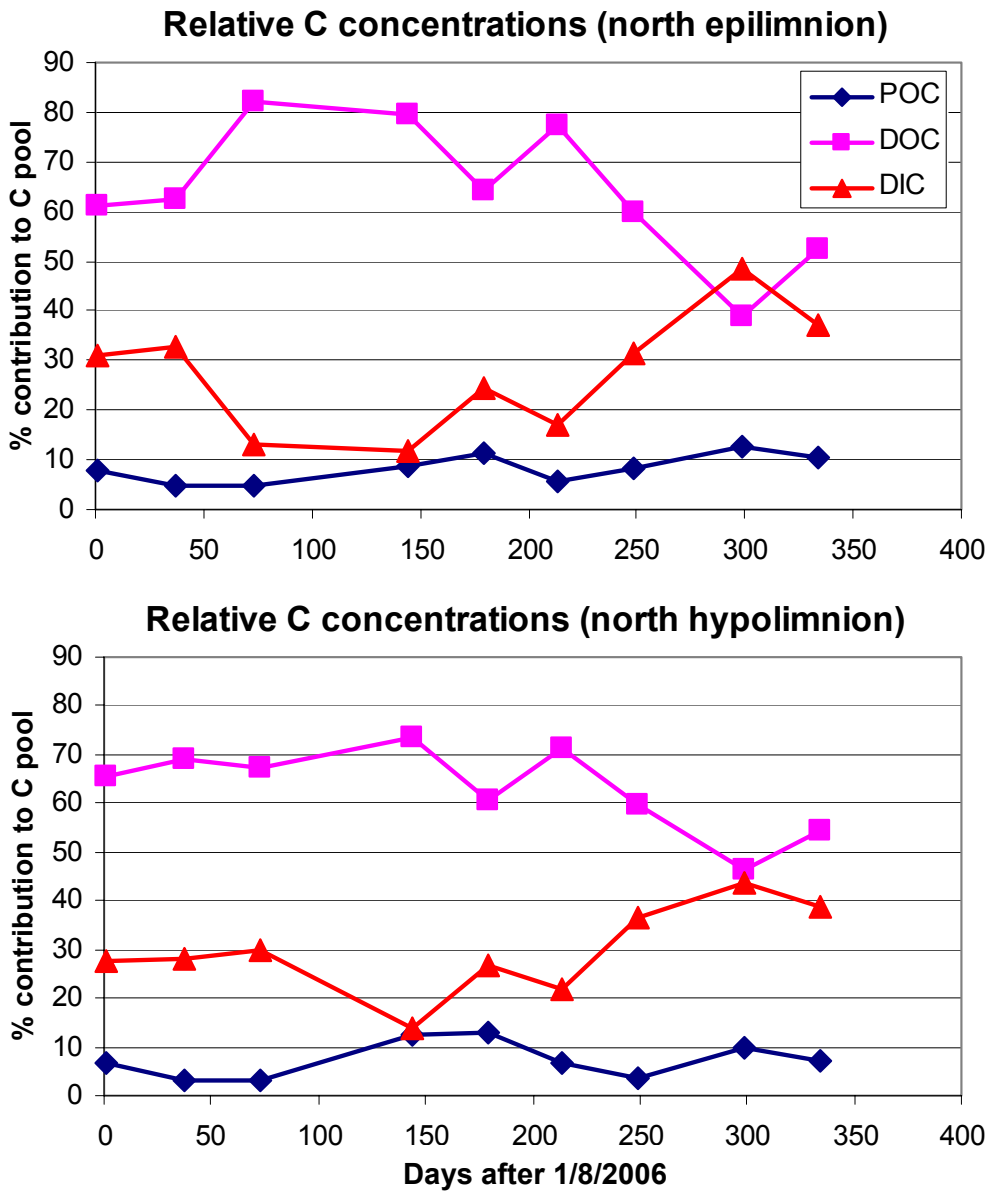

Figure 61: Percentage contributions of POC, DOC and DIC to total carbon pool in the south basin a) epilimnion and b) hypolimnion.

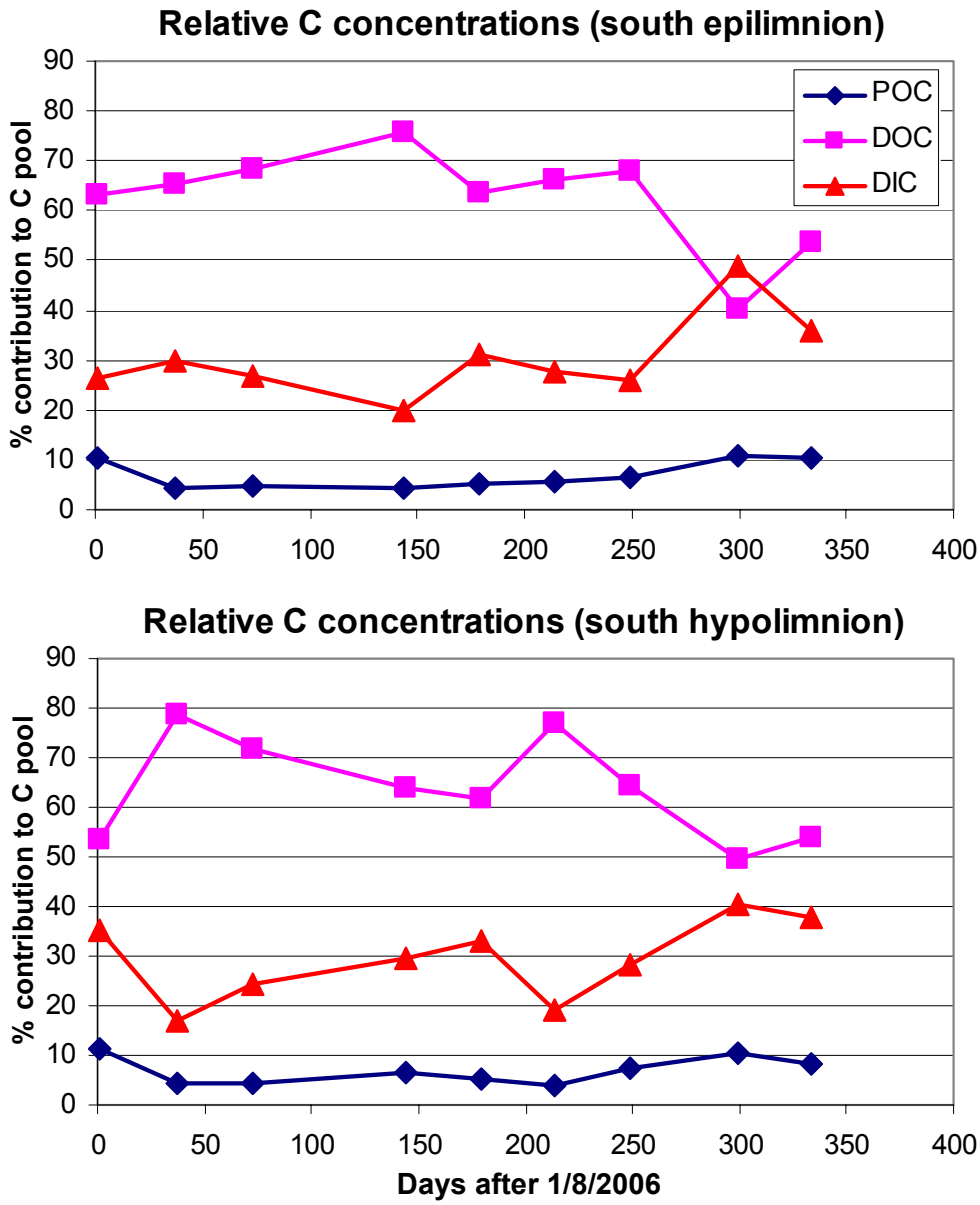

Figure 62: Percentage contributions of POC, DOC and DIC to total carbon pool in the south basin a) epilimnion and b) hypolimnion.

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