On the community and ecosystem level consequences of warming

Yvon-Durocher, Gabriel

On the Community and Ecosystem Level Consequences of Warming

Gabriel Yvon-Durocher

School of Biological and Chemical Sciences
Queen Mary, University of London

Submitted for the degree of
Doctor of Philosophy
of the University of London
July 2010

Abstract

The carbon cycle modulates climate change, via the regulation of atmospheric CO_2, and it represents one of the most important ecosystem services of value to humans. However, considerable uncertainties remain concerning potential feedbacks between the biota and the climate. I used an ecosystem-level manipulative experiment in freshwater mesocosms to test novel theoretical predictions derived from the metabolic theory of ecology (MTE), in an attempt to understand the consequences of warming for aquatic communities and ecosystems. The year-long experiment simulated a warming scenario (A1B) expected by the end of the century. The experiment revealed that (1) Ecosystem respiration increased at a faster rate than primary production, reducing carbon sequestration by 13%. These results confirmed my theoretical predictions based on the different activation energies of these two processes. Furthermore, I provided a theoretical prediction that accurately quantified the precise magnitude of the reduction in carbon sequestration observed experimentally, based simply on the activation energies of these metabolic processes and the relative increase in temperature. (2) Methane efflux increased at a faster rate than ecosystem respiration and photosynthesis in response to temperature. This phenomenon was well described by the activation energies of these metabolic processes. Therefore, warming increased the fraction of primary production emitted as methane by 21%, and methane efflux represented a 9% greater fraction of ecosystem respiration. Moreover, because methane is 21 times more potent as a greenhouse gas, relative to CO_2, this work suggests that warming may increase the greenhouse gas efflux potential of freshwater ecosystems, revealing a previously unknown positive feedback between warming and the carbon cycle. (3) Warming benefited smaller organisms and increased the steepness of the slope of the

community size spectrum. As a result the mean body size of phytoplankton in the warmed systems decreased by an order of magnitude. These results were down to a systematic shift in phytoplankton community composition in response to warming. Furthermore, warming reduced community biomass and total phytoplankton biomass, although zooplankton biomass was unaffected. This resulted in an increase in the zooplankton to phytoplankton biomass ratio in the warmed mesocosms, which could be explained by faster turnover within the phytoplankton assemblages. Warming therefore shifted the distribution of phytoplankton body size towards smaller individuals with rapid turnover and low standing biomass, resulting in a reorganisation of the biomass structure of the food webs. The results of this thesis suggest that as freshwater ecosystems warm they become increasingly carbon limited, resulting in a reduced capacity for carbon sequestration, elevated greenhouse gas efflux potential, and altered body size and biomass distribution.

Acknowledgements

This thesis is dedicated to my father, who nurtured my fascination for science from a very early age and continues to support my academic endeavours with unwavering enthusiasm and excitement.

A special thank you must go to my first supervisor, Jose Montoya for giving me the opportunity to work on this unique project and teaching me the philosophy of scientific study. Thank you also for all the good times in London, Dorset and Barcelona (though I'm still awaiting a visit to the fabled absinth bar).

Thanks also to Mark Trimmer for your bounding enthusiasm about any scientific question and for teaching me the importance of rigorous and detailed measurement (or in Mark's words "belts and braces") when studying biogeochemical cycles.

Thank you also to Guy Woodward, who was the initial catalyst of my scientific career and has continued to support my academic progress by giving me numerous unique opportunities along the way.

Thanks to Drew Allen and Dan Reuman for many fascinating discussions and helping me with theoretical aspects of this thesis. I look forward to our exciting collaborations in the very near future.

A big thanks to my dear friends, flatmates and office-mates Daniel and Jacob. We've shared some great times over the past four years, and without your much needed distraction, completing this work would have been a much more painful (although maybe slightly quicker) task.

I would also like to thank the rest of my family for your unconditional support and inspiration.

Finally and most importantly, thank you to Joanna, for helping me through all of this, for rebuilding my enthusiasm in difficult times and tempering my hyperbole where necessary.

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General Introduction: On the Community and Ecosystem Level Consequences of Warming

The Ecosystem: Reconciling Structural and Functional Components

"*.....the more fundamental conception is as it seems to me, the whole system (in the sense of physics), including not only the organism-complex, but also the whole complex of physical factors forming what we call the environment..... Though the organisms may claim our primary interest, when we are trying to think fundamentally we cannot separate them from their special environment, with which they form one physical system..... Our natural human prejudices force us to consider the organisms as the most important parts of these systems, but certainly the inorganic factors are also parts – there could be no systems without them, and there is constant interchange of the most various kinds within each system, not only between the organisms but between the organic and the inorganic. These ecosystems.....are of the most various kinds and sizes.* " A. G. Tansley, 1935 (pg 299)

Since its inception by Arthur Tansley in 1935, the concept of the ecosystem has borne many guises (Tansley 1935, Lindeman 1942, Odum 1953, O'Niel *et al.* 1986, Jones & Lawton 1992, Likens 1992, Willis 1997), though the fundamental tenet that organisms and their physical, biological and chemical environment are inextricably linked has remained the cornerstone of ecology for almost a century. This definition is suitably broad, as it must encompass the multitude of biogeochemical processes that occur within the ecosystems of the biosphere. However, its brevity has brought about a significant dichotomy in ecological thinking and has hindered its conceptual unification. Ecosystems are frequently conceptualised and studied from one of two overarching perspectives. The "structural" or "community" perspective emphasises the importance of the organisms within the ecosystem and focuses on the interactions, dynamics and diversity of the populations that comprise a community, a viewpoint enormously influenced by the early ideas of Robert MacArthur (MacArthur 1955, 1960, 1972). On the other hand, the

"functional" or "ecosystem" approach advocates the importance of the fluxes and transformations of energy and matter through ecosystems, which are governed by the physical laws of thermodynamics and chemical stoichiometry. This perspective was largely developed by the ideas of Eugene Odum (Odum 1953, Odum *et al.* 1962). These two seemingly different branches of enquiry into the nature of ecosystems are, in reality, inextricably bound to one another and the generality of the nature of this interrelationship is a cornerstone of modern ecology, although one that is often implicit rather than explicit in our thinking. For example, it seems obvious that the distribution of the abundances of different populations in an ecosystem is related to the flux of energy from autotrophs (organisms which synthesise their own energy either from the sun – the photoautotrophs – or from inorganic compounds – the chemoautotrophs) through the community food web. What is less obvious however is the ubiquity of the mechanism (or mechanisms) that governs the flux of energy through ecosystems, and ultimately couples "structural" (i.e. the abundance of populations in this case) with "functional" (i.e. energy flux) attributes.

This fundamental gap in our knowledge has long been a "Holy Grail" for ecologists, and one that has assumed increasing prominence in the last twenty years, as exemplified in the landmark publication "*Linking Species and Ecosystems*" by Jones and Lawton (1992). Understanding how the structure and functioning of ecosystems might be related has taken on increased urgency in light of forecasted global warming due to anthropogenic inputs of CO_2 to the atmosphere (IPCC 2007), and the unprecedented rates of biodiversity loss related to the human domination of the Earth's ecosystems (Vitousek *et al.* 1997). Significant progress has been made thanks to the realisation that the biodiversity within an ecosystem is typically correlated with its ability to perform vital biogeochemical processes (e.g. CO_2 sequestration,

decomposition, primary production, nutrient cycling) (Naeem *et al.* 1994). Biodiversity-ecosystem functioning relationships have provided considerable insight into the linkages between structural and functional components of ecosystems (Loreau *et al.* 2001), though these effects have often lacked generality, owing to the idiosyncratic responses to biotic and abiotic variables (Emmerson *et al.* 2001, Hooper *et al.* 2005) that reflect trait differences among species (McGill *et al.* 2006). These limitations have hindered the development of a single general, mechanistic theory that links biodiversity to ecosystem functioning, suggesting that this approach might not be the most fruitful avenue for developing a more general understanding of the connections between structure and function. Here I approach this problem from a new perspective, by adopting a holistic view of the carbon cycle within an ecosystem and the response of its constituent structural and functional components to an important perturbation – "environmental warming" – in a freshwater mesocosm experiment.

Carbon Cycling within an Ecosystem: A Tractable Model

The cycling of carbon between the organic (i.e. the biotic) and inorganic (i.e. the abiotic) pools of an ecosystem (Fig. 1.1) is a useful place to begin to build an understanding of the interrelatedness of the structure of the biotic communities and the transformations and fluxes of energy, of which carbon is the universal currency (Baird & Ulanowicz 1989). In a simplified view of the carbon cycle of a lentic freshwater ecosystem, biomass is synthesised either from the capture of photons (light energy) by photosynthesis or from the exergonic redox reaction of inorganic chemical compounds by chemosynthesis (i.e. methanogenesis). This biomass, which is predominantly composed of organic carbon compounds, is transferred through the food web

from autotrophs (i.e. the photo- and chemosynthesisers) to heterotrophic primary consumers and then to secondary consumers, and so on. Along each trophic transfer in a food chain a substantial proportion of the energy converted to biomass by an organism is lost as work (i.e. respiration and the production of heat), a result of the 2nd law of thermodynamics (i.e. it is energetically expensive to maintain negative entropy), and is not transferred to its consumer (Lindeman 1942). This is why large organisms at the top of food chains tend to be scarce (Hutchinson 1959): I will revisit this concept again in *chapter five*. At each node in the food web a certain portion of the biomass senesces, and along each link a certain portion of the biomass consumed is excreted. This "dead" biomass is then remineralised by aerobic and anaerobic respiration back to its inorganic constituents by the vast microbial consortium in the benthic and pelagic zones of the lake.

The carbon cycle of an ecosystem forms an energetic feedback loop, where in an idealised "closed" system the energy that enters via autotrophic processes is balanced by the energy released from the system by heterotrophic processes (again a result of thermodynamics). In terms of carbon this is called the "metabolic balance" of the ecosystem, and is determined by the balance between the gross absorption of CO_2 from the atmosphere by photosynthesis (gross primary production) and the total respiration (ecosystem respiration) of fixed carbon which then effluxes back to the atmosphere as CO_2. Because CO_2 is a greenhouse gas and has radiative forcing potential, the net exchange of CO_2 between ecosystems and the atmosphere is crucial in the regulation of global temperature (Lovelock 1972). The response of the metabolic balance of ecosystems to warming is therefore likely to be important for the strength of biotic-atmospheric feedbacks on a global scale and forms the principal theme of *chapter three*. The greenhouse gas efflux potential of an ecosystem is further complicated by considering CH_4 in conjunction with

CO_2, which are the two most important gaseous end products of the remineralisation of organic carbon. The fraction of carbon fixed by the ecosystem that is respired and released as either CO_2 or CH_4 determines its greenhouse gas efflux potential and, because CH_4 has 21 times the radiative forcing potential of CO_2 (Rodhe 1990), the balance of the efflux of these gases may be decisive in determining the propagation of future global warming: I will consider this topic in detail in *chapter four*.

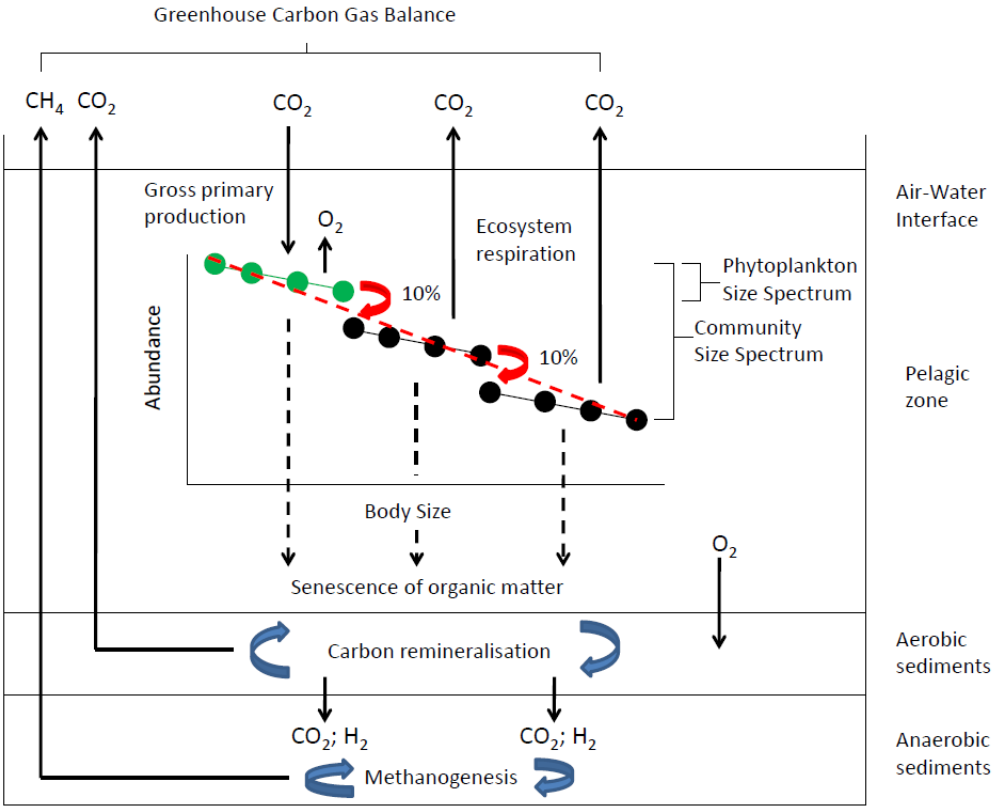

Fig. 1.1. Simplified view of the carbon cycle in a model aquatic ecosystem. The structure of the biotic community is illustrated by the size spectrum. Here the community size spectrum is denoted by the dashed red line, and the phytoplankton size spectrum is donated by the green circles and line, while the heterotrophic size spectrum is given by the black circles and lines. According to energetic equivalence and food web theory the slope of the community size spectrum is expected to be steeper than the slope of the size spectra within trophic levels because ~only 10% of production is transferred between trophic levels and production to biomass ratios vary with trophic level. The biogeochemical processes are denoted by the bold arrows.

A significant advance in developing an understanding of how the structural and functional components of an ecosystem are related has come with the appreciation that the gross

photosynthetic and respiratory fluxes of an ecosystem are the product of the fluxes of all the individual organisms in that ecosystem (Enquist *et al.* 2003, Allen *et al.* 2005). This is a fundamental tenet of the "metabolic theory of ecology" (Brown *et al.* 2004), which offers a foundation for a deeper understanding of the nature of ecosystems and will be discussed in detail later in this chapter. For now it is sufficient to note that metabolism (i.e. the rate at which energy is transformed and allocated within an organism) determines the overall fluxes of energy and materials through communities and ecosystems. The relationship between the abundance and body size of all the organisms in an ecosystem is an important "structural property" (White *et al.* 2007, Reuman *et al.* 2008), which is a useful candidate for linking structure with function. This is because the abundance of organisms (grouped either by the similarity of their body size or by taxonomy) is determined by the amount of energy they receive, either through their interaction with other members of the food web, or by the amount of energy they can synthesise themselves (i.e. in the case of autotrophs). In aquatic ecosystems the relationship between abundance and body size is typically conceptualised as a frequency distribution of individual body sizes, regardless of taxonomy, which has been dubbed the "size spectrum" (Sheldon *et al.* 1972, Gaedke 1993, Kerr & Dickie 2001, Jennings & Mackinson 2003, Blanchard *et al.* 2009). Generally, abundance declines as body size increases and this gives size spectra their characteristically negative slopes (in a log-log relationship). In aquatic ecosystems, especially in the pelagic zones of lakes and oceans, feeding interactions are strongly size structured: small organisms are fed on by larger organisms, which are consumed by progressively larger organisms, and so on (i.e. energy flows from a suite of small, abundant and diverse organisms to larger, rarer consumers) (Cohen *et al.* 2003). Consequently, the dissipation of energy along food chains means that larger organisms become progressively scarcer. The slope and the elevation of the size spectrum (see Fig. 1.1) are key aspects of community structure and provide important

clues to the partitioning of production and biomass and also the efficiency of energy transfer through food webs. They are also likely to be intrinsically related to the abiotic drivers (e.g. environmental temperature), as well as the rates of biogeochemical cycling of key elements (C, N and P) in the ecosystem, though the mechanisms that might drive these interrelations are as yet poorly understood and are a central aim of this thesis to be explored in detail in *chapters five* and *six*.

The Ecological Consequences of Global Warming

The biosphere is in the midst of a pronounced warming trend: global surface temperature has risen by ~ 0.74°C in the last century and is projected to increase by a further 3-5°C over the next 100 years (IPCC 2007). The ecological consequences of recent athropogenic global warming have been far reaching, pervading all levels of ecological organisation, from individuals to ecosystems (Walther *et al.* 2002, Montoya & Raffaelli 2010). Indeed, an ever increasing catalogue of species experiencing altitudinal or pole-ward range shifts, alterations in phenological cycles, and local extinction or extirpation punctuate the literature (Parmesan & Yohe 2003). However, although the ecological impacts of global warming on numerous examples of single taxa are now unequivocal, the potential responses of whole ecosystems remain uncertain. This is often ascribed to the perceived complexity of ecological networks, since the apparently frequent indeterminacy of ecological interactions often appears to preclude clear predictions at these higher levels of organisation (Montoya *et al.* 2006).

Alterations to the carbon cycle are regarded as one of the greatest potential impacts of global warming on ecosystem services (e.g. nutrient cycling, CO_2 sequestration, crop pollination)

supply (Schroter *et al.* 2005, Montoya & Raffaelli 2010). These changes include direct effects – e.g. on productivity, CO_2 sequestration, and resource quality-, but also indirect effects –e.g. on precipitation patterns, water availability, and crop production. Specifically, the carbon sequestration capacity and the greenhouse gas efflux potential of ecosystems have the potential to be dramatically altered by changes in temperature. This is because the metabolic rates of organisms (e.g. their photosynthetic, respiratory and methanogenic rates) are strongly dependent on temperature (Gillooly *et al.* 2001). Furthermore, warming has the potential to shift the size structure of aquatic communities by two main mechanisms, which are not necessarily mutually exclusive. First, organisms might exhibit a degree of phenotypic plasticity to changes in temperature. This hypothesis has been termed the temperature-size rule and posits that reduced organism size at higher temperatures is an adaptive plastic response that results from selection for earlier reproduction as population growth rate increases. The accelerated completion of the life cycle occurs at the expense of maturation size (Atkinson *et al.* 2003). Second, warming has the potential to alter the prevailing selection pressures in the environment which determines the outcome of interspecific competition and therefore the species composition of the assemblages (Finkel *et al.* 2005). Changes in the distribution of body size in aquatic communities could alter carbon sequestration rates in pelagic ecosystems profoundly because the mean particle size of phytoplankton is an important determinant of their sinking rates, and therefore their potential to "bury" atmospheric carbon in sediments. In marine ecosystems this phenomenon is commonly referred to as the biological pump (Fujii *et al.* 2005).

Recent evidence has highlighted the potential for strong positive feedbacks between warming and the carbon sequestration capacity of terrestrial ecosystems, due to the temperature dependence of soil respiration (Knorr *et al.* 2005, Davidson & Janssens 2006). However, this

topic remains controversial as others have suggested that the long term sequestration capacity of soils is unaffected by temperature: for instance, the initial stimulation of soil respiration eventually acclimates as the labile carbon stored in the sediment is consumed (Luo *et al.* 2001). Nevertheless, the longevity of the incipient elevation of soil respiration can last for years (Trumbore 2000), which on a global scale, and in combination with anthropogenic greenhouse gas emissions is likely to alter atmospheric chemistry. There is also increasing concern that the large quantities of stored carbon and CH_4 currently locked up in permafrost regions may thaw if mean global temperatures continue to rise, which could result in a potentially catastrophic emission of greenhouse gases (Mastepanov *et al.* 2008).Given the emerging evidence for strong positive feedbacks between biogeochemical cycles and future global warming, the need to develop accurate and widely applicable coupled climate-carbon models, linking the biotic components of the C cycle to the multiple aspects of climate change has increased in urgency in recent years (Cox *et al.* 2000, Friedlingstein *et al.* 2006). In addition to the mounting evidence for feedbacks between biogeochemical processes and warming, new results suggest that "*reduced body size is the third universal response to global warming*, *besides range, and phenological shifts*" (Daufresne *et al.* 2009), and changes in the size-structure of communities in response to warming are beginning to be documented across a range of ecosystem types and spatial scales (Finkel *et al.* 2005, Winder *et al.* 2009, Moran *et al.* 2010) which are also likely to be important for determining the propagation of future global warming.

Despite the abundance of ecological evidence of recent global warming across a diverse range of spatio-temporal scales and levels of biological organisation, we still understand relatively little about the underlying mechanisms driving this change, and as a result our predictive capabilities remain limited. Further, a coherent comprehension of the potential

synergies between the multifarious effects of warming at the ecosystem level is urgently required if we are to successfully address the challenges brought about by environmental change. It is the overarching goal of this thesis to make a first step in developing a better understanding of the feedbacks between warming, community size structure and the biogeochemical cycling of carbon in aquatic ecosystems. I have taken a multidisciplinary approach to tackle this problem, by developing bioenergetic models based on the metabolic theory of ecology which use the physical and chemical laws that govern the flux of energy through biological systems to make robust predictions. I have combined this theoretical approach with an ecosystem scale experimental manipulation that attempted to simulate the effects of future warming on shallow lake ecosystems and to test the predictions of my models.

The Metabolic Theory of Ecology and Global Change

Metabolism is the rate at which an organism uptakes energetic and material resources from its environment, transforms them into useable forms and provisions them to the biochemical processes necessary for growth, survival, and reproduction (Brown *et al.* 2004). Metabolic rate therefore "sets the pace of life" and determines the reciprocal interactions between organisms and their environment. Over the past decade the rich history of research into the comparative physiology of organisms (Klieber 1961, Peters 1983) has been combined with a more mechanistic, theoretical perspective taken from physics (West *et al.* 1997) that attempts to explain biological phenomena in terms of physical and chemical laws: this integration underpins the "metabolic theory of ecology" (Brown *et al.* 2004).

The metabolic theory of ecology (MTE) has developed two broad types of models. The first set of models attempt to explain the size and temperature dependence of metabolic rate. The relationship between body size and metabolic rate has had a long tradition in biology that can be traced back to Max Rubner (1883), who was the first to demonstrate that whole organism metabolic rate typically scales as the $^{2}/_{3}$ power of body size, and was thought to be related to the rate at which heat generated from metabolism was dissipated through the body surface (Rubner 1883). However, Max Kleiber's influential monograph demonstrated that metabolic rate actually scaled as the ¾ power of body size, which has been known since as Klieber's law (Klieber 1961). This scaling relationship has subsequently been extended to encompass a diverse array of prokaryotic and eukaryotic life, from a single mitochondrion to a blue whale (West *et al.* 2002). In 1997 a model developed by West *et al*., proposed to account for the quarter power scaling of metabolism (mass specific metabolic rate scales as the -¼ power of body size) with body size.

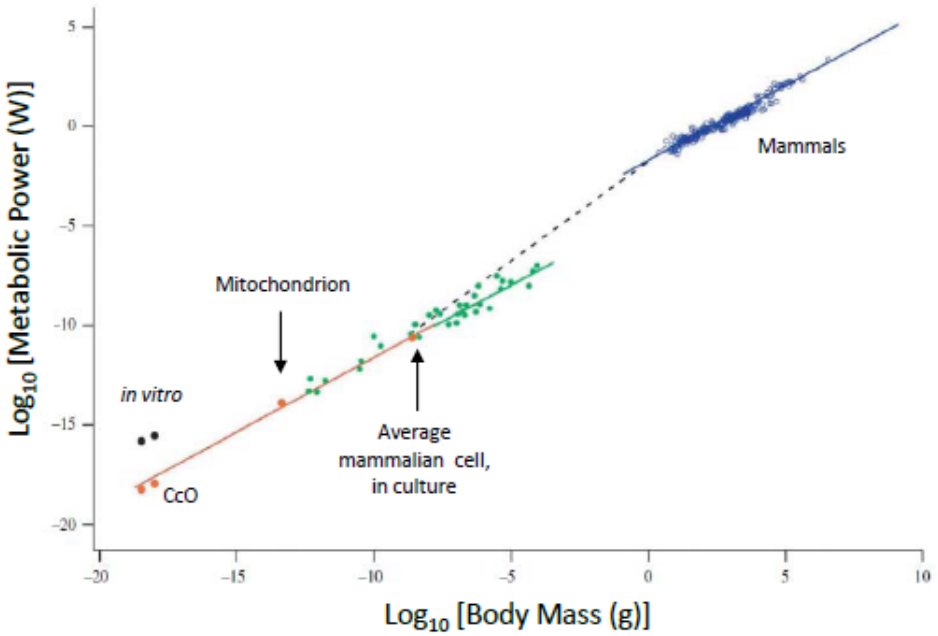

Fig. 1.2. An extension of Kleiber's ¾ power law for the metabolic rate of mammals, to cover 27 orders of magnitude from individuals (blue circles) to uncoupled mammalian cells, mitochondria and terminal oxidase molecules, CcO, of the respiratory complex (red circles). Also shown are data from unicellular organisms (green circles). Figure redrawn from West *et al*., (2002).

In their model, West *et al*., (1997) made three simplifying assumptions: (i) the biological distribution networks that deliver energy and materials to cells are fractal-like in their geometry; (ii) the terminal metabolic units (i.e. mitochondria and chloroplasts) of these networks are energetically invariant, meaning that the energy flux per metabolic unit is independent of body size and (iii), the energy required to distribute resources through the network is minimized. The "universality" of quarter power scaling laws in biology is however shrouded in controversy and there have been numerous contravening examples to the ¾ or the -¼ scaling of metabolism (Glazier 2005, Makarieva *et al.* 2008) as well as criticisms of the mathematical foundations of the theory, which have polarised the field (Kozlowski & Konarzewski 2005, Makarieva *et al.* 2005). In fact, new research (DeLong *et al.* 2010) has demonstrated that the ¾ power scaling of metabolic rate does not hold across all evolutionary domains of life, for prokaryotes the relationship is super-linear (i.e. >1), while for protists it is linear (i.e. =1), suggesting that the theory of fractal distribution networks is also invalid for these groups.

The exponential increase in biological rates with temperature, including metabolic rate has been well established for over a century. The kinetics of metabolism are well described by the Boltzmann factor or the Van't Hoff-Arrhenius relation $e^{-E/kT}$, where E is the activation energy of metabolism, k is Boltzmann's constant and T is absolute temperature. This exponential form describes the temperature dependence of metabolism of almost all organisms, from unicellular organisms to the largest multicellular plants and animals (Gillooly *et al.* 2001). Together, the joint effects of body size and temperature can be combined in the general model of the MTE to describe the metabolic rate of an individual organism:

$$B_i = b_0 e^{-E/kT} M_i^{3/4} \quad (1)$$

where B_i is the metabolic rate of an individual i, b_0 is a normalisation constant that is independent of body size and temperature and M_i the individual's body size. This simple model is the foundation of the MTE and provides a potential platform for a deeper understanding of the structure and functioning of individuals, populations, communities and ecosystems, whose dynamical interactions with their environment can be explained, to a large degree, by metabolism.

The second class of models that have been derived from the MTE have built upon these foundations to explore the consequences of metabolism across almost all levels of biological organisation, from genomes and subcellular organelles (Gillooly *et al.* 2005, Savage *et al.* 2007, Allen & Gillooly 2009) to the structure and dynamics of populations (Savage *et al.* 2004), communities and ecosystems (Enquist *et al.* 2003, Allen *et al.* 2005, Allen & Gillooly 2009). Arguably one of the greatest steps forward in developing a mechanistic understanding of the structure and functioning of ecosystems offered by the MTE, and of the greatest direct relevance to this thesis, is the appreciation that the biogeochemical fluxes at the ecosystem level (e.g. gross primary production, ecosystem respiration) are the product of the sum of all the metabolic rates of all the organisms within the system under study (Enquist *et al.* 2003, Allen *et al.* 2005). For example:

$$B_{tot} = \frac{1}{V} B_0 e^{-E/kT} \sum_{i=1}^{n} M_i^{3/4} \quad (2)$$

where B_{tot} is the metabolic flux of the ecosystem (i.e. gross photosynthesis or respiration) B_0 is a normalisation constant that is independent of mass and temperature and n is the number of

individuals of i in the volume V of the ecosystem. This realisation provides great predictive power and underpins the development much of the theory presented in this thesis. For example, equation 2 is particularly useful for exploring the consequences of warming on ecosystem metabolism because it is implicit in equation 2 that the temperature dependence of ecosystem metabolism is equal to the activation energy of metabolic rate at the individual level (Enquist *et al.* 2003, Allen *et al.* 2005). This can be seen best by taking logarithms of both sides of equation 2 (Fig. 1.3):

$$\ln(B_{tot}) = -E\left(\frac{1}{kT}\right) + \ln\left(\frac{1}{V} B_0 \sum_{i=1}^{n} M_i^{-3/4}\right) \quad (3)$$

In equation 3 the natural logarithm of ecosystem metabolism is a linear function of the inverse of absolute temperature ($1/kT$), with a slope (i.e. the temperature dependence) given by the activation energy of metabolism.

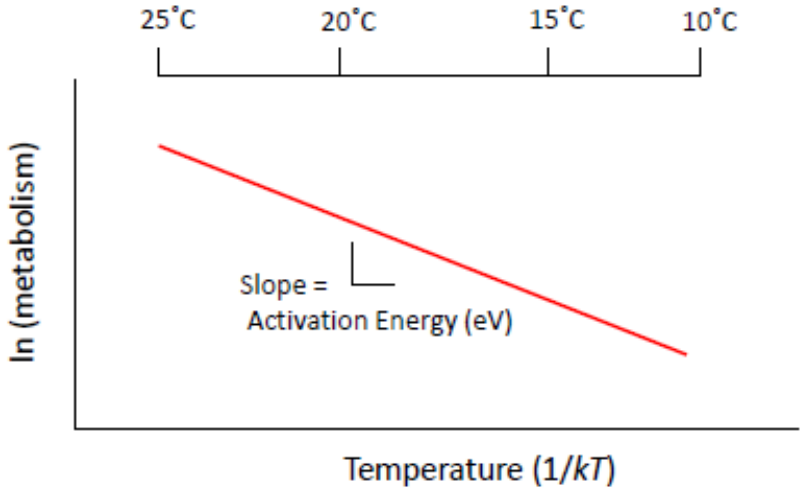

Fig. 1.3. Example of a typical Boltzmann-Arrhenius plot. Here the natural logarithm of metabolic rate is plotted against the reciprocal of absolute temperature. Note that temperature increases in the opposite direction on the x axis. The slope provides an estimate of the of the activation energy (eV; 1eV = 96.49 KJ mol^{-1}) of metabolism, i.e., the average amount of energy required to catalyse the reactions involved in metabolism

Equations of this general form will be used frequently throughout this thesis to make predictions on the effects of warming on the fundamental biotic components of the carbon cycle.

Despite its obvious appeal – it provides a simple general mechanism for explaining diverse ecological phenomena – the MTE has come under heavy criticism mainly for its perceived oversimplification and tendency to ignore, rather than incorporate, its exceptions (Glazier 2005, Kozlowski & Konarzewski 2005, Makarieva *et al.* 2005, Hawkins *et al.* 2007, Makarieva *et al.* 2008, Glazier 2010). One of the major criticisms of the MTE is that it is anchored to a "universal ¾ power-law". Recent work has demonstrated that there is often considerable inter- and intraspecific variation which surrounds the ¾ power mass scaling of metabolic rate (Glazier 2005), and it has been argued that the focus of the current model [i.e. the model of (West *et al.* 1997)], which explains the average exponent, should be shifted to explain extreme boundary limits of this exponent (Glazier 2010). However, proponents of the MTE have responded with the counter-argument that to find general laws in science with broad predictive capabilities it is sometimes necessary to give less credence to the variability and focus emphasis on the underlying patterns (Allen & Gillooly 2007). Whatever its shortcomings the MTE has provided ecology with a quantitative set of predictions derived from first principals that can be tested with empirical data. Therefore, the MTE has generated a huge amount of research over the past decade in an attempt to either prove or disprove its predictions and this has been a significant positive influence on ecology, by stimulating debate and providing a framework within which to attempt to link previously seemingly disparate disciplines (e.g. community and ecosystem ecology).

Ecosystem Level Manipulations: Simulating Warming

The experimental component of this study involved a replicated, ecosystem level manipulation of freshwater mesocosms which simulated the potential effects of future warming on aquatic ecosystems (Fig. 1.4): this experimental set-up is described in detail in *chapter two*. Briefly, the experiment consisted of twenty freshwater mesocosms: ten replicates remained at ambient temperature, whilst the other ten were maintained at 3-5°C (mean 4°C) above ambient, in line with the A1B warming scenario predicted for temperate latitudes by the end of the 21st century (IPCC 2007). Mesocosm experiments represent an inevitable compromise between the control and replication of laboratory studies and the realism of descriptive field surveys but, despite their limitations, they can provide a useful tool for predicting how global change scenarios might affect ecosystem level processes (Benton *et al.* 2007).

Fig. 1.4. (a) Aerial view of the global warming mesocosm experiment in April 2008. The experimental plot consisted of 20 mesocosms: 10 heated and 10 unheated. (b) Close-up of mesocosm 1 (heated) in April 2008, highlighting the presence of diverse floral and faunal assemblages.

In particular, they afford the opportunity to isolate the effects of temperature from other potentially confounding variables (e.g. latitude, altitude, nutrient avialability) on the structure of entire replicated ecosystems, and permit direct comparisons to be made between the structure

and functioning of ecosystems under ambient conditions with those of their "future" warmed counterparts (Table 1.1). This thesis can therefore be understood from two perspectives. First, the theoretical and experimental results represent an "applied" approach that can provide much-needed insight into the potential future consequences of warming on aquatic ecosystems and aid in developing more mechanistic, predictive capabilities to tackle future climate change. Second, warming can be viewed as a perturbation experiment within a purely fundamental scientific perspective that aims to provide a deeper understanding of the intricate and reciprocal nature between community structure and ecosystem functioning.

Study Scale	Advantages	Disadvantages
Lab Microcosms	-Easily to manipulate environmental variables -High level of replication -Ability to focus on specific mechanisms or variables	-Difficult to scale up to natural systems -Only focus on a sub-set of species/traits/individuals -Short temporal duration -Cannot incorporate natural stochasticity
Field Mesocosms	-Intermediate level of replication -Large enough to reproduce realistic community structures -Ability to focus on specific mechanisms/variables	-Closed systems -Often short temporal duration
Field Manipulations	-Natural community compositions -No issues with realism	-Low level of replication -Perturbation may be unrealistic -Confounded by co-variables (e.g. nutrient limitation)
Spatial Field Surveys	-Natural community compositions -Incorporate natural stochasticity	-Confounded by co-variables (e.g. biogeographical effects) -Long temporal series required

Table 1. Advantages and disadvantages of different scales of ecological experiments used to answer global change questions. This thesis focuses on field mesocosm experiments.

Goals of the Thesis

The overarching goal of this thesis was to understand the reciprocal relationship between community structure and ecosystem functioning and to provide a first glimpse of the potential ecosystem level responses to future warming. To achieve this I addressed the following

subsidiary aims and hypotheses using the experimental and theoretical approach described above:

1) To gain predictive capabilities to assess how the metabolic balance of aquatic ecosystems might respond to future warming scenarios (*chapter three*).

    a) I expect the ratio of ecosystem respiration (ER) to gross primary production (GPP), which determines the net carbon sequestration capacity of the ecosystem, to increase with increases in temperature. This is because the activation energy for respiration (E_r) is greater than that of photosynthesis (E_p), therefore respiration should increase faster than photosynthesis with increasing temperature. I expect that this will reduce the ability of warmer ecosystems to sequester carbon.

2) To gain a mechanistic understanding of how the rate of methane efflux in relation to primary production (i.e. CO_2 absorption) and ecosystem respiration (CO_2 efflux) will be affected by warming (*chapter four*).

    a) Similarly to hypothesis 1a, I expect the ratio of methane emission (ME) to GPP and ME to ER to increase with increases in environmental temperature. The ratio of ME/GPP represents the proportion of carbon absorbed by the ecosystem that is subsequently released as methane due to the anaerobic remineralisation of organic matter. The ratio of ME/ER represents the proportion of ER that is effluxed to the atmosphere as methane and gives an indirect estimate of the balance between CH_4 and CO_2 emission. In both cases I expect these ratios to increase with temperature, because methanogenesis should have a higher activation energy than aerobic respiration or photosynthesis. This is because the entropy change in the biochemical reaction is particularly great (Conrad & Wetter 1990).

3) To explore how the size structure and distribution of biomass in aquatic ecosystems might be expected to respond to future warming (*chapter five*).

    a) In line with recent observations in aquatic ecosystems I expect warming to favour small sized organisms (Daufresne *et al.* 2009), which will drive an increase in the steepness of the slope of the size spectrum (see section carbon cycling within an ecosystem: a tractable model, for a definition).

    b) If the resource supply rates of limiting resources remains unchanged with increases in temperature by applying the MTE I would expect total community biomass to decline with temperature according to $e^{-E/kT}$.

I will develop these predictions in greater detail and test them empirically at the ecosystem scale using a replicated freshwater mesocosm experiment in chapters three, four and five.

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General Methods: The Mesocosm Experiment

Experimental Design

The field-based mesocosm experiment was established in December 2005 by Dr Jose Montoya as part of a NERC fellowship (NE/C002105/1), and the research presented in my thesis is focused on a 12-month period of intensive sampling I undertook between April 2007 to April 2008. The experiment was based at the Freshwater Biological Association River Laboratory (2°10`W, 50°13`N) East Stoke, Dorset, UK. Twenty artificial ponds, each holding $1m^3$ of water were set up to mimic shallow lake ecosystems (Fig. 2.1): this scale of mesocosm reproduces the key elements of community structure (e.g. diversity, trophic complexity) and functioning (e.g. nutrient cycling) of shallow lake ecosystems (Jones *et al.* 2002, McKee *et al.* 2003, Ventura *et al.* 2008).

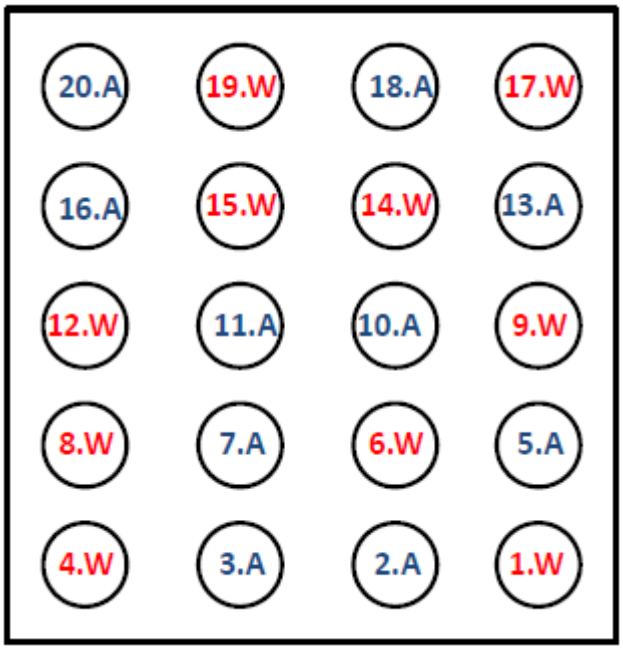

Fig. 2.1. Schematic diagram of the design of the mesocosm experiment, highlighting the position of warmed (W) and ambient (A) treatments.

Ten of the 20 ponds were warmed 3-5°C above ambient temperature, in accordance with the IPCC A1B global warming projections for the next 100 years for temperate areas in the northern hemisphere (IPCC 2007). Experimental warming was achieved by an electronic heating element connected to a thermocouple which monitored the temperature in a given heated and unheated treatment pair of mesocosms. Temperatures were logged every 5 minutes over the entire year using HOBO temperature-irradiance data loggers and regular adjustments were made to ensure that temperature differences between treatments were ~4°C. The mean annual temperature difference between treatments was 4.1°C (±SE 0.01; Table 2.1)

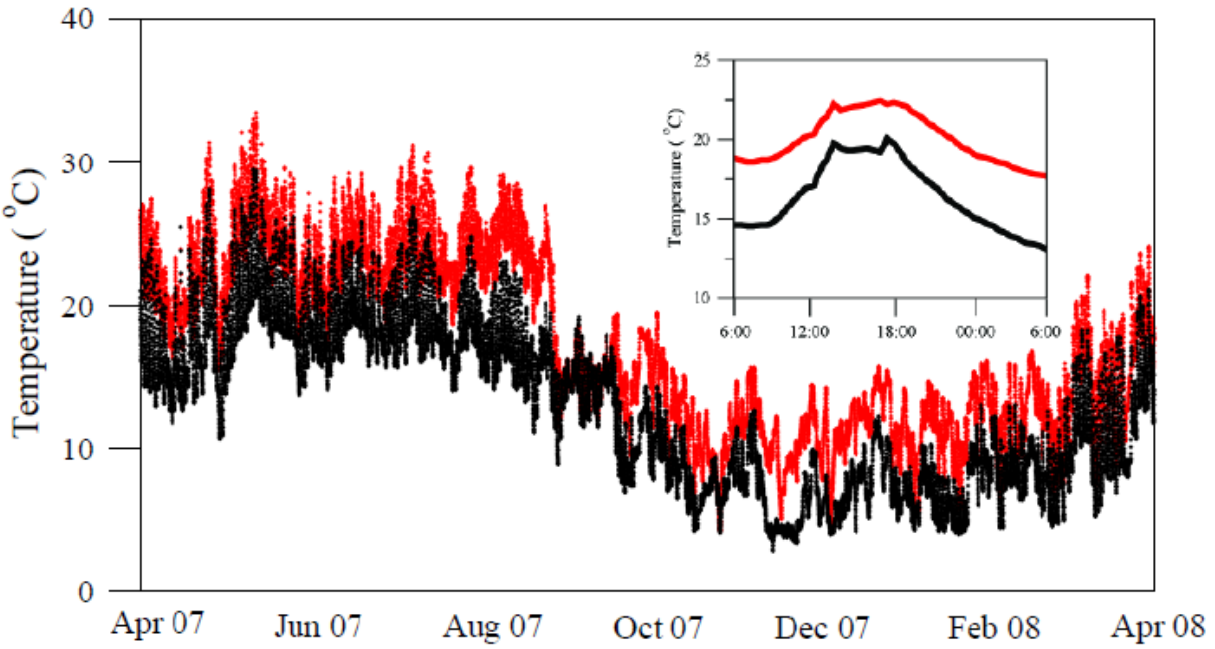

Fig. 2.2. Annual temperature differences between heated and unheated treatments. Example of temperature regimes over the course of the experiment for a heated and unheated treatment pair**.** Pond 1 (Heated: Red; see fig. 2.1) and Pond 2 (Unheated: Black; see fig. 2.1). The mean temperature difference over the year was 4.8°C, ± 0.0096. Insert, diel temperature differences between heated and unheated treatments. Example of diel temperature regimes for a heated and unheated treatment pair. Pond 1 (Heated: Red) and Pond 2 (Unheated: Black).

The mesocosms were seeded in December 2005 with organic substrates and a suite of organisms, representing a pelagic and benthic community that contained representative species from primary producers to top predators (Roach, *Rutilus rutilus*), and a suite of intermediate invertebrate

consumers (zooplankton, including *Daphnia* and *Bosmina*, and benthic macroinvertebrates, including Mollusca, Malacostraca, Trichoptera, Ephemeroptera, and Odonata) to mimic, as far as possible, the organismal composition, trophic complexity and physical structure of shallow lake ecosystems. The submerged macrophytes *Elodea canadensis* Michaux, Myriophyllum spicatum L. and *Ceratophyllum spicatum* L. were added to each pond in equal quantities (250 g wet weight) and *Chara contraria* A. Braun ex Kutz colonized all 20 ponds during the experiment. The biota was left to establish for ten months prior to the onset of experimental warming, which commenced in September 2006, thereby allowing time for further natural colonisation before the start of the annual sampling period on the 11th April 2007. Populations of the introduced top predator, *R. rutilus* were maintained at constant densities [two individuals (age 1+) per mesocosm (~12 g C m^{-3})] in all mesocosoms and monitored via regular eletro-fishing surveys. Because the fish were maintained at predetermined biomass-densities they merely served to "complete" the food webs to mimic natural shallow lakes and were not considered further in the analyses.

Mesocosm Pair	Mean Temperature difference (°C)	± SE
P1+P2	4.802	0.010
P3+P4	N/E	N/E
P5+P6	3.862	0.013
P7+P8	4.700	0.012
P9+P10	4.050	0.015
P11+P12	2.959	0.016
P13+P14	3.885	0.011
P15+P16	4.337	0.013
P17+P18	3.832	0.018
P19+P20	4.727	0.016
Overall mean	4.128	0.010

Table. 2.1. Summary of the temperature differences between heated and unheated treatments over the course of the experiment (April 2007-April 2008). No data were available for ponds 3 and 4 due to failure of the data loggers (N/E).

General Equipment and Techniques

(a) Measuring Dissolved Oxygen

Changes in the concentration of dissolved oxygen were used to measure rates of ecosystem metabolism (either gross photosynthesis or respiration). Measurement of dissolved oxygen was achieved by deploying YSI 600XLM multiparameter Sondes equipped with 6562 rapid pulse™ dissolved oxygen sensors in the mesocosms 24 hours (Fig. 2.4).

Fig. 2.4. Photograph of the YSI600XLM dissolved oxygen sensor deployed in the centre of the mesocosm.

The Sondes measure dissolved oxygen by means of a Clark-type sensor which is polarized at a voltage sufficiently negative to cause oxygen to be reduced to hydroxide ion at the cathode and silver metal to be oxidized to silver chloride at the anode. The oxygen diffuses through the Teflon membrane. The current associated with this process is proportional to the oxygen present in the solution outside the membrane. A unique feature of this sensor is the rapid pulse™ technology which prevents significant consumption of oxygen by the sensor during electrolysis. To minimise oxygen depletion, the probe electrodes are rapidly polarized and depolarized during a measurement sequence and the sensor measures the charge associated with the reduction of oxygen during a carefully controlled time interval. The net charge is therefore proportional to the oxygen partial pressure in the medium. The oxygen sensors were calibrated in water saturated air using a specially designed calibration capsule attached to the probe. Calibration accuracy was determined against know oxygen saturation for the given temperature and pressure prior to

deployment. The YSI 600XLM oxygen probe consisted of an internal data logger which facilitated the deployment of the probe for 24 hours.

(b) Measuring Methane on the Gas Chromatogram

To measure methane (CH_4) emission from the mesocosms in-situ gas samples were taken from custom built chambers positioned at the air-water interface (details of the precise sampling protocol are given in *chapter four*) and were stored in 3 mL gas tight vials (Exetainers; Labco, High Wycombe, UK). The CH_4 concentration in the headspace of the sample was determined by gas chromatography. Samples (50 µl) were withdrawn from the headspace of the sample vials and injected using an auto-sampler (Multipurpose Sampler MSP2, Gerstel, GmbH, Germany) into a gas-chromatograph fitted with a flame ionising detector (GC/FID; Agilent Technologies, UK). Gases (CO, CH_4, CO_2) were separated on a stainless steel column (length 6' x 1/8″∅) packed with Porapak (Q 80/100) at 30^{o}C with zero grade N_2 (British Oxygen Company, BOC) as the carrier gas (14 ml min^{-1}). CH_4 was combusted in H_2 and zero grade air (40 and 450 ml min^{-1} respectively) and measured with a FID detector heated to 300°C. Headspace concentrations of CH_4 were calculated from peak areas calibrated against known standards (Scientific and Technical gases, Staffs, UK) and were converted into µmol m^{-2} accordingly (Fig. 2.3).

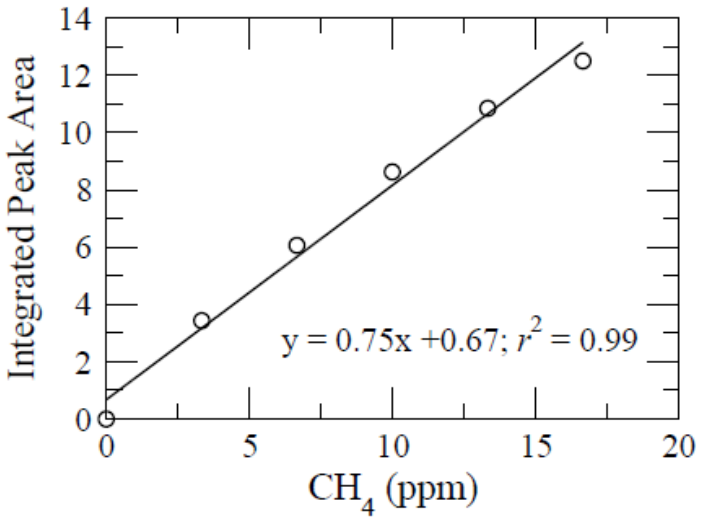

Fig. 2.3. Example calibration for CH_4. The detected peak area from the gas chromatogram is regresses against known standards, formula is then used to determine the actual concentration of CH_4 in each sample.

Potential Confounding Variables

(a) Inorganic Nutrient Regime

The fundamental goal of this thesis was to determine the potential effects of warming on the carbon cycle of aquatic ecosystems. Therefore, it was crucial to isolate the effects of warming *per se* from any other potentially confounding variables that might influence rates of C cycling. One such potentially confounding variable is the extent of inorganic nutrient limitation, because this can strongly influence rates of primary production (Woodward 2007) and also the size structure of phytoplankton communities (Finkel *et al.* 2005, Winder *et al.* 2009). To determine whether the experimental treatment (i.e., warming) affected the extent of nutrient limitation in the mesocosms, I made detailed seasonal measurements of the major inorganic nutrients which might be expected to regulate primary production: water samples for measuring dissolved inorganic nutrient concentrations were collected from mid depth in each mesocosm at 9am on each sampling occasion. Samples were filtered (Whatmann GF/F) and stored frozen (-20˚C) for subsequent determination of NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} and Si ($Si(OH)_4$) using a

segmented flow auto-analyser (Skalar, San++, Breda, Netherlands), according to (Kirkwood 1996).

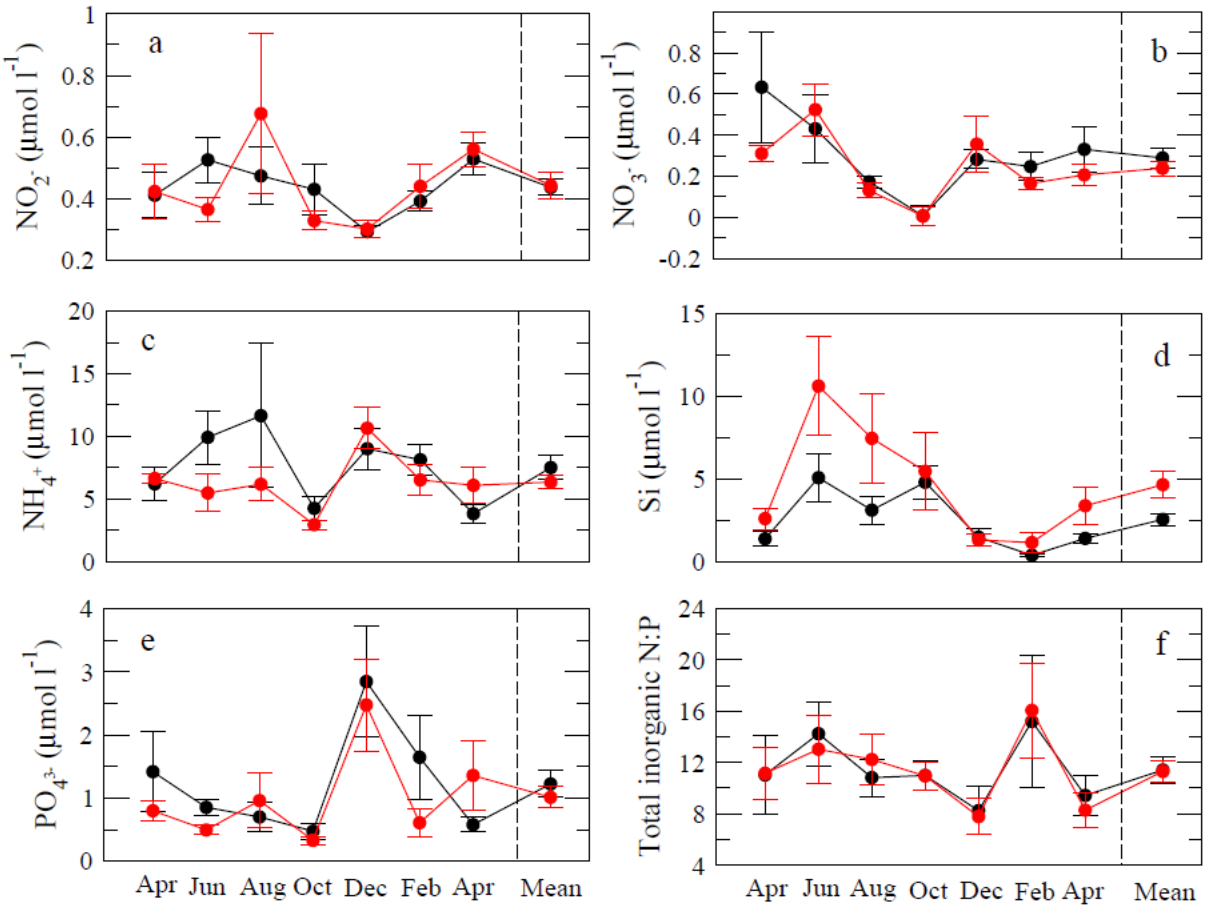

Fig. 2.5. Seasonality of inorganic nutrients in the warmed (red lines) and ambient (black lines) mesocosms. (a) Nitrite, (b) Nitrate, (c) Ammonium, (d) Silicate, (e) Phosphate, (f) the stoichiometry of the inorganic nutrient pool, N:P.

Inorganic nutrients (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-} & Si) exhibited strong seasonal trends (Fig. 2.5). For example, NO_3^- concentrations peaked in spring and declined progressively throughout the summer, and were depleted to ~0.005 µmol l^{-1} by October, before remineralisation in the winter. Concentrations of NO_3^-, NO_2^-, NH_4^+ and PO_4^{3-} showed identical seasonal patterns in the warmed and ambient treatments, with no significant differences in the overall mean annual

concentrations of these nutrients (Table 2.2). Furthermore, the stoichiometry of the inorganic nutrient pool exhibited remarkable similarity between treatments, with a mean annual ratio of total inorganic N to P of ≈11:1 in both heated and ambient mesocosms. The only inorganic nutrient which differed markedly between treatments was Si, which exhibited the greatest differences between warmed and ambient mesocosms during the spring and summer (Table 2.2): I explore the possible explanations for this pattern in detail in *chapter 5*.

Importantly, consistency of the dissolved inorganic nutrient concentrations between experimental treatments meant that this potentially confounding variable, because it represents the potential resource supply rate for primary production, could be discounted from further analyses: i.e., any changes in carbon cycling in the mesocosms could be ascribed to the effects of warming *per se*.

Inorganic Nutrient	DF	*F*	*P*
NO_2^-	1, 120	0.06	0.812 (NS)
NO_3^-	1, 120	0.65	0.420 (NS)
NH_4^+	1, 120	0.23	0.632 (NS)
Si	1, 120	6.08	**0.015**
PO_4^{3-}	1, 120	0.68	0.412 (NS)
Total inorganic N to P	1, 120	0.009	0.922 (NS)

Table. 2.2. Results of the linear mixed effects model testing for differences in the concentration of inorganic nutrients between heated and ambient mesocosms. A linear mixed effects model was conducted with restricted maximum likelihood methods using the *lme* (linear mixed-effects model) function in R, treatment (heated or unheated) was the fixed effect, and temporal pseudo-replication from repeated sampling of the mesocosms over the year was accounted for by including mesocosm identity nested within sampling occasion as random effects.

(b) Air-Water Gas Exchange due to Advection and Diffusion

Apart from biological metabolic activity, gas exchange with the atmosphere due to diffusion and advection is an additional factor that might affect the DO concentration and may also affect the interpretation of my results (Cole & Caraco 1998). The flux of oxygen across the

air-water interface is dependent on the concentration gradient between the water and the overlying air, and the gas transfer velocity, *k*, (otherwise known as the piston velocity). Gas flux across the air-water interface is described by the following equation (Cole & Caraco 1998):

$$f = k(C_{water} - C_{eq}) \qquad (1)$$

where k is the gas transfer velocity (cm h^{-1}), and $C_{water} - C_{eq}$ is the concentration gradient of the gas between the water and the concentration that would be at equilibrium with the atmosphere. In running waters characterised by turbulent flow, reaeration due to physical processes is typically a crucial determinant of the concentration of dissolved gases, and must therefore be accounted for in calculation of metabolism from changes in the concentration of dissolved gases (Marzolf *et al.* 1994, Mulholland *et al.* 2001). In still waters however, reaeration due to turbulence is less important and k is typically determined by wind velocity (Cole & Caraco 1998) which determines surface water turbulnce. In the present study measured wind velocities were typically very low (average 0.53 m s^{-1}), with only 2.22% of measurements above 3 m s^{-1}. Importantly, k is largely independent of wind velocity at wind speeds < ~3 m s^{-1} (Cole & Caraco 1998) therefore, enhanced gas exchange due to the turbulence created by wind was not considered in the calculations of ecosystem metabolism. However, advective processes which also determine k at low winds might still have been influenced by the heating of the mesocosms (i.e. through convection). To determine whether experimental warming systematically altered the gas transfer velocity I estimated k from simultaneous measurements of the efflux of CH_4 and dissolved CH_4 from:

$$k = f/(C_{water} - C_{eq}) \qquad (2)$$

where *f* is the measured efflux of CH_4 across the air-water interface, C_{water} - C_{eq} is the concentration gradient of the gas in the water and the concentration in the water at equilibrium with the atmosphere (C_{eq}). C_{eq} was calculated using the equations of (Yamamoto *et al.* 1976) and the measured mixing ratio for CH_4 in the air and temperature of the water on each occasion. Details of the precise methodology employed in the measurement of CH_4 efflux and the concentration of dissolved CH_4 are given in detail in *chapter four*, however, a discussion of the potentially confounding effects of advective gas exchange are relevant at this early stage because they have the potential to influence the findings of all aspects of ecosystem metabolism discussed in this thesis.

The gas transfer velocity, *k*, exhibited no clear seasonal variability (i.e. it was independent of seasonal changes in temperature; Fig. 2.6) and was not significantly different between treatments on average over the course of the experiment (Fig. 2.6; $F_{1,123} = 3.46$; $P = 0.068$). This evidence suggests that the physical influence of heating the mesocosms by ~4˚C had little discernable effect on advective processes. Consequently, this potentially confounding variable can be discounted from further analyses of the biogeochemical processes in the experiment, and changes in the concentration of oxygen and methane in the water column and the efflux of methane across the air-water interface can be ascribed to biological metabolism.

In addition, gas exchange due to diffusive flux alone (i.e. molecular diffusion) was considered insignificant and similarly excluded from further analyses for a number of reasons. Firstly, the diffusive capacity of oxygen into and out of water is extremely low compared with other processes (i.e. advection or biological consumption/production) (Strumm & Morgan 1996). For example, the diffusive flux (calculated using Fick's 1st law: Flux = $da[C_{sat}-C_{wat}]$, where *d*

is the diffusivity constant and *a* is the surface area to volume ratio) was typically a vanishingly small proportion of the total oxygen pool of the mesocosms.

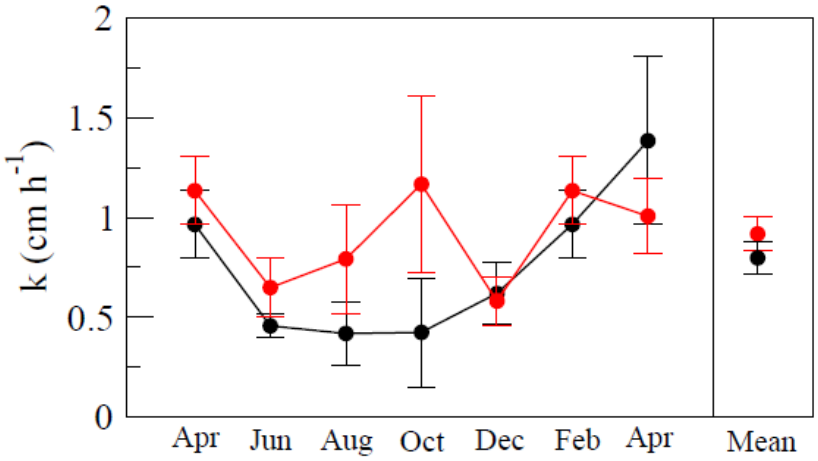

Fig. 2.6. Seasonal trends in the gas transfer velocity (*k*), between heated (red lines) and unheated (black lines) treatments. The gas transfer exhibited little seasonal variability and was not systematically affected by the heating of the mesocosms (Table 4.1). Data were natural log transformed for statistical analysis but presented here untransformed for ease of interpretation. A linear mixed effects model was conducted with restricted maximum likelihood methods using the *lme* (linear mixed-effects model) function in R, treatment (heated or unheated) was the fixed effect, and temporal pseudo-replication from repeated sampling of the mesocosms over the year was accounted for by including mesocosm identity nested within sampling occasion and block as random effects.

For example, in August in pond 1 (heated) the mean ratio of gas transfer due to diffusion relative to the total oxygen pool (i.e. the concentration of O_2 multiplied by the volume of the mesocosm) was 0.0013 (95% confidence interval, 0.0011 to 0.0015; i.e. 0.13%). Therefore, changes in oxygen transfer due to molecular diffusion were less than the error associated with oxygen measurement using the probe (±1% of saturation). Taken together these analyses provide firm evidence to suggest that abiotic factors that might affect the concentration of dissolved gases such as diffusion and re-aeration through the turbulent effects of wind velocity and convection are comparatively insignificant relative biological metabolic activity and vary little between treatments. Therefore, because heated and unheated treatments were measured simultaneously,

they were assumed to experience equivalent atmospheric gas exchange regimes over the diel cycle.

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Warming Alters the Metabolic Balance of Ecosystems

A modified version of this chapter is published in *Philosophical Transactions of the Royal Society of London Series-B Biological Sciences*, **365**, 2117-2126, (2010)

Abstract

The carbon cycle modulates climate change, via the regulation of atmospheric CO_2, and it represents one of the most important services provided by ecosystems. However, considerable uncertainties remain concerning potential feedbacks between the biota and the climate. In particular, it is unclear how global warming will affect the metabolic balance between the photosynthetic fixation and respiratory release of CO_2 at the ecosystem scale. Here I present a combination of experimental field data from freshwater mesocosms, and theoretical predictions derived from the metabolic theory of ecology to investigate whether warming will alter the capacity of ecosystems to absorb CO_2. The manipulative experiment simulated the temperature increases predicted for the end of the century and revealed that ecosystem respiration increased at a faster rate than primary production, reducing carbon sequestration by 13%. These results confirmed my theoretical predictions based on the differential activation energies of these two processes. Using only the activation energies for whole ecosystem photosynthesis and respiration I provide a theoretical prediction that accurately quantified the precise magnitude of the reduction in carbon sequestration observed experimentally. The combination of whole-ecosystem manipulative experiments and ecological theory is one of the most promising and fruitful research areas to predict the impacts of climate change on key ecosystem services.

Introduction

The biosphere is in the midst of a pronounced warming trend. Global surface temperature has risen by ~ 0.74°C in the last century and is projected to increase by a further 3-5°C over the next 100 years (Houghton 2001, IPCC 2007). Evidence for the ecological impacts of global warming on individual taxa is now unequivocal, as represented by range expansions and poleward migrations (Walther *et al.* 2002, Parmesan & Yohe 2003, Rosenzweig 2008) but the potential responses of whole ecosystems are uncertain (Walther *et al.* 2002). This may be at least partially due to the perceived difficulties of dealing with such seemingly complex systems (Walther *et al.* 2002, Montoya *et al.* 2006, Memmott *et al.* 2007).

Changes to the carbon cycle are generally considered as being one of the greatest potential impacts on ecosystem services associated with climate change (Schroter *et al.* 2005). These changes compries direct effects –e.g. on productivity, CO_2 sequestration, resource quality-, but also indirect effects –e.g. on precipitation patterns, water availability, and crop production. Of special interest are those changes in the biogeochemical cycling of carbon that could potentially alter the "metabolic balance" of ecosystems, because this determines the carbon sequestration capacity of ecosystems. This balance is defined as the rate of carbon absorption by photosynthesis relative to remineralisation by respiration, and it determines whether an ecosystem acts as a source or a sink for atmospheric CO_2 (Woodwell *et al.* 1998, del Giorgio & Duarte 2002, Woodward 2007).

Some recent evidence has highlighted the potential for feedbacks between warming and ecosystem CO_2 sequestration (Cox *et al.* 2000, Canadell *et al.* 2007, Piao *et al.* 2008). For instance, in terrestrial ecosystems there is a strong positive feedback between temperature and

CO_2 emission due to elevated rates of soil respiration (Lloyd & Taylor 1994, Cox *et al.* 2000, Knorr *et al.* 2005, Davidson & Janssens 2006, Arnone *et al.* 2008), and it has also been suggested that as the oceans warm their ability to sequester CO_2 from the atmosphere may weaken (del Giorgio & Duarte 2002, Lopez-Urrutia *et al.* 2006).

Recently, several attempts have been made with coupled climate-carbon models to incorporate some of the key biotic components of the carbon cycle (Cox *et al.* 2000, Friedlingstein *et al.* 2006). However, there is little agreement as to exactly how this should be done in a systematic and predictive manner. In relation to this point the two fundamental questions that I address here, are:

(i) How will the metabolic balance of ecosystems respond to warming?

(ii) Is it possible to predict the precise magnitude of such changes for any likely warming scenario?

To answer these questions I combined a whole-system experiment with predictive ecological theory. In particular, the experimental component permits direct comparisons to be made between contemporary ecosystems with their "future" warmed counterparts, and also gives the opportunity to explore the underlying drivers behind the observed responses. Furthermore, by using materially closed systems (i.e. the only inputs of carbon are through gaseous exchange with the atmosphere) I am able to avoid the confounding effects of changes in the movements of allochthonous carbon into and out of the system and focus on the mechanisms affecting changes in the balance of autochthonous carbon.

Here, I first present and test the Metabolic Theory of Ecology (MTE) [*sensu* (Brown *et al.* 2004)] by attempting to establish the temperature dependence of the fundamental components of the carbon cycle [net and gross primary production and ecosystem respiration (*NPP*, *GPP* and

ER respectively)] and their dependence on metabolism. I then use the theoretical platform of the MTE to explore whether warming will alter carbon sequestration rates in ecosystems.Finally, through extension of the MTE, I attempt to predict quantitative changes in the metabolic balance of ecosystems in response to a likely warming scenario predicted for the end of the next century (IPCC 2007). I then test my predictions at the ecosystem scale using a whole system manipulative experiment in aquatic mesocosms that mimicked this degree of warming.

Lentic freshwater ecosystems are tractable as mesocosms because the unit of the ecosystem is easily delimited and replicable. Importantly, these systems enable the assembly of functioning ecosystems which, although simplifications of their natural counterparts, allow an understanding the mechanisms behind the ecosystem level changes that may occur as a result of warming. Furthermore, freshwater ecosystems (e.g. wetlands) are fundamental components of the global carbon cycle with respect to carbon sequestration (Whiting & Chanton 2001). Therefore, understanding how carbon sequestration rates behave in response to warming in these systems is critical.

Theoretical Framework

Metabolism is a fundamental process that regulates the flux of energy and matter through multiple levels of biological organisation, from individuals to ecosystems (West *et al.* 1997, Brown *et al.* 2004). According to the MTE, individual metabolic rate (i.e. the power required to sustain an organism), can be explained by the general metabolic model (West *et al.* 1997, Gillooly *et al.* 2001, Brown *et al.* 2004):

$$B_i = b_0 e^{-E/kT} M_i^{\alpha} \qquad (1)$$

where B_i is the basal metabolic rate of an individual i, b_0 is a normalisation constant independent of body size and temperature, $e^{-E/kT}$ is the Boltzmann factor that describes the temperature, T, dependence of metabolic rate, where k is Boltzmann's constant ($8.62 \cdot 10^{-5}$ eV K^{-1}) and E is the activation energy of metabolism. M_i corresponds to the body mass of an individual i, and α is the allometric scaling exponent (West *et al.* 1997, Brown *et al.* 2004). By summing the individual metabolic rates of all the organisms within an ecosystem it is possible to predict total ecosystem metabolic rates (Enquist *et al.* 2003, Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006). This general metabolic model has been extended to describe three ecosystem processes that underpin the carbon cycle: net primary production (*NPP*), gross primary production (*GPP*), and ecosystem respiration (*ER*) (Enquist *et al.* 2003, Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006).

The rate of Gross Primary Production (*GPP*) for a whole ecosystem can be estimated from the sum of the individual photosynthetic rates of all of its autotrophic organisms (Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006):

$$GPP = \frac{1}{V}\sum_{i=1}^{na} P_i = \frac{1}{V} n_0 e^{-E_p / kT} \sum_{i=1}^{na} M_i^{\alpha} \qquad (2)$$

where na is the number of autotrophic organisms in volume V, n_0 is a normalisation constant independent of body size M_i and temperature T, E_p is the "effective" activation energy governing the temperature dependence of photosynthetic reactions reported in the literature (≈ 0.32 eV) (Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006), and α is the allometric scaling exponent. The parameter E_p, which is the "effective" activation energy of photosynthesis, approximates the hyperbolic temperature dependence of photosynthesis with an exponential function over the temperature range (0-30°C) to permit direct comparison with the exponential temperature dependence of respiration. (Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006). The photosynthesis-

temperature response is typically hyperbolic, slowing at high temperatures due to deactivation of the component reactions (Medlyn *et al.* 2002). However, photosynthetic temperature optima are generally correlated with the environmental temperature range experienced by plants and deactivation is uncommon within the annual environmental temperature range experienced by plants in their natural environment (Larcher 1995). I therefore approximate the hyperbolic photosynthesis-temperature relationship with E_p, following Allen *et al.* (2005) using a well established model of leaf photosynthesis (Farquhar *et al.* 1980) and reasonable assumptions (internal CO_2 concentrations are about 70% of ambient, co-limitation of photosynthesis by Rubisco, similar kinetic properties for Rubisco across species) that are frequently used in carbon cycling models (Farquhar *et al.* 1980). It is important to note here, that the derivation of E_p is based on the expected concentrations of CO_2 at the sites of photosynthesis in terrestrial plants (Allen *et al.* 2005). Therefore, potential differences between aquatic and terrestrial photosynthesis, for instance, changes in the concentration gradient of CO_2 at the site of photosynthesis, due to Henry's law or slight differences in Rubisco kinetics between aquatic and terrestrial plants, may result in a divergence from the expected $E_p \sim 0.32$ eV in aquatic ecosystems, a point that has been previously neglected in tests of MTE in aquatic systems (Lopez-Urrutia *et al.* 2006).

The *GPP* of an ecosystem is the gross absorption of CO_2 and thus accounts for the photosynthate respired by autotrophs. Because autotrophic respiration is ultimately limited by, and tightly coupled to, photosynthate production within individual autotrophs (i.e. by substrate availability) (Dewar *et al.* 1999, Atkin & Tjoelker 2003) the temperature dependence of autotrophic respiration should be constrained by the photosynthetic activation energy over relatively short temporal scales. This process is called type I respiratory acclimation, and has

been observed empirically (Atkin & Tjoelker 2003) and experimentally (Dewar *et al.* 1999). In the model for *GPP* I therefore assume that autotrophic respiration (*AR*) has an activation energy equivalent to E_p (Allen *et al.* 2005).

$$AR = \frac{1}{V}(1-\varepsilon)GPP = \frac{1}{V}(1-\varepsilon)n_0 e^{-E_p/kT}\sum_{i=1}^{na} M_i^{\alpha} \qquad (3)$$

where (1-ε) is the fraction of photosynthate respired by autotrophs.

The Net Primary Production (*NPP*) of an ecosystem is defined as its *GPP* minus the carbon respired by autotrophs, *AR* (i.e. it is the net fixation of CO_2 into plant biomass) (Allen *et al.* 2005, Woodward 2007):

$$NPP = \frac{1}{V}\varepsilon GPP = \frac{1}{V}\varepsilon n_0 e^{-E_p/kT}\sum_{i=1}^{na} M_i^{\alpha} \qquad (4)$$

where ε is the is the fraction of photosynthate allocated to the net primary production of producer biomass.

In a similar way, the rate of ecosystem respiration (*ER*) can be estimated from the individual respiratory rates of all of its autotrophic (*AR*) and heterotrophic (*HR*) organisms (Enquist *et al.* 2003, Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006):

$$ER = \frac{1}{V}\left[AR + HR\right] = \frac{1}{V}\left[(1-\varepsilon)n_0 e^{-Ep/kT}\sum_{i=1}^{na} M_i^{\alpha,a} + r_0 e^{-Er/kT}\sum_{i=1}^{nh} M_i^{\alpha,h}\right] \qquad (5)$$

where *na* is the total number of autotrophic organisms and *nh* is the number of heterotrophic organisms in a volume *V*, r_0 is a normalisation constant which is independent of M_i and *T*. The scaling exponent α is the same for autotrophs, *a*, and heterotrophs, *h*, (West *et al.* 1997, Gillooly

et al. 2001, Brown *et al.* 2004). The average activation energy governing the temperature dependence of respiratory reactions, E_r is ≈ 0.65 eV (Gillooly *et al.* 2001, Enquist *et al.* 2003).

In equation (5), unlike *NPP* or *GPP*, because *ER* is the sum of both heterotrophic and autotrophic respiration it does not have a simple exponential temperature dependence governed by a single activation energy. At steady state, in a closed system, *ER*, is limited for substrate and must equal *GPP* over the course of a year. Therefore, under conditions of substrate limitation the activation energy for heterotrophic metabolism, E_r should approach the activation energy for photosynthetic reactions, E_p resulting in equivalent temperature dependences between *GPP* and *ER* over the relevant temporal scale (Allen *et al.* 2005). However, when an ecosystem deviates from steady state (i.e. $ER < GPP$ or $ER > GPP$), *ER* is not constrained by *GPP*. During non-steady state dynamics, such as those likely to be occurring in this experiment, providing there is sufficient stored carbon, heterotrophic respiration may exceed *NPP* (i.e. the potential contemporary carbon substrate) over temporal scales dependent on the turnover time of the carbon stores. Under such conditions heterotrophic metabolism can proceed at maximum capacity. Therefore, during non-steady state dynamics, because $E_r > E_p$, *ER* should have a temperature dependence approaching that of heterotrophic metabolism, E_r and therefore, greater than the activation energy for *GPP*.

Equations (2), (4) and (5) yield general expressions for the temperature dependence of *NPP*, *GPP* and *ER* and highlight the importance of the activation energies for individual metabolism in controlling the temperature response of whole ecosystem metabolic rates. Importantly, the theory outlined above differs from previous work modelling the temperature dependence of the carbon cycle based on individual metabolism (Allen *et al.* 2005). Here I do not make the assumption of steady state. Rather, because I am simulating the consequences of

global warming on ecosystem metabolism (i.e. a perturbation), I attempt to understand what might happen to the metabolic balance of ecosystems during the transitory phase between steady states. As such, *GPP* and *ER* have the potential to go out of balance. In a scenario where *ER*>*GPP*, *ER* may be fuelled by baseline respiration (i.e. respiration uncoupled from contemporary primary production) that is dependent on the carbon stored within the system (Trumbore 2000, del Giorgio & Williams 2005). On the other hand, when *ER*<*GPP*, *ER* is not substrate limited. In either case *ER* is not constrained by *GPP* and can exhibit non-steady state dynamics in response to warming, and increase exponentially with temperature according to the activation energy of heterotrophic metabolism (E_r ~0.65 eV).

The theory above provides a platform from which a mechanistic understanding of the potential consequences of global warming on the metabolic balance of ecosystems can be drawn and leads to a number of important predictions, which I tested experimentally. First, the temperature dependence of *NPP* is governed by the effective activation energy that characterises photosynthetic reactions, and the relationship between ln(*NPP*) and 1/*k*T should approximate a slope of $E_p \sim 0.32$ eV . Second, the temperature dependence of *GPP* is constrained by the activation energy for photosynthetic reactions because of the acclimation of autotrophic respiration and the slope of the relationship between ln(*GPP*) and 1/*k*T should be indistinguishable from that of *NPP*. Third, assuming non-steady state dynamics, the temperature dependence of *ER* should be greater than that of *GPP* and the slope of the relationship between ln(*ER*) and 1/*k*T should approach the activation energy of heterotrophic metabolism, $E_r \approx 0.65$ eV. Finally, and most importantly, because of the differential temperature dependences of the two processes, ecosystem respiration should increase more rapidly than primary production as ecosystems warm, which has the potential to alter rates of carbon sequestration.

With an understanding of the mechanisms controlling the temperature dependence of *NPP*, *GPP* and *ER* it is possible predict how the metabolic balance (*ER*/*GPP*) - which is the ability of an ecosystem to sequester carbon - will respond to warming. I define $R_{H:U}$ as the ratio of the metabolic balance (ER_H/GPP_H) in the heated, i.e. "future", systems to the ratio of the metabolic balance (ER_U/GPP_U) in the unheated, i.e. "contemporary", ecosystems, which is given by:

$$R_{H:U} = \frac{ER_H / GPP_H}{ER_U / GPP_U} = e^{\frac{[(Er-Ep)(T_H - T_U)]}{kT_H T_U}} \quad (6)$$

where E_r and E_p are the activation energies for ecosystem respiration and photosynthesis, respectively, and T_H and T_U are the temperatures of heated and unheated ecosystems (see appendix 1 for a full derivation of equation 6). Equation (6) suggests that the response of the metabolic balance of an ecosystem to warming can be predicted and quantified from the knowledge of two parameters: the difference between the activation energies of respiration and photosynthesis (E_r - E_p) and the temperature increase affecting the system (T_H-T_U).

Materials and Methods

Experimental Set-up

I tested these predictions by comparing ecosystem metabolism rates in freshwater mesocosms designed specifically for ecosystem scale manipulations (see *chapter two* for details of the experimental set-up).

Calculation of Metabolic Parameters

Ecosystem metabolic fluxes (*NPP*, *GPP* and *ER*) were measured over a 24h diel cycle for each replicate of each treatment on alternate months over the course of one year (April 2007 to April 2008) using the dissolved oxygen (DO) change technique (Marzolf *et al.* 1994, Mulholland *et al.* 2001), resulting in 140 measurements of each. This technique assumes that changes in DO concentration over a diel cycle represent the metabolic activity (photosynthetic and respiratory) of an aquatic ecosystem. To measure the concentration of DO in the mesocosms, YSI 600XLM multiparameter Sondes equipped with 6562 rapid pulse™ dissolved oxygen sensors were deployed for 24 hours in each heated and unheated treatment pair on each of the seven sampling occasions over the year. Measurements of DO, temperature and pH were taken every 15 minutes for 24 hours at mid depth (0.25m) in the water column of each pond. At the beginning of each sampling occasion the calibration of each Sonde was tested by deploying both Sondes in the same pond for 1 hour to ensure equivalence in DO readings, re-calibration was carried out when necessary. Subsequently, prior to deployment in each treatment pair, the Sondes were calibrated in water-saturated air with a correction for barometric pressure. Calibration accuracy was verified by monitoring the DO concentration of water-saturated air for 10 min and checking against 100% O_2 saturation for the measured temperature and pressure.

The record of continuous DO measurements was used to calculate the *NPP*, *GPP* and *ER* for each pond on each sampling occasion. The dissolved oxygen change (ΔDO) for each 15 minute time interval was calculated as the difference in O_2 concentration between t_1 and t_2 (i.e., $t_2 - t_1$) (Fig. 3.1). The daylight and night-time analysis periods were

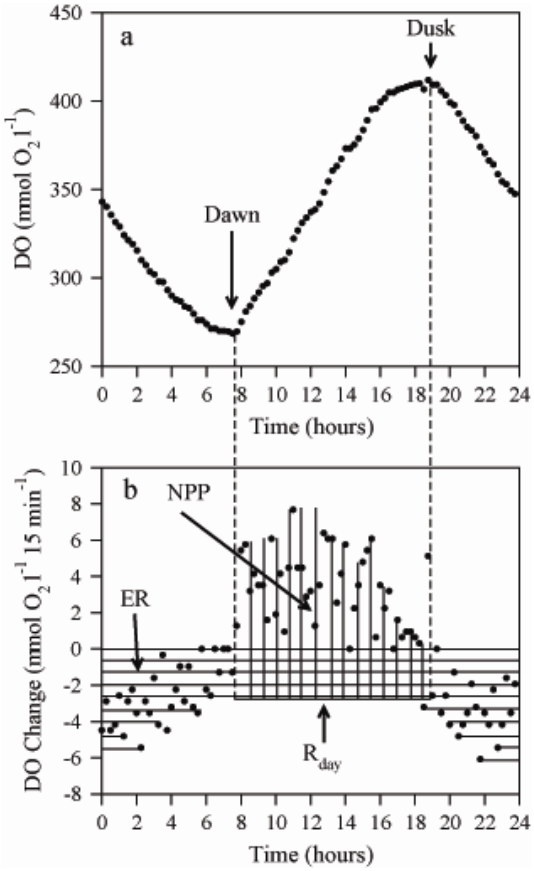

Fig. 3.1. Calculation of *NPP*, *GPP*, and *ER* from diel oxygen profiles. Diel Oxygen profiles for Pond 1 (heated) on the 5th June 2007. (a): Dissolved oxygen concentration (μmol l^{-1}) (b) dissolved oxygen change (μmol O_2 l^{-1} 15$mins^{-1}$). Net primary production (*NPP*) was calculated as the sum of all oxygen change values during the day (i.e. vertical lines above zero). Gross primary productivity (*GPP*, vertical lines) was calculated by the addition of *NPP* and day respiration (R_{day}, cross hatched area) (i.e. sum of all vertical lines). Ecosystem respiration (*ER*) was calculated as the integral of the region indicated by horizontal lines.

delimited as follows: the total analysis period was defined from the minimum O_2 concentration on the 1st night and extended for 24 hours to include the minimum O_2 concentration on the 2nd night. Photosynthetic dawn was identified as the minimum O_2 concentration after which all subsequent values were greater than it. Photosynthetic dusk was defined as the maximum O_2 concentration after which all subsequent values were lower (Fig. 3.1) (Bales 2007). Each O_2 change value was then assigned to a day or night-time category. Subsequently the metabolic parameters were calculated by numerical integration. *NPP* was calculated as:

$$NPP = \sum \Delta O_{2\,day} \quad (7)$$

GPP was calculated as:

$$GPP = NPP + R_{day} \quad (8)$$

where R_{day} is day-time respiration. Since it is impossible to directly measure R_{day}, it was estimated, in keeping with the literature, by extrapolating the mean night time respiration value across the hours of daylight (Marzolf *et al.* 1994, Mulholland *et al.* 2001, Bales 2007). *ER* was calculated as:

$$ER = R_{day} + \sum \Delta O_{2\,night} \quad (9)$$

The metabolic balance of each replicate of each treatment was then determined as the ratio of *ER/GPP*. In the rare event of significant instrument drift or failure, the entire replicate was removed from the final analysis (9 measurements were removed from a total of 140; n = 131). Current biogeochemical techniques presently preclude the disentanglement of autotrophic and heterotrophic respiration at the ecosystem level (Mulholland *et al.* 2001) and rule out the estimation of photorespiration (Marzolf *et al.* 1994). Consequently measures of *GPP* using the DO change technique may be slightly overestimated given the inclusion of heterotrophic respiration in calculation of R_{day}. Furthermore, as with other studies it was not possible to isolate heterotrophic respiration (resulting in O_2 consumption) from my measures of *NPP* (Bales 2007), so *NPP* estimates may be slightly underestimated.

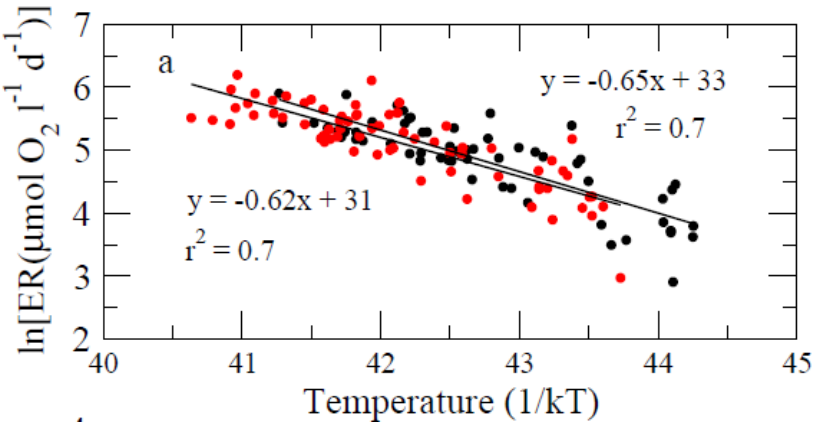

Fig. 3.2. Temperature (1/kT) dependence of ecosystem respiration (ER) separated by treatment (heated = red circles; unheated = black circles). Data exhibit the identical temperature dependence of ER in both treatments (see Table 3.1 for ANCOVA analysis), which provides substantial evidence to suggest that the gas transfer velocity (*k*) was unaffected by the heating of the mesocosms.

Apart from biological metabolic activity, as discussed in *chapter two*, gas exchange with the atmosphere due to diffusion and advection are additional factors that might affect the DO concentration in running waters (Marzolf *et al.* 1994, Mulholland *et al.* 2001) and large lakes (Cole & Caraco 1998) but are relatively insignificant in the small still water mesocosms used in this study. Nevertheless, to provide further quality control I analysed the data for *ER*, determined by the O_2 change technique, to test for homogeneity of the slopes of the temperature - *ER* relationship between treatments. The slopes were indistinguishable between treatments (Fig. 3.2; Table 3.1). If gas transfer velocities (i.e. *k* in equation VII) were significantly elevated in the heated mesocosms due to convection caused by heating I would expect the slope of the temperature dependence of *ER* to be steeper because *ER* would be over estimated at higher temperatures due to faster de-gassing of O_2. This was not the case. Therefore, gas exchange due to advective processes (i.e. turbulence caused by wind or convection caused by heating) was

considered to be equivalent between treatments and was not considered in the estimation of *GPP*, *NPP* or *ER*.

Statistical Analyses

The activation energy of metabolism is given by the slope of the relationship of an Arrhenius plot between ln(*x* flux) and 1/*k*T, where *k* is Boltzmann's constant and T is absolute temperature (*K*). The temperature dependence of ln(*NPP*), ln(*GPP*) and ln(*ER*) (i.e. the activation energy) was determined by ANCOVA. Furthermore, I used ANCOVA to test for statistical differences in the slopes and intercepts of these relationships between treatments and months (i.e. $n = 7$ sampling occasions), to identify the most parsimonious model for determining the activation energy (i.e. the temperature dependence). Model comparison was carried out using the Akaike Information Criterion (AIC). In the ANCOVA, temperature was delimited as a continuous variable and defined as 1/*k*T. To account for temporal pseudo-replication in the statistical model pond identity ($n = 131$) was nested within sampling occasion. ANCOVA computations were carried out in R statistical software (R. Development. Core. 2006).

Comparison of mean annual *NPP*, *GPP*, *ER* and the ratio of *ER/GPP* amongst treatments (treating temperature as a categorical factor) was conducted with restricted maximum likelihood methods (PROC MIXED) in SAS, using a blocked, factorial design with repeated measures (Wolfinger 1998). In the model, treatment (heated or unheated) was the fixed effect, and temporal pseudo-replication from repeated sampling of the mesocosms over the year was accounted for by including mesocosm identity nested within block and sampling occasion as random effects. The repeated measures model was used to test for overall statistical differences between treatments in the mean annual values of the above parameters.

Results

The Temperature Dependence of NPP, GPP and ER

NPP, *GPP* and *ER* all increased with temperature (Fig 3.3 a, b, and c, Table 3.1). There were no significant differences in the slopes or intercepts of the temperature dependences of *NPP*, *GPP* or *ER* between heated and unheated mesocosms (Table 3.1). Furthermore, there were no significant interactions between temperature, treatment or pond identity and sampling occasion for *NPP*, *GPP* or *ER* suggesting that temporal random effects were not important (Table 3.1). This facilitated the use of a single linear model to characterise each of the empirically determined temperature dependences of *NPP*, *GPP* and *ER* (Fig 3.3 a, b, and c). Empirical measures of photosynthetic and respiratory activation energies were close to theoretical expectations and those values reported in the literature. For *NPP,* E_p was 0.41 eV (95% confidence interval 0.32 to 0.5 eV, n = 131) which is steeper than the predicted value ($E_p \approx 0.32$ eV) (Allen *et al.* 2005) (Fig 3.3a). This small overestimate may be ascribed to the fact that *NPP* measures based on O_2 production are inevitably influenced to some extent by heterotrophic metabolism and may therefore more accurately be described as "net ecosystem production" (Bales 2007). Because it is currently impossible to completely disentangle autotrophic and heterotrophic processes in a systematic way at the ecosystem level (Baldocchi *et al.* 2001) heterotrophic metabolism could not be isolated from the measurements of O_2 production. Nevertheless, the effective activation energy of *NPP* reported in the literature ($E_p \approx 0.32$ eV) (Allen *et al.* 2005) falls within the 95% confidence limits of the empirically determined activation energy for *NPP*. For *GPP*, E_p was 0.45 eV (95% confidence interval 0.38 to 0.53 eV, n = 131) (Fig 3.3b), which was statistically indistinguishable from the activation energy from *NPP* (Table 3.1) though slightly steeper than predicted from the activation energy of

photosynthesis. Nevertheless, these results provide substantial evidence for the assumption that the temperature dependence of *GPP* is governed by the activation energy for photosynthesis due to the type I acclimation of autotrophic respiration to photosynthate production over periods of months to years (e.g., (Dewar *et al.* 1999, Atkin & Tjoelker 2003)).

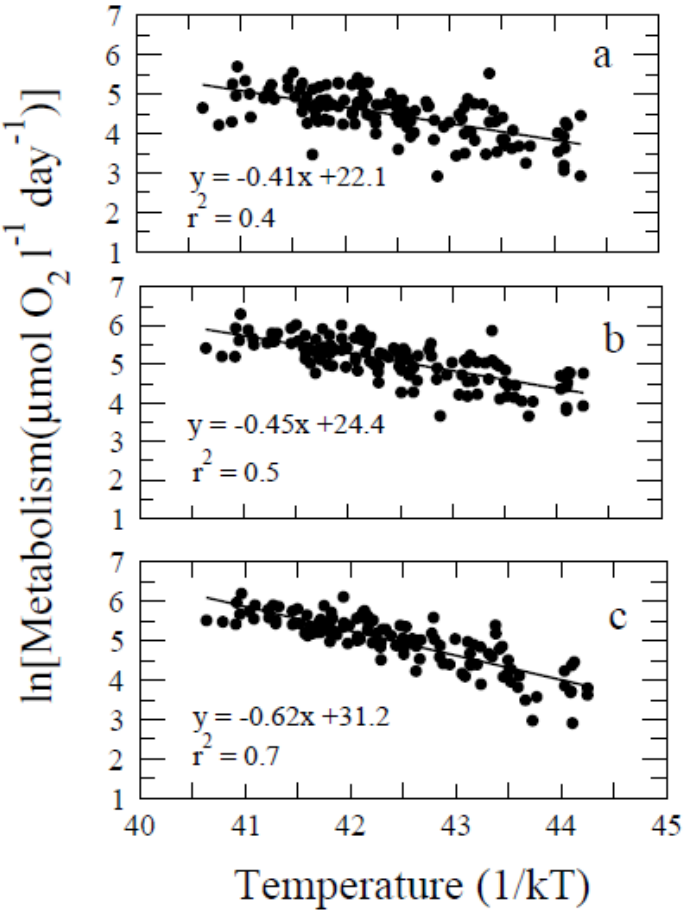

Fig. 3.3. Temperature dependence of whole ecosystem net primary production *NPP* (a), gross primary production, *GPP* (b) and whole ecosystem respiration *ER* (c). The slope of the temperature response equates to the activation energy of the respective process rate. Each data point corresponds to either, the *NPP*, *GPP* or *ER* of a single mesocosm on each of the seven sampling occasions. The slope of the temperature dependence of *ER* was more sensitive to increases in temperature than *NPP* and *GPP* (see main text).

Relationship	d.f.	*F*-ratio	*P*-value
ln(*NPP*) vs 1/kT	1,123	85.9	**<0.0001**
ln(*GPP*) vs 1/kT	1,123	146.6	**<0.0001**
ln(*ER*) vs 1/kT	1,123	294.85	**<0.0001**
Difference in slope of ln(*NPP*) vs 1/kT between treatments	1,123	0.51	0.47
Difference in intercept of ln(*NPP*) vs 1/kT between treatments	1,123	2.05	0.15
Difference in slope of ln(*GPP*) vs 1/kT between treatments	1,123	0.23	0.82
Difference in intercept of ln(*GPP*) vs 1/kT between treatments	1,123	2.55	0.11
Difference in slope of ln(*ER*) vs 1/kT between treatments	1,123	0.56	0.46
Difference in intercept of ln(*ER*) vs 1/kT between treatments	1,123	2.27	0.13
Difference in slope between ln(*GPP*) x ln(*NPP*) vs 1/kT	1,254	0.45	0.5
Difference in slope between ln(*ER*) x ln(*NPP*) vs 1/kT	1,254	12.88	**<0.001**
Difference in slope between ln(*ER*) x ln(*GPP*) vs 1/kT	1,254	3.2	**0.0015**

Table 3.1. Results from Analysis of Co-Variance (ANCOVA). The first ANCOVA tests for relationships between ecosystem level metabolic rates (*NPP*, *GPP* or *ER*) and temperature, parallelism between treatments, and difference between intercepts. Metabolic rates are used as dependent variables, temperature (1/*k*T) as the covariate, and treatment (heated or control) as the factor. The second ANCOVA tests for differences in the slope of the temperature dependence between metabolic rates (i.e., *ER* x *NPP* and *ER* x *GPP*). Here metabolic rate is used as the dependent variable, temperature (1/kT) as the covariate and metabolic rate ID (e.g. *NPP* or *ER*) as the factor.

For *ER*, the activation energy was 0.62 eV (95% confidence interval 0.55 to 0.69 eV $n = 131$), and approached the activation energy expected for heterotrophic metabolism ($E_r \approx 0.65$ eV) (Gillooly *et al.* 2001, Enquist *et al.* 2003, Allen *et al.* 2005) (Fig 3.3c). The activation energy for *ER* was greater than that of *GPP*, supporting the assumption that *ER* and heterotrophic metabolism were not limited by *GPP* (i.e. the mesocosms exhibit non-steady-state dynamics). Further, the empirically determined temperature dependence of *NPP*, and *GPP* differed from *ER* (Table 3.1) and, as predicted, *ER* was more sensitive to temperature increases than *NPP* and *GPP*, further substantiating the modelling assumption of non-steady state dynamics. Moreover,

because *ER* responded more rapidly to rising temperatures than *NPP* and *GPP*, warming has the potential to alter the metabolic balance (i.e., the balance between gross primary production [*GPP*] and *ER*) and carbon sequestration rates within ecosystems.

The Metabolic Balance: Quantitative Predictions

GPP and *ER* were consistently elevated (both within and across seasons) in the warmed mesocosms (Fig 3.4a and 3.4b). Correspondingly, mean annual *GPP* ($F_{1,113}$=9.58, P=0.0025, Fig. 3.4a) and mean annual *ER* ($F_{1,113}$=33.37, P<0.0001, Fig. 3.4b) were significantly higher in the warmed mesocosms, but the magnitude of their responses to warming differed markedly. In agreement with my qualitative theoretical predictions, *ER* increased at a faster rate under experimental warming than did *GPP*. As such, experimental warming increased *ER* considerably more than *GPP* which showed smaller differences between warmed and control mesocosms (Fig 3.4a and 3.4b).

Given the differential responses of *GPP* and *ER* to warming, which were governed by their activation energies at the individual level, I then sought to predict how the metabolic balance of the mesocosms would respond to warming (i.e. equation 6; $R_{H:U}$). In Figure 3.5, I show how the metabolic balance of a given ecosystem should change quantitatively with increasing temperatures. For a constant reference value of T_U (e.g. present-day temperatures), carbon sequestration is reduced ($R_{H:U}$ increases) as T_H increases. The magnitude of the increase (i.e. the slope) is governed by the difference in the activation energies of respiration and photosynthesis (see equation 6; E_r - E_p).

I tested this general prediction with data from the experiment. Here, E_r and E_p were the empirically observed values of 0.62 and 0.45 eV, respectively; T_H and T_U were the mean annual absolute temperatures in the heated and unheated mesocosms (290.9 and 286.1 K respectively). After substituting the empirical values into equation (6) I would expect the ratio $R_{H:U}$ to be 1.12, i.e. carbon sequestration will be reduced by a 12% in the warming scenario. The empirically measured $R_{H:U}$ (mean annual ratio) was 1.13 (95% confidence interval 1.07 to 1.19),

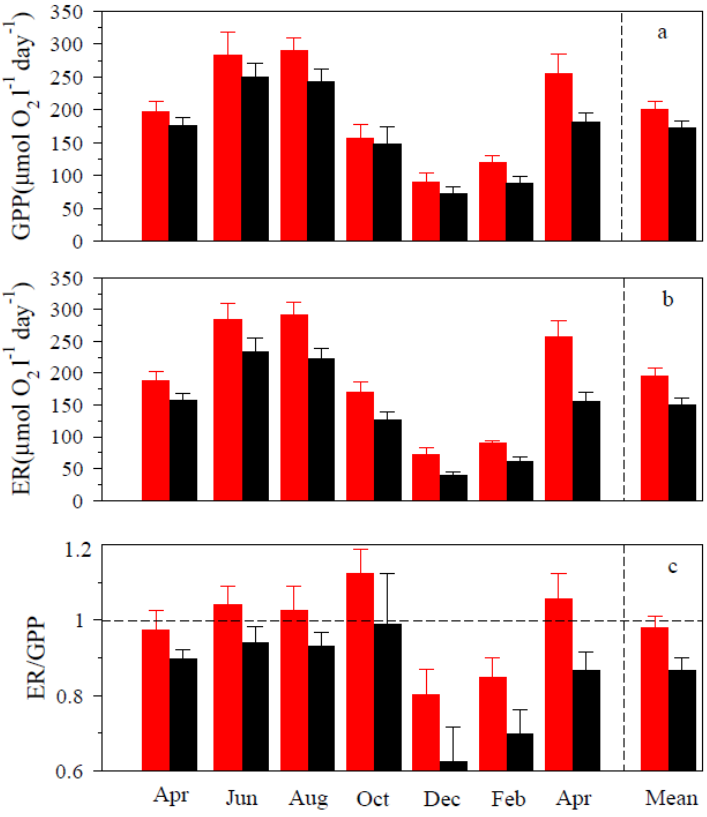

Fig. 3.4. Differences in ecosystem metabolism (±SE) between heated (red) and unheated (black) experimental treatments. Both Gross Primary Production, *GPP* (a), and ecosystem respiration, *ER* (b), were consistently elevated in warmed treatments. The magnitude of the increase in *ER* between warmed and unheated systems was markedly greater than the increase in *GPP*, reflecting its stronger temperature dependence. Correspondingly, there was a highly significant treatment effect on the *ER:GPP* ratio (c), such that the metabolic balance of the warmed mesocosms shifted towards heterotrophy, both seasonally and over the whole year (represented by mean annual values). The dotted line represents the metabolic balance (*ER* = *GPP*). Warmed ecosystems were net sources of CO_2 to the atmosphere in June, August, October and April (i.e., *ER*:*GPP*>1).

and was statistically indistinguishable from my theoretical prediction (Fig 3.5). Accordingly, the metabolic balance (*ER*:*GPP* ratio) of the warmed mesocosms was significantly elevated over the course of the year ($F_{1,113}$=12.71, P<0.005, Fig. 3c). In fact, in four months during the study (June, August, and October 2007 and April 2008) the *ER*/*GPP* ratio was greater than one, suggesting that the warmed systems became net sources of CO_2 to the atmosphere over the growing season.

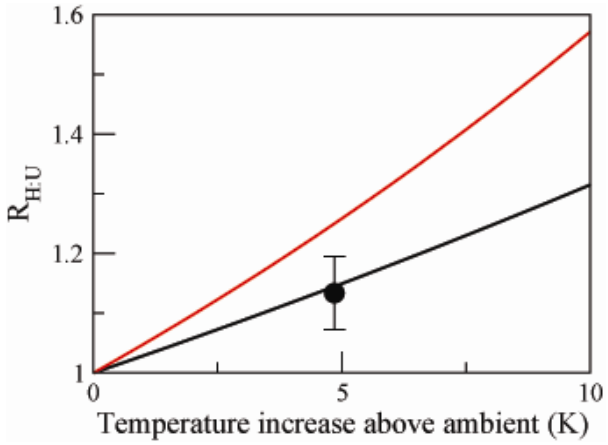

Fig. 3.5. Quantitative changes in the ratio of the metabolic balance between warmed and ambient ecosystems ($R_{H:U}$ in equation 10) as temperature, T_h, increases. The black line corresponds to the prediction for the experimentally-observed activation energies for respiration, E_r, and photosynthesis, E_p (0.62 and 0.42 eV, respectively). The red line is the prediction for the mean value reported in the literature, based on E_r of 0.65 eV and E_p of 0.32 eV. The dot corresponds to the mean annual value (and 95% confidence intervals) measured empirically in the mesocosms, which is undistinguishable from the theoretical prediction.

Discussion

These results suggest that the temperature dependences of whole ecosystem respiration and primary production are fundamentally different, and are governed by their respective activation energies, providing strong support for aspects of the MTE which predict that the temperature dependence of metabolism can be scaled from the individual to the ecosystem level

(Enquist *et al.* 2003). This finding provides a simple mechanistic platform with strong predictive power for understanding how global warming might alter carbon sequestration rates within ecosystems. Because the activation energy for ecosystem respiration is higher than that of primary production, ecosystem respiration increased proportionately more than production under the experimentally induced global warming scenarios predicted for the end of the century. The shift in the metabolic balance of the warmed ecosystems in my experiment suggests that a larger fraction of the carbon fixed by photosynthesis was remineralised and released as CO_2, thus compromising the capacity of these systems to sequester carbon as they warm.

In this experiment both warmed and control mesocosms were net sinks for CO_2 on average over the year. However, the carbon sequestration capacity of the warmed systems relative to the control systems was severely compromised. In both warmed and control mesocosms the carbon balance deviated from steady state because *ER*/*GPP* was <1 averaged over the year, validating the assumptions of my theoretical models. Importantly, in the control mesocosms, at ambient temperature, *ER*/*GPP* averaged over the year was considerably lower than 1, indicating that these systems were strong sinks for CO_2. In the warmed mesocosms *ER*/*GPP* was <1 when averaged over the year, however, during the summer and autumn months these systems were net CO_2 sources (i.e. ER/GPP >1) indicating that a portion of heterotrophic metabolism was fuelled by stored organic carbon. Because the mesocosms were not at steady state (i.e. *ER*/*GPP* <1 or *ER*/*GPP* >1) *ER* was not substrate limited by contemporary *NPP*. As such, heterotrophic metabolism increased in response to warming, and was unconstrained by the weaker temperature dependence of *GPP*. This corroborates the assumption that the activation energy for *ER* closely reflected the activation energy for heterotrophic metabolism in response to warming. In the mesocosm experiment the temperature response of *ER* was not constrained by

GPP and warming increased the fraction of absorbed carbon (*GPP*) that was respired (*ER*), thereby reducing carbon sequestration.

In general, caution must be exercised when extrapolating from mesocosm experiments to natural ecosystems. Having a general theoretical framework that is supported by experimental observations may assist this extrapolation. In particular, the effects of temperature on the metabolic balance observed in this whole-ecosystem manipulation should be treated somewhat cautiously when extrapolating to other systems where limiting resources (e.g. light, nutrients, organic carbon) might alter the temperature response of primary production and, to a lesser extent, ecosystem respiration (Woodwell *et al.* 1998). For instance, in both marine (Lopez-Urrutia & Moran 2007) and terrestrial (Woodwell *et al.* 1998) systems it has been suggested that resource limitation may override the effects of temperature on primary production at the ecosystem level. However, if at higher temperatures resource limitation were to curtail the temperature response of primary production to a greater extent than respiration, as seen in oceanic carbon cycling (Lopez-Urrutia & Moran 2007), the shift in the metabolic balance might be further amplified over temporal scales relevant to the turnover times of stored organic carbon pools (i.e. years). This is because the large stores of organic carbon in these systems will be available to fuel *ER* even if contemporary primary production is reduced.

Acclimation is of fundamental importance to any discussion of the potential effects of warming on the metabolic balance of ecosystems (Dewar *et al.* 1999, Melillo *et al.* 2002, Atkin & Tjoelker 2003, Allen *et al.* 2005). It has often been suggested that over temporal scales relevant to the study of the effects of global warming *ER* must balance *GPP* (i.e. the ecosystems reach steady state) (Gifford 2003, Allen *et al.* 2005). The acclimation of *ER* to *GPP* arises from the assumption that oxidative metabolism is ultimately limited by carbon from *GPP* (Gifford

2003, Allen *et al.* 2005). If this is correct, the consequences of warming revealed in this study may be viewed as transient non-steady state effects which, in natural ecosystems, would eventually reach metabolic equilibrium. However, the consequences of warming for the carbon balance of natural ecosystems depend fundamentally on the turnover times of the organic carbon pools. For instance, studies of soil organic carbon (SOC) pools suggest that the majority of contemporary respiration is driven by organic matter fixed more than two years but less than thirty years ago (Trumbore 2000). Furthermore, the effects of warming on soil respiration are most pronounced on the non-labile SOC pools that have large turnover times (decades to centuries), which increases the potential for strong long term positive feedbacks to warming (Knorr *et al.* 2005). Given the considerable reserves of "stored" organic carbon in natural ecosystems (Trumbore 2000, del Giorgio & Williams 2005), particularly in soils and aquatic sediments, any increase in baseline respiration (i.e. respiration uncoupled from contemporary primary production) relative to primary production driven by the differential activation energies of heterotrophic and autotrophic processes could shift the carbon balance of many ecosystems from being net sinks for atmospheric CO_2 to becoming net sources.

Importantly, and as I have shown in this experiment, ecosystems are likely to exhibit non-steady state dynamics with respect to carbon sequestration in response to warming, which is a long-term, ongoing press perturbation. Over geological time scales these "transient" dynamics must reach steady state because ultimately *ER* requires fixed carbon as a substrate. However, understanding the effects of global warming on the carbon sequestration of ecosystems is crucial over much shorter temporal scales, and those which are relevant to the manifestations of positive feedbacks which may hasten global warming (i.e. decades). In this context, the use of

manipulative experiments to inform short term consequences of warming can be very useful (Benton *et al.* 2007).

Conclusion

The biotic regulation of atmospheric CO_2 constitutes one of the most important "ecosystem services" of value to humans (Schroter *et al.* 2005). It is perhaps surprising then, that there is still no general consensus as to how the metabolic balance of ecosystems will respond to projected global warming (del Giorgio & Duarte 2002, Knorr *et al.* 2005, Lopez-Urrutia *et al.* 2006, Lopez-Urrutia & Moran 2007). In addressing these problems I have used a combination of ecological theory, tested explicitly in experimental ecosystems. My approach revealed a fundamental mechanism, ultimately driven by the metabolic rates of individuals, which dictated the effects of temperature on the metabolic balance of ecosystems. Furthermore, my results demonstrate that predicting how the metabolic balance of ecosystems respond to environmental warming may not require a bespoke model plagued with detail and numerous parameters specific to the system under study. A significant portion of the biological complexity of an ecosystem (e.g. community composition, trophic architecture) may be reduced to two fundamental parameters: the activation energies for metabolic processes and temperature. However, given the inherent complexity and diversity of biotic and abiotic factors influencing the dynamics of carbon cycling in natural ecosystems caution should be exercised in extrapolating my findings in mesocosms to natural systems. The generality of the quantitative predictions developed here to other systems may be achieved after verification in other natural ecosystem types (e.g. terrestrial and marine). Nevertheless, the models developed here and their experimental verification

provide an important baseline and foundation for understanding the mechanisms dictating the effects of temperature on the metabolic balance of ecosystems, and for predicting future change.

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Warming Increases the Proportion of Primary Production Emitted as Methane from Freshwater Mesocosms

A modified version of this chapter is in-press in *Global Change Biology*

Abstract

Methane and carbon dioxide are the dominant gaseous end products of the remineralisation of organic carbon and also the two largest contributors to the anthropogenic greenhouse effect. I investigated whether warming altered the balance of methane efflux relative to primary production and ecosystem respiration in a freshwater mesocosm experiment. Whole ecosystem CH_4 efflux was strongly related to temperature with an apparent activation energy of 0.85eV. Furthermore, CH_4 efflux increased faster than ecosystem respiration or primary production with temperature, with all three processes having sequentially lower activation energies. Warming of 4°C increased the fraction of primary production effluxing as methane by 20% and the fraction of ecosystem respiration as methane by 9%, in line with the offset in their respective activation energies. Because methane is 21 times more potent as a greenhouse gas, relative to CO_2, these results suggest freshwater ecosystems could drive a previously unknown positive feedback between warming and the carbon cycle.

Introduction

The two most important gaseous end products of the remineralisation of organic carbon, carbon dioxide (CO_2) and methane (CH_4), are also the two largest contributors to the anthropogenic greenhouse effect (IPCC 2007). The net emission of greenhouse carbon gases from an ecosystem is the balance between the CO_2 absorbed by the ecosystem by gross primary production (GPP) and the carbon that is respired and released as CO_2 and/or CH_4 (Whiting & Chanton 2001). Further, the fraction of fixed carbon that is respired and released as either CO_2 or CH_4 may be decisive for future global warming, as shifts in this balance will affect the greenhouse gas efflux potential of ecosystems, because CH_4 has 21 times the radiative forcing potential of CO_2 over periods of up to 20 years (Rodhe 1990, Lelieveld *et al.* 1991, Whiting & Chanton 2001).

Methanogenesis in freshwater ecosystems is the result of complex and often interrelated biotic and abiotic processes (Christensen *et al.* 2003a). Methane is produced under strictly anaerobic conditions during organic matter mineralisation but the net efflux of CH_4 from ecosystems can be considerably reduced through oxidation by methanotrophs, which can consume significant quantities of the CH_4 produced in the sediments of lakes (Kuivila *et al.* 1988) and wetlands (Bartlett & Harriss 1991, Segers 1998). Primary production by plants also influences CH_4 production (Joabsson *et al.* 1998, 1999, Christensen *et al.* 2003b), and this has been attributed to the co-variability of organic carbon through root exudation (Chanton *et al.* 1995), the turnover of labile carbon, and/or litter production (Joabsson *et al.* 1999, Christensen *et al.* 2003b). These various lines of evidence are supported by isotopic data which have shown that a large fraction of the organic material that fuels methanogenesis in wetlands is derived from recently synthesised carbon (Chanton *et al.* 1995, Joabsson *et al.* 1999). Vascular plants can also enhance emissions of CH_4 to the atmosphere via root aerenchyma that act as conduits across

zones of potential CH_4 oxidation in soils and sediments (Kelker & Chanton 1997, King *et al.* 1998).

Clearly the mechanisms that influence CH_4 efflux are diverse, however, when all other limiting factors (e.g. substrate limitation, water-table depth) are equal, temperature, via the physiological stimulation of microbial metabolism, has been shown to exert strong control on CH_4 efflux (Schutz *et al.* 1990, Christensen *et al.* 2003a, Gedney *et al.* 2004). Recently, considerable attention has been given to the temperature dependence of the biotic components of the carbon cycle and how the "metabolic balance" of ecosystems, that is the balance between the gross sequestration and release of CO_2, might respond to future global warming (Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006, Yvon-Durocher *et al.* 2010). Emerging evidence suggests that autotrophic and heterotrophic metabolisms (e.g. photosynthesis and respiration) have different temperature dependencies (or activation energies when depicted in an Arrhenius plot) at the ecosystem level, such that respiration increases more rapidly with temperature than does photosynthesis (Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006, Yvon-Durocher *et al.* 2010). In *chapter three* I demonstrated that the differential temperature dependence of these two processes reduced the ability of the warmed systems to sequester CO_2 because more of the carbon fixed by primary production was respired (Yvon-Durocher *et al.* 2010). The response of the greenhouse gas efflux potential of aquatic ecosystems to warming is further complicated by considering the balance of CH_4 efflux in relation to carbon sequestration and CO_2 emission.

A substantial body of work over the last two decades has established the strong temperature dependence of methanogenesis in a wide range of ecosystems (i.e. from landfill sites to high latitude wetlands) and from pure cultures of methanogens to whole ecosystem-level production (Schutz *et al.* 1989, Westermann *et al.* 1989, Conrad & Wetter 1990, Schutz *et al.*

1990, Walter & Heimann 2000, Christensen *et al.* 2003a, Gedney *et al.* 2004). Studies of the temperature dependence of methanogenesis in pure cultures (under optimal conditions) have revealed that its activation energy is typically higher than for other forms of metabolism, due to the relatively large entropy change of the reaction (Westermann *et al.* 1989, Conrad & Wetter 1990, Segers 1998). Methanogenesis and potentially CH_4 efflux may, therefore, be especially sensitive to increases in temperature which raises a number of important unanswered questions. First, does the temperature dependence of CH_4 efflux at the ecosystem scale differ from that of primary production and ecosystem respiration? Second, how will the balance between carbon sequestration, ecosystem respiration and CH_4 efflux respond to warming?

Although our knowledge of CH_4 efflux and its regulation by temperature is extensive, it is largely based on seasonal field surveys (e.g. in wetlands, soils, lakes) and laboratory experiments (e.g. with peat monoliths and rice paddy-soil incubations) which cannot fully address these unanswered questions. I sought to extend this knowledge by generating quantitative predictions of the effects of warming on the greenhouse gas balance using a novel extension of the metabolic theory of ecology (MTE) and testing my predictions in controlled a freshwater mesocosm experiment where I compared the efflux of CH_4 with rates of gross primary production (GPP) and whole ecosystem respiration (ER).

Theoretical Framework

The fundamental ecosystem processes involved in the maintenance of the greenhouse gas carbon balance involve distinct metabolic pathways which are carried out by fundamentally different organisms. For instance, the GPP of an ecosystem is determined by the photosynthetic rates of

all its autotrophs (see *chapter three* also), while ER is governed by the rates of aerobic respiration of both autotrophs and heterotrophs. The CH_4 efflux of an ecosystem is controlled by a distinct metabolic pathway and group of organisms: methanogenesis by anaerobic methanogenic Archaea.

To predict how the net emission of greenhouse gases from freshwater ecosystems will respond to global warming, we first need to understand the mechanics of the temperature dependence of individual metabolism. I applied recent developments of the MTE to define these constraints. The metabolism of individuals exhibit predictable size- and temperature-dependencies (Enquist *et al.* 2003, Brown *et al.* 2004, Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006, Yvon-Durocher *et al.* 2010). Furthermore, recent advances toward a metabolic theory of ecology have demonstrated that whole ecosystem-level fluxes (i.e. GPP and ER) can be predicted based on the sums of individual-level fluxes (Enquist *et al.* 2003, Brown *et al.* 2004, Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006, Yvon-Durocher *et al.* 2010). Using MTE as a theoretical platform, I sought to predict quantitatively how warming will affect the fraction of carbon absorbed by the ecosystem (GPP) that is respired via the methanogenic pathway and released to the atmosphere as CH_4. Furthermore, I attempted to predict how the proportion of ER due to methanogensis will respond to the elevated temperatures predicted for the end of the century; this balance is likely to be decisive in determining the greenhouse gas efflux potential of freshwater ecosystems. Importantly, in the theoretical framework (see *chapter three*) I do not make the assumption of steady state. Rather, because I am simulating the consequences of global warming on ecosystem metabolism (i.e. a long-term press perturbation away from steady state) I attempt to understand what happens to the carbon balance of ecosystems during the transitory phase between steady states.

The flux of CH_4 from an individual methanogen is determined by its metabolic rate, which has a predictable mass M_i and temperature T (K) dependence, and can be described by the following equation (Brown *et al.* 2004):

$$A_i = A_0 e^{-Ea/kT} M_i^{3/4} \quad (1)$$

where A_i is the rate of CH_4 flux of individual i. The normalisation constant, $A_{0,}$ is independent of mass M_i and temperature T. The Boltzmann factor $e^{-Ea/kT}$ describes the temperature dependence of metabolic rate, where k is Boltzmann's constant ($8.62 \cdot 10^{-5}$ eV K^{-1}) and E_a is the activation energy of methanogenesis. By summing the individual metabolic fluxes of all the organisms within an ecosystem it is possible to predict total ecosystem metabolic fluxes (Enquist *et al.* 2003, Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006, Yvon-Durocher *et al.* 2010). The rate of CH_4 production (a) can therefore be estimated from the sum of the individual CH_4 fluxes of all the methanogens in the community:

$$a = \left(\frac{1}{A}\right)\sum_{i=1}^{na} A_i = a_o \left(\frac{na}{A}\right)\langle M^{3/4}\rangle_n e^{-Ea/kT} \quad (2)$$

where na is the number of methanogens in the sediment area, A, a_0 is a normalisation constant independent of body mass, M_i, and temperature T, and E_a is the apparent activation energy governing the temperature dependence of methanogenesis. The normalisation constant:

$$a_0 = A_0 \left(\frac{na}{A}\right)\langle M^{3/4}\rangle_n \quad (3)$$

where $<M^{3/4}>_n$ is the average $M_i^{3/4}$ for individuals ($=(1/n)\sum^{n}_{i=1} M_i^{3/4}$) (Allen *et al.* 2005), and accounts for the total standing ecosystem biomass of methanogens ($((na/A) <M^{3/4}>_n$) and

represents the intrinsic capacity for methanogenesis of the ecosystem. Equation 2 can be rewritten as:

$$\ln(a) = \frac{-E_a}{k}\left(\frac{1}{T}\right) + \ln(a_0) \quad (4)$$

Here, I am attempting to understand how biogeochemical feedbacks will respond to global warming and to predict how the net efflux of CH_4 to the atmosphere behaves in response to temperature. There is, however, an important distinction between CH_4 efflux and CH_4 production (*a*). CH_4 efflux to the atmosphere is the net result of the difference between CH_4 production in the anaerobic zone of the sediment and the oxidation of CH_4 by methanotrophs in the oxic layers of the sediment and the overlying water column (Segers 1998). CH_4 oxidation can in the presence of sufficient concentrations of oxygen, oxidize ~90% of CH_4 production in the sediment (Schutz *et al.* 1989) and substantially reduce net CH_4 efflux relative to its production. In my model I make the simplifying assumption that although CH_4 oxidation can typically considerably reduce net CH_4 efflux it has little effect on the its temperature dependence. As such, I expect CH_4 efflux to be a constant proportion of CH_4 production with temperature, therefore the temperature dependence of CH_4 efflux should be equivalent to the activation energy for methanogenesis.

Here I am attempting to understand the mechanisms controlling the temperature dependence of CH_4 efflux not predict the absolute amounts of CH_4 efflux or CH_4 production. Given the above assumptions, equation (4) yields a general expression for the temperature dependence of CH_4 efflux, and leads to a number of important predictions. First, the temperature dependence of CH_4 efflux should be governed by the activation energy of individual methanogenesis. Thus, the slope of the relationship between ln(CH_4 efflux) and the reciprocal of

absolute temperature $1/kT$ should approximate a slope equal to the activation energy of methanogenesis in pure culture. Second, $\ln(a_0)$ should be lower in ecosystems which are substrate limited. This is a result of the effects of resource limitation on the intrinsic methanogenic capacity of the ecosystem (a_0), which is dependent on the abundance of methanogens (n/A) and the relative rate of methanogenesis per individual methanogen (A_0). Finally, $\ln(a_0)$ may be higher in warmed ecosystems as a consequence of greater oxygen consumption from respiration and the reduction in oxygen solubility with temperature both of which may increase the extent of the anaerobic zone in sediments facilitating a greater biomass of methanogens.

The rate of photosynthesis also has a predictable size M_i and temperature T dependence (Brown *et al.* 2004, Allen *et al.* 2005), and can be described by the following equation:

$$P_i = P_0 e^{-Ep/kT} M_i^{3/4} \qquad (5)$$

where P_i is the rate of photosynthesis of individual i. The normalisation constant, $P_{0,}$ accounts for the rate of photosynthesis per chloroplast and the density of chloroplasts per unit body mass (Allen *et al.* 2005). E_a is the "effective" activation energy for photosynthesis (0.32 eV) (Allen *et al.* 2005), which describes the temperature dependence of the rate limiting step in photosynthesis, Rubisco carboxylation (see *chapter three*). Similarly, the GPP (p) for a whole ecosystem can be described by the sum of the photosynthetic rates of all of the autotrophic organisms in the ecosystem (Allen *et al.* 2005, Yvon-Durocher *et al.* 2010):

$$\ln(p) = \frac{-Ep}{k}\left(\frac{1}{T}\right) + \ln(p_0) \qquad (6)$$

Here the normalisation constant:

$$p_0 = P_0\left(\frac{np}{A}\right)\langle M^{3/4}\rangle_a \quad (7)$$

where *np* is the number of autotrophs in the area, *A*. The temperature dependence of GPP (*p*) is governed by the effective activation energy for individual photosynthesis, E_p, ~ 0.32 eV (Allen *et al.* 2005).

Similarly, individual respiration has a predictable size M_i and temperature *T* (K) dependence (Brown *et al.* 2004, Allen *et al.* 2005), which can be described by the following equation:

$$R_i = R_0 e^{-Er/kT} M_i^{3/4} \quad (8)$$

where R_i is the rate of respiration of an individual *i*. The normalisation constant, R_0, accounts for the rate of respiration per respiratory complex and the density of mitochondria per unit body mass (Allen *et al.* 2005). Here, E_r is the apparent activation energy for respiration (~0.65 eV) (Gillooly *et al.* 2001). Like CH_4 efflux and GPP, the rate of ER *(r)* can be estimated from the sum of the individual respiratory rates of all its autotrophic and heterotrophic organisms (Enquist *et al.* 2003, Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006, Yvon-Durocher *et al.* 2010):

$$\ln(r) = \frac{-Er}{k}\left(\frac{1}{T}\right) + \ln(r_0) \quad (9)$$

The normalisation constant:

$$r_0 = R_0\left(\frac{nr}{A}\right)\langle M^{3/4}\rangle \quad (10)$$

accounts for the numbers of heterotrophs and autotrophs in the ecosystem, *nr*. ER is the sum of both heterotrophic and autotrophic respiration. Here, as in *chapter three* I assume a transient, non-steady state, (Yvon-Durocher *et al.* 2010) with respect to the balance between ER/GPP because I am attempting to understand ecosystem dynamics in response to perturbations away from steady state (i.e. warming). Therefore, I assume that the temperature dependence of ER is unconstrained by NPP, a portion of which can be sustained by baseline respiration of stored organic carbon (del Giorgio & Williams 2005) and is therefore governed by the activation energy of heterotrophic respiration $E_r \sim 0.65$eV, as has been shown previously in *chapter three*.

GPP represents the total absorption of CO_2 by autotrophic biomass. Previous work has shown that primary productivity exerts a strong control on methanogenesis as it provides a significant portion of its labile substrate, therefore the two biogeochemical processes are often tightly coupled (Whiting & Chanton 1993, Joabsson *et al.* 1999, Christensen *et al.* 2003b). The fraction of GPP that is respired via the methanogenic pathway and emitted from the ecosystem to the atmosphere as CH_4 is important in determining the greenhouse gas efflux potential of the system and its response to warming might be crucial in determining the extent of future biotic feedbacks. I define the fraction of GPP respired via the methanogenic pathway in the warmed (a_H/p_H) versus contemporary (a_U/p_U) ecosystems as R_{fixed}H:U, and it is given by:

$$R_{fixed}H:U = \frac{a_H / p_H}{a_U / p_U} = e^{\frac{[(Ea-Ep)(T_H - T_U)]}{kT_H T_U}} \qquad (11)$$

where E_a and E_p are the empirically determined activation energies for CH_4 efflux and GPP, respectively, and T_H and T_U are the temperatures of the heated and unheated ecosystems (see appendix 2 for the full derivation of equation 11). Similarly the proportion of ecosystem respiration that is due to methanogenesis is an important determinant of the balance between CH_4

and CO_2 efflux. Therefore, to predict the relative offset between warmed and ambient treatments with respect to the balance between ecosystem respiration and CH_4 efflux I define the fraction of ecosystem metabolism respired via the methanogenic pathway in warmed (a_H/r_H) versus contemporary (a_U/r_U) ecosystems as $R_{emitted}$H:U, and it is given by:

$$R_{emitted}H:U = \frac{a_H/r_H}{a_U/r_U} = e^{\frac{[(Ea-Er)(T_H-T_U)]}{kT_HT_U}} \quad (12)$$

where E_a and E_r are the empirically determined activation energies for CH_4 efflux and ER, respectively (see appendix 3 for the full derivation of equation 12). Importantly, equations (11 and 12) suggest that the balance of the net emission of greenhouse gases from an ecosystem can be predicted from the knowledge of the differences in the activation energies for the metabolic process under question and the degree of expected warming.

Material and Methods

Dissolved Methane

The concentration of dissolved CH_4 was measured by removing a water sample (30 mL in a gas-tight syringe) and gently transferring it to a gas tight vial (12.5 mL Exetainers, Labco, High Wycombe, UK), allowing it to overflow, fixing it with a bactericide (100 µL 50 % w/v $ZnCl_2$) and sealing it. Samples were collected at hourly time intervals (in total 6 to 10 hours depending on the time of year) over a day for each replicate on alternate months for one year (April 2007 to April 2008, n = 1416 individual measurements). Upon return to the laboratory, a headspace (2 mL analytical grade helium) was introduced to the gas tight vial and the sample was shaken

vigorously for 0.5 minutes and then allowed to stand for a further 30 minutes to allow for headspace equilibration, before analysis of the headspace concentration of CH_4 using a gas chromatograph as described in *chapter two* (Sanders *et al.* 2007). Samples (50 μL) were withdrawn from the headspace of the sample vials and injected into a gas-chromatograph fitted with a flame ionising detector (GC/FID; Agilent Technologies, UK). Headspace concentrations of CH_4 were calculated from peak areas calibrated against known standards (Scientific and Technical gases, Staffs, UK) and the total amount of CH_4 in the gas tight vial (water plus headspace) was calculated using the appropriate solubility coefficients (Yamamoto *et al.* 1976). Finally, the 1,416 individual measurements were pooled in each case to give an average daily concentration of dissolved CH_4 for each pond (140 measures over the year for 70 heated and 70 ambient).

Methane Efflux

Measurements of the efflux of CH_4 were made simultaneously to those of dissolved CH_4. A single gas chamber was positioned at the water surface of each mesocosm on each sampling occasion. The chambers were made of polycarbonate and enclosed a headspace (300 mL) of ambient air at the air-water interface of the mesocosm (Fig. 4.1). The lid of the gas chamber was equipped with a Teflon septum port, through which samples of gas (1 mL) were removed using a gas-tight syringe (2 mL VICI gas tight syringe) every 15 minutes for the first hour of the incubation, then hourly for up to 10 hours thereafter. The samples were then transferred to water-filled gas-tight vials (3 mL, Exetainers; Labco, High Wycombe, UK) through a two way valve

venting through a narrow bore needle. The gas-tight vials were then stored upside down prior to analysis.

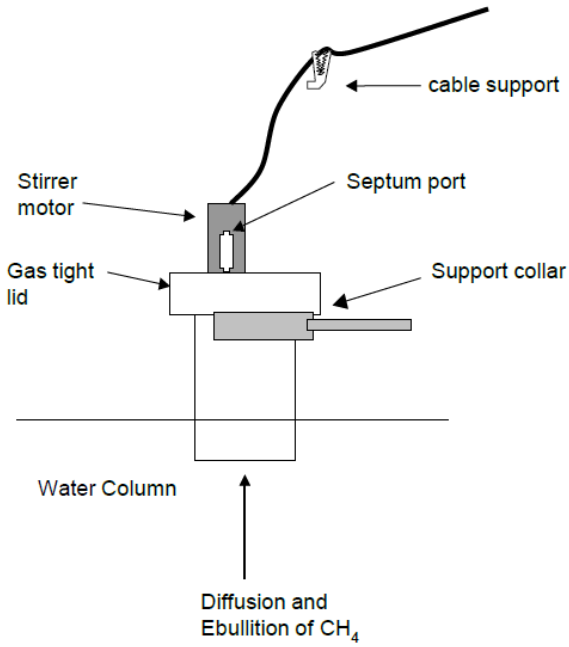

Fig. 4.1. Diagram of the gas trap sampler used to measure CH_4 efflux.

The concentration of CH_4 in the headspace of the sample was determined by gas chromatography as described above and *chapter two*. The efflux of CH_4 across the water-air interface was calculated by regression analysis of the change in concentration of CH_4 in the chamber headspace over time. Subsequently, one hour was used as an appropriate duration for accurately estimating the flux of CH_4 (Lambert & Frechette 2005) (Fig. 4.2). Regression slopes with a significance of $P > 0.05$ and/or an R-squared of below 0.9 were considered non-significant and were excluded from further analyses (9 from the 140 individual flux measurements).

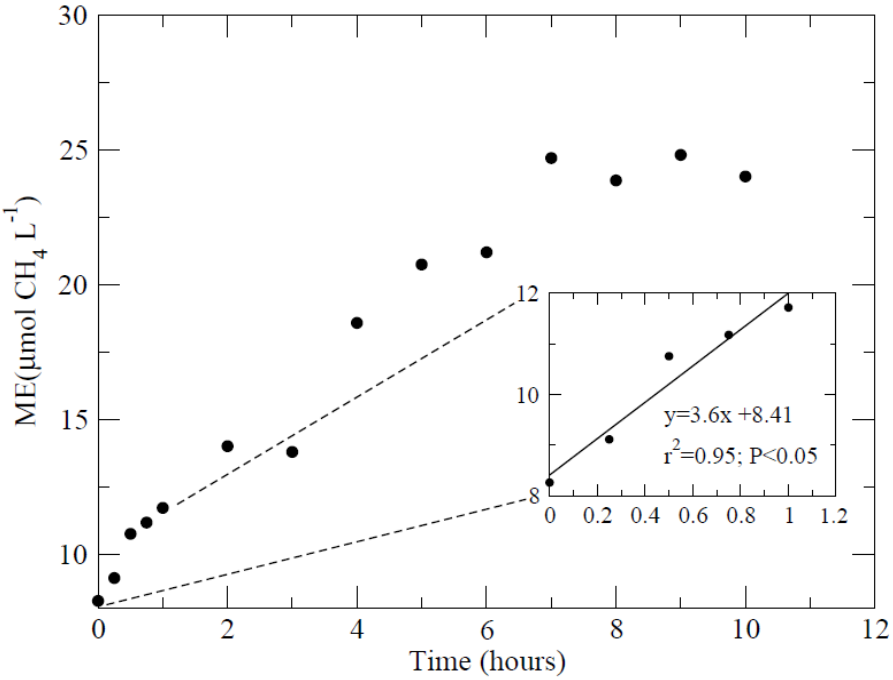

Fig. 4.2. Example of field measurements of methane efflux (ME) from mesocosm 1 (June 2007) to the atmosphere. After approximately 7 hours ME reaches an asymptote (dashed line). This might be an artefact of the gas trap enclosure and artificial changes in partial pressure and/or temperature, but may also include a decline in photosynthesis and gas transfer through the aerynchema of the macrophytes in early afternoon as photosynthesis declines. As a result the shortest possible time (typically 1 hour) to generate a significant (r^2>0.9; P<0.05) flux (insert) was used for determination of flux rates.

Determination of GPP and ER

GPP and ER were estimated simultaneously with the measurements of dissolved CH_4 and CH_4 efflux, by applying a standard single station dissolved oxygen (DO) change technique (Odum 1956, Marzolf *et al.* 1994, Mulholland *et al.* 2001). This technique is described in detail in *chapter three*.

Meta-analysis of literature data on methanogenesis in pure culture

To determine the average activation energy of methanogenesis in pure cultures of methanogens under controlled laboratory conditions I performed a meta-analysis of the available literature data on rates of CH_4 production in pure cultures of methanogens conducted at a range temperatures. Because I was interested in determining the "true" activation energy of methanogenesis it was important that temperature was the only variable affecting the rate of methanogenesis in the experiments selected for reanalysis. Therefore, only studies in which methanogenic substrates were supplied in non-limiting quantities were chosen. The studies were (Vandenberg *et al.* 1976, Huser *et al.* 1982, Westermann *et al.* 1989)). Data were removed from the temperature-methane production figures in these manuscripts using GrabIT™ software. Only values within the exponentially increasing parts of the temperature-methanogenesis curves up to the optimum temperature were used for subsequent analysis because at high temperatures (+35˚C) in these studies rates of CH_4 production were inhibited. The activation energies in each study were determined by plotting the natural logarithm of the rate of CH_4 production against the reciprocal of absolute temperature ($1/kT$), where the slope of the relationship describes the activation energy.

Statistical Analyses

All data were checked for normality using the Shapiro Wilks test for normality and were natural log transformed prior to statistical analysis where necessary. The activation energy of metabolism is given by the slope of the relationship of an Arrhenius plot between ln(x flux) and $1/kT$, where k is Boltzmann's constant and T is absolute temperature (K). The temperature

dependence of ln(CH_4 efflux), ln(GPP) and ln(ER) (i.e. the activation energy) was determined by ANCOVA. Furthermore, I used ANCOVA to test for statistical differences in the slopes and intercepts of these relationships between treatments and months (i.e. $n = 7$ sampling occasions), to identify the most parsimonious model for determining the activation energy (i.e. the temperature dependence). Model comparison was carried out using the Akaike Information Criterion (AIC). In the ANCOVA, temperature was delimited as a continuous variable and defined as $1/kT$. To account for temporal pseudo-replication in the statistical model pond identity ($n = 131$) was nested within sampling occasion. ANCOVA computations were carried out in R statistical software (R. Development. Core. 2006).

Categorical analyses (treating temperature as a fixed factor i.e. heated or unheated) of CH_4 efflux, dissolved CH_4 pool, k gas transfer, CH_4 efflux /GPP and CH_4 efflux /ER was conducted with restricted maximum likelihood methods using the *lme* (linear mixed-effects model) function in R (R. Development. Core. 2006). In the model, treatment (heated or unheated) was the fixed effect, and temporal pseudo-replication from repeated sampling of the mesocosms over the year was accounted for by including mesocosm identity nested within block and sampling occasion as random effects. The repeated measures model was used to test for overall statistical differences between treatments in the mean annual values of the above parameters.

Results

The concentration of CH_4 exhibited clear and near identical seasonal trends in the water of both the heated and ambient mesocosms and, on average, over the year, was not significantly different

between treatments (Fig. 4.3a and Table 4.1). The concentration of CH_4 ranged from 0.04 µmol L^{-1} to 6.8 µmol L^{-1}, though the distribution of CH_4 concentration exhibited strong positive skew (Shapiro Wilks test; $W = 0.63$; $P < 0.005$), such that 75% of all measurements were less than 0.66 µmol L^{-1} over the seasonal cycle. Using the 75th percentile for dissolved CH_4 as a conservative estimate, and the average concentration of CH_4 that would be at equilibrium with the atmosphere (~3.55 $\times 10^{-3}$ µmol L^{-1}), I estimated that the mesocosms were typically about 128 times supersaturated with respect to the atmosphere.

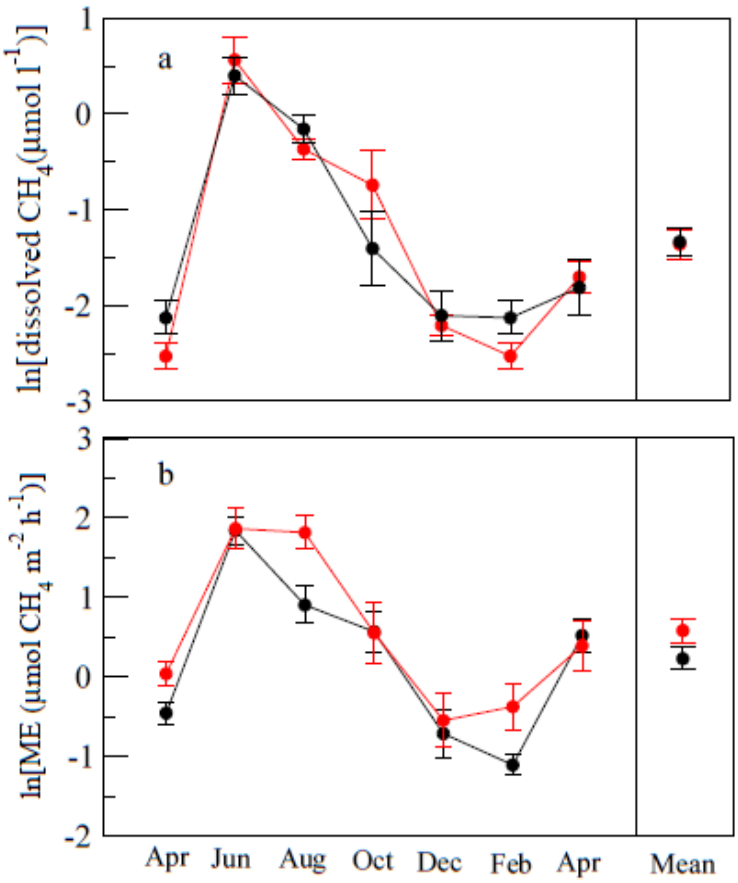

Fig. 4.3. (a) Differences in the pool of dissolved methane [ln(CH_4)] (±SE) between heated (red lines) and unheated treatments (black lines). The pool of dissolved methane exhibited strong seasonal trends and which were identical between treatments. Furthermore, the average annual pool of dissolved methane was identical between treatments (Table 1). (b) Differences in methane efflux, ln(ME) (±SE) between heated (red lines) and unheated (black lines) treatments. Methane efflux showed a strong seasonal pattern and was elevated, on average, over the annual cycle in warmed treatments reflecting its strong temperature dependence (Table 1).

Similarly, the rate of CH_4 efflux showed strong seasonal trends with peaks in early summer and lowest rates in winter (Fig. 4.3b) and was strongly positively correlated ($r = 0.94$; $P<0.005$) with the concentration of CH_4 in the water column. The rate of CH_4 efflux ranged from 0.35 to 7.02 µmol m^{-2} h^{-1} in the ambient mesocosms and from 0.96 to 8.14 µmol m^{-2} h^{-1} in the warmed mesocosms. The distribution of CH_4 efflux between heated and ambient mesocosms was also strongly positively skewed (Shapiro Wilks test; $W = 0.74$; $P < 0.005$), with 75% of measurements falling below 3.6 µmol m^{-2} h^{-1}. Furthermore, the rate of CH_4 efflux was elevated in the warmed mesocosms over parts of the seasonal cycle (April, August and February), and, on average, the mean annual rate of CH_4 efflux was significantly greater in the warmed mesocosms (Table 4.1).

Variable	d.f.	*F*-ratio	*P* – Value
ln(CH_4 Pool)	1,123	0.0001	0.992(NS)
ln(CH_4 efflux)	1,123	6.22	**0.014**
ln (*k*)	1,123	3.46	0.068(NS)
ln(CH_4 efflux) / ln(GPP)	1,123	6.33	**0.013**
ln(CH_4 efflux) / ln(ER)	1,123	6.23	**0.0139**

Table 4.1. Linear mixed effects model analysis. Analysing differences between heated and unheated treatments in the annual means of methane efflux [ln(CH_4 efflux)], the dissolve methane pool [ln(CH_4 Pool)], the ratio of methane efflux to GPP [ln(CH_4 efflux)/ln(GPP)], and the ratio of methane efflux to ER [ln(CH_4 efflux)/ln(ER)]. Significant *P*-values are given in bold.

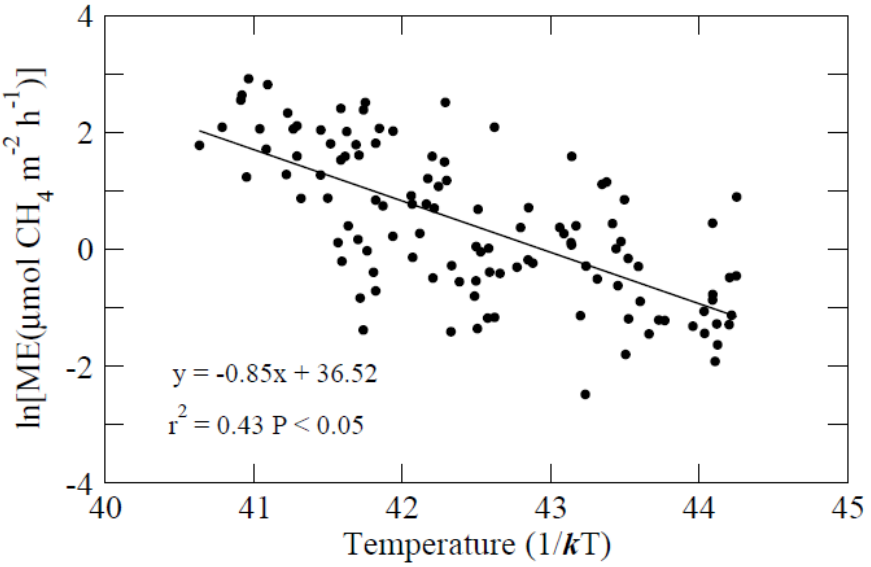

Fig. 4.4. Temperature dependence of whole ecosystem methane efflux. The slope of the temperature response of methane efflux in the experiment was equivalent to the activation energy of methanogenesis. Each data point corresponds to the CH_4 efflux from a single mesocosm on each of the seven sampling occasions (n = 131). There were no significant differences in the slopes of the temperature dependences of CH_4 efflux between heated and unheated mesocosms nor any effects due to repeatedly sampling individual ponds (Table 4.2): this facilitated the use of a single model to characterise the activation energy.

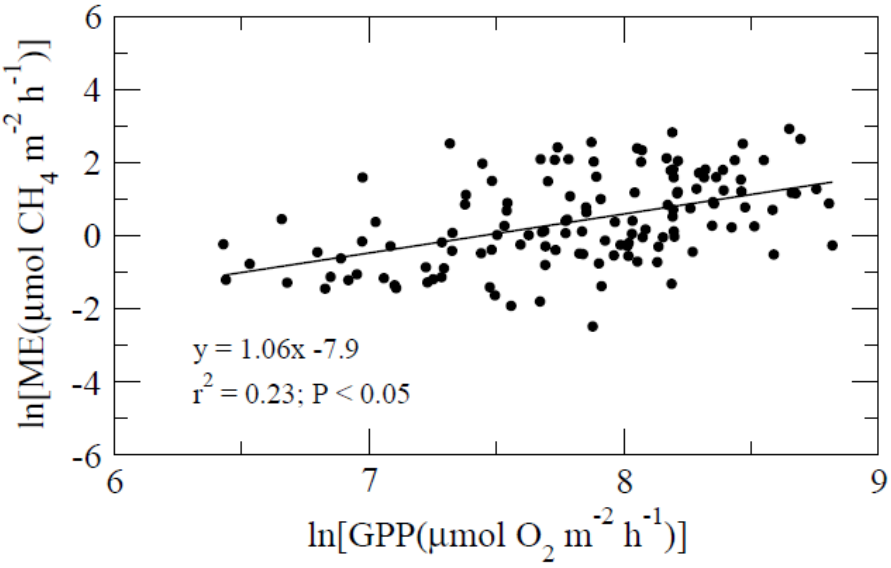

Fig. 4.5. Positive correlation between methane efflux [ln(ME)] and gross primary production and [ln(GPP)]. Each data point corresponds to the CH_4 efflux and GPP of a single mesocosm on each of the seven sampling occasions (n = 131). There were no significant differences in the slope or intercepts of the relationship between ln(ME) vs ln(GPP) between heated and unheated mesocosms nor any effects due to repeatedly sampling individual ponds (Table 2), facilitating the use of a single model to characterise the relationship.

CH_4 efflux was strongly related to water temperature ($1/kT$) (Table 4.2 and Fig. 4.4), with an apparent activation energy in the order of 0.85 eV (95% confidence interval: 0.64 to 1.02 eV). In addition, CH_4 efflux was also related to GPP, though much more weakly (Table 4.2 and Fig. 4.5), with temperature explaining 43% of the variance in rate of CH_4 efflux, while GPP explained only 23% (Fig. 4.4 and Fig. 4.5).

Relationship	d.f.	*F*-ratio	*P* – Value
ln(CH_4 efflux) vs $1/kT$	1,127	97.98	**<0.0001**
Difference in slope of ln(CH_4 efflux) vs $1/kT$ between treatments	1,127	1.23	0.23
Difference in intercept of ln(CH_4 efflux) vs $1/kT$ between treatments	1,127	2.29	0.132
ln(CH_4 efflux) vs ln(GPP)	1,127	38.61	**<0.0001**
Difference in intercept of ln(CH_4 efflux) vs ln(GPP) between treatments	1,127	1.28	0.26
Difference in slope of ln(CH_4 efflux) vs ln(GPP) between treatments	1,127	0.38	0.54
Difference in slope between ln(CH_4 efflux) vs $1/kT$ and ln(GPP) vs $1/kT$	1,258	21.61	**<0.0001**
Difference in slope between ln(CH_4 efflux) vs $1/kT$ and ln(ER) vs $1/kT$	1,258	8.36	**0.0042**

Table 4.2. Analysis of Co-Variance table for the relationships between ln(CH_4 efflux), ln(GPP) and ln(ER) vs $1/kT$. Significant *P*-values are given in bold.

Methanogenesis in pure cultures of methanogens under non-limiting conditions, determined from a meta-analysis of previous published data revealed that it was also strongly temperature dependent (Fig. 4.6). The activation energy of methanogenesis in pure cultures was (mean E_a = 0.88 eV, 95% confidence interval: 0.80 to 0.96 eV). Furthermore, as predicted this was indistinguishable from that of the activation energy of CH4 efflux at the ecosystem level (E_a = 0.85 eV, 95% confidence interval: 0.64 to 1.02 eV; Fig. 4.4).

GPP and ER were also strongly related to temperature, as has been described previously in *chapter three*, with apparent activation energies in the order of 0.45 eV (95% confidence interval 0.38 to 0.53 eV) and 0.62 eV (95% confidence interval 0.55 to 0.69 eV) for each, respectively. The temperature dependence of CH_4 efflux was significantly higher than that for either GPP or ER (Table 4.2) and, correspondingly, CH_4 efflux increased more rapidly in response to warming than did either GPP or ER.

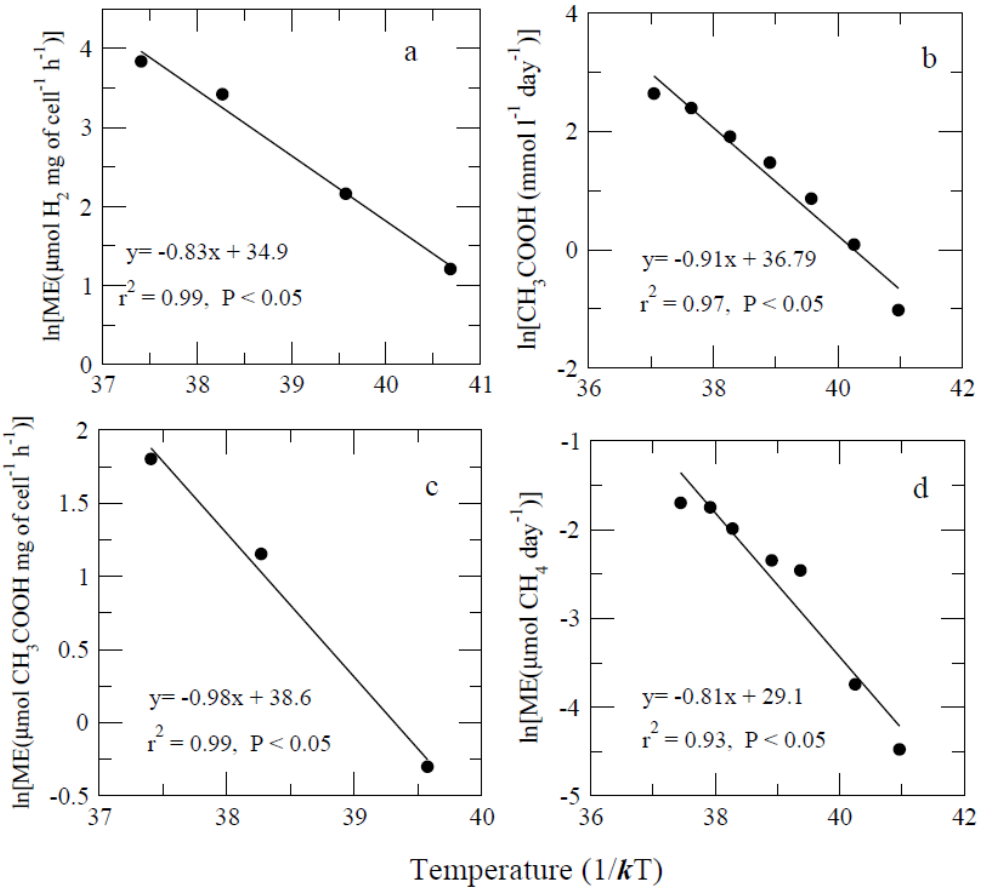

Fig. 4.6. Temperature dependence of methanogenesis in pure cultures of the methanogens. (a) Methanogenesis of *Methanosarcina barkeri* using H_2 as a substrate; (b) methanogenesis of an enrichment culture using CH_3COOH as a substrate; (c) methanogenesis of *M. barkeri* using CH_3COOH as a substrate; and (d) methanogenesis of *Methanothrix soehngenii* using CH_3COOH as a substrate. Data in a and c were reanalysed from V_{max} values and temperatures reported in Westermann *et al*. (1989). Data for b was reanalysed from data on rates of CH_3COOH conversion to CH_4 in Van den Berg *et al*., (1976), and data for c was reanalysed from data on rates of CH_4 production in Huser et al., (1982). In each case, the slope of the temperature response equates to the activation energy of methanogenesis and agree very well with that derived in Fig. 4.5.

Given the large differences between the activation energies of GPP, ER and CH_4 efflux I attempted to predict how the fraction of GPP respired via the methanogenic pathway and emitted as CH_4 (i.e. $R_{fixed}H:U$ in equation 11) and the balance between CH_4 and CO_2 emission (i.e. $R_{emitted}H:U$ in equation 12) would respond to experimental warming. The balance between carbon absorption and CH_4 emission is given by the ratio of CH_4 efflux to GPP, which was significantly elevated in the heated mesocosms, on average, over the year (Table 4.1 and Fig 4.7a). The mean annual ratio of CH_4 efflux to GPP was elevated by ~20 % in response to the ~4˚C experimental warming. Substituting the empirically derived activation energies for CH_4 efflux, E_a, and GPP, E_p, (0.85 and 0.45 eV respectively) and the mean annual absolute temperatures in the heated and unheated mesocosms (290.9 and 286.1 K respectively) into equation (11) I would expect a 1.30 fold (range 1.18 to 1.38; based on the 95% confidence intervals of the respective empirically measured activation energies) increase in $R_{fixed}H:U$. The empirically measured value of $R_{fixed}H:U$ was 1.20, close to my theoretical prediction.

Similarly, the ratio of CH_4 efflux to ER was significantly elevated in the heated mesocosms, on average, over the annual cycle (Table 4.1 and Fig 4.7b), with warming elevating the mean annual ratio of CH_4 efflux to ER by 9%. By substituting the empirically derived activation energies for methanogenesis, E_m, and *ER*, E_r, (0.85 and 0.62 eV respectively) and the mean annual absolute temperatures in the heated and unheated mesocosms (as in equation 11) into equation (12), I would expect a 1.16 fold (range 0.17 to 1.24; based on the 95% confidence intervals of the respective empirically measured activation energies) increase in $R_{emitted}H:U$. Again, my empirically measured value of $R_{fixed}H:U$ was 1.09 (9%) (±SE = 0.22) and very close to my theoretical prediction.

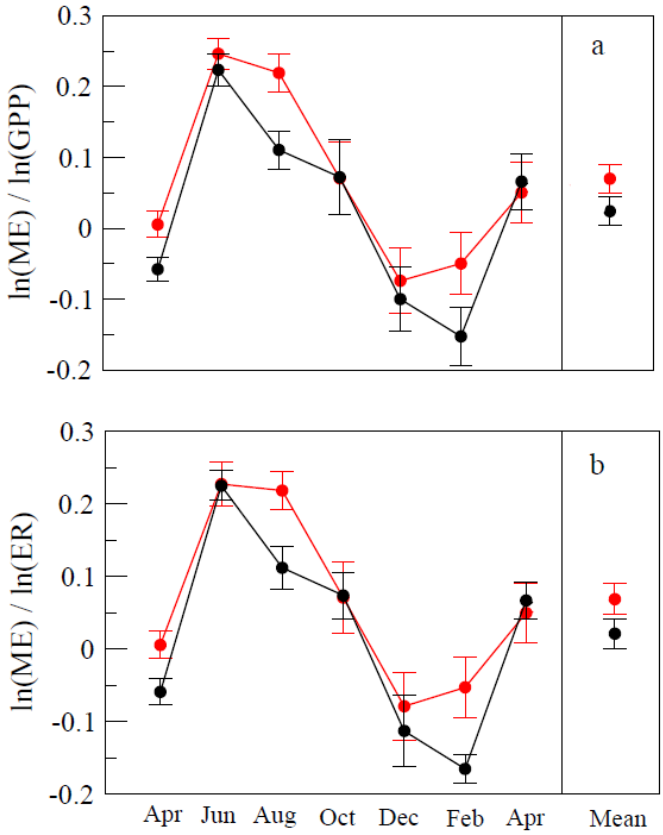

Fig. 4.7. (a) Differences in the ratio of methane efflux to GPP [ln(ME)/ln(GPP)] (±SE) and (b) methane efflux to ER ln(ME)/ln(ER) (±SE) between heated (red lines) and unheated (black lines) experimental treatments. Both ln(ME)/ln(GPP), and ln(ME)/ln(ER), were elevated, on average over the annual cycle in the warmed treatments. The magnitude of the increase in ln(ME)/ln(GPP) and ln(ME)/ln(ER) reflected the differences in activation energies of these three metabolic processes. Correspondingly, the fraction of GPP respired via the methanogenic pathway increased by 20% in the warmed mesocosms. Furthermore, the fraction of ER due to methanogenesis was 9% greater in the heated treatment.

Discussion

Mesocosm experiments represent a compromise between the control and replication of laboratory studies and the realism of descriptive field surveys but, despite their limitations, can provide a fundamental tool for predicting how global change scenarios might affect ecosystem level processes (Benton *et al.* 2007). Measured rates of mesocosm CH_4 efflux (0.35 – 8.14 µmol CH_4 m^{-2} h^{-1}) were comparable to those measured in natural shallow lakes (Rudd & Hamilton

1978, Bastviken *et al.* 2004), suggesting that the experimental scale was sufficiently realistic to reproduce the fundamental components of biogeochemical cycling of carbon observed in natural ecosystems.

To provide further quality control to rule out the influence of elevated gas transfer in the warmed mesocosms due to advection I simultaneously measured the concentration of dissolved CH_4. If the gas transfer velocity was systematically enhanced by artificial warming, I would have expected to observe considerable differences in the concentration of dissolved CH_4 between treatments, because of more rapid de-gassing of dissolved CH_4 in the warmed mesocosms. This was not the case. At first glance, the consistency in the pool size of dissolved CH_4 appears at odds with the elevated efflux of CH_4 measured in the warmed mesocosms because the gas transfer velocity and the concentration gradient (i.e. between the water and the atmosphere) drive the efflux of gas across the air-water interface (Cole & Caraco 1998). This discrepancy can be explained when the relative magnitudes of the respective processes and pool sizes are taken into consideration: using the 75^{th} percentiles for both CH_4 efflux and dissolved CH_4 (scaled to a whole mesocosm) of 11 μmol CH_4 $mesocosm^{-1}$ h^{-1} and 662 μmol CH_4 $mesocosm^{-1}$, respectively, 75% of the measurements of CH_4 efflux represented <1.7% of the total pool of dissolved CH_4. The subtle differences detected in the efflux of CH_4 between treatments would have been masked when analysing for treatment effects at the level of the pool, because the overall magnitude of the pool size of dissolved CH_4 was much greater than the flux. Therefore, any error associated with the measurement of the CH_4 pool would likely overwhelm the detection of any subtle statistical differences between treatments. This evidence suggests, therefore, that the physical influence of heating the mesocosms by ~4˚C had little discernable effect on advective processes.

Consequently, the biogeochemical patterns revealed by the experiment could be ascribed to the biological consequences of warming on the experimental ecosystems.

My experimental results have implications for understanding the mechanisms controlling CH_4 efflux from freshwater ecosystems, and how CH_4 dynamics in relation to carbon sequestration rates might be affected by future global warming. The experiment revealed that temperature was the dominant driver of CH_4 efflux from the mesocosms. This result agrees with other studies from a range of natural ecosystems, from soils to wetlands, which also highlight the strong temperature dependence of CH_4 efflux (Schutz *et al.* 1990, Whiting & Chanton 2001, Christensen *et al.* 2003a, Gedney *et al.* 2004). Because of the overriding influence of temperature, the overall rates of CH_4 efflux were elevated on average in the warmed mesocosms relative to ambient conditions over the course of the annual study, presumably reflecting the strong physiological response to the temperature stimulation of methanogenesis.

The activation energy for CH_4 efflux at the ecosystem level was indistinguishable from the activation energy of methanogenesis in pure cultures, in line with my predictions. The consistency of these results across experimental scales and organisational levels suggests that much of the potential complexity associated with ecosystem level efflux of CH_4 might be reduced to the first principals of individual/cellular kinetics. This result suggests that whole ecosystem metabolic fluxes can be scaled from the individual to the ecosystem level, in line with my predictions derived from the "metabolic theory of ecology" (Enquist *et al.* 2003, Allen *et al.* 2005, Lopez-Urrutia *et al.* 2006). This study, therefore, contributes to the growing body of evidence which suggests that metabolism is a fundamental driver of the dynamics of ecological processes across multiple levels of organisation, by demonstrating that CH_4 efflux at the ecosystem level appears to be constrained by the activation energy of methanogenesis. The

models presented in this study and those derived from the metabolic theory of ecology might therefore provide additional insight into the dynamics and temperature response of whole ecosystem CH_4 efflux in aquatic and other ecosystems.

As well as temperature, primary production has been shown to regulate the efflux of CH_4 to the atmosphere. Such regulation stems from the whole autotrophic assemblage providing structural, labile carbon compounds in the form of dead biomass (Whiting & Chanton 1993), and vascular plants producing root exudates in the form of organic acids (Chanton *et al.* 1995, Joabsson & Christensen 2001, Christensen *et al.* 2003b). Furthermore, rooted aquatic vascular plants can act as conduits for the transport of CH_4 from the anaerobic zone of the sediment to the atmosphere, bypassing the zones of potential CH_4 oxidation in the sediment and water column (Joabsson *et al.* 1999, Joabsson & Christensen 2001). In the experiment, however, the efflux of CH_4 did not appear to be limited by substrates from GPP, as suggested by three lines of evidence. Firstly, the weak correlation and gentle slope of the relationship between the efflux of CH_4 and GPP indicated that the flux of CH_4 was relatively independent of the simultaneous rate of photosynthesis and carbon fixation. Secondly, warming had no effect on the intercept of the relationship between ln(CH_4 efflux) vs $1/kT$ between treatments (Table 4.2). In equation 6, I predicted that if organic substrates were limiting for CH_4 production and efflux, I would expect to see a lower intercept in the warmed treatments because elevated physiological rates would be expected to reduce organic substrates more rapidly, resulting in faster substrate limitation and thus a reduced intrinsic capacity for methanogenesis. Thirdly, on average, the efflux of CH_4 represented a very small fraction of GPP (mean annual value = 0.01%). If oxidation of methane production is assumed to be 95% (King *et al.* 1990), from the average annual CH_4 efflux measures (3 μmol m^{-2} h^{-1}), I estimate the mean annual CH_4 production to be ~290 μmol m^{-2} h^{-1}

which would represent only 10% of the mean annual rate of GPP (2862 μmol m^{-2} h^{-1}). Therefore, carbon sequestration and fixation by photosynthesis is likely to vastly exceed the demand of methanogenesis throughout the annual cycle.

The response of the greenhouse gas carbon balance of freshwater ecosystems to warming could affect the strength of biotic feedbacks on a potentially global scale (Woodwell *et al.* 1998). In my experiment, the fraction of carbon absorbed by GPP and subsequently remineralised via the methanogenic pathway to efflux as CH_4 increased by 20% in response to the simulated global warming scenarios projected for the end of the century. In addition, the efflux of CH_4 as a proportion of ER was 9% greater in the warmed mesocosms. If, as aquatic ecosystems warm, carbon remineralisation becomes increasingly dominated by methanogenesis this could result in more CH_4 being emitted to the atmosphere relative to CO_2 emission and carbon draw-down. Using a novel extension of the metabolic theory of ecology I was able to show that these patterns could be explained by the differential activation energies of the three metabolic processes involved in the greenhouse carbon balance of ecosystems. For instance, here, and in *chapter three*, I have demonstrated that the three key ecosystem level carbon fluxes have progressively higher activation energies (i.e., GPP = 0.45eV; ER = 0.62eV; CH_4 efflux = 0.85eV). Furthermore, using, equations 11 and 12 I was able to predict the direction of change in the relative offset of the greenhouse carbon gas balance between the ambient and warmed mesocosms. This finding suggests that the response of the main components of the carbon cycle to warming can be predicted by the differences in activation energies of the metabolisms and the degree of expected warming. In addition, this result highlights the potential for a positive feedback between warming and the carbon cycle of freshwater ecosystems, especially given the greater radiative forcing potential of CH_4 (Rodhe 1990, Lelieveld *et al.* 1991, Whiting &

Chanton 2001). Finally, accepting the caveats associated with mesocosms, the close agreement between the activation energy of methanogenesis in pure culture and that of whole system CH_4 efflux, suggests that much of the complexity of ecosystem level fluxes can be reduced to produce simpler predictive models.

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Warming Alters the Size Spectrum and Shifts the Distribution of Biomass in Aquatic Ecosystems

A modified version of this chapter is in-press in Global Change Biology

Abstract

Body size is one of the key determinants of community structure. The relationship between abundance and body size can explain how community biomass is partitioned among the biota of an ecosystem. I used an aquatic mesocosm experiment to explore how warming of ~4˚C would affect the distribution of body size and biomass in planktonic communities. I found that warming increased the steepness of the slope of the community size spectrum, primarily by altering the phytoplankton size spectrum. Warming also reduced the mean and maximum body size of phytoplankton by approximately one order of magnitude. The observed shifts in phytoplankton size structure were reflected in large shifts in phytoplankton community composition, though zooplankton taxonomic composition was unaffected by warming. Furthermore, warming reduced community biomass and total phytoplankton biomass, although zooplankton biomass was unaffected. This resulted in an increase in the zooplankton to phytoplankton biomass ratio in the warmed mesocosms, which could be explained by faster turnover within the phytoplankton assemblages. Overall, warming shifted the distribution of phytoplankton body size towards smaller individuals with rapid turnover and low standing biomass, resulting in a reorganisation of the biomass structure of the food webs. These results indicate future environmental warming may have profound effects on the structure and, by extension, the functioning of aquatic communities.

Introduction

Body size can play a key role in determining community structure (Elton 1927, Lindeman 1942, Damuth 1981, Peters 1983, Brown *et al.* 2004, Petchey *et al.* 2008) because it influences ecological processes across multiple levels of organisation; from individuals (Peters 1983, Brown *et al.* 2004), and their interactions (Emmerson & Raffaelli 2004, Berlow *et al.* 2009), to populations (Damuth 1981, Jennings & Mackinson 2003, Reuman *et al.* 2008), communities and ecosystems (Petchey *et al.* 2008). Understanding how this "size-structure" might then be altered by human impacts is an important contemporary challenge for ecology, especially in light of recent concerns over accelerating rates of biodiversity loss and climate change (Walther *et al.* 2002).

The relationship between abundance and body size (≈ body mass; terms are interchangeable hereafter) is potentially a very powerful descriptor of how energy and nutrients are partitioned within the biomass of an ecosystem (White *et al.* 2007). It is also a result of size structure at lower levels of organisation: for example, body size can be important for determining the presence and strength of trophic interactions between individuals because it constrains their metabolic requirements (Berlow *et al.* 2009). The trophic architecture of the community determines the amount of energy available to an organism of a given size, and therefore its population abundance (Damuth 1981). The relationship between abundance and body mass therefore integrates size-structure over many levels of organisation.

Since the pioneering work of Sheldon *et al*. (1972) the relationship between abundance and body size in pelagic food webs has typically been conceptualised as a frequency distribution of individual body sizes, largely irrespective of taxonomy (Sheldon *et al.* 1972). This

relationship has been dubbed the "size spectrum" (Kerr & Dickie 2001). The negative slopes of size spectra describe how quickly abundance decreases with size, and have often been used to assess the ecological status of aquatic ecosystems impacted by fisheries (Rice & Gislason 1995) and, more recently, terrestrial systems impacted by agricultural practices (Mulder & Elser 2009). For example, in the former case, steep size spectra with negative slopes in marine ecosystems are indicative of over-fishing because the relative abundance of large organisms is suppressed by size-selective harvesting (Pauly *et al.* 1998).

Understanding how the distribution of biomass in aquatic ecosystems might respond to warming is crucial for predicting the robustness and functioning of these ecosystems in the warmer climates predicted for the coming decades. New evidence suggests that "*reduced body size is the third universal response to global warming*, *besides range, and phenological shifts*" (Daufresne *et al.* 2009). Changes in the size-structure of communities in response to warming are now being documented across a range of ecosystem types and spatial scales. For instance, experiments on aquatic micro-organisms have found that warmed communities appear to be dominated by smaller bacteria (Daufresne *et al.* 2009). Macroecological studies across latitudinal temperature gradients (Moran *et al.* 2010), and palaeoecological studies (Finkel *et al.* 2005) in the open ocean have also revealed an increased prevalence of small phytoplankton in warmer oceanic regions. These studies suggest that the underlying size structure of aquatic ecosystems might not be robust to global warming (Finkel *et al.* 2005, Falkowski & Oliver 2007, Daufresne *et al.* 2009, Winder *et al.* 2009, Finkel *et al.* 2010, Moran *et al.* 2010). This has important implications for the carbon sequestration capacity of oceanic ecosystems because plankton particle size can be an important determinant of sinking rates which drive carbon export in the biological pump (Fujii *et al.* 2005).

However, these studies have either focused on the effects of warming on restricted subsets of species (e.g. diatoms or phytoplankton) within an ecosystem (Finkel *et al.* 2005, Winder *et al.* 2009) or documented changes in community size-structure across latitudinal gradients where other factors (i.e. nutrient limitation) are potentially confounded with temperature (Moran *et al.* 2010). At present, we still lack sufficient data documenting the effects of warming *per se* on the size-structure of entire local communities to be able to isolate its effects at this level of biological organisation.

Here I attempt to address this current knowledge gap by characterising the community size spectrum and the distribution of biomass in planktonic food webs from 20 replicated freshwater mesocosms. These experimental systems were maintained at either ambient temperature (n = 10), or ~ 4ºC above ambient (n = 10), in line with warming scenarios predicted for temperate latitudes by the end of the 21st century (IPCC 2007), as part of a long-term field experiment. Mesocosm scale experiments such as these afford the opportunity to isolate the effects of temperature from other potentially confounding variables (e.g. spatial gradients in available nutrients) on the structure of entire replicated communities. They also permit direct comparisons to be made between communities under ambient conditions with those of their "future" warmed counterparts. I used this experiment to test the following hypotheses:

(i) Warming will shift the distribution of body size by increasing the prevalence of small sized organisms, resulting in an overall steepening of the slope of the community size spectrum. This effect is predicted to be most pronounced in the phytoplankton assemblages because phytoplankton size structure tends to be strongly related to the prevailing physical and chemical environment (Reynolds 1984) and recent observations in aquatic ecosystems suggest that

warming tends to favour smaller phytoplankton (Finkel *et al.* 2005, Falkowski & Oliver 2007, Daufresne *et al.* 2009, Winder *et al.* 2009, Moran *et al.* 2010).

(ii) Warming will reduce total standing community biomass. Again, this effect is predicted to be most pronounced for phytoplankton for two reasons. First, a shift in the community size spectrum towards smaller individuals should result in an overall reduction in the standing biomass. Second, theoretical expectations from the metabolic theory of ecology (MTE) suggest total standing biomass should decline with increasing temperature (Allen *et al.* 2002), such that the total standing biomass in a community (B_{tot}) is predicted to vary as $B_{tot} = r_0 e^{-E/kT} M^{1/4}$ where r_0 is the resource supply rate, $e^{-E/kT}$ is the Boltzmann factor where E is the activation energy of metabolism, k is Boltzmann's constant and T is absolute temperature. Therefore, holding r_0 constant (i.e. if the supply rate of limiting resources does not vary with T), B_{tot} should decline with increases in environmental temperature according to $e^{-E/kT}$.

(iii) Warming will alter the relative distribution of biomass between phytoplankton and zooplankton assemblages. This shift in phytoplankton size structure and a concomitant reduction in standing biomass will result in elevated zooplankton-to-phytoplankton biomass ratios in the warmed mesocosms. I also predict that relatively high zooplankton biomass will be retained in the warmed mesocosms, because phytoplankton turnover rates should increase in response to metabolic stimulation by warming and by a shift towards smaller individuals with faster generation times. Comparable shifts in the organisation of plankton communities have been observed in the open ocean (Gasol *et al.* 1997) and in lakes (del Giorgio & Gasol 1995) along large scale spatial gradients of nutrient limitation.

Materials and Methods

Measuring the Size Spectrum

The plankton communities from each of the 20 mescosms were sampled at the beginning and end of the growing season in April and October 2007 respectively (Yvon-Durocher *et al.* 2010) to limit the potential disturbance caused by the destructive sampling of the biota which was necessary to characterise the size spectra. The entire water column (depth 0.5m) from the sediment surface to the water surface was sampled using a 0.8m – long tube sampler (Volume: 2L), which was positioned at random in each mesocosm on each date. Each sample was divided into two size categories for preservation and subsequent analyses, via filtration through a 80µm sieve: organisms that were retained were preserved in 4% Formalin, and of the remaining sample (i.e. organisms <80 µm), a 100ml sub-sample was preserved in 1% Lugol's iodine for microscopy analyses.

Plankton >80 µm were counted and measured by microscopy (using a Nikon SMZ1500 dissection microscope). Planktonic Organisms <80µm were enumerated and measured by inverted microscopy. Organisms were settled for 24 h in a 10ml Utermöhl sedimentation chamber before viewing under an inverted light microscope (Leica DMIRE2). An initial scan of the sample, viewed under low magnification (150×), of a fixed area (50 mm^2) was used to count and measure large, rare organisms. At higher magnification (630×), *n* fields of view were chosen at random and all organisms were counted, sized and identified until a minimum of 400 individuals were measured from each sample. This was sufficient to estimate 95% of the variance in the distribution of body size (Fig. 5.1) given that settlement of organisms followed a Poisson distribution within the sedimentation chamber (Fig 5.2).

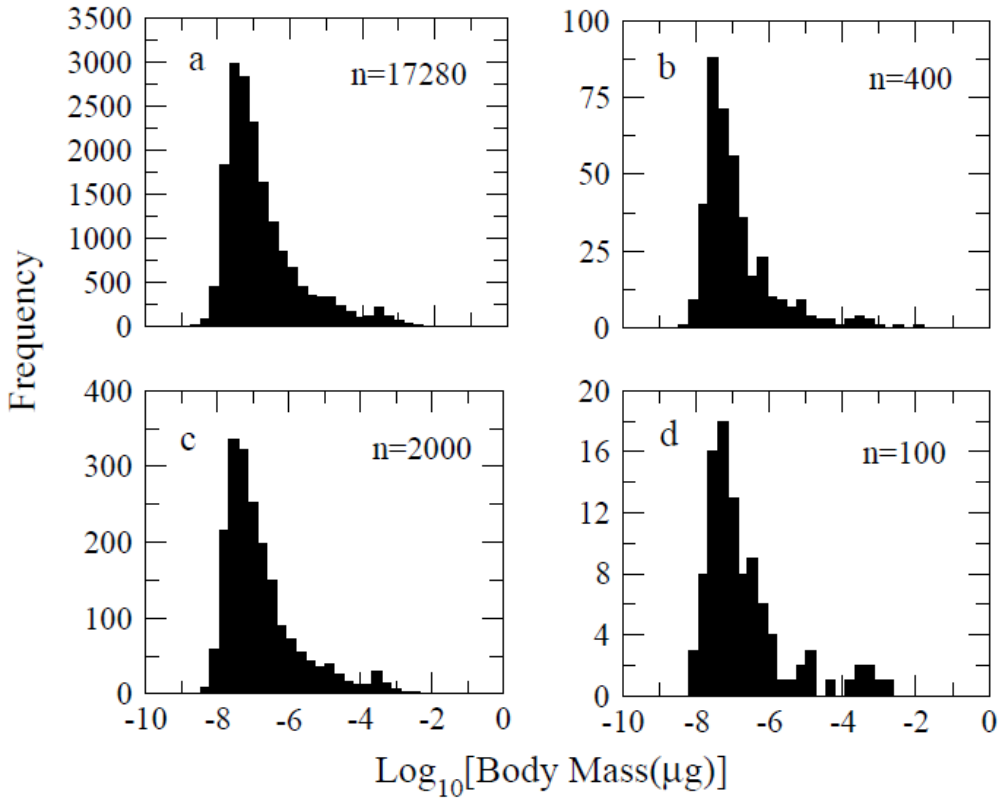

Fig. 5.1. Frequency distributions of individual body mass for (*a*) all individuals measured, (*b*) a random sample of 400 (i.e. the number of individuals actually measured in a sample) from *a*, (*c*) a random sample of 2000 from *a*, (*d*) a random sample of 100 from *a*. Data highlight that a sample of 400 individuals is sufficient to estimate the variance in the distribution of body size comparable to the whole community. When measuring the phytoplankton a minimum of 400 individuals from any given pond were measured over the number of fields of view required to count 400 from the sample in the sedimentation chamber. It is also clear that a sample of 100 is not sufficient to accurately reproduce the variance in the body mass distribution of the whole community. Assuming that organisms of a given body mass are Poisson distributed (figure S2, table S3) on the surface of the sedimentation chamber, the measurement of 400 individuals should be sufficient to attain an error of 5% [if error = 1/sqrt(*n*)].

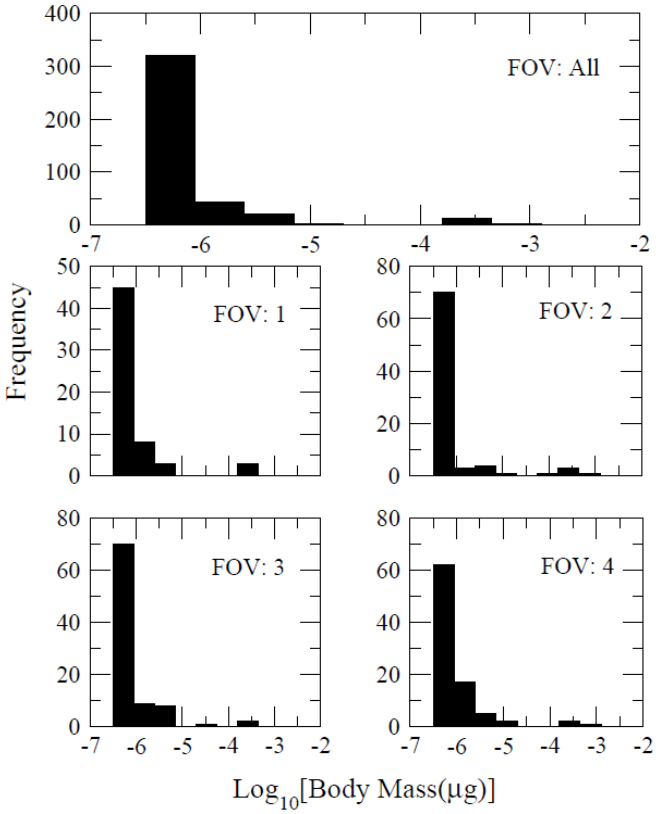

Fig. 5.2. Size-frequency distribution for phytoplankton in pond 14 from April 2007. Panels show the size-frequency distribution after analysing all fields of view (FOV) taken to measure ~400 individuals in the sedimentation chamber, 1 FOV, 2 FOVs, 3 FOVs and 4 FOVs. Data highlight the equitable distribution of body size among fields of view which reflects the random settlement of phytoplankton cells in the sedimentation chamber. Tests for dispersion were carried for all samples and settlement conformed to Poisson statistics in every case.

Linear body dimensions were determined with an interactive image analysis system (Hamamatsu C4742-95 camera and Openlab software). Body size of all organisms was expressed in units of carbon (µg C). For organisms >80µm (typically zooplankton), biovolumes were determined by assigning organisms to geometric shapes (see appendix 6) that closely represented the real shape of the organism (Ruttner-Kolisko 1977, Reiss & Schmid-Araya 2008). Body mass was determined by converting biovolume to freshweight using a factor of 1.1, and carbon content was then estimated from a dry/wet weight ratio of 0.25 and a dry carbon content of 40% (Reiss & Schmid-Araya 2008). For organisms <80µm (typically phytoplankton) biovolumes were

similarly estimated from geometric shapes (see appendix 6) that were most similar to the shape of the organism (Hillebrand *et al.* 1999). Biovolume was then converted into carbon units assuming a multiplication factor of 0.109 (Montagnes *et al.* 1994). In total 47,699 individual organisms of both phytoplankton and zooplankton were measured.

Phytoplankton turnover

Turnover rates of the phytoplankton assembalges ($\mu g\ C\ m^{-3}\ d^{-1}/\mu g\ C\ m^{-3}$) were estimated for each mesocosm on each sampling occasion ($n = 40$). Phytoplankton turnover was calculated as the quotient of primary production and standing phytoplankton biomass after Gasol *et al*. (1997). This gives an estimate of the biomass specific production, or the rate at which the carbon in the assemblage turns over. Measurements of primary production were made simultaneously using the dissolved oxygen change technique and are presented in detail in *chapter three*.

Constructing the Size Spectrum

The community size spectrum ($n = 40$), which included phytoplankton and zooplankton, and the phytoplankton assemblage size spectrum ($n = 40$) were constructed for each mesocosm on each sampling occasion. The size spectrum of the zooplankton assemblage alone could not be constructed accurately due to the relatively small body mass range and the low number of individuals present in some samples. Size spectra were constructed by logarithmic binning of the body masses (M) of the individuals measured in each mesocosm (either the entire community or just the phytoplankton). The total range of $\log_{10}(M)$ values was divided into 10 bins of equal width and the $\log_{10}$ of the total population abundance of all organisms with $\log_{10}(M)$ in each bin

were regressed against the bin centres (Reuman *et al.* 2008, White *et al.* 2008). The slope of the linear model describes how quickly the abundance of individuals declines with increasing size in the size spectrum (see appendix 4 and 5). I also derived two normalisation constants of the linear model. The intercept at $x = 0$: its variation between warmed and ambient treatments gives information on the relative abundance of large organisms, and the intercept at $x = -8$: its variation provides information on the differences among treatments in the relative abundance of the smallest organisms. For both the community and the phytoplankton size spectrum, non-significant coefficients of the linear models (i.e. at $P>0.05$) were excluded from further analyses ($n = 5$ out of 40 for the phytoplankton size spectrum).

Statistical Analyses

Between treatment differences in size spectrum slopes and intercepts, total community biomass, phytoplankton and zooplankton biomass and mean individual body mass, were analysed by ANOVA, using treatment (either warmed or ambient) and sampling occasion (April or October) as fixed factors. The relationships between phytoplankton and zooplankton biomass and the biomass ratio of zooplankton to phytoplankton were determined using ANCOVA, again using treatment and sampling occasion as factors. In all statistical modelling procedures the most parsimonious model was identified using the Akaike Information Criterion (AIC). Statistical analyses were performed using R statistical software (R. Development. Core. 2006).

Multivariate analysis of phytoplankton taxonomic composition was conducted using the vegan package in R. Redundancy analysis (RDA) was used to test for a significant linear trend in community composition. RDA is a constrained form of principal components analysis and

assesses the variation in taxonomic composition that can be explained by specific environmental variables defined as the constraints. Here, the first RDA axis quantified the linear component of the between treatment variation in phytoplankton taxonomic composition. Consequently, it was used to assess the strength of the trend and its significance was tested using permutation tests. The *F*-ratio of the first RDA axis was compared with those of 999 permutations, to assess the statistical significance of the linear trend. As well as treatment (warming), NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, and total inorganic N:P (see *chapter two* for details on nutrient measurements) were tested as constraining environmental variables. Phytoplankton taxon biomass was transformed prior to the construction of the RDA by taking the proportional contribution of a given taxa as a fraction of the total biomass in a given mesocosm. Furthermore, rare genera defined as those occurring in less than two mesocosms per sampling date were excluded from the RDA analysis to reduce noise in the data.

Results

Warming alters the Size Spectrum

Warming significantly increased the steepness of the slope of the community size spectrum from -0.86 (95% CI -0.83 to -0.89) in the systems at ambient temperature to -0.95 (95% CI -0.92 to -0.98) in the warmed mesocosms (Fig. 5.3 a, b & c; Table 5.1), i.e., smaller organisms were relatively more abundant than large organisms in the warmed communities. Furthermore, the intercept of the community size spectrum at $x = 0$ (i.e. at large body masses) was significantly reduced, whilst the intercept at $x = -8$ (i.e. at small body masses) was significantly elevated in the warmed mesocosms (Table 5.1). Thus, the abundance of larger organisms declined on average, whereas the abundance of small organisms increased in response to warming.

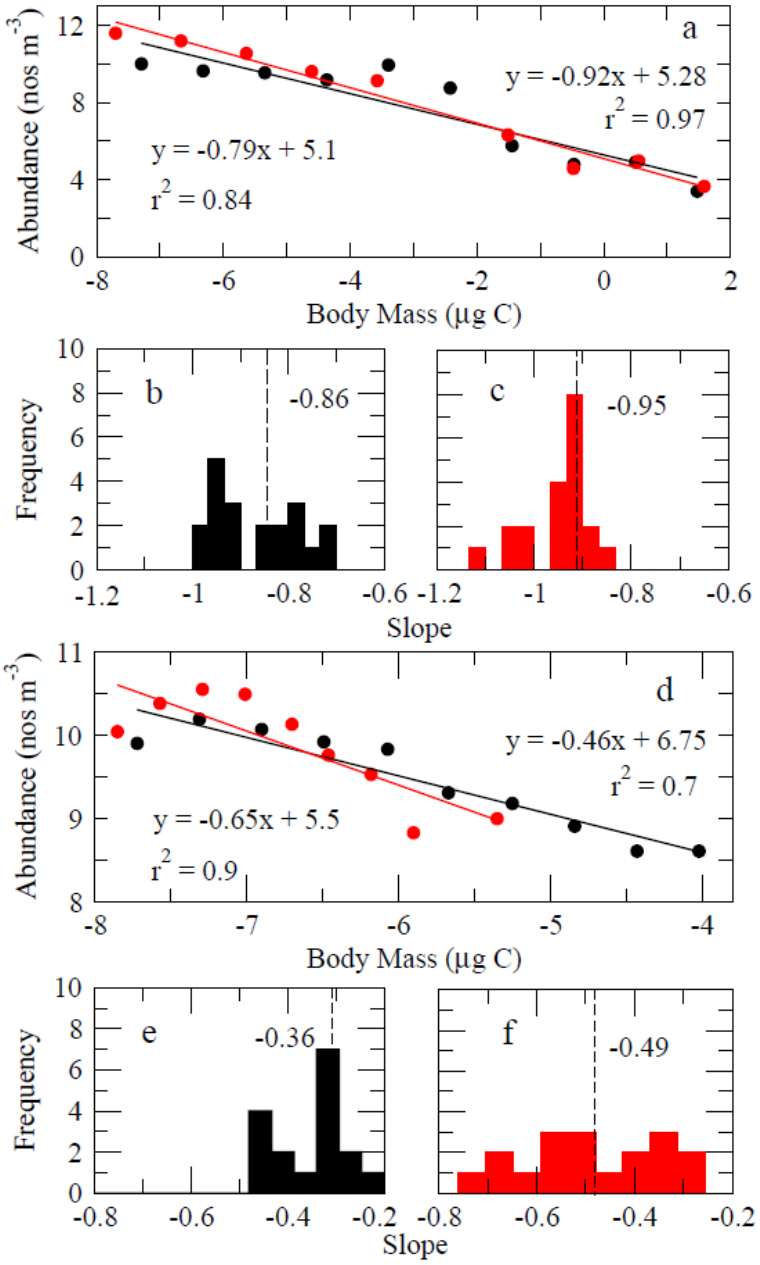

Fig. 5.3. The size spectrum. (a) The community size spectrum of a heated (red circles) and ambient (black circles) mesocosm, highlighting the increase in the steepness of the slope in the warmed mesocosm. (b) Frequency distribution of the slope of the community size spectrum in the ambient mesocosoms (*n*=20), (c) frequency distribution of the slope of the community size spectrum in the warmed mesocosoms (*n*=20). On average the slope of the community size spectrum in the warmed mesocosms was significantly steeper than the ambient mesocosms (Table 1). (d) The phytoplankton size spectrum of a heated (red circles) and ambient (black circles) mesocosm, highlighting the increase in the steepness of the slope and the truncation of large sized individuals in the warmed mesocosm. (e) Frequency distribution of the slope of the phytoplankton size spectrum in the ambient mesocosoms (*n*=17), (f) frequency distribution of the slope of the community size spectrum in the warmed mesocosoms (*n*=18).

Comparable patterns were observed for the phytoplankton size spectrum (Fig. 5.3 d, e & f). Warming significantly increased the steepness of the slope of the phytoplankton size spectrum from -0.36 (95% CI -0.32 to -0.40) in the systems at ambient temperature to -0.49 (95% CI -0.43 to -0.55) in the warmed mesocosms (Table 5.1; Fig. 5.3 d, e & f). Warming also significantly reduced the intercept of the phytoplankton size spectrum (Table 5.1). Therefore, small organisms were relatively more abundant than large organisms in the warmed mesocosms. Additionally, warming truncated the upper size classes of the phytoplankton size spectrum (Fig. 5.3 d).

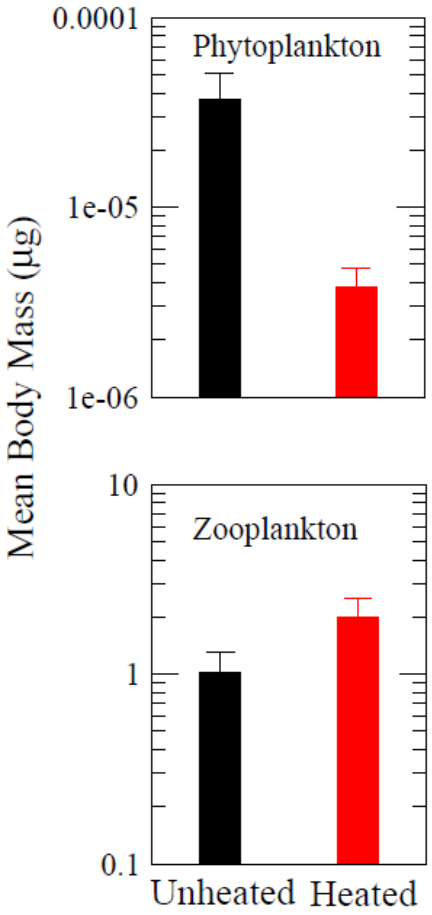

Fig. 5.4. Effects of warming on mean body mass (±1 s.e.m) of phytoplankton (a) and zooplankton (b) individuals. Data are presented as the overall average of the mean body mass of phytoplankton and zooplankton individuals over 20 mesocosms for each treatment. The mean cell mass of phytoplankton is significantly reduced in response to warming while there is no significant difference in the mean body mass of zooplankton between heated and unheated treatments (table 5.1).

The maximum phytoplankton body mass in the ambient mesocosms was 1.36×10^{-2} µg C, while in the heated the maximum body mass was only 3.88×10^{-3} µg C. Furthermore, the average body mass of an individual phytoplankter was almost an order of magnitude smaller in the warmed mesocosms relative to the ambient systems (Fig. 5.4; Table 5.1), while the average size of an individual zooplankter was unaffected by warming (Fig. 5.4; Table 5.1).

Community Property	DF	*F*	*P*
CSS slope	1, 38	11.1	**0.002**
CSS intercept ($x = 0$)	1, 38	8.2	**0.007**
CSS intercept ($x = -8$)	1, 38	4.2	**0.047**
PSS slope	1, 33	11.8	**0.002**
PSS intercept	1, 33	8.27	**0.007**
Total community biomass	1, 38	10.8	**0.002**
Total phytoplankton biomass	1, 38	13.1	**<0.001**
Total zooplankton biomass	1, 38	0.47	0.5 (NS)
Mean phytoplankton body mass	1, 38	18.9	**<0.001**
Mean zooplankton body mass	1, 38	1.4	0.2 (NS)
Z:P Biomass ratio	1, 38	4.82	**0.034**

Table 5.1. The effect of treatment (heated or ambient) on community-level properties. CSS is the community size spectrum and PSS is the phytoplankton size spectrum. ANOVAs were used to isolate treatment effects on individual community-level properties. In each ANOVA month (either April or October) was added as a factor. For each community-level property there was no significant effect of month, which was removed from the model using the AIC score.

Effects of Warming on Community Composition

Redundancy analysis of the phytoplankton taxa revealed that the composition of the phytoplankton assemblages were significantly different between warmed and ambient treatments in both April (Fig. 5.5; *F*-ratio = 5.72; *P* = 0.011; permutation number = 999) and October (Fig. 5.6; *F*-ratio = 5.87; *P* = 0.001; permutation number = 999). RDA1 which was constrained by treatment, explained 24.1% and 24.6% of the variation in the taxonomic composition of the

phytoplankton assemblages in April and October respectively, which in both cases was greater than the variation explained by PCA1, indicating that treatment effects were the dominant predictor of phytoplankton taxonomic composition. We also tested for significant relationships between phytoplankton taxonomic composition and other environmental variables (NO_3^-, NO_2^-, NH_4^+, PO_4^{3-}, total inorganic N:P) using permutation tests, though none of these variables significantly predicted taxonomic composition. Certain taxa were strongly associated with either warmed or ambient treatments. For example, in both April and October, the large chlorophyte, *Botryococcus* clustered towards the ambient treatment centroid, while the small cyanophyte *Synechocystis*, and the small chlorophyte *Monoraphidium*, typically clustered towards the heated centroid. The phytoplankton assemblages consisted of many rare, generalist taxa that were present in both treatments; however, in most of the mesocosms the biomass was dominated by a few indicator taxa (named above) that were associated with either the heated or the ambient treatments. Furthermore, figures 5.5 and 5.6 show that a large core contingent of the phytoplankton assemblages were present in both April and October and that only a few taxa were present in only one month, suggesting that temporal succession was less important than treatment effects in determining phytoplankton community composition.

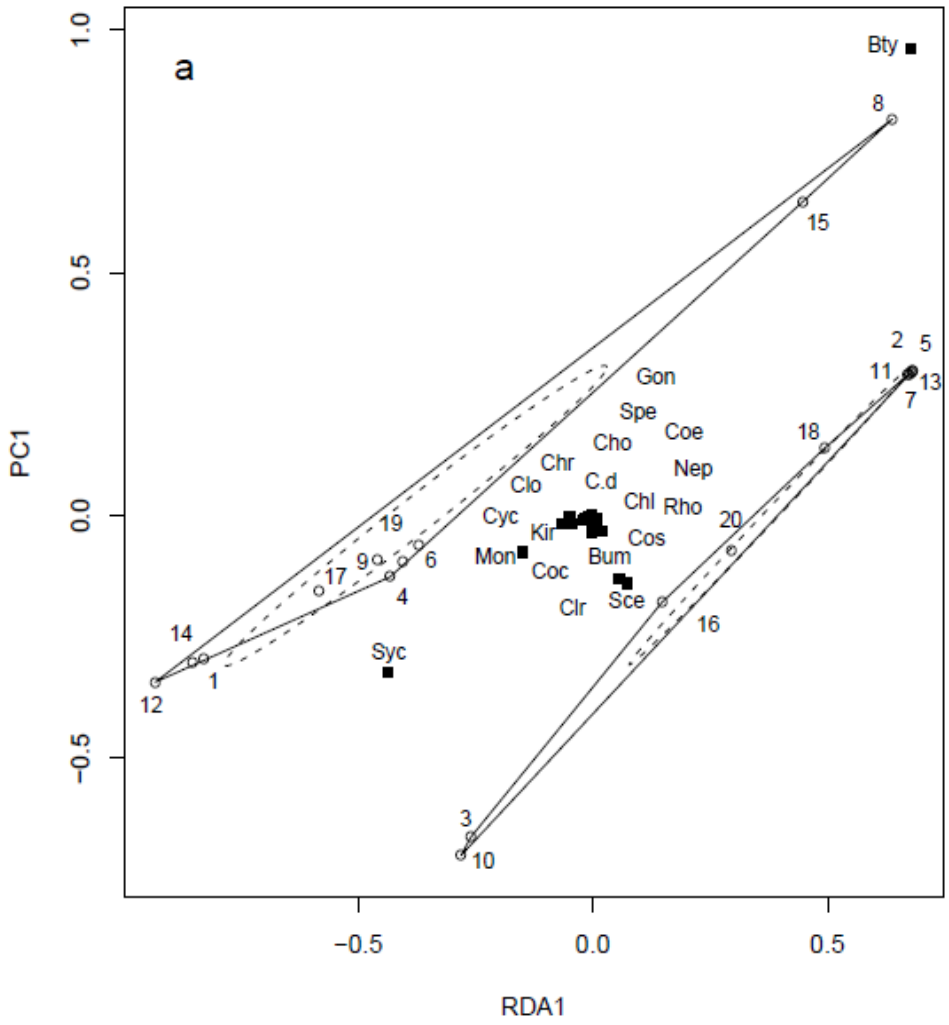

Fig. 5.5. Redundancy analysis (RDA) biplot for sites (i.e. mesocosms) and species scores for phytoplankton taxa recorded in the mesocosm experiment in April. RDA 1 was constrained by treatment and accounted for 24.1% of the variation in the taxonomic composition of the mesocosms. In the plot the dotted lines denote the 95% confidence ellipses around the centroids for both treatments. Note that these ellipses do not overlap indicating that the community composition was significantly different between warmed and ambient treatments. The solid lines enclose all mesocosms that belong to a particular treatment; in both cases heated treatments (1, 4, 6, 8, 9, 12, 14, 15, 17, 19) cluster to the left, while ambient treatments (2, 3, 5, 7, 10, 11, 13, 16, 18, 20) cluster to the right. Genus abbreviations are as follows: *Aphanothece* (Aph), *Asterococcus* (Ast), *Botryococcus* (Bty), *Bumilleriopsis* (Bum), *C.dinobryonis* (C.d), *Chlorella* (Chl), *Chlorococcum* (Coc), *Chroococcus* (Chr), *Chroomonas* (Cho), *Coencococcus* (Coe), *Cosmarium* (Cos), *Cryptomonas* (Cry), *Goniochloris* (Gon), *Kirchneriella* (Kri), *Monoraphidium* (Mon), *Navicula* (Nav), *Nephrocytium* (Nep), *Rhodomonas* (Rho), *Scenedesmus* (Sce), *Synechococcus* (Syn), *Synechocystis* (Syc), *Spermatozopsis* (Spe).

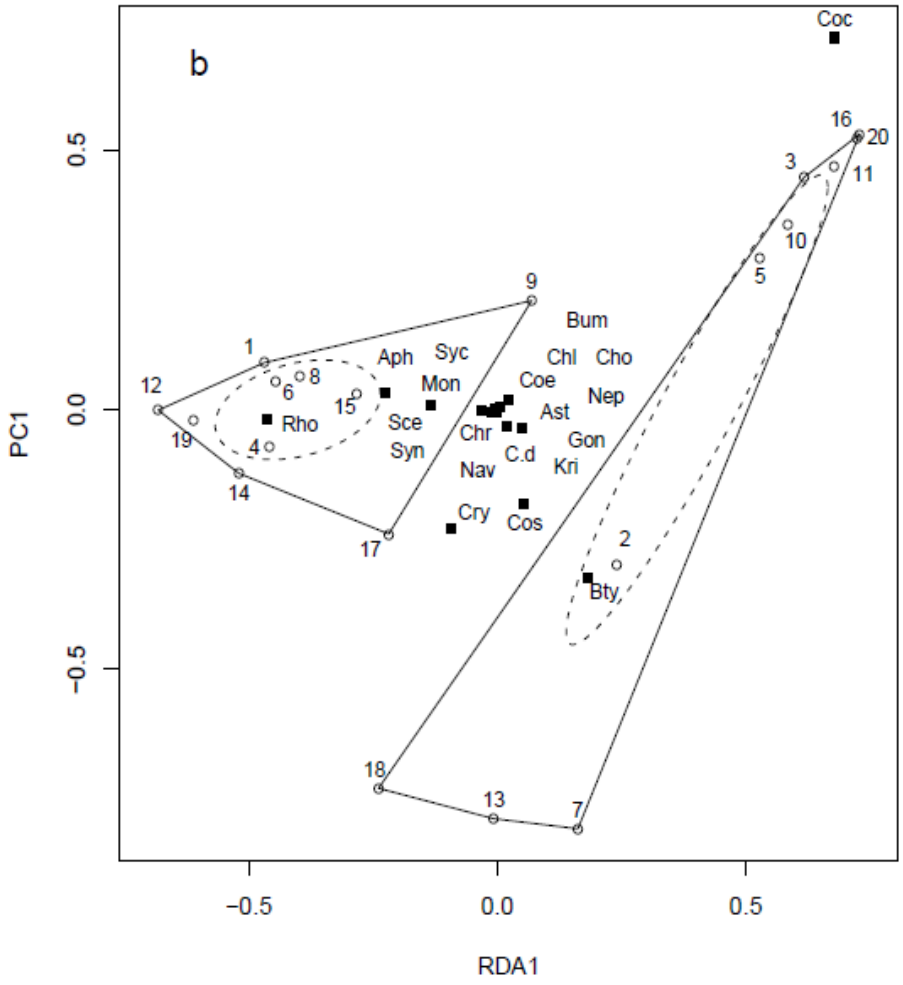

Fig. 5.6. Redundancy analysis (RDA) biplot for sites (i.e. mesocosms) and species scores for phytoplankton taxa recorded in the mesocosm experiment in April. RDA 1 was constrained by treatment and accounted for 24.6% of the variation in the taxonomic composition of the mesocosms. In the plot the dotted lines denote the 95% confidence ellipses around the centroids for both treatments. Note that these ellipses do not overlap indicating that the community composition was significantly different between warmed and ambient treatments. The solid lines enclose all mesocosms that belong to a particular treatment; in both cases heated treatments (1, 4, 6, 8, 9, 12, 14, 15, 17, 19) cluster to the left, while ambient treatments (2, 3, 5, 7, 10, 11, 13, 16, 18, 20) cluster to the right. Genus abbreviations are as follows: *Aphanothece* (Aph), *Asterococcus* (Ast), *Botryococcus* (Bty), *Bumilleriopsis* (Bum), *C.dinobryonis* (C.d), *Chlorella* (Chl), *Chlorococcum* (Coc), *Chroococcus* (Chr), *Chroomonas* (Cho), *Coencococcus* (Coe), *Cosmarium* (Cos), *Cryptomonas* (Cry), *Goniochloris* (Gon), *Kirchneriella* (Kri), *Monoraphidium* (Mon), *Navicula* (Nav), *Nephrocytium* (Nep), *Rhodomonas* (Rho), *Scenedesmus* (Sce), *Synechococcus* (Syn), *Synechocystis* (Syc), *Spermatozopsis* (Spe).

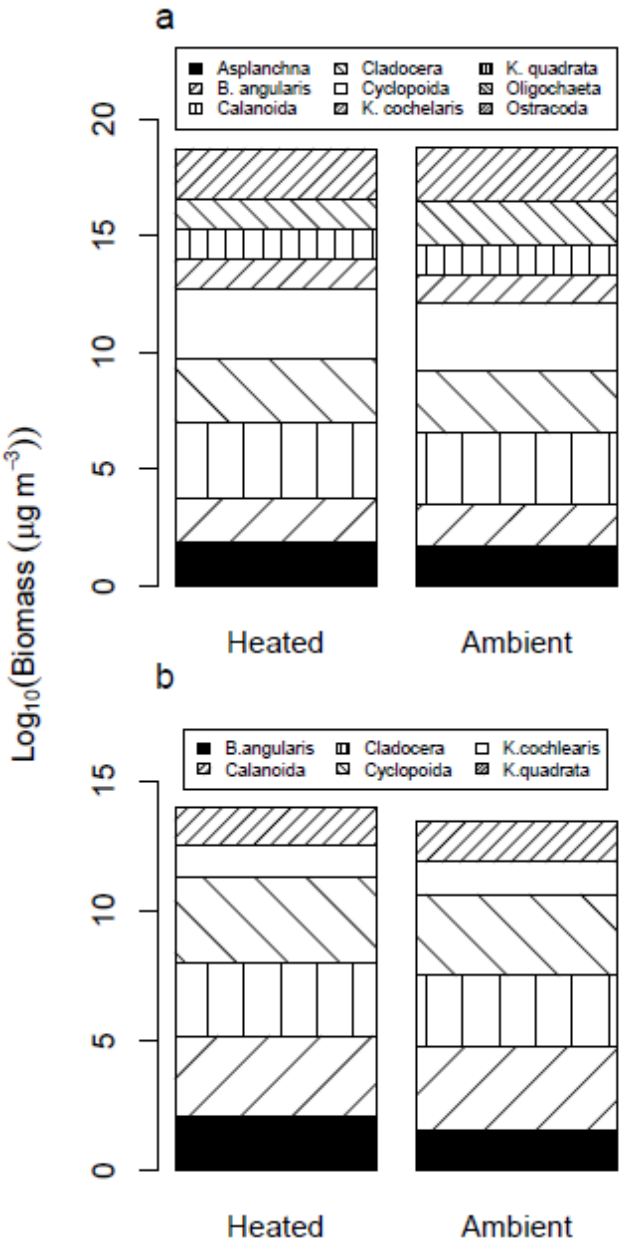

Fig. 5.7. Mean biomass of the major zooplankton taxonomic groups documented in the mesocosms in (a) April and (b) October. Note that there is very little difference in the biomass contribution of the different zooplankton taxa between treatments suggesting that the zooplankton community composition was unaffected by warming.

In contrast to the phytoplankton assemblages the taxonomic composition of the zooplankton assemblages differed very little between treatments in both April and October (Fig. 5.7a & b). In heated and ambient treatments calanoid and cyclopoid copepods dominated

zooplankton biomass with cladocerans and rotifers forming a smaller secondary contingent of the assemblages. These patterns were consistent between April and October, though ostracods, oligochates and the rotifer *Asplanchna* were absent from the zooplankton assemblage in October.

Warming Shifts the Distribution of Biomass

Total planktonic community biomass differed between April and October in the ambient but not in the warmed mesocosms (Fig. 5.8). Overall, warming significantly reduced total community biomass (Fig. 5.8; Table 5.1). This was principally driven by a considerable reduction in total phytoplankton biomass in the warmed mescocosms (Fig. 5.8; Table 5.1). Overall, warming shifted the distribution of biomass and body size of phytoplankton from assemblages comprised of large individuals with high standing biomass to assemblages with low standing biomass and many small individuals. In contrast, warming appeared to have no effect on the biomass of the zooplankton assemblages (Fig. 5.8; Table 5.1).

Zooplankton and phytoplankton biomass were not correlated (Fig. 5.9a; Table 5.2). The former varied by about two orders of magnitude and the latter by three orders of magnitude among mesocosms (Fig. 5.9a). The ratio of zooplankton to phytoplankton biomass (Z:P) was, however, significantly and negatively correlated with phytoplankton biomass (Fig. 5.9b; Table 5.2). In general, zooplankton biomass exceeded phytoplankton biomass (i.e. Z:P >1) when phytoplankton biomass was low and vice versa (i.e. Z:P <1) when phytoplankton biomass was high. Warming significantly increased the ratio of Z:P biomass (Table 5.1). Furthermore, the ratio of Z:P biomass was strongly and positively correlated with the estimated turnover rates of the phytoplankton assemblages, which exhibited distinct variation between warmed and ambient

mesocosms (Fig. 5.9c; Table 5.2). In summary, the warmed mesocosms were characterised by phytoplankton assemblages comprised of small individuals with low standing stocks of biomass and rapid turnover rates which supported relatively high standing stocks of zooplankton, exemplified by high Z:P biomass ratios.

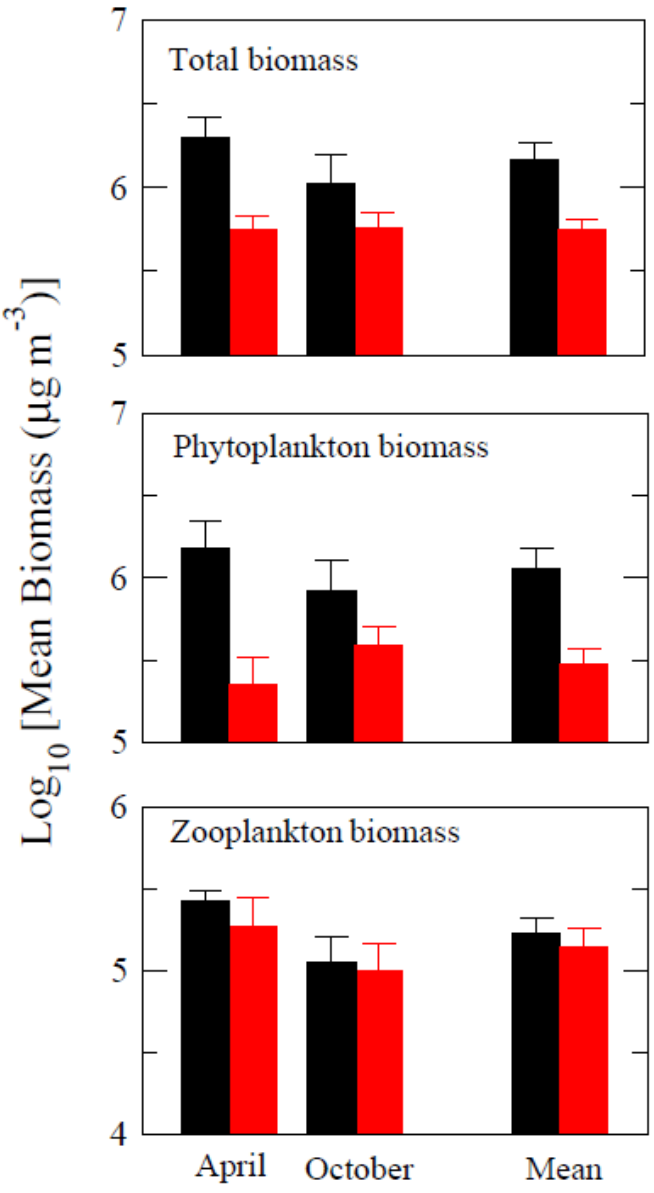

Fig. 5.8. Effects of warming on mean total planktonic biomass (±1 s.e.m). Data are presented as the averages of the total biomass of either phytoplankton and/or zooplankton across the mesocosms for each treatment (n=20 per treatment for the overall mean; n=10 per treatment for each sampling occasion). Total biomass is significantly reduced by warming. This is mainly driven by a reduction in phytoplankton biomass, while there is no significant difference in the biomass of zooplankton in response to warming (table 1).

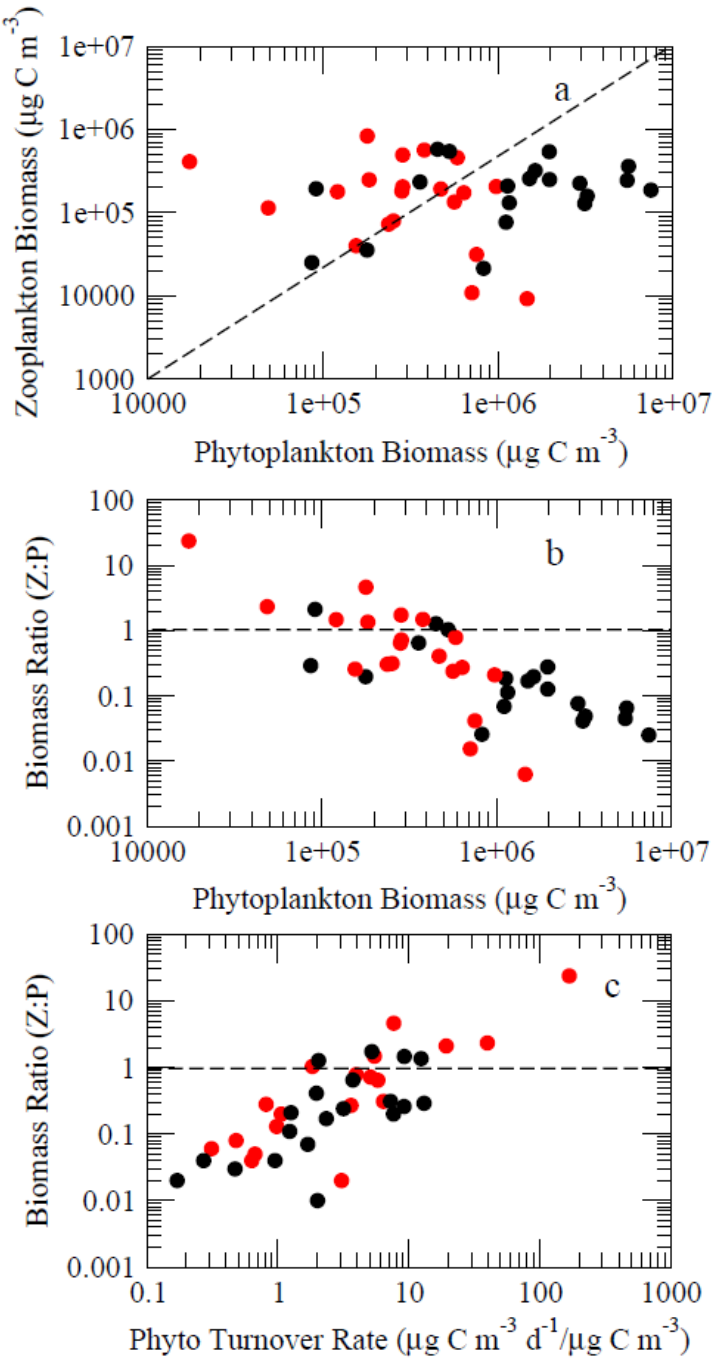

Fig. 5.9. (a) Relationship between zooplankton and phytoplankton biomass. (b) Relationship between the ratio of zooplankton to phytoplankton biomass (Z:P) and total phytoplankton biomass. (c) The relationship between Z:P and the turnover rate of the phytoplankton communities. Each data point corresponds to either the total zooplankton or phytoplankton biomass or the Z:P in either a heated (red circles) or ambient mesocosm (black circles).

Relationship	DF	*F*	*P*	r^2
Log_{10}(Zoo biomass) vs Log_{10} (Phyto biomass)	1, 38	0.062	0.805 (NS)	0.002
Difference in slope	1, 38	3.021	0.073(NS)	N/A
Difference in intercept	1, 38	0.195	0.661 (NS)	N/A
Log_{10}(Z:P) vs Log_{10} (Phytoplankton biomass)	1, 38	32.65	**<0.0001**	0.58
Difference in slope	1, 38	1.806	0.187 (NS)	N/A
Difference in intercept	1, 38	0.002	0.956 (NS)	N/A
Log_{10}(Z:P) vs Log_{10} (Phytoplankton turnover)	1, 38	52.51	**<0.0001**	0.58
Difference in slope	1, 38	2.171	0.147 (NS)	N/A
Difference in intercept	1, 38	0.538	0.468 (NS)	N/A

Table 5.2. Analysis of covariance for the relationships between zooplankton and phytoplankton biomass, the Z:P biomass ratio and phytoplankton biomass, and the Z:P biomass ratio and phytoplankton turnover time.

Discussion

There is ample evidence that ecological responses to recent climate change are already occurring at the species (and by extension the population) level (Walther *et al.* 2002), but scaling from populations to communities and ecosystems is challenging because of the perceived indeterminacy of ecological interactions (Yodzis 1988). As a result, there is an increasingly urgent need to explore the higher-level effects of the principal components of climate change (e.g., warming) on community structure and ecosystem functioning (Tylianakis *et al.* 2008). My results broadly supported my experimental hypotheses: i.e., that warming would increase the steepness of the size spectrum slope, reduce total community biomass, and increase the zooplankton to phytoplankton biomass ratio. These findings could provide some novel insights into how future warming might change the distribution of body size and biomass in aquatic ecosystems. The size structure of plankton communities in aquatic ecosystems is a key driver of rates of carbon sequestration and nutrient cycling (Laws *et al.* 2000), and therefore changes in the distribution of planktonic body size and biomass could alter the regulation of biotic feedbacks with warming on a potentially global scale (Falkowski *et al.* 1998).

The general increase in the prevalence of small organisms with increases in environmental temperature that I observed experimentally agrees well with recent studies that have either focused on specific taxa, or subsets of taxa (Atkinson *et al.* 2003, Finkel *et al.* 2005, Daufresne *et al.* 2009, Winder *et al.* 2009), or described correlational trends in community structure across latitudinal gradients in temperature (Moran *et al.* 2010). The increase in the dominance of small phytoplankton and the truncation of the larger size classes in their size spectrum resulted in a general increase in the steepness of the slope of the community size spectrum in the warmed mesocosms. Changes in the distribution of organism size might arise from at least two broad mechanisms, which are not necessarily mutually exclusive. Firstly, organisms might exhibit a degree of phenotypic plasticity to changes in temperature, as described by the "temperature-size rule" (Atkinson *et al.* 2003) that posits reduced organism size at higher temperatures is an adaptive plastic response resulting from selection for earlier reproduction as population growth rate increases: i.e., the accelerated completion of the life cycle occurs at the expense of maturation size (Atkinson *et al.* 2003). In the second mechanism, changes in the physicochemical environment created by warming select for smaller species. In this case, changes in community size structure occur as an indirect effect of warming, mediated for example by concomitant nutrient limitation, resulting in the competitive exclusion of larger species (Finkel *et al.* 2005, Irwin *et al.* 2006, Falkowski & Oliver 2007, Winder *et al.* 2009, Finkel *et al.* 2010). Here, small cell size increases the efficiency of the acquisition of limiting nutrients because of a higher surface area to volume ratio and is therefore competitively advantageous under conditions of nutrient limitation (Litchman *et al.* 2009).

The results presented here support the second mechanism. Redundancy analysis revealed that warming dramatically shifted the taxonomic composition of the phytoplankton assemblages.

Moreover, warming favoured smaller phytoplankton genera, resulting in a reduction in mean and maximum body size by almost an order of magnitude. For example, the large cholorophyte *Botryococcus* dominated the biomass of the ambient mesocosms in both April and October, but was almost entirely absent from the warmed mesocosms. Similarly the small cyanophyte *Synechocystis* and the small chlorophyte *Monoraphidium* were strongly associated with the warmed mesocosms but were only peripheral members of the assemblages in the ambient mesocosms. Warming therefore resulted in the establishment of phytoplankton assemblages dominated by small species, rather than reducing the body size of the same species composition present in the ambient mesocosoms.

The relatively infrequent but highly replicated sampling regime adopted in my experimental design was a necessary compromise. For example, I documented the size, biomass and taxonomic structure of 20 replicated experimental ecosystems on two separate sampling occasions at the beginning and end of the growing season (identified from measures of primary production; see Yvon-Durocher *et al*., (2010) and *chapter three* for details) rather than focusing on the complex temporal dynamics of the plankton assemblages of one or two systems, as would typically be logistically feasible within such a study. As a result, these findings come with an associated caveat: I am unable to discern the effects of warming on the temporal succession of the plankton communities. However, analysis of the phytoplankton taxonomic composition suggests that a large, core contingent of these assemblages are present in both April and October but which differ markedly between treatments in both months. These results suggest that temporal succession in the plankton communities was less important than the effect of treatment (i.e. warming) in determining the taxonomic and therefore the body size and biomass structure of these assemblages.

Inorganic nitrogen was limiting throughout the experiment (N:P ratios were ≈11:1, and were below the 16N:1P expected at Redfield; see *chapter two* for further details) but to the same extent in both warmed an ambient treatments: i.e., warming did not exacerbate nutrient limitation. It is therefore unlikely that a direct effect of nutrient limitation induced by warming caused the observed shifts in phytoplankton size structure that have been frequently documented in the open ocean and in lake ecosystems (Finkel *et al.* 2005, Falkowski & Oliver 2007, Winder *et al.* 2009, Finkel *et al.* 2010). The observed shift in phytoplankton size structure in the warmed treatments might simply reflect the fact that smaller phytoplankton have lower specific nitrogen requirements than large phytoplankton (Litchman *et al.* 2007). Litchman *et al*. (2007) found that the minimum nitrogen quota required to support growth, Q_{min}, across a wide range of phytoplankton taxa increases allometrically, resulting in a disproportionate increase in cellular nitrogen quota with size. Metabolic rates and nutrient uptake rates increase with temperature and size (Gillooly *et al.* 2001, Allen & Gillooly 2009), so under conditions of nutrient limitation small cell size should provide a competitive advantage as environmental temperatures rise because species with lower Q_{min} will be better able to balance the increased demand for limiting nutrients imposed by temperature driven elevated metabolic rates.

An alternative mechanism for the shifts in phytoplankton size and taxonomic structure in the warmed mesocosms is that warming served to increase "top down" control of the phytoplankton community by increasing zooplankton grazing rates. We have previously demonstrated that heterotrophic metabolism increased more rapidly than autotrophic metabolism with increasing temperature in the same experimental system (measurements made simultaneously; see Yvon-Durocher *et al*., (2010) and *chapter three* for details). Therefore, because ingestion rates increase in proportion with metabolic rates (Berlow *et al.* 2009),

warming might have increased the strength of top down control of phytoplankton populations by zooplankton grazing. Moreover, zooplankton are often size selective when feeding on phytoplankton, typically consuming the largest size classes possible (Porter 1973, Hall *et al.* 1976, Katechakis *et al.* 2002). Warming might therefore have increased the prevalence of small sized phytoplankton indirectly, by elevating grazing pressure on the larger size classes of the phytoplankton community due to the elevated metabolic demands of zooplankton at higher temperature. Importantly, both the "top down" and "bottom up" hypotheses stated here are not mutually exclusive: both bottom up regulation of phytoplankton competitive ability for limiting nutrients, and top down control of large phytoplankters by zooplankton grazing could occur simultaneously, and combine with the direct effects of warming on metabolism to produce the observed shifts in size, biomass taxonomic structure.

Warming reduced total standing community biomass, largely via a reduction in phytoplankton biomass, in line with my qualitative theoretical predictions. For example, because the potential resource supply rate (i.e. the concentrations of limiting inorganic nutrients) remained constant, I predicted that elevated metabolic demands at higher temperatures should have resulted in a decline in standing community biomass in the warmed mesocosms. Assuming $B_{tot} = r_0 e^{-E/kT} M^{1/4}$ and that r_0 (i.e. the resource supply rate) and $M^{1/4}$ (i.e. the allometric scaling of biomass with body mass) are constant with temperature, for ~4˚C rise in temperature, standing community biomass should decline approximately 1.54 fold according to: $e^{-E/kTh} / e^{-E/kTa}$ where T_h and T_a are the mean annual temperatures of the heated and ambient mesocosms (290.9 and 286.1 K, respectively) and E is the activation energy of metabolism ~0.65eV (Gillooly *et al.* 2001). In the experiment average total community biomass declined 2.53 fold (i.e. the ratio of mean

biomass in the heated and ambient mesocosms), almost double that predicted by metabolic costs alone, suggesting that additional factors might be operating.

The large shift in the distribution of body size from large to small phytoplankton might further reduce standing biomass. For example, the above prediction assumes that the allometric scaling of biomass with body mass (i.e. $B_{tot} = M^{1/4}$) remains constant with warming. However, the slope of the community size spectrum (i.e. the log-log relationship), which is equivalent to the exponent of $N = M^{-\alpha}$, where N is abundance (White *et al.* 2007, Reuman *et al.* 2008, White *et al.* 2008), changes from -0.86 to -0.95 in response to warming. Therefore, because $B_{tot} = N \times M$, the allometric scaling of B_{tot} declined from $B_{tot} = M^{0.14}$ in the ambient mesocosms to $B_{tot} = M^{0.05}$ in the warmed mesocosm: i.e., more standing biomass was retained in larger body size classes in the ambient relative to the warmed mesocosoms. The effects of increased metabolic costs associated with warmer temperatures and the shift in the distribution of body size caused by changes in the balance of competition between large and small phytoplankton might therefore have acted synergistically to reduce total community biomass in the warmed mesocosms.

The ratio of zooplankton to phytoplankton biomass, Z:P, declined as a function of phytoplankton biomass, in line with my third experimental hypothesis. These results are qualitatively similar to the findings of Gasol *et al*. (1997) who also demonstrated that the ratio of heterotroph to autotroph biomass (H:A) was a declining function of autotroph biomass in the open ocean and coastal seas, although they attributed the relationship to a nutrient gradient rather than temperature. In this experiment, the large shifts in community size structure and the distribution of biomass between zooplankton and phytoplankton were independent of the inorganic nutrient status of the mesocosms and appear to have been driven largely by the effects

of temperature on metabolism and the relative competitive abilities of large and small phytoplankton.

A strong, positive correlation was evident between the Z:P biomass ratio and the turnover rate of the phytoplankton assemblages, which differed profoundly between warmed and ambient treatments. The inverted pyramid or squared biomass distributions (i.e. Z>P or Z=P) in the warmed mesocosms contrasted markedly with the pyramidal biomass structure (i.e. Z<P) of the mesocosms at ambient temperature, suggesting that warming of ~4˚C fundamentally altered both the structure and functioning (i.e. energy transfer) of the experimental ecosystems. In the heated mesocosms the high relative biomass of zooplankton may have been supported by a fast turnover rate of the phytoplankton assemblage: for the low standing stocks of phytoplankton biomass in the warmed mesocosms (2.93×10^5 µg C m^{-3} in heated; 1.12×10^6 µg C m^{-3} in ambient) to sustain the equivalent biomass of zooplankton as the mesocosms at ambient temperature (1.71×10^5 µg C m^{-3} in ambient; 1.36×10^5 µg C m^{-3} heated), the turnover rate of the phytoplankton community would need to increase by a factor of ~4. The average turnover rates of the phytoplankton community in the warmed treatments were actually elevated by a factor of ~5 (i.e. 40.9 µg C m^{-3} d^{-1} / µg C m^{-3} in heated; 8.25 µg C m^{-3} d^{-1} / µg C m^{-3} in the ambient) and were therefore likely to be sufficient to support the biomass of zooplankton in these systems. Taken together, these results suggest that warming might dramatically increase the rate of carbon flux between autotrophs and heterotrophs, primarily via the relative increase in small phytoplankton, which should have faster turnover times (Gillooly *et al.* 2002, Brown *et al.* 2004), and also the direct stimulation of metabolism and generation time by temperature (Gillooly *et al.* 2002).

Conclusion

Warming pushed planktonic communities towards greater heterotrophy, and the results of this experiment reflected empirical patterns observed in phytoplankton communities over macroevolutionary time (Finkel *et al.* 2005, Finkel *et al.* 2007), across latitudinal gradients in temperature (Moran *et al.* 2010), and across gradients of nutrient regime and productivity (del Giorgio & Gasol 1995, Gasol *et al.* 1997). My results represent the first experimental evidence for a shift in the distribution of body size and biomass of whole plankton communities that can be attributed directly to the effects of warming via a controlled and replicated whole ecosystem manipulation. Although these results offer some of the first tantalising hints as to the underlying mechanism, the elucidation of the relative importance of phenotypic versus taxonomic effects requires future research that can integrate both size-based and species-based approaches to community and ecosystem ecology. Furthermore, these results raise a further unanswered question for ecology and the science of global change: what is the mechanistic basis of the relationship between temperature and the competition of large and small phytoplankton?

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Chapter Six

General Discussion: On the Community and Ecosystem Level Consequences of Warming

Overview

Ecosystems are composed of thousands to millions of individuals, which often belong to hundreds to thousands of species, and which interact with one another in a multitude of different ways - competition, mutualism and predation being the three most familiar examples. This inevitably results in the emergence of ecosystems that display often seemingly bewildering complexity. The array of different species in an ecosystem typically also express great diversity in their traits and attributes, which are often strongly linked to body size. The open ocean is a striking example of this, where the body size range spans from picoplankton (10^{-13} g) to the largest animal that has ever lived, the blue whale (10^{8} g): a range of ~20 orders of magnitude within a single ecosystem (Brown & West 2000, McGill *et al.* 2006). A range of ecosystem processes (e.g., nutrient recycling, primary production) are overlain on this taxonomic and functional complexity, and these are carried out by the vast biotic consortium which maintains the normal functioning of ecosystems that provide important goods and services to human civilization (Loreau *et al.* 2001, Schroter *et al.* 2005). It is the organisms that define the structure of a community and carry out the functions of the ecosystem. Given this obvious link between structure and functioning, it is perhaps surprising that a general, comprehensive, mechanistic understanding of the linkages between these levels of ecological organisation is still lacking (Allen & Gillooly 2009). Three relatively new fields in ecology, "*metabolic ecology*", which views metabolism as being a unifying biological mechanism that determines a diverse range of ecological phenomena (Brown *et al.* 2004); "*ecological stoichiometry*", which demonstrates that the mass balance of essential elements in biological systems are key in determining their structure and function (Sterner & Elser 2002); and "*biodiversity-ecosystem functioning*", which focuses on understanding the role of species and their traits in determining ecosystem level processes (Loreau *et al.* 2001, Hooper *et al.* 2005), have made significant advances toward this

end. However, a far more complete understanding of the interrelations between structure and function within a general predictive framework, capable of successfully addressing the challenges posed by global change is most likely to be achieved through integration of these approaches.

Evidence for the ecological impacts of the multifarious components of climate change, most notably global warming, has now been amassed for a great number of individual taxa (Walther *et al.* 2002, Parmesan & Yohe 2003, Montoya & Raffaelli 2010). Far less is known, however, about the likely responses of entire communities and ecosystem level processes. Moreover, the potential feedbacks between warming, community structure and ecosystem functioning remain largely unexplored. In this thesis, I attempted to take a first step towards addressing this important knowledge gap, by measuring the effects of a simulated potential future warming scenario (A1B; IPCC 2007) on community structure and biogeochemical cycling in a freshwater mesocosm experiment. The effects were marked. Warming of ~4˚C dramatically impaired the ecosystems' carbon sequestration capacity, increased the efflux rates of CH_4 relative to primary production, and reorganised the distribution of biomass, body size and taxonomic composition.

The integrated experimental and theoretical approach, which focused on the metabolic theory of ecology, provided insight into the mechanistic basis for the experimentally observed changes and may also provide predictive tools for the wider scientific community to explore the potential consequences of changes in temperature in other ecosystems. For example, two important new findings of this thesis are that not only did warming reduce carbon sequestration capacity by 13%, but that the precise magnitude of this shift could also be predicted by the differences in the activation energies of photosynthesis and respiration at the cellular (or even

organelle) level. The activation energy of ecosystem respiration (ER, E_r = 0.62eV) was greater than that of gross primary productivity (GPP, E_p = 0.45eV), therefore ER increased proportionately more rapidly than GPP as temperatures increased. Another discovery made here for the first time, with potentially far reaching consequences, was that the activation energy that describes the temperature dependence of CH_4 efflux (E_a = 0.85eV) was significantly greater than that of either GPP or ER. This meant that not only was the carbon sequestration capacity reduced but the warmed systems also emitted relatively more CH_4, which is up to 21 times more potent as a greenhouse gas in the atmosphere compared to CO_2 (Rodhe 1990). This discovery identifies the potential for a previously unknown positive feedback between warming and the carbon cycle. Again, the relative offset of these ecosystem level fluxes could be predicted by the models developed here which were derived from the metabolic theory of ecology.

The large shifts in ecosystem level carbon flux changed in parallel with the size structure of the plankton communities in the mesocosms: warming shifted the distribution of body size of phytoplankton to assemblages dominated by small individuals with low standing biomass but rapid turnover rates. This resulted in a redistribution of biomass within the plankton, to communities that were dominated by heterotrophic biomass in the warmed mesocosms, reflecting the patterns observed with the changes in ecosystem metabolism. The warmed mesocosms not only shifted towards heterotrophy with respect to the dominant pool of standing biomass, but also in the balance between ER and GPP. These findings point towards fundamental similarities in the response of both the structural and the functional components of the experimental systems to warming and provide substantial evidence for the reciprocal relationship between these levels of organisation. I will now explore these relations in greater detail and hypothesise on the potential mechanisms that determine them.

The Ecosystem: Reconciling Structural and Functional Components

There were significant correlations between several structural attributes of the communities in the mesocosms and their associated ecosystem processes (Fig. 6.1, 6.2). Although a correlation between two variables does not necessarily imply cause-and-effect, it may be that the underlying mechanism is related to a co-variable (in this case the most likely one is temperature). Nevertheless, if we have an *a priori* reason to expect the correlation based on a mechanistic hypothesis, such relationships may provide important insight into the nature of the correlation. Furthermore, the consequences of environmental warming are likely to result in complex feedbacks between levels of organisation, whereby changes in one level caused directly by warming result in changes in another level, which are then amplified by the press perturbation. In this case, the ecosystem may exist in alternative stable states where the effects of the perturbation propagate over all levels of ecological organisation resulting in a hysteresis towards an alternative equilibrium state under the newly imposed environmental conditions (Scheffer *et al.* 2001, Scheffer & Carpenter 2003). Phase transition dynamics between alternative equilibria have been frequently documented in lakes subjected to heavy nutrient loading or by removal of large piscivores (Scheffer *et al.* 2001, Scheffer & Carpenter 2003). Environmental warming has the potential to induce similar changes in the prevailing stable state of shallow lakes, though such changes have not yet been documented (McKee *et al.* 2003). The results from this experiment however, appear to provide the tentative evidence that warming has the potential to induce shifts in both community structure and ecosystem functioning that might, over time constitute a shift towards a new equilibrium ecosystem state.

The average annual slopes of the community and the phytoplankton size spectra were negatively correlated with the average annual metabolic balance (ER/GPP) of the mesocosms

(Fig. 6.1a & b), such that systems characterised by a high ratio of ER/GPP tended to have communities with steeper size spectra, especially among the warmed mesocosms.

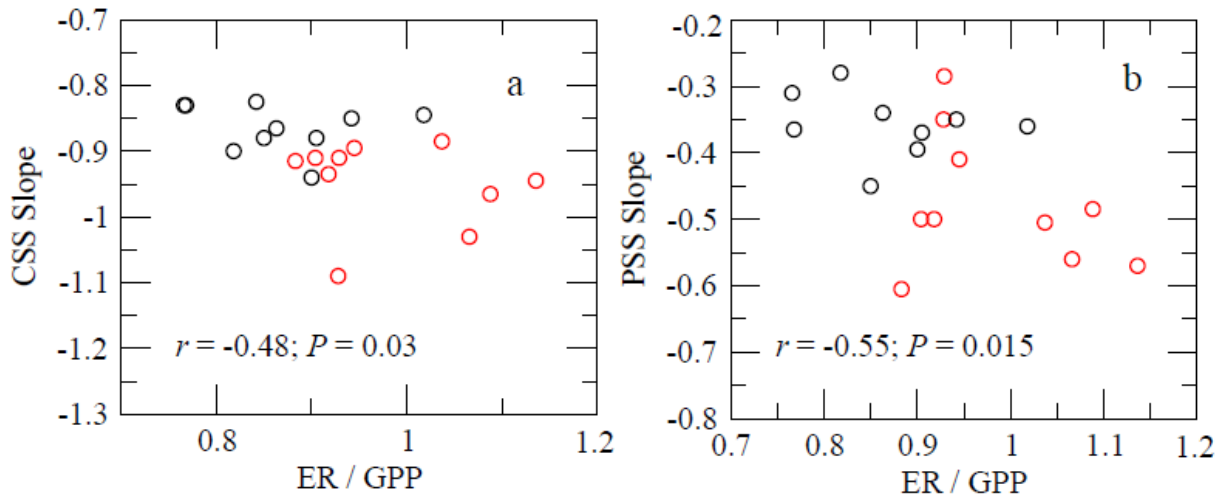

Fig. 6.1. Linkages between community structure and ecosystem functioning, black circles denote ambient treatments and red circles denote the warmed treatments. All data points are the mean annual values for a particular mesocosm error bars are not shown to ease interpretation of the correlation. (a) The negative correlation between the slope of the community size spectrum (CSS) and the metabolic balance (ER/GPP). (b) The negative correlation between the slope of the phytoplankton size spectrum (PSS) and the metabolic balance (ER/GPP).

This correlation between ecosystem and community level attributes might have a mechanistic basis. For example, high ratios of ER/GPP suggest that heterotrophic metabolism respires (or consumes) a greater proportion of primary production (i.e. phytoplankton biomass) averaged over the year. Because the activation energy for heterotrophic metabolism was greater than that of autotrophic production, and because consumption rates are proportional to metabolic rates (Emmerson *et al.* 2005, Berlow *et al.* 2009), this suggested the intensity of grazing pressure in the warmed systems with elevated ratios of ER/GPP was greater. Zooplankton grazing is typically size selective, whereby the largest and most nutritious phytoplankton are consumed preferentially (Porter 1973, Hall *et al.* 1976, Katechakis *et al.* 2002). This might indicate that increased grazing pressure brought about by elevated rates of heterotrophic relative to

autotrophic metabolism resulted in a suppression of large phytoplankton, thereby truncating the phytoplankton size spectrum and increasing its slope. This points towards a direct mechanism linking changes in community structure with ecosystem functioning, mediated by the differential temperature dependence of photosynthesis and respiration.

The mean annual ratio of zooplankton to phytoplankton biomass (Z:P) was negatively correlated with both the concentration of PO_4^{3-} and the concentration of NH_4^+ (Fig. 6.2). Therefore, in systems with relatively high concentrations of PO_4^{3-} and NH_4^+ phytoplankton biomass exceeded zooplankton biomass. This finding appeared to be independent of temperature and therefore of the experimental treatment, because neither PO_4^{3-} nor NH_4^+ varied systematically with temperature (Fig. 6.2; see *chapter two* also). The correlation between Z:P biomass and PO_4^{3-} or NH_4^+ is perhaps not surprising, considering phosphorous and nitrogen are typically nutrients that limit phytoplankton growth in aquatic ecosystems (Elser *et al.* 2000a).

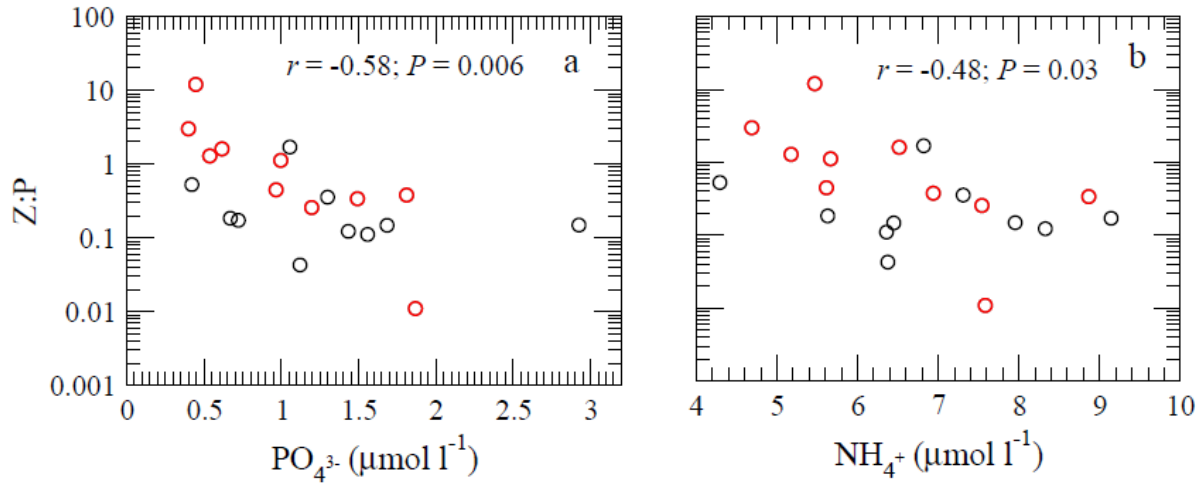

Fig. 6.2. (a) Positive correlation between the mean annual ratio of zooplankton to phytoplankton biomass and the mean annual concentration of dissolved PO_4^{3-}. (b) Positive correlation between the mean annual ratio of zooplankton to phytoplankton biomass and the mean annual concentration of dissolved NH_4^+.

What is surprising, however, is the lack of co-variability between phytoplankton biomass, temperature and PO_4^{3-} and/or NH_4^+. For example, the reciprocal of mean annual temperature ($1/kT$) and the natural logarithm of phytoplankton biomasses were correlated (Fig.6.3a). However, it is evident from figure 6.2a that PO_4^{3-} might also be important for regulating phytoplankton. To assess this I plotted the residuals from the temperature-phytoplankton biomass relationship (Fig. 6.3a) against the mean annual concentration of PO_4^{3-} (Fig. 6.3b). The residuals in figure 6.3a were positively correlated with the mean annual concentration of PO_4^{3-}, therefore in the systems where phytoplankton biomass was greater than the average for a given temperature (i.e. positive residuals), concentrations of PO_4^{3-} were typically relatively high and might have facilitated elevated phytoplankton biomass production. Taken together, these findings suggest that temperature and rates of recycling of limiting elements might have jointly, but independently, regulated phytoplankton in the experiment. An important caveat to bear in mind here is that during the study I did not directly measure rates of PO_4^{3-} flux, rather instantaneous concentrations were measured seasonally. However, because the mesocosms used in this study were materially closed systems (i.e. allochthonous subsidies were improbable) I assumed that the mean annual pool of PO_4^{3-} is reflective of the relative rate of its regeneration as this is its only potential source. Nevertheless, this result again provides tantalising evidence for building an understanding of the potential mechanisms that will determine the feedbacks between warming and changes in community structure and biogeochemical cycling.

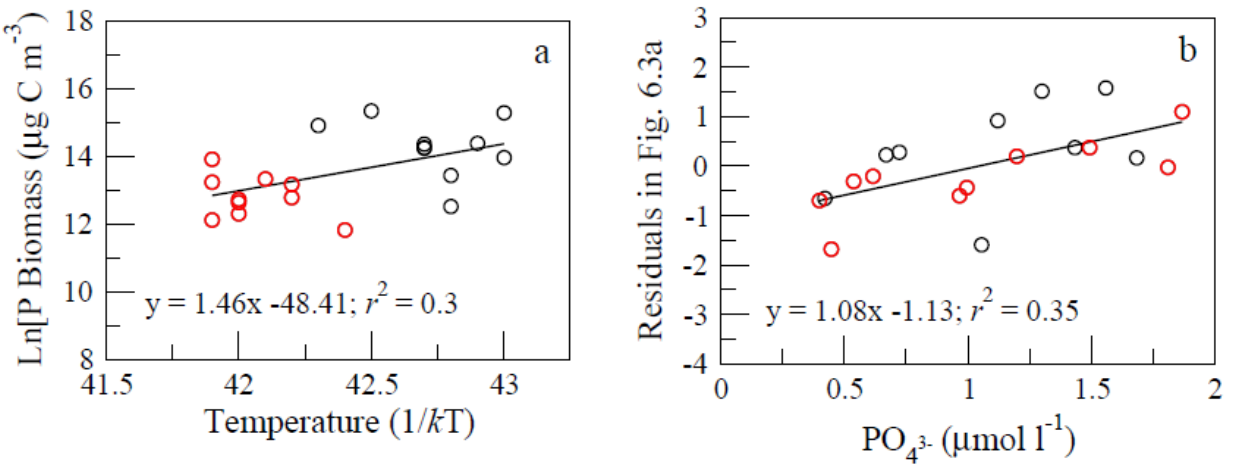

Fig. 6.3. (a) Relationship between the natural logarithm of mean annual phytoplankton biomass and the reciprocal of the mean annual temperature of the mesocosm. (b) Relationship between the residuals from the fitted line in Fig. 6.3a and the mean annual concentrations of PO_4^{3-} in the mesocosms. Red circles denote heated treatments; black circles denote treatments at ambient temperature.

Conclusions, Caveats and Future Directions

In conclusion, this thesis has demonstrated that the warming scenario (A1B) projected for temperature latitudes by the end of this century (IPCC 2007) has the capacity to profoundly alter both the structure and the functioning of aquatic ecosystems. Indeed, in this experiment warming reduced the capacity of the ecosystems to sequester carbon, which was driven by the greater activation energy of respiration relative to photosynthesis. Furthermore, warming increased the greenhouse gas efflux potential of the ecosystems by increasing rates of CH_4 efflux relative to primary production and ecosystem respiration elucidating a previously unknown potential positive feedback between warming and the carbon cycle. Again, this was driven by the fact that the activation energy of methanogenesis and, therefore, the temperature dependence of CH_4 efflux were greater than that of primary production and ecosystem respiration. These findings reveal a general pattern with potentially broad application: the temperature dependence of the ecosystem level fluxes involved in the biogeochemical cycling of carbon are different and are

predictable from their activation energies at the cellular level. These shifts in ecosystem functioning were mirrored by a reorganisation of the body size and biomass distributions in the warmed mesocosms. Warmed communities were dominated by phytoplankton assemblages with low standing biomass, smaller average body size, and rapid turnover rates. This resulted in a shift in the biomass structure of the food webs whereby the warmed systems were characterised by inverted or squared biomass pyramids, in which zooplankton biomass often exceeded phytoplankton biomass, but was sustained by the elevated turnover rates of the smaller, warmed phytoplankton assemblages (Fig. 6.4).

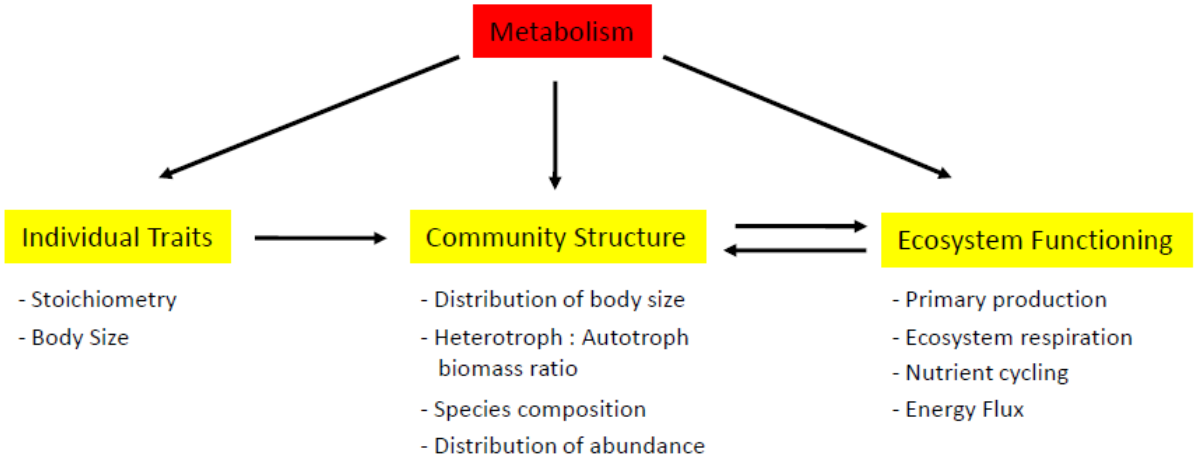

Fig. 6.4. Conceptual diagram illustrating how the different levels of biological organisation are simultaneously controlled by metabolism and reciprocally related to one another. Many of the attributes of these levels were measured in this study and are discussed in the text.

The results presented in this thesis portray a convincing story of the potential future consequences of warming for aquatic ecosystems, and how they might be predicted in natural ecosystems using a general ecological theory. However, it is important to bear in mind the caveats associated with both the experimental approach (i.e. mesocosm scale experiments) and the application of the metabolic theory of ecology in the interpretation of these results and their

relevance for natural ecosystems. The use of mesocosm experiments in ecology represents an inevitable compromise between the control and specificity of laboratory studies and the realism of field surveys (Benton *et al.* 2007). Mesocosm experiments cannot replicate the full range of the complexities of the ecosystem processes and aspects of community structure that occur in nature. For example, this experiment, which attempted to mimic shallow lake ecosystems, was a materially closed-system, therefore, allochthonous subsides which are often important in lakes both for community structure (del Giorgio & Gasol 1995) and ecosystem metabolism (del Giorgio & Peters 1994, del Giorgio *et al.* 1999) were not considered. Although this may be considered a shortcoming of the experiment, in that it might not truly replicate the dynamics of natural systems to warming, it did offer the opportunity to focus solely on the mechanisms that govern the changes in autochthonous carbon, without the extra complexity associated allochthonous subsides that can be highly variable both temporally and spatially (Matthews & Mazumder 2006). Furthermore, the temporal and spatial scales of the experiment are clearly an abstraction of the potential dynamics of future warming on natural systems. For example, the warming of the mesocosms from ambient to plus 4˚C in this experiment occurred almost instantaneously, whereas in reality this level of warming is likely to occur over decades (IPCC 2007). Therefore, the potential for acclimation of natural communities to warming is likely to be far greater than can be replicated in this experiment. However, the temporal scale of the experimental perturbation spanned multiple generations of many of the planktonic organisms that were the focus of this investigation. Experiments such as these offer the only opportunity to provide a "best guess" approach to predicting the future consequences of global change.

The application of the metabolic theory of ecology in combination with the mesocosm experiment allowed a more mechanistic understanding of the effects of elevated temperature on

ecosystem metabolism to be gained. However, there are also a number of caveats associated with the application of metabolic theory because, as with any other theory, it is an oversimplification of complex natural phenomena. For example, in chapters *three* and *four* I demonstrated that primary production, ecosystem respiration and CH_4 efflux increased with temperature according to their respective activation energies at the cellular level. Therefore, the temperature dependences of the ecosystem level fluxes were equivalent to their activation energies at the cellular level, as predicted by the metabolic theory of ecology (Gillooly *et al.* 2001). However, potential divergence between the temperature and mass scaling with metabolic rate within species and across species (such as the ones presented in this thesis) have caused considerable debate in the ecological literature (Clarke 2004, Clarke & Fraser 2004). Here I will concentrate primarily on the debate surrounding the temperature scaling of metabolic rate, because it most closely relates to the analyses and approach presented in this thesis. Gillooly *et al*., (2001) suggested a universal temperature dependence for the scaling of metabolism (respiration) with temperature, which is governed by the Boltzmann-Arrhenius factor taken from statistical thermodynamics $b_0 e^{-E/kT}$, where b_0 is a normalisation constant, E is the average activation energy of metabolism (i.e. the average for all of the component reactions involved in metabolism and assumed to range between 0.6-0.7 eV), k is Boltzmann's constant (8.62×10^{-5} eV K^{-1}) and T is absolute temperature. In this equation the parameter E, the activation energy, drives the rate of change in the reaction rate (i.e. the metabolic rate) with temperature. The main point of contention concerns the constancy of E within and across species (Clarke 2004, Clarke & Fraser 2004). For example, Gillooly *et al*., (2001) propose that E is consistent because the same mechanism governs the temperature dependence of metabolism: i.e., the speed of the reaction (metabolism) is governed by the kinetic energy of the system, which takes the form of temperature, and determines rate of collisions between molecules and the probability that any

given collision will lead to a reaction. Any differences between species should be reflected by changes in b_0, which may be related to thermal acclimation or adaptation (Gillooly *et al.* 2006). The counter-argument put forward by Clarke & Fraser (2004) and Clarke (2004) refute this claim of a "universal temperature dependence", and propose an alternative "evolutionary trade off hypothesis" in which they suggest that the temperature dependence of metabolism in every species represents an evolutionary optimisation to the energetic demands of maintaining metabolism at its environmental temperature and ecological mode. They posit that the across species relationship is merely a statistical description of "quasi-independent evolutionary optimisations to temperature and ecology" (pg 253) and therefore there is no mechanistic *a priori* reason to suggest the temperature dependence of respiratory metabolism should be universal. The work presented in this thesis, however, appeared to support the ideas of Gillooly *et al*., (2001) and the metabolic theory of ecology, because the temperature dependence of the ecosystem level fluxes were equivalent to their respective activation energies at the individual level. Moreover, in *chapter four* I demonstrated with a literature compilation that the temperature dependence of CH_4 efflux at the ecosystem level was identical to the activation energy of methanogenesis in pure cultures, which varied little between species.

The approach adopted in this thesis, which involved the simultaneous analysis of both the community and ecosystem level consequences of warming and not only offered insight into the effects of warming on these levels of organisation in isolation, but also the opportunity to understand the mechanisms that drive the linkages between the structure of communities and the functioning of ecosystems as a whole. For example, I found that the size structure of the plankton communities, exemplified by the slope of the size spectrum, was well correlated with the metabolic balance of the ecosystem. Moreover, this relationship might be explicable by the

cascading effects of the elevated temperature dependence of heterotrophic relative to autotrohic metabolism, resulting in a shift in the size spectrum in concert with that of the metabolic balance. Further work is clearly needed in this field to truly elucidate the ultimate mechanisms that link community structure to ecosystem functioning. For example, the application of environmental metagenomics (He *et al.* 2010), and the combination of ecological stoichiometric and metabolic theories in ecology (Allen & Gillooly 2009) are emerging promising avenue of research. This thesis has provided some evidence to suggest that a new perspective emphasising the importance of the size structure of communities, rather than the classic paradigm focused on the role of biodiversity (Raffaelli 2007) might offer a promising avenue for future research.

Emerging research at the intersection of the metabolic theory of ecology and ecological stoichiometric theory could potentially offer a useful platform to build a more predictive understanding of linkages between community structure and biogeochemical cycles based on the flux, storage and turnover of elements (Gillooly *et al.* 2005, Allen & Gillooly 2009). For example, the efficiency of energy transfer across the autotroph to heterotroph interface is crucial for determining the overall biomass structure of food webs (Cebrian 1999, 2004, Cebrian & Lartigue 2004). Typically, the efficiency of secondary production is strongly related to the stoichiometry of autotrophic resources (e.g., CNP), because this determines its nutritional quality for the herbivore (Cebrian *et al.* 2009). Combining aspects from the metabolic theory of ecology with ecological stoichiometric theory could lead to predictions of how autotrophic stoichiometry might change with temperature. For instance, a significant but variable fraction of whole body P is dependent on the RNA concentration within tissues, and because ~85% of RNA is in the form of ribosomal rRNA changes in cellular ribosome density reflect changes in whole body P. This is part of Elser's growth rate hypothesis from ecological stoichiometric theory, which posits that

changes in the density of rRNA, and thus whole body P, reflect differences in an organism's capacity for growth; i.e., the more rRNA present for protein synthesis the greater the capacity for rapid growth (Elser *et al.* 2000b). Extensions of the metabolic theory of ecology have indeed shown that the activation energy for protein synthesis by ribosomes is equivalent to the activation energy for respiration, ~0.65 eV (Gillooly *et al.* 2005), while the activation energy of photosynthesis per chloroplast is ~0.32 eV (Allen *et al.* 2005). Thus, at higher temperatures, less ribosomes are required per chloroplast to keep pace with rates of cellular photosynthesis (Andrew Allen; *pers. com*), therefore, in plants, which have the capacity to regulate their sub-cellular constituents to a much greater extent than animals (Sterner & Elser 2002), the ratio of N:P should increase with increases in temperature. In fact, in a recent compilation of data from field surveys and experiments using aquatic and terrestrial plants from all over the Earth, I have demonstrated that the ratio of plant N:P increases with temperature according to the difference in the activation energies of protein synthesis by ribosomes and photosynthesis by chloroplasts (Fig. 6.5; Yvon-Durocher; *unpublished data*). This suggests that, on average, at higher temperatures the nutritional quality of autotrophic biomass is lower, which might have important implications for the efficiency of energy transfer through food webs, and provide a mechanistic link between community structure, rates of nutrient cycling, sub-cellular metabolism, and elemental composition. Furthermore, if the gross growth efficiencies of herbivores are predictable from the elemental ratio of their resources (i.e. using the threshold elemental ratio theory) (Frost *et al.* 2006) then changes in the slope of the community size spectrum in response to altered stoichiometry at the base of food webs should be predictable by combining aspects of the metabolic theory of ecology (Reuman *et al.* 2008) with ecological stoichiometric theory. This work I hope to form the basis of my future research.

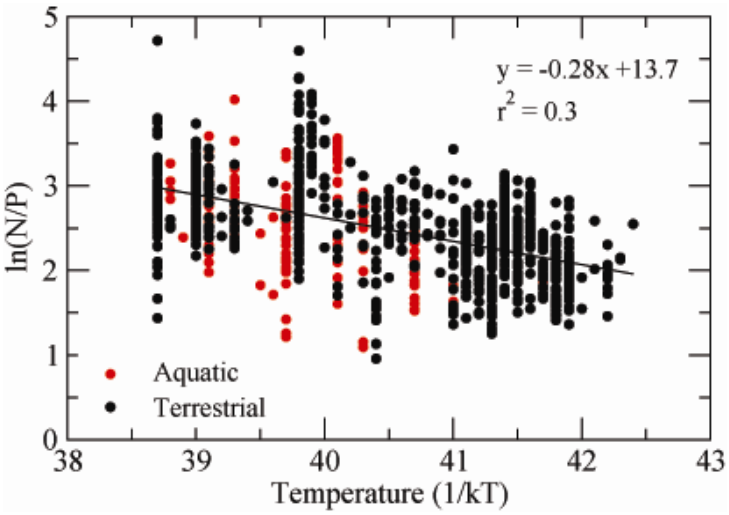

Fig. 6.5. N/P ratio of aquatic (red circles) and terrestrial (black circles) autotrophs as a function of the inverse of absolute average growing season temperature (1/*k*T). The slope of this relationship is indistinguishable to the difference between the activation energies of protein synthesis by ribosomes (0.65 eV) and photosynthesis by chloroplasts (0.32 eV) suggesting a potential biochemical explanation for this biogeographical phenomena. Clearly more experimental work is needed to characterise the temperature dependence of the potential changes in the densities of the sub-cellular constituents that might drive this relation, though the overall pattern is tantalisingly consistent. Data have been compiled and reanalysed from Cebrain *et al*., (2009) PLOS one, 4, 4129-4135; Reich & Oleksyn (2006) PNAS, 101, 11001-11006; Brey *et al*., (in-press), J. Sea. Res.

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Appendix 1. Quantitative prediction for changes in the metabolic balance in response to warming

The ratio $R_{H:U}$ of the metabolic balance between heated and unheated systems can be expressed as:

$$R_{H:U} = \frac{ER_H}{GPP_H} / \frac{ER_U}{GPP_U} = \frac{ER_H}{GPP_H} \cdot \frac{GPP_U}{ER_U} \qquad \text{(I)}$$

Substituting *ER* and *GPP* by their allometric equations I get:

$$R_{H:U} = \frac{\frac{1}{V}\left[(1-\varepsilon)n_0 e^{-Ep/kT}\sum_{i=1}^{na} M_i^{\alpha,a} + r_0 e^{-Er/kT}\sum_{i=1}^{nh} M_i^{\alpha,h}\right]}{\frac{1}{V}\sum_{i=1}^{na} e^{-Ep/kTh} M_i^{\alpha,a}} \cdot \frac{\frac{1}{V}\sum_{i=1}^{na} e^{-Ep/kTu} M_i^{\alpha,a}}{\frac{1}{V}\left[(1-\varepsilon)n_0 e^{-Ep/kT}\sum_{i=1}^{na} M_i^{\alpha,a} + r_0 e^{-Er/kT}\sum_{i=1}^{nh} M_i^{\alpha,h}\right]}$$

(II)

ER is the sum of both heterotrophic and autotrophic respiration. For simplicity, and in order to get a quantitative prediction that does not require many parameters, I assume that that during non-steady state dynamics, as was the case in the mesocosm experiment, the temperature dependence of heterotrophic respiration is unconstrained by *NPP* (i.e. the available contemporary carbon substrate; see main text for empirical justification). This implies that the temperature response of ecosystem respiration is mainly driven by heterotrophic metabolism because $E_r > E_p$ as has been shown for marine oceanic ecosystem (Lopez-Urrutia *et al.* 2006, Lopez-Urrutia & Moran 2007). During non-steady state dynamics heterotrophic metabolism can increase at maximum capacity, getting ahead of *NPP* and dominating the respiratory response of the ecosystem. Thus, given this assumption, I can remove the term for autotrophic respiration from equation (II). As such the ratio $R_{H:U}$ is given now by:

$$R_{H:U} = \frac{\frac{1}{V} r_0 e^{-Er/kT_H} \sum_{i=1}^{nh} M_i^{\alpha}}{\frac{1}{V} n_0 e^{-Ep/kT_H} \sum_{i=1}^{na} M_i^{\alpha}} \cdot \frac{\frac{1}{V} n_0 e^{-Ep/kT_U} \sum_{i=1}^{na} M_i^{\alpha}}{\frac{1}{V} r_0 e^{-Er/kT_U} \sum_{i=1}^{nh} M_i^{\alpha}} \quad \text{(III)}$$

I then simplify equation (III) to get:

$$R_{H:U} = \frac{e^{-Er/kT_H}}{e^{-Ep/kT_U}} \cdot \frac{e^{-Ep/kT_U}}{e^{-Er/kT_H}} \quad \text{(IV)}$$

In simplifying equation III I make the fundamental assumption that total biomass and the distribution of body mass are independent of temperature. By rearranging terms in equation (IV) I get:

$$R_{H:U} = e^{[(-Er+Ep)/kT_H]+[(-Ep+Er)/kT_U]} \quad \text{(V)}$$

Which can be rearranged to get equation (6) in the main text as follows:

$$R_{H:U} = e^{\frac{(Er-Ep)\cdot(T_H-T_U)}{kT_H T_U}} \quad \text{(VI)}$$

Equation (6) provides a simple yet informative approximation for the behaviour of the metabolic balance between heated and unheated systems while using minimal parameterisations, and making the assumption that heterotrophic respiration dictates ecosystem respiration in transient dynamics between different steady-states. Importantly, at steady state, where *ER* is limited by contemporary primary production I expect to see no shift in the metabolic balance of an ecosystem. This may be the case over geological time scales, but for temporal scales relevant to

the effects of global warming (i.e. decades) an understanding of transient non-steady state dynamics is fundamental.

Appendix 2. Quantitative prediction for changes in the balance between CH_4 efflux and *GPP*.

The ratio $R_{fixed\mathrm{H:U}}$ between heated and unheated systems can be expressed as:

$$R_{H:U} = \frac{a_H / p_H}{a_U / p_U} = \frac{a_H}{P_H} \cdot \frac{p_U}{a_U} \qquad \text{(I)}$$

Where *a* and *p* are the allometric equations for CH_4 efflux and GPP respectively and H and U denote the heated and unheated mesocosms. Substituting *a* and *p* by their allometric equations gives:

$$R_{H:U} = \frac{\left(\frac{1}{A}\right)\sum_{i=1}^{na} A_0 \langle M^{3/4} \rangle_{na} e^{-E_a / kTh}}{\left(\frac{1}{A}\right)\sum_{i=1}^{np} p_0 \langle M^{3/4} \rangle_{np} e^{-E_p / kTh}} \cdot \frac{\left(\frac{1}{A}\right)\sum_{i=1}^{np} p_0 \langle M^{3/4} \rangle_{np} e^{-E_p / kTu}}{\left(\frac{1}{A}\right)\sum_{i=1}^{n} A_0 \langle M^{3/4} \rangle_{na} e^{-E_a / kTu}} \qquad \text{(II)}$$

Equation (2) can then be simplified to give:

$$R_{H:U} = \frac{e^{-Ea/kT_h}}{e^{-Ep/kT_h}} \cdot \frac{e^{-Ep/kT_u}}{e^{-Ea/kT_u}} \qquad \text{(III)}$$

In simplifying equation II I make the fundamental assumption that total biomass and the distribution of body mass are independent of temperature. By rearranging terms in equation (III) we get:

$$R_{H:U} = e^{[(-Ea+Ep)/kT_H]+[(-Ep+Ea)/kT_U]} \quad \text{(IV)}$$

Which can be rearranged to get equation (11) in the main text as follows:

$$R_{H:U} = e^{\frac{(Ea-Ep)\cdot(T_H - T_U)}{kT_H T_U}} \quad \text{(V)}$$

Appendix 3. Quantitative prediction for changes in the fraction of *ER* taken up by whole ecosystem methanogenesis, *ME*.

The ratio $R_{emitted\text{H:U}}$ between heated and unheated systems can be expressed as:

$$R_{H:U} = \frac{a_H / r_H}{a_U / r_U} = \frac{a_H}{r_H} \cdot \frac{r_U}{a_U} \quad \text{(I)}$$

a is as equation (II) above. Substituting a and r by their allometric equations we get:

$$R_{H:U} = \frac{\left(\frac{1}{A}\right)\sum_{i=1}^{na} A_0 \langle M^{3/4} \rangle_{na} e^{-Ea/kTh}}{\left(\frac{1}{A}\right)\sum_{i=1}^{nr} R_0 \langle M^{3/4} \rangle_{nr} e^{-E_r/kTh}} \cdot \frac{\left(\frac{1}{A}\right)\sum_{i=1}^{nr} R_0 \langle M^{3/4} \rangle_{nr} e^{-E_r/kTu}}{\left(\frac{1}{A}\right)\sum_{i=1}^{na} A_0 \langle M^{3/4} \rangle_{nr} e^{-E_m/kTu}} \quad \text{(II)}$$

Equation (II) can be simplified to give:

$$R_{H:U} = \frac{e^{-Ea/kT_h}}{e^{-Er/kT_h}} \cdot \frac{e^{-Er/kT_u}}{e^{-Ea/kT_u}} \quad \text{(III)}$$

In simplifying equation II I make the fundamental assumption that total biomass and the distribution of body mass are independent of temperature. By rearranging terms in equation (III) we get:

$$R_{H:U} = e^{[(-Ea+Er)/kT_H]+[(-Er+Ea)/kT_U]} \quad \text{(IV)}$$

Which can be rearranged to get equation (12) in the main text as follows:

$$R_{H:U} = e^{\frac{(Ea-Er)\cdot(T_H - T_U)}{kT_H T_U}} \quad \text{(V)}$$

Appendix 4. Regression statistics for the community size spectrum.

Regression statistics for the community size spectrum of each mesocosm for the relationship: log (N_i) = b * log (M_i) + a. Where N_i is the abundance of the size class i and is the mass at the centre of the i^{th} size bin, b and a are the slope and the intercept respectively. These data highlight that the size spectrum was linear for each of the mesocosms and that the individual size distribution was a power law.

Pond	Treatment	Month	Slope	Intercept	r^2	*P*-value
1	Heated	April	-0.92	4.64	0.91	0.00020
2	Ambient	April	-0.94	4.58	0.81	0.00040
3	Ambient	April	-0.93	4.80	0.86	0.00030
4	Heated	April	-0.93	4.50	0.90	0.00003
5	Ambient	April	-0.79	5.28	0.84	0.00020
6	Heated	April	-1.12	3.30	0.78	0.00060
7	Ambient	April	-0.83	5.09	0.84	0.00020
8	Heated	April	-0.90	4.39	0.80	0.00040
9	Heated	April	-1.03	3.94	0.85	0.00040

10	Ambient	April	-0.92	3.89	0.80	0.00100
11	Ambient	April	-0.86	4.71	0.78	0.00200
12	Heated	April	-0.90	4.52	0.97	0.00004
13	Ambient	April	-0.98	4.21	0.74	0.00100
14	Heated	April	-0.88	4.25	0.91	0.00080
15	Heated	April	-0.94	4.64	0.80	0.00100
16	Ambient	April	-0.91	4.90	0.93	0.00001
17	Heated	April	-1.05	4.05	0.88	0.00050
18	Ambient	April	-0.71	5.58	0.81	0.00030
19	Heated	April	-0.92	5.09	0.97	0.00000
20	Ambient	April	-0.75	5.49	0.90	0.00010
1	Heated	October	-0.87	4.58	0.94	0.00001
2	Ambient	October	-0.94	4.15	0.95	0.00001
3	Ambient	October	-0.72	5.46	0.70	0.00200
4	Heated	October	-0.94	4.02	0.90	0.00001
5	Ambient	October	-0.94	4.10	0.81	0.00040
6	Heated	October	-1.06	3.68	0.85	0.00100
7	Ambient	October	-0.97	4.12	0.83	0.00020
8	Heated	October	-0.92	4.50	0.89	0.00040
9	Heated	October	-0.90	4.50	0.84	0.00040
10	Ambient	October	-0.84	5.09	0.87	0.00070
11	Ambient	October	-0.80	5.27	0.83	0.00020
12	Heated	October	-0.93	4.42	0.88	0.00006
13	Ambient	October	-0.78	5.30	0.88	0.00010
14	Heated	October	-0.94	3.75	0.82	0.00080
15	Heated	October	-0.95	4.17	0.93	0.00010
16	Ambient	October	-0.79	5.00	0.87	0.00020
17	Heated	October	-1.01	4.25	0.93	0.00010
18	Ambient	October	-0.95	3.77	0.93	0.00009
19	Heated	October	-0.85	4.70	0.89	0.00010
20	Ambient	October	-0.94	4.24	0.92	0.00004

Appendix 5. Regression statistics for the phytoplankton size spectrum.

Regression statistics for the phytoplankton size spectrum of each mesocosm for the relationship: $\log(N_i) = b * \log(M_i) + a$. Where N_i is the abundance of the size class i and is the mass at the centre of the i^{th} size bin, b and a are the slope and the intercept respectively.

Pond	Treatment	Month	Slope	Intercept	r^2	*P*-Value
1	Heated	April	-0.41	7.50	0.42	0.040000
2	Ambient	April	-0.31	8.20	0.70	0.005000
4	Heated	April	-0.50	6.89	0.90	0.000092
6	Heated	April	-0.35	7.70	0.71	0.002200
7	Ambient	April	-0.22	8.39	0.57	0.012000
8	Heated	April	-0.27	8.03	0.55	0.014000
9	Heated	April	-0.55	6.78	0.88	0.000160
10	Ambient	April	-0.47	6.72	0.91	0.000020
11	Ambient	April	-0.30	7.82	0.53	0.018000
12	Heated	April	-0.76	5.08	0.62	0.011000
13	Ambient	April	-0.34	7.77	0.56	0.013000
14	Heated	April	-0.65	5.52	0.70	0.004600
15	Heated	April	-0.57	6.86	0.87	0.000200
16	Ambient	April	-0.45	7.28	0.75	0.001200
17	Heated	April	-0.52	7.20	0.67	0.004000
18	Ambient	April	-0.27	7.90	0.48	0.030000
19	Heated	April	-0.65	6.36	0.93	0.000006
20	Ambient	April	-0.34	7.60	0.77	0.000900
2	Ambient	October	-0.48	6.65	0.82	0.000300
4	Heated	October	-0.50	6.41	0.78	0.000680
5	Ambient	October	-0.34	7.70	0.85	0.000300
7	Ambient	October	-0.34	7.70	0.62	0.007000
8	Heated	October	-0.30	8.14	0.68	0.004000
9	Heated	October	-0.42	7.16	0.94	0.000005
10	Ambient	October	-0.43	7.50	0.79	0.001400
11	Ambient	October	-0.32	7.90	0.77	0.000880
12	Heated	October	-0.45	7.09	0.87	0.000240
13	Ambient	October	-0.40	7.43	0.90	0.000099
14	Heated	October	-0.35	7.41	0.58	0.010000
15	Heated	October	-0.57	6.25	0.80	0.000400
16	Ambient	October	-0.25	8.20	0.60	0.023000

17	Heated	October	-0.60	6.70	0.91	0.000200
18	Ambient	October	-0.46	6.70	0.82	0.000300
19	Heated	October	-0.36	7.49	0.69	0.003000
20	Ambient	October	-0.38	7.38	0.86	0.000300

Appendix 6. Formulas and geometric shapes used to estimate biovolumes of zooplankton and phytoplankton.

Taxon	**Biovolume (V)**	**Reference**
Rotifera – Bdelloidea	$V= ¼(l\times w^2\times \Pi)/6$	Ruttner-Kolisko, 1977
Rotifera – Monogononta	$V= ¼(l\times w^2\times \Pi)/6$	Ruttner-Kolisko, 1977
Oligochaeta	$V= ¼(l\times w^2\times \Pi)\times 530$	Ruttner-Kolisko, 1977
Copepoda – Nauplii	$V= ¼(l\times w^2\times \Pi)/6$	Reiss and Schmid-Araya, 2007
Copepoda – Harpacticoida	$V= ¼(l\times w^2\times \Pi)\times 560$	Reiss and Schmid-Araya, 2007
Copepoda-Cyclopoida	$V= ¼(l\times w^2\times \Pi)\times 560$	Reiss and Schmid-Araya, 2007
Ostracoda	$V= ¼(l\times w^2\times \Pi)\times 450$	Reiss and Schmid-Araya, 2007
Cladocera	$V= ¼(l\times w^2\times \Pi)/6$	Reiss and Schmid-Araya, 2007
Phytoplankton	$V = (\Pi\times l\times w^2)/6$	Ellipse; Hillebrand *et al*. 1998
	$V = (\Pi\times w^3)/6$	Sphere; Hillebrand *et al*. 1998
	$V = \Pi\times w^2\times l$	Cylinder; Hillebrand *et al*. 1998
	$V = 1/3\times\Pi\times w^2\times l$	Cone; Hillebrand *et al*. 1998
	$V = \Pi/4\times l\times w$	Half elliptic prism; Hillebrand *et al*. 1998