A multi-scale investigation into the effects of permanent inundation on the flood pulse, in ephemeral floodplain wetlands of the River Murray.

The Murray-Darling Junction during the drought of 1914
Photo, taken by Sydney Milne, was kindly donated by his son Robin Milne for use in this thesis.

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Abstract

Using a multi-scale experimental approach, the research undertaken in this thesis investigated the role of the flood pulse in ephemeral floodplain wetlands of the River Murray, in order to better understand the impact of river regulation (and permanent inundation) on these wetlands.

An ecosystem-based experiment was conducted on the River Murray floodplain, to compare changes in nutrient availability and phytoplankton productivity in three ephemeral wetlands (over a drying/re-flooding cycle) with three permanently inundated wetlands. In the ephemeral wetlands, both drying and re-flooding phases were associated with significant increases in nutrient availability and, in some cases, phytoplankton productivity. It was demonstrated that the 'flood pulse', as described by the Flood Pulse Concept (FPC), can occur in ephemeral wetlands in dryland river-floodplain systems, although considerable variation in the nature of the pulse existed amongst these wetlands. Results of this experiment suggest that factors such as the degree of drying and length of isolation during the dry phase, the rate of re-filling, timing of re-flooding and the number of drying/re-flooding cycles may be potentially important in producing the variation observed.

Permanent inundation of ephemeral wetlands effectively removed these periods of peak nutrient availability and phytoplankton productivity, resulting in continuously low levels (of nutrient availability and phytoplankton productivity). It was concluded that alteration of the natural hydrological cycle in this way can significantly reduce nutrient availability, primary production and secondary production, essentially changing the structure and function, the ecology, of these wetlands. Equally, the results of this experiment indicate that some of the changes resulting from river regulation and permanent inundation can be somewhat reversed, within a relatively short period of time, given re-instatement of a more natural hydrological regime.

A mesocosm experiment was used to examine the influence of the dry phase, specifically the effect of the degree of wetland drying, on patterns of nutrient availability and primary productivity comprising the flood pulse. Compared to permanent inundation, re-flooding of completely desiccated sediments increased carbon (C) and nitrogen (N) availability while partial drying generally decreased, or had little

effect on, C and N availability after re-flooding. However, degree of drying had little effect on phosphorus availability or rates of primary production measured after re-flooding, and it is possible that these two factors are related. Partial drying reduced rates of community respiration after re-flooding, possibly a reflection of the reduced carbon concentrations measured in these mesocosms in this phase of the experiment. Degree of drying also influenced the macrophyte community (measured after three months of flooding), with plant biomass generally decreasing and species diversity increasing as the degree of drying increased (with the exception of complete sediment desiccation which had lasting negative effects on both macrophyte biomass and species diversity).

The results of the ecosystem and mesocosm experiments were utilised, in addition to results collected from the same experiment conducted at two smaller scales (minicosms and microcosms), to assess whether the effects of hydrological regime on nutrient availability at the 'wetland' scale could be replicated in smaller-scale experiments. None of the smaller-scaled experiments included in this investigation were able to replicate the specific response to hydrological regime recorded at the ecosystem scale, however the mesocosm experiment did produce results that were more similar to those at the ecosystem scale than those produced by the mini and microcosm experiments. The results of this study indicated that extrapolation of results from small-scale experiments should be undertaken with caution, and confirmed that a multi-scale approach to ecological research is wise, where large-scale field experimentation and/or monitoring provides a check on the accuracy, and hence relevance, of conclusions reached via mesocosm experiments.

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1

Introduction

River systems in arid and semi-arid regions of the world (dryland river systems) are characterised by highly variable flow regimes and as such, are often extensively regulated to provide a reliable source of water in an unreliable climate (e.g. Walker *et al*., 1995). In addition to disrupting longitudinal gradients within a riverine system (c.f. the Serial Discontinuity Concept; Ward and Stanford, 1983, 1995), dams and weirs used to regulate river flows can also significantly alter lateral connections within river-floodplain systems (Ward and Stanford, 1995; Thoms, 2003). The River Murray, a dryland river-floodplain system in eastern Australia, has not escaped regulation: dams and weirs in the Murray have, among other things, caused the permanent inundation of associated floodplain wetlands that were ephemeral under natural conditions (e.g. Pressey, 1986). Floods are known to be central in determining the structure and function of tropical, lowland river-floodplain systems, as encapsulated by the Flood Pulse Concept (FPC) (Junk *et al*., 1989). Although the FPC is widely cited in the literature as relevant to the functioning of river-floodplain systems, including dryland systems (e.g. Davies *et al*., 1994; Walker *et al*., 1995; Puckridge *et al*., 1998; Kingsford, 2000a), little rigorous experimentation has been undertaken to confirm its applicability in systems outside of the tropics (e.g. Thorp *et al*., 1998; Tockner *et al*., 1999; Douglas *et al*., 2005). Using a multi-scale experimental approach, the research undertaken in this thesis investigates the role of the 'flood pulse' in ephemeral floodplain wetlands of the River Murray, in order to better understand the impact of river regulation (and permanent inundation) on these wetlands.

1.1 River regulation

Human intervention, particularly the building of dams and diversion of water to support agriculture, has impacted greatly on the hydrology of most large river systems in the world (Dynesius and Nilsson, 1994; Sparks, 1995). Damming of a river can have a catastrophic effect on the riverine ecosystem

and has been identified as “one of the most dramatic and widespread deliberate impacts of humans on the natural environment” (Dynesius and Nilsson, 1994; Ligon *et al*., 1995). Waters of the Murray-Darling Basin (MDB), in eastern Australia, have not been spared; the MDB, receiving less than 5% of the continents total runoff, supports approximately three quarters of Australia’s water requirements (Hillman, 1995). It has been described as the most developed river basin on the driest inhabited continent in the world (Kingsford, 2000b).

Flows in the River Murray, one of the major rivers within the MDB, are directly controlled by a number of large dams (total capacity >10 000 GL), as well as a series of barrages and weirs (Pressey, 1986). In addition to storages, flow control structures, surface- and ground-water diversions, large, privately owned off-river storages and levee banks (Close, 1990; Evans *et al*., 1990; Kingsford, 1999), flows in almost all tributaries of the River Murray are also regulated by dams and weirs (Reid and Brooks, 1998).

For most of its length the River Murray and its floodplain, estimated to cover an area of up to 10 000 square kilometres, exist in semi-arid and arid regions (Pressey, 1986; Pressey, 1990; Roberts and Ludwig, 1990). Consequently, the River Murray has been classified as a ‘dryland’ river (Walker *et al*., 1995). As is characteristic of other dryland river systems, the natural flow regime of the River Murray is highly variable and it is this variation that has necessitated extensive development to provide a reliable water resource in an unreliable climate (Molles *et al*., 1992; Walker *et al*., 1995; Puckridge *et al*., 1998; Kingsford, 2000b).

The impact of regulation on the natural flow regime of the River Murray has been significant and complex. Storage and regulation of flows in the Murray has not only disrupted longitudinal gradients within the river system (e.g. the Serial Discontinuity Concept; Ward and Stanford, 1983, 1995), but by altering in-channel flows and the nature of flooding events, has also greatly modified lateral connections between the river and its floodplain (Walker *et al*., 1995).

The flow control structures in the Murray have raised water levels to heights previously only reached during minor flooding events, creating a continuous series of pools along a significant length of the river (Pressey, 1986; Close, 1990). In addition, the delivery of water from reservoirs to meet downstream irrigation requirements means that for a considerable length of the river (particularly below Hume Dam), peak flows now occur in summer/autumn (typically periods of low flow under natural conditions) and low flows during winter/spring when normally higher flows are absorbed by water storage facilities (Close, 1990). Consequently, many of the associated floodplain wetlands which were ephemeral under natural flow conditions have been permanently inundated since completion of infrastructure in the 1920s and 1930s (Jacobs, 1990; Pressey, 1990; Kingsford 2000a). Similar phenomena have been reported in other river systems following the construction of dams and weirs (e.g. the Mississippi River; Sparks and Spink, 1998).

The impact of dams and weirs, and corresponding changes to river-floodplain connectivity, on food webs and other ecological processes in floodplain wetlands is poorly understood, but could be severe (Kingsford, 2000a). Anecdotal evidence regarding Moira Lake, an ephemeral wetland on the floodplain of the River Murray (near Mathoura, New South Wales), supports the suggestion that river regulation can have a dramatic impact on floodplain wetland ecology. The ephemeral nature of Moira Lake was drastically altered immediately following the construction of Hume Dam in the 1930s (Leslie, 1995), when regular summer/autumn drying events ceased to occur and Moira Lake became more or less permanently inundated (Leslie and Lugg, 1994). Reports have indicated that the lake, in the 1800's and early 1900's, was a wildlife haven supporting an abundance and great diversity of native flora and fauna (e.g. Leslie and Lugg, 1994; Leslie, 1995). During this period the lake supported major commercial industries utilizing the native fish, snake and leech populations, and was a popular destination for bird-watchers (Leslie, 1995). In the decades following river regulation, changes to the native vegetation, and a conspicuous loss of fauna associated with the lake (including fish, waterbirds, snakes, frogs, leeches and water rats) have been documented (see Leslie and Lugg, 1994; Leslie, 1995). Although numerous pressures may have contributed to these changes, altered hydrology is likely to be one of the major factors responsible for the decline in the health of the lake.

1.2 The flood pulse concept

The Flood Pulse Concept (FPC) highlights the importance of lateral connections in the functioning of river-floodplain systems (Junk *et al*., 1989). In fact, the FPC suggests that the 'flood pulse' is the primary driver of structure and function in these ecosystems (Junk *et al*., 1989). Junk *et al*. (1989) observed that periodic inundation of a floodplain increases the availability of nutrients and rates of organic matter recycling, increasing the potential for primary and hence secondary production. As such, the floodplain is able to support an increased biomass and diversity of biota which have adapted to exploit this periodically available habitat and resource, producing a characteristic community structure (Junk *et al*., 1989).

While the FPC arose from observations of large, tropical lowland river-floodplain systems with regular, predictable flood pulses, numerous extensions to the concept have been proposed to broaden its applicability to systems outside of the tropics. Indeed, the 'flood pulse advantage' has long been recognised by civilizations around the world, for example, the ancient Egyptians based tax rates on the extent of annual flooding of the Nile (Tockner and Standford, 2002). Tockner *et al*. (2000), using data collected from three semi-natural European river-floodplain systems, have illustrated that the FPC is useful in describing patterns and processes driving temperate river-floodplain systems. It has also been proposed that the FPC, with some modification, may be applicable to dryland river systems where flooding can be irregular, unpredictable and highly variable in nature (e.g. Davies *et al*., 1994; Walker *et al*., 1995; Puckridge *et al*., 1998).

Since its conception the FPC has been widely cited as a model relevant to the functioning of river-floodplain systems, including those in semi-arid and arid environments (e.g. Davies *et al*., 1994; Walker *et al*., 1995; Puckridge *et al*., 1998; Kingsford, 2000a), although few attempts have been made to rigorously test and quantify its applicability (e.g. Thorp *et al*., 1998; Tockner *et al*., 1999; Douglas *et al*., 2005). Nonetheless, there is evidence in the literature that indicates the flood pulse may play an important role in the functioning of dryland river-floodplain systems. For example, the periods of flood and drought experienced in dryland river-floodplain systems are commonly associated in the literature with 'boom' and 'bust' phases of resident biota, respectively (Kingsford *et al*., 1999; Kingsford, 2000b;

Timms, 2001; Choy *et al*., 2002; Jenkins and Boulton, 2003; Kingsford *et al*., 2004). These 'boom' and 'bust' phases are clearly analogous to the 'flood pulse' described by the FPC.

The major difference between the FPC and the functioning of dryland river-floodplain systems, is however, the nature of flooding events (Walker *et al*., 1995). The tropical systems on which the FPC was based are characterised by flooding events that return at regular, predictable intervals (Junk *et al*., 1989), whereas floods in dryland systems are typically irregular, unpredictable and are interspersed with periods of drought that are equally as erratic (e.g. Walker *et al*., 1995). The FPC states that regularity is central to the importance of the flood pulse in tropical systems, allowing biota to evolve adaptations that enable exploitation of newly accessible habitat and the 'pulse' in nutrient availability and primary productivity associated with floodplain inundation (Junk *et al*., 1989).

However, it has since been demonstrated that biota have developed adaptations to the highly variable flow regime characteristic of dryland river systems. For example, both aquatic plant and zooplankton species in temporary wetlands in Australia have evolved complex life-history traits which ensure resilience to drought and allow rapid recovery when inundation of the floodplain occurs (e.g. Brock *et al*., 2003). These species produce large reservoirs of dormant propagules which are long-lived and capable of surviving extended periods in dry sediments, ensuring that a species-rich, multiple generation seed and egg 'bank' exists at any one point in time (Brock *et al*., 2003). While species-rich communities of aquatic plants and zooplankton re-establish rapidly in temporary wetlands following re-flooding, cues for breaking dormancy in these propagules are complex and diverse (Brock *et al*., 2003). This guarantees the seed and egg bank is not exhausted by a single re-flooding event (or even successive floods) which may not be long enough in duration to allow successful hatching/germination and reproduction, thus ensuring a substantial proportion of the species pool remains in reserve to survive the next dry period (Brock *et al*., 2003). In addition, many of these species are able to germinate/hatch, reach maturation and replenish the egg and seed banks within a short period of time (e.g. 8-12 weeks for aquatic plants and within days for some zooplankton) (Brock *et al*., 2003), allowing these species to reproduce even during floods of relatively short duration.

The other essential component of the FPC to be assessed in relation to dryland river systems then, is the pulse in nutrient availability and primary productivity which occurs following inundation of the floodplain. This 'pulse' is fundamental to the concept, as it stimulates a chain-reaction of increased productivity which is transferred up the food-chain, so that "the overwhelming bulk of the riverine animal biomass derives directly or indirectly from production within the floodplains" (Junk *et al*., 1989). There is evidence, accumulated from a number of controlled laboratory experiments, to suggest that re-flooding of dried wetland sediments can result in a significant release of both nitrogen and phosphorus into the water column (Briggs *et al*., 1985; Fabre, 1988; Qiu and McComb, 1994, 1996; Turner and Haygarth, 2001). However, these findings have been contradicted by numerous other laboratory studies where no significant release of nutrients into the water column occurred following the re-flooding of dried wetland sediments (Qiu and McComb, 1994; Mitchell and Baldwin, 1998; Mitchell and Baldwin, 1999; Baldwin *et al*., 2000).

Few relevant studies of dryland floodplains have been conducted in the field, fewer still have tried to link nutrient availability with primary productivity. An exception is the study of two ephemeral wetlands of the MDB, monitored over a drying and re-flooding cycle. Nitrate and phosphate concentrations in the water column of these wetlands were generally higher after re-flooding, compared to concentrations recorded during the drying process (Briggs *et al*., 1985) and although the highest levels of macrophyte production were recorded approximately three months after re-flooding (Briggs and Maher, 1985), the re-flooding phase was characterized by the lowest levels of phytoplankton productivity (Briggs *et al*., 1993). These findings may indicate that in dryland systems the changes resulting from inundation of the floodplain, particularly in ephemeral floodplain wetlands, may differ from those in tropical systems described by the FPC. While the 'pulse' in tropical systems is transferred up the food-chain resulting in significant increases in animal biomass, in dryland systems competition by aquatic macrophytes for available nutrients may limit production of phytoplankton and algae, altering the trophic pathway of the pulse and resulting in increased vegetation, rather than animal biomass.

These findings also demonstrate that while laboratory experiments have substantially furthered our knowledge of the effects of drying and re-flooding on wetland and floodplain nutrient cycle processes, micro-scale experiments conducted under controlled conditions may not allow for reproduction of the many complex abiotic and biotic interactions that occur in wetlands (Baldwin, 1996). As such, recommendations have been made in the literature for *in situ* studies (Baldwin and Mitchell, 2000) taking an experimental approach, involving manipulations of key variables at the ecosystem-level (Walker *et al*., 1995).

1.3 Experimental scale

Explicit consideration of scale in experimental design is not common in ecological research, rather experiments are regularly conducted at scales that are chosen arbitrarily or based on tradition (e.g. Allen and Starr, 1982; Wiens, 1989; Sugihara and May, 1990; Levin, 1992). As the patterns and processes observed in many ecological systems have been demonstrated to be scale-dependent (see Wiens, 1989; Cooper *et al*., 1998), any inferences based on experimental results are constrained by the extent and grain (scale) of that experiment and extrapolation of results to other scales should be undertaken with caution (Wiens, 1989).

Despite this knowledge, there is a growing trend in many fields of biology and ecology toward small-scale, laboratory based experimentation. For example, a review of the field of marine microbial ecology found that most experiments performed in the 5 years prior to 1997 were conducted under closely controlled conditions in the laboratory, with just 3% undertaken in the field (Duarte *et al*., 1997). Just 19 of 92 studies (20%) published in soil biological journals (1993 – 1998) were performed in the field (Kampichler *et al*., 2001) and of 44 studies on food-web biomanipulation in lakes, only half were experimental in nature and just 4 (9%) were conducted at the ecosystem scale (e.g. lake) (DeMelo *et al*., 1992). Likewise, a comprehensive review of the ecotoxicology literature (162 000 papers, computer printouts, abstracts and citations) found that of the small proportion of studies conducted in the field, most were simply extrapolations or confirmations of laboratory studies made under field conditions, remarkably not a single experiment of those reviewed had been conducted at the ecosystem/community scale (see Schindler, 1987).

A review of research into the effects of drying and re-flooding on nutrient availability (and cycling) and phytoplankton productivity in wetlands reveals that as in other ecological fields, the majority (>65%) of the research has been conducted at scales smaller than the ecosystem, primarily in meso- and micro-cosm laboratory experiments (see Chapter 4). Presumably, this bias toward laboratory based studies in ecology is due to the numerous virtues of small-scale experiments. Not only are small-scale experiments relatively cheap and quick to complete, but they generally also allow for greater replication, manipulation and control of relevant variables compared to ecosystem-scale experiments (e.g. Carpenter, 1996; Petersen *et al*., 1997; Culp *et al*., 2000; Ahn and Mitsch, 2002). Greater replication and control generally reduces the variability of data, increasing precision, which in turn improves statistical power to test hypotheses – seemingly generating a greater insight and understanding of the processes underlying the particular patterns of interest at larger scales (see Wiens, 1989; Culp *et al*., 2000).

While small-scale experiments have many advantages, they do have some significant limitations. Firstly, unless the phenomena of interest is known to be scale-independent, extrapolation of results from small-scale experiments to infer patterns and processes occurring in natural systems is dubious (e.g. Wiens, 1989). The ability to extrapolate results from small-scale experiments is further complicated by artifacts inherent in the 'containerization' process (e.g. Schindler, 1987; Carpenter, 1996; Sarnelle, 1997; Cooper *et al*., 1998; Berg *et al*., 1999; Kampichler *et al*., 2001). Because of these artifacts, environmental conditions in small-scale experiments will often diverge from those in natural conditions (Cooper *et al*., 1998). Together, these factors can combine to produce patterns and processes that are inconsistent with those occurring in natural systems. For example Skelly (2002), studying interactions between two larval amphibian populations found that a significant (negative) interaction was observed in artificial mesocosms, however in small freshwater ponds no interaction was observed. Similarly, Hendry *et al*. (2001) reported that measurements of microbial respiration in minicosm and mesocosm experiments were similar, however these measurements were far in excess of any recorded in the field.

Consequently a multi-scale approach to ecological investigations is now recommended by a number of scientists, where ecosystem scale experiments should be integrated with smaller scale experiments in order to determine the most appropriate experimental scales for particular questions (e.g. Wiens, 1989; Levin, 1992; Walker *et al*., 1995; Cooper *et al*., 1998; Mac Nally and Quinn, 1998; Drenner and Mazumder, 1999; Culp *et al*., 2000; Kampichler *et al*., 2001). This approach should allow us to define the extent to which experimental scale can be reduced while maintaining the ability to predict natural phenomena (e.g. Sarnelle, 1997), so that smaller-scale experiments can produce information that is both precise and relevant.

1.4 Research aims and thesis structure

The primary objective of this research was to investigate the impact of river regulation, specifically permanent inundation, on ephemeral floodplain wetlands of the River Murray. The Flood Pulse Concept provided a potential framework to describe the fundamental processes driving the structure and function of these wetlands, and against which the effects of permanent inundation could be assessed.

As such, this study has three major aims:

1. Investigation, using an experimental ecosystem-scale approach, of the role of a 'pulse' in nutrient availability and phytoplankton productivity in the functioning of ephemeral floodplain wetlands of the River Murray. The flood pulse, as outlined by the FPC, has not previously been quantified and tested in a rigorous fashion in dryland river-floodplain systems.
2. Quantification of changes to the functioning of ephemeral floodplain wetlands of the River Murray, in relation to nutrient availability and phytoplankton productivity, resulting from permanent inundation.
3. Assessment of the potential for patterns and processes in ephemeral and permanently inundated floodplain wetlands of the River Murray to be reproduced at smaller experimental scales, addressing the lack of a multi-scale experimental approach to date in this field of research.

The thesis is organised into three data chapters (Chapters 2 – 4) as well as a general discussion chapter (Chapter 5). Chapter 2 describes the ecosystem-scale experiment that investigated the flood pulse (nutrient availability and phytoplankton productivity) in, and the effects of permanent inundation on, ephemeral floodplain wetlands of the River Murray. Chapter 3 investigates the effect of degree of drying on nutrient availability and community metabolism after re-flooding via a mesocosm experiment, while Chapter 4 examines the ability of meso, mini and micro-cosm experiments to reproduce the patterns of nutrient availability in ephemeral floodplain wetlands of the River Murray.

2

Examining the nature of the flood pulse in ephemeral floodplain wetlands of the River Murray: An ecosystem-scale experiment

2.1 Introduction

The Flood Pulse Concept (FPC), cited as a model relevant to the functioning of dryland river-floodplain systems (e.g. Davies *et al.,* 1994; Walker *et al.*, 1995; Puckridge *et al.*, 1998; Kingsford, 2000a), focuses on the chain of events that take place following floodplain inundation. However, there is a growing opinion that the drying process is of equal importance (to the flooding process) in the functioning of ephemeral wetlands and floodplains in dryland regions (Humphries and Baldwin, 2003; McMahon and Finlayson, 2003; Kingsford *et al.*, 2004). This research examines the role of drying and re-flooding in the functioning of ephemeral floodplain wetlands of the River Murray, in order to assess the impact of permanent inundation on their ecology.

Of the few examples in the literature where ephemeral wetlands have been monitored over a drying and re-flooding cycle, most have focused on the responses of zooplankton, macroinvertebrate or waterbird communities (e.g. Maher, 1984; Maher and Carpenter, 1984; Crome and Carpenter, 1988; Boulton and Lloyd, 1991; Briggs et *al.*, 1997; Leeper and Taylor, 1998; Timms, 2001; Hillman and Quinn, 2002; Kingsford *et al.*, 2004). *In situ* studies of water quality, nutrient availability and phytoplankton productivity in wetlands over a drying/re-flooding cycle, although limited in number, suggest that conditions may become increasingly harsh once a floodplain wetland is disconnected from its parent channel and a dry phase is initiated: ionic concentrations, pH, turbidity, and salinity in

the water column may increase dramatically as water levels in a wetland drop due to evaporation (Briggs *et al*., 1985; Gabellone *et al*., 2001; Scholz *et al*., 2002). While total nitrogen (TN), total phosphorus (TP) and dissolved organic carbon (DOC) concentrations in the water column can also increase as water levels drop, the inorganic nutrients readily utilized by biota (e.g. dissolved inorganic nitrogen and ortho-phosphate) may become less available (Briggs *et al*., 1985; Briggs *et al*., 1993; Scholz *et al*., 2002).

As the drying phase progresses and wetland sediments become increasingly oxygenated and eventually desiccated, some significant changes to nutrient and organic matter cycling have been reported. Increased oxygen availability can stimulate a significant increase in the mineralization of organic carbon, nitrogen and phosphorus by microbes (up to 40%, 50% and 50%, respectively) (De Groot and Van Wijck, 1993; Mitchell and Baldwin, 1998; van Oorschot *et al*., 2000). The increased availability of inorganic nitrogen (NH_4^+) produced by the mineralization of organic N, along with higher oxygen concentrations, can promote nitrification (conversion of NH_4^+ to NO_X^-) (De Groot and Van Wijck, 1993; van Oorschot *et al*., 2000). While an oxic sediment layer overlies a deeper anoxic layer, coupled nitrification-denitrification (conversion of NO_X^- to N_2) may also be promoted, facilitating a significant loss of N from a wetland system (Figure 1) (De Groot and Van Wijck, 1993; van Oorschot *et al*., 2000). Phosphate produced by the mineralization of organic P can flux towards the surface sediments (related to the upward flux of pore-water as sediments dry), can be assimilated by microbes or adsorbed onto Fe hydroxides associated with sediments (De Groot and Van Wijck, 1993). While De Groot and Van Wijck (1993) showed that the drying process may increase sediment affinity for P sorption (associated with changes in the Fe cycle), Baldwin (1996) demonstrated that exposure to oxygen and progressive desiccation can actually alter phosphate binding sites (e.g. sediment-mineral associations such as Fe hydroxides) within the sediment and hence reduce sediment affinity for P sorption when re-flooding occurs (Figure 2).

As sediments progressively dry, denitrification and other anaerobic processes may be limited, slowing/stoping loss of N from the system (van Oorschot *et al*., 2000). Nutrient cycling in general will slow and then cease as the sediment becomes desiccated and the resident microbial consortia experience moisture stress and eventually death (Baldwin and Mitchell, 2000). Complete sediment

desiccation has been shown to kill up to 75 percent of the resident microbial community (Qiu and McComb, 1995), with cell lysis resulting in a release of nutrients into the surrounding sediments (e.g. Baldwin and Mitchell, 2000).

Changes to the cycling of nutrients and organic matter during a drying phase are likely to be critical in determining the sequence of events that take place after the river and floodplain are re-connected, when the floodplain and associated wetlands become inundated during a flooding event. Flow control structures used to regulate the River Murray have raised water levels, created a continuous series of pools along a significant length of the river and produced a seasonal reversal of flows, effectively removing the dry phase in many of the associated floodplain wetlands (Pressey, 1986; Close, 1990). Consequently, many of these wetlands (~35%) which were ephemeral under natural flow conditions, have been permanently inundated since completion of flow control infrastructure in the 1920s and 1930s (Jacobs, 1990; Pressey, 1990; Kingsford 2000a). Elimination of the dry phase may have a large impact on nutrient availability and cycling in these wetlands, as it not only prevents the significant changes associated with the dry phase (described above) from occurring, but is also likely to limit changes associated with re-flooding.

The FPC describes an increase in nutrient availability and phytoplankton productivity and subsequent increases in secondary production (animal biomass) on the floodplain following inundation (Junk *et al*., 1989). Research to date, comprising laboratory-based experiments and *in situ* monitoring of ephemeral wetlands has suggested that re-flooding of desiccated sediments can result in a large release of nitrogen (N) and phosphorus (P), significantly increasing the availability of these nutrients in the overlying water column (Briggs *et al*., 1985; Fabre, 1988; Qiu and McComb, 1994; Qiu and McComb, 1996; Sánchez-Carrillo and Álvarez-Cobelas, 2001; Turner and Haygarth, 2001; Scholz *et al*., 2002). However, results have been variable with a number of laboratory experiments demonstrating that sediment desiccation does not necessarily result in a 'pulse' in nutrient availability after re-flooding (Qiu and McComb, 1994; Mitchell and Baldwin, 1998; Mitchell and Baldwin, 1999), suggesting that results may differ depending on particular characteristics of the wetland sediments under investigation and the specific microbial consortia they support (e.g. Qiu and McComb, 1994; Mitchell and Baldwin, 1998). Furthermore, a number of studies have suggested that drying/re-flooding

of wetland sediments may bring about carbon limitation in some wetlands after re-flooding, due to increased mineralization of organic matter during the dry phase (Briggs *et al*., 1993; Bianchi *et al*., 1996; Mitchell and Baldwin, 1998).

Few relevant studies of dryland floodplains have been conducted in the field, fewer still have tried to link changes in nutrient availability with primary productivity. An exception is the study by Briggs *et al*., (1985, 1993) where two ephemeral wetlands in the MDB were monitored over a drying and re-flooding cycle. In accordance with predictions of the FPC, nitrate and phosphate concentrations in the water column of these wetlands were generally higher after re-flooding (compared to concentrations recorded during the drying process) (Briggs *et al*., 1985). However, the re-flooding phase in these dryland floodplain wetlands was characterized by the lowest levels of phytoplankton productivity recorded throughout the study (Briggs *et al*., 1993).

Therefore, while research to date has produced some conflicting results, there is an indication that in dryland river-floodplain systems a 'flood pulse' may occur following inundation of the floodplain and as such, the FPC provides a potential framework within which the nature and role of the flood pulse in these wetlands can be assessed. The primary objective of this research was to investigate the impact of river regulation, specifically permanent inundation, on ephemeral floodplain wetlands of the River Murray. The Flood Pulse Concept was used to generate hypotheses, specifically relating to changes in nutrient availability and phytoplankton productivity during a flood pulse in these wetlands. Once the nature of the flood pulse in these wetlands is identified, an assessment can be made of the impact of permanent inundation on the ecology of these wetlands.

As such, the research described in this chapter has two major aims:

1. Investigate, using a replicated, controlled ecosystem-scale experiment, the nature of a 'pulse' in nutrient availability and phytoplankton productivity in ephemeral floodplain wetlands of the River Murray. Therefore, the following hypotheses were tested:

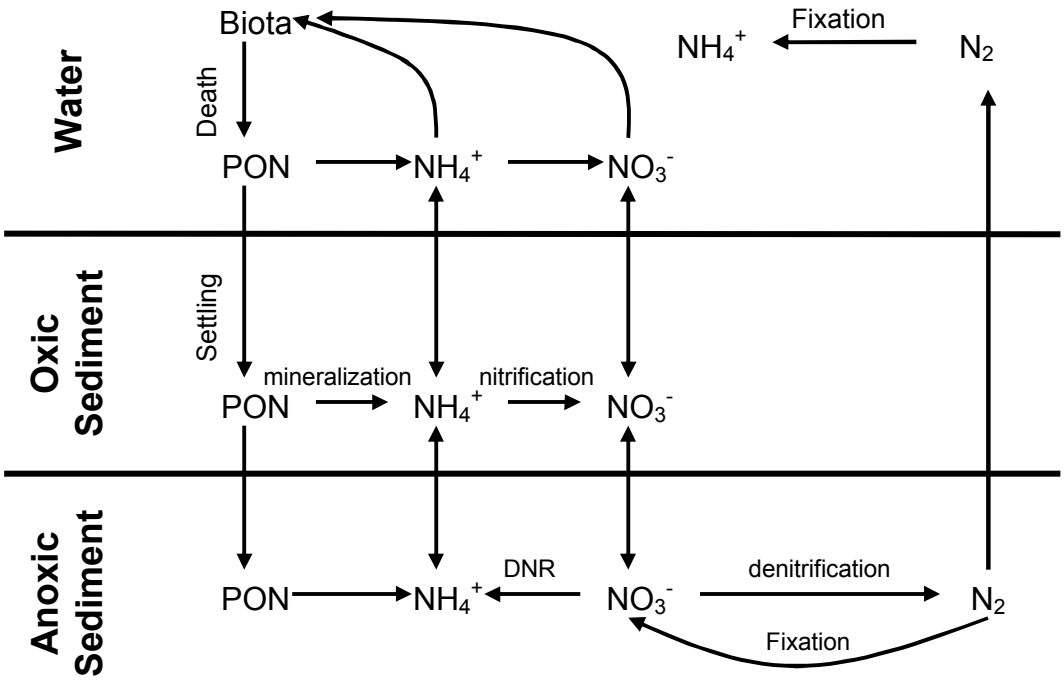
> $\mathbf{H_O}$: ephemeral floodplain wetlands function as predicted by the FPC, namely an increase in nutrient availability occurs in the water column following drying and re-flooding (e.g. = FPC)
> $\mathbf{H_A}$: ephemeral floodplain wetlands do not function as predicted by the FPC, namely no increase in nutrient availability occurs in the water column following drying and re-flooding (e.g. ≠ FPC)

> $\mathbf{H_O}$: ephemeral floodplain wetlands function as predicted by the FPC, namely an increase in phytoplankton productivity (biomass) occurs in the water column following drying and re-flooding (e.g. = FPC)
> $\mathbf{H_A}$: ephemeral floodplain wetlands do not function as predicted by the FPC, namely no increase in phytoplankton productivity (biomass) occurs in the water column following drying and re-flooding (e.g. ≠ FPC)

2. Quantify changes to the functioning of ephemeral floodplain wetlands of the River Murray, in relation to nutrient availability and phytoplankton productivity, resulting from permanent inundation. Therefore, the following hypotheses were tested:

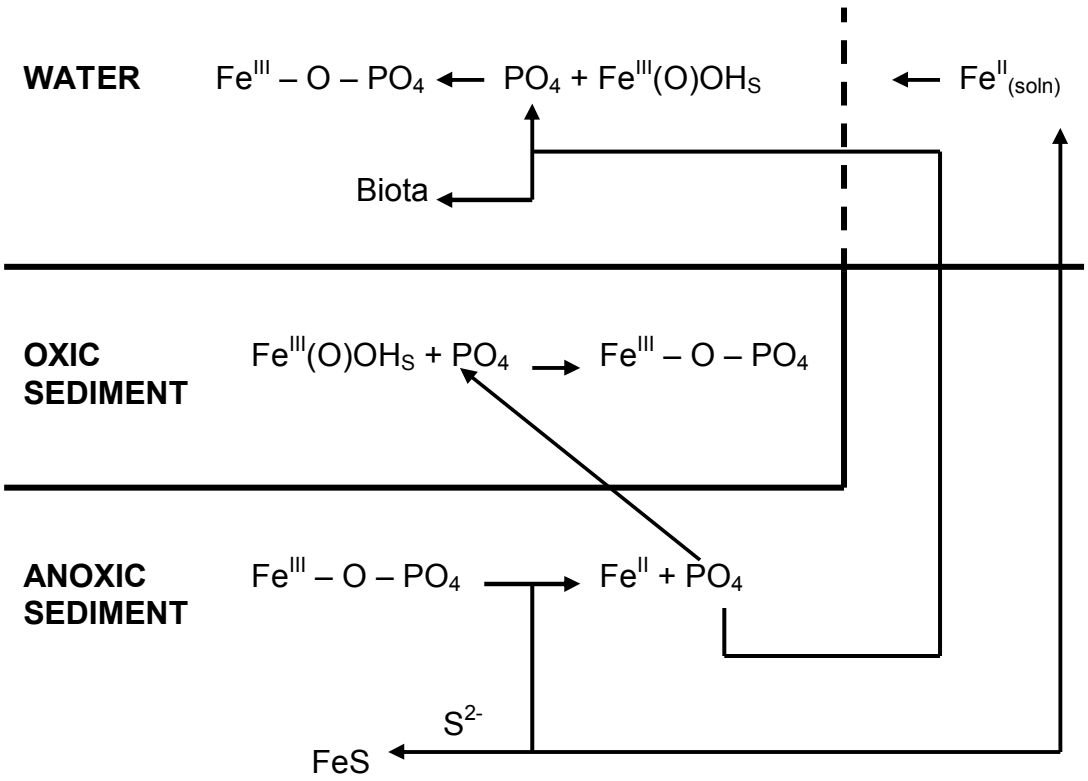
> $\mathbf{H_O}$: nutrient availability and phytoplankton productivity (biomass) in the water column of ephemeral floodplain wetlands over a drying and re-flooding cycle is not different to that in permanently inundated wetlands
> $\mathbf{H_A}$: nutrient availability and phytoplankton productivity (biomass) in the water column of ephemeral floodplain wetlands over a drying and re-flooding cycle differs to that in permanently inundated wetlands

Figure 1: The nitrogen cycle in wetland systems, indicating the affects of oxygen availability in different sediment layers (reproduced from Baldwin and Mitchell, 2000).



PON = particulate organic nitrogen

Figure 2: The phosphorus cycle in wetland systems, indicating interactions with the iron cycle (reproduced from Baldwin and Mitchell, 2000).



Subscript ‘s’ = solid phase

Subscript ‘soln’ = dissolved in solution

2.2 Materials and methods

2.2.1 Experimental design

This experiment involved six wetlands, comprising three pairs, located on the floodplain of the River Murray (Figure 3). One wetland in each pair was assigned as a treatment, the other as a control:

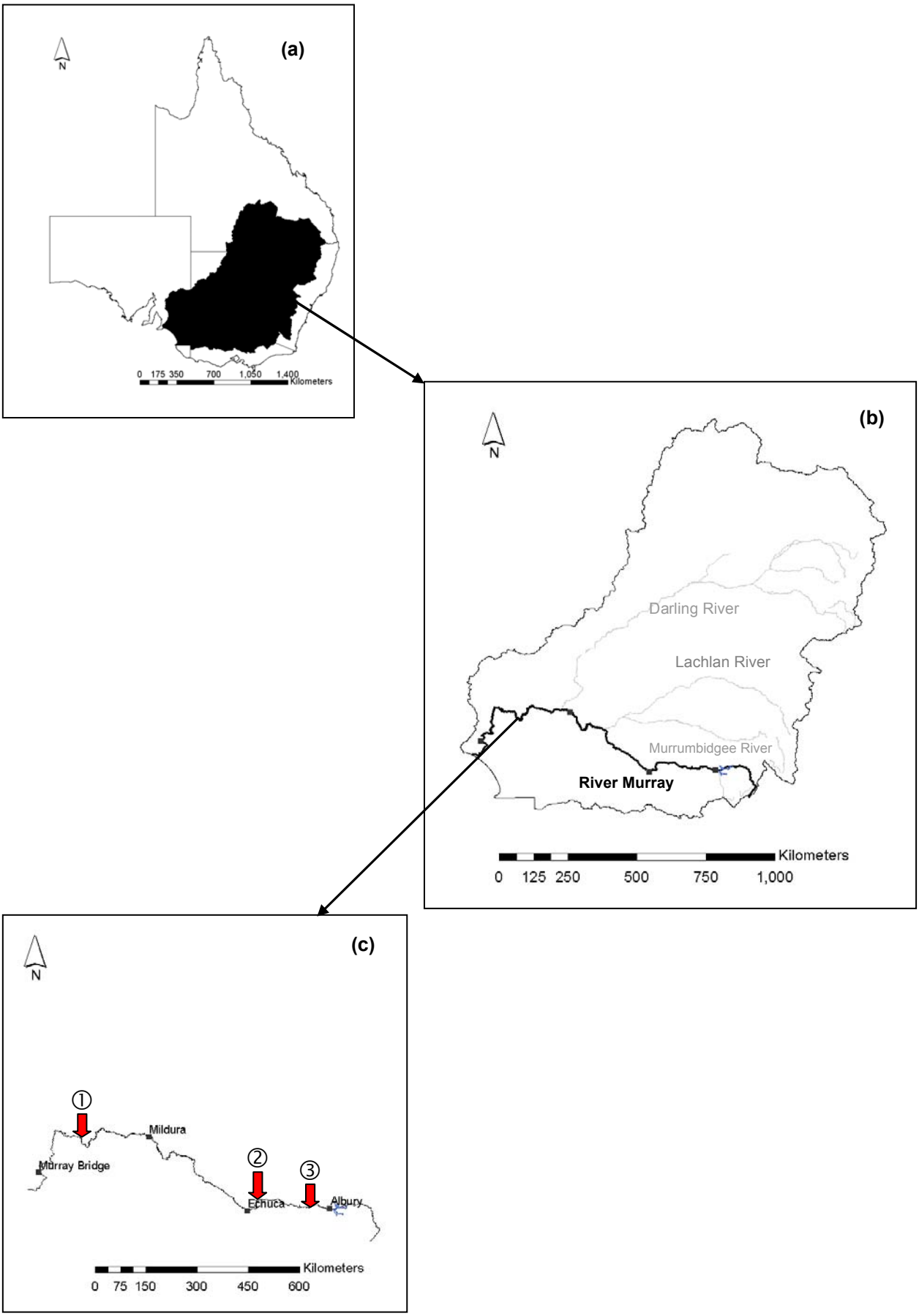
- TREATMENT WETLAND: Flow regulating structures (regulators), located on watercourses connecting the treatment wetland with the River Murray, enable manipulation of water levels in the wetland independently of the River so that a more natural hydrological regime can be restored.
- CONTROL WETLAND: the control wetland is permanently inundated as a result of raised water levels in the River Murray caused by river regulation.

Therefore, the control and treatment in this experiment were each replicated three times.

Wetlands selected for use in the experiment, and the allocation of wetlands to treatments, was not random. Of all the ephemeral wetlands affected by permanent inundation along the River Murray floodplain, only three had regulators installed with managers aiming to reinstate a more natural hydrological regime during the experimental period. Therefore, these wetlands were selected as the treatment wetlands. Control wetlands were selected on the basis of:

- Hydrology – the control wetland had to be permanently inundated as a result of river regulation;
- Location – the control wetland had to be located as close as possible to the treatment wetland; and
- Size – the control wetland had to be as similar in size to the treatment wetland as possible.

Figure 3: Maps of (a) eastern Australia indicating the Murray-Darling Basin (shaded), (b) the Murray-Darling Basin indicating the River Murray (bold), (c) the River Murray indicating the location of wetland pairs included in the experiment (indicated by ↓).



2.2.2 Study sites

The three pairs of wetlands investigated in this experiment were:

- Mussel Lagoons (T) and Chambers Creek Wetland (C) in South Australia (Figure 3c, ①);
- Moira Lake (T) and Barmah Lake (C) in New South Wales/Victoria (Figure 3c, ②); and
- Croppers Lagoon (T) and Lake Moodemere (C) in New South Wales/Victoria (Figure 3c, ③).

2.2.2.1 Mussel Lagoons and Chambers Creek Wetland

Big Mussel Lagoon and Little Mussel Lagoon are directly connected and together form Mussel Lagoons. Mussel Lagoons are located in the Riverland Region of South Australia and cover an area of approximately 145 ha (Jensen *et al*., 1996). In the survey of wetlands on the River Murray floodplain (Pressey, 1986) Mussel Lagoons were classified under Hydrological Category 1: previously free-draining areas of the floodplain now connected to the river at minimum regulated flow or at pool level (e.g. permanently inundated).

Mussel Lagoons are managed by the South Australian Field and Game Association (SAFGA). A gradual but significant reduction in the diversity and abundance of bird species utilizing the wetland had been recorded by the Association over a period of approximately thirty years (Peter Schram, 1997 – Personal Communication). This prompted SAFGA to undertake a number of management actions aimed at restoring the Lagoons and the associated bird fauna. These actions included re-vegetation programs and the installation of a regulator on the inlet channel to allow a more natural hydrological regime to be re-instated (SAFGA, 1991). In 1995 the Lagoons were dried for the first time since Weirs 3 and 4 in the River Murray had been operational (1925 and 1929, respectively) (Jacobs, 1990; Jensen *et al*., 1996).

Chambers Creek Wetland is located just upstream of Mussel Lagoons, on the same side of the river. The area of Chambers Creek Wetland included in this study covers approximately 100 ha (Jensen *et al*., 1996) and is most likely to have been ephemeral under natural conditions, although is now permanently inundated as a result of river regulation (Jensen *et al*., 1996). As such, this wetland was also classified under Hydrological Category 1 in the survey by Pressey (1986).

Chambers Creek Wetland is connected to Lake Bonney. Prior to 1994, in an attempt to reduce the salinity of Lake Bonney, water level manipulations were undertaken at Weir No. 3 in the River Murray. This management action was aborted due to increases in salinity in Chambers Creek Wetland (which also affected irrigators) (Jensen *et al*., 1996). No management actions were undertaken during the experimental period that affected water levels in Chambers Creek Wetland.

2.2.2.2 Moira Lake and Barmah Lake

Moira and Barmah Lakes, together with several other wetlands, areas of grass and sedge plain and red gum forest, form the area historically referred to as the *Moira Marshes* (Leslie, 1995). The Moira Marshes cover an area of approximately 25 000 ha and are contained within the larger Barmah-Millewa River Red Gum Forest, located in the Murray Riverine Plains Region (Eastburn, 1990; Leslie, 1995).

Moira Lake is located south of the township of Mathoura in New South Wales (NSW), and the Moira Lake complex identified by Pressey (1986) covers an area of approximately 1 382 ha. Leslie (1995) modeled natural *vs* current flooding and drying cycles for Moira Lake and demonstrated that, under natural conditions, the Lake did not dry every year although drying was nonetheless a regular occurrence - beginning in summer and continuing through until winter/spring when re-flooding usually occurred (Leslie, 1995). The ephemeral nature of Moira Lake was drastically altered immediately following the construction of Hume Dam in the 1930s (Leslie, 1995). The regular summer/autumn drying events ceased to occur, and the ever-present winter/spring re-flooding was occasionally replaced with short-term low-flow cycles (Leslie, 1995). Consequently, Moira Lake is now more or less permanently inundated (Leslie and Lugg, 1994).

A proposal to dam Moira Lake for use as a water storage facility in 1991 was opposed, and prompted a series of management actions to restore the Moira Lake ecosystem (Leslie and Lugg, 1994; Leslie, 1995). Altered hydrology was not the only factor responsible for the documented decline in native plant and animal populations associated with Moira Lake, however re-instating a more natural

hydrological cycle was seen as key to the successful rehabilitation of the Lake (Leslie and Lugg, 1994; Leslie, 1995).

The rehabilitation project included the placement of regulators on numerous inlet/outlet creeks associated with the lake, to allow the hydrology of Moira Lake to be controlled independently of water levels in the River Murray (except in major flooding events) (Leslie, 1995). Active management of the hydrological cycle in Moira Lake (regulators on all inlet/outlet creeks closed) would occur between November – August to allow Moira Lake to drain and dry (complete drying every 6-7 years in 10) independently of flows in the River Murray during the irrigation seasons (Leslie, 1995). Over the 1997/98 summer Moira Lake completely dried for the first time, possibly since the construction of the Hume Dam in the 1930s. Under the management plan, inundation of the lake would occur between August – November each year with water levels in the lake expected to vary on a yearly basis, depending on flows in the River Murray.

Barmah Lake is located north of the township of Barmah in Victoria, and the Barmah Lake complex identified by Pressey (1986) covers an area of approximately 1,304 ha. Barmah Lake is immediately adjacent to Moira Lake, on the opposite side of the River Murray (Leslie, 1995). Although Barmah Lake is slightly shallower than Moira Lake, as expected by their close vicinity they have similar hydrological, biological and ecological characteristics (Leslie, 1995). As cited in Leslie (1995, p71), a field naturalist visiting Barmah Lake in 1893 recorded thousands of birds utilizing Barmah Lake, which at the time consisted of a “moist tract in the centre of the dry lake” (from the *Australasian*, 29/4/1893). The effects of river regulation on the Barmah Lake ecosystem can be expected to be similar to those documented for Moira Lake. In the survey of wetlands on the River Murray floodplain (Pressey, 1986) both Moira and Barmah Lakes were classified under Hydrological Category 2: areas of the floodplain now actually (or potentially) connected to the river at or below maximum regulated flows but above minimum regulated flows. As such, Barmah Lake now experiences high water levels in Summer/Autumn when normally the wetland would have low or no surface water, and often water levels in Winter/Spring are low when under natural conditions water levels in the wetland would have been high. Effectively, Barmah Lake is now permanently inundated.

2.2.2.3 Croppers Lagoon and Lake Moodemere

Croppers Lagoon is located on the floodplain of the River Murray near Corowa in NSW. In this area, the River Murray and its floodplain make a transition from upland origins into the Riverine Plains region (Eastburn, 1990). Croppers Lagoon covers an area of approximately 86 ha and is connected to the River Murray by a single, narrow inlet-outlet channel (Lugg, 1993). The lagoon is located only a short distance downstream of Hume Dam and is therefore exposed to the full impact of river regulation. A study by Beovich *et al*. (1992) indicated that river regulation has had two major impacts on the hydrology of Croppers Lagoon, specifically: the timing and duration of inundation. Flow regulation has resulted in higher water levels in the Lagoon during late Spring – early Autumn, and lower water levels in winter (Beovich *et al*., 1992). This represents a reversal of natural fluctuations in the water levels of the Lagoon. A drain on the northern side of the Lagoon also delivers between 200 – 1500 ML/year of stormwater run-off and treated sewage effluent from surrounding areas (Lugg, 1993). As a result, Croppers Lagoon will now rarely, if ever, undergo natural periods of drying (Lugg, 1993).

A management strategy for Croppers Lagoon, drafted in 1992, aimed to return a more natural hydrological regime to the Lagoon (Beovich and Lloyd, 1992). A regulator was installed on the inlet /outlet creek during 1996, and in following years several attempts were made to dry the Lagoon and re-instate a more natural hydrological regime (Beovich and Lloyd, 1992; Lugg, 1993; Lloyd, pers.comm. 1997).

Lake Moodemere is located near Rutherglen (Victoria) and is just upstream of Croppers Lagoon, on the opposite side of the River. Lake Moodemere covers an area approximately 150 ha and is directly connected to the River Murray via two natural inlet/outlet creeks and one constructed channel (Pressey, 1986). Beovich (1993) reported that Lake Moodemere was previously ephemeral, drying over summer months and re-flooding when River levels rose in spring. Increasing demand for the delivery of un-seasonal irrigation flows resulted in permanent inundation of the Lake. The Lake is now also kept inundated over the summer months for recreational purposes including water skiing and rowing (Beovich, 1993).

2.2.3 Sampling regime

The field experiment was conducted over a period of approximately three years, between December 1997 - November 2000. A dry phase was attempted in each of the treatment wetlands by closing the regulator/s over summer and autumn months. Re-flooding of the treatment wetlands occurred when the regulator/s were opened, usually during winter/spring months. Control wetlands were to remain inundated throughout the experimental period.

Where possible, wetland pairs were sampled regularly prior to, and during, the drying process in the treatment wetland. Following re-flooding of the treatment wetland, the pair of wetlands were sampled 1 day, 1 week, 1 month and 3 months after re-flooding. The sampling regime of each pair of wetlands is detailed below.

2.2.3.1 Mussel Lagoons and Chambers Creek Wetland

In 1995, Mussel Lagoons were dried and then re-flooded reportedly for the first time since river regulation (Jensen *et al*., 1996; P. Schram, 1997, personal communication). During the summer of 1997/98, the managers of the Lagoons had initiated a second drying phase by using the regulator to dis-connect the Lagoons from the river. Sampling of Mussel Lagoons began in December 1997 (Table 1). At this time, the drying process was well underway and the water level in the Lagoons had dropped substantially as a result of evaporation. A suitable control, Chambers Creek Wetland, was identified shortly after this initial sampling date and another sampling date was planned for later in the drying process. However, due to a mis-communication the regulator on the inlet/outlet creek was opened without notification sometime after the sampling date in December, and the proposed post-re-flooding sampling regime could not be undertaken.

The managers of Mussel Lagoons agreed to attempt to dry the Lagoons the following year, over the summer of 1998/99. As such, sampling of Mussel Lagoons and Chambers Creek Wetland was undertaken throughout 1998 (on four occasions) in order to collect data on these wetlands between a drying/re-flooding cycle in the treatment wetland (Table 1). During 1998 widespread, above-average rainfall and flooding occurred in the northern part of the Murray-Darling Basin and the managers of the lagoons received advice that the floodwaters, although taking several months to reach South

Australia, were likely to result in extensive flooding of the Lagoons and surrounding areas (P. Schram, 1998, personal communication). Consequently, the managers believed any attempt to dry the Lagoons would be futile, and the regulator remained open to prevent possible damage to the structure. As such, drying of the Lagoons was postponed until the following Summer, 1999/2000.

Mussel Lagoons and Chambers Creek wetland were sampled on two occasions during 1999: in March and then one day prior to the re-flooding of Mussel Lagoons (Table 1). As in 1997/98, the Lagoons did not completely dry out prior to re-flooding. As managers of the Lagoons, SAFGA attempt to balance the requirements of their association with the aim of improving the health of the wetland and its associated flora and fauna. Therefore, re-flooding of Mussel Lagoons took place on the 19 December 1999, rather than the following Winter/Spring as would occur under natural conditions. This allowed the Lagoons to contain water for a sufficient period of time to attract associated bird-life for the opening of the hunting season (which takes place in March). Both Mussel Lagoons and Chambers Creek Wetland were sampled 1 day, 1 week, 1 month and 3 months after re-flooding (Table 1; Figures 4 and 5). Samples were also taken from the River Murray, just upstream of the inlet creek, on the first two sampling dates after re-flooding commenced.

Table 1: Mussel Lagoons and Chambers Creek Wetland, dates sampled. (● = sampled; ○ = not sampled; ▭ = post re-flooding)

Sampling Date	Mussel Lagoons	Chambers Creek Wetland
26/12/1997	●	○
6/2/1998	●	●
16/4/1998	●	●
17/9/1998	●	●
16/12/1998	●	●
18/3/1999	●	●
18/12/1999	●	●
20/12/1999 **1 day after re-flooding**	●	●
26/12/1999 **1 week after re-flooding**	●	●
20/11/1999 **1 month after re-flooding**	●	●
13/3/2000 **3 months after re-flooding**	●	●

2.2.3.2 Moira Lake and Barmah Lake

Over the summer of 1997/98 Moira Lake was successfully dried after an extended period of permanent inundation. It was possible to sample Moira and Barmah Lakes on two occasions prior to the complete desiccation of Moira Lake (Table 2). In accordance with the management plan, Moira Lake was re-flooded towards the end of winter 1998. Both Moira and Barmah Lakes were sampled 1 day, 1 week, 1 month and 3 months after re-flooding (Table 2; Figures 6 and 7).

Over the following Summer (1998/1999) NSW State Forests initiated a second drying phase in Moira Lake. The Lakes were sampled at the end of January 1999 (six months after re-flooding) when the drying phase was significantly progressed. Moira Lake was re-flooded towards the end of winter 1999, and both the treatment and control wetland were again sampled 1 day, 1 week, 1 month and 3 months after re-flooding (Table 2). In addition, samples were taken from the River Murray just upstream of the two wetlands, 1 day and 1 week after re-flooding commenced in Moira Lake (1999).

Table 2: Moira Lake and Barmah Lake, dates sampled. (● = sampled; ○ = not sampled; = post re-flooding).

Sampling Date	Moira Lake	Barmah Lake
14/1/1998	●	●
24/2/98	●	●
4/8/98 **1 day after re-flooding**	●	●
11/8/98 **1 week after re-flooding**	●	●
6/9/98 **1 month after re-flooding**	●	●
5/11/98 **3 months after re-flooding**	●	●
28/1/1999	●	●
4/8/1999* **1 day after re-flooding**	●	●
13/8/1999 **1 week after re-flooding**	●	●
2/9/1999 **1 month after re-flooding**	●	●
3/11/1999* **3 months after re-flooding**	●	●

2.2.3.3 Croppers Lagoon and Lake Moodemere

The Murray Wetlands Working Group (managers of Croppers Lagoon) planned to use the regulator installed on the inlet/outlet creek to impose a dry phase in the Lagoon during Summer 1997/98. This first attempt was unsuccessful, however the Management Plan proposed the Lagoon be dried on an annual basis so another drying phase was planned for the upcoming summer 1998/99. This presented an opportunity to collect data in the treatment and control wetlands prior to dyring/re-flooding of the treatment wetland and sampling was undertaken throughout 1998 (on three occasions) (Table 3).

Under natural conditions, some water would drain out of Croppers Lagoon when River levels were low (during summer) and then water remaining in the Lagoon would evaporate over the hot summer months. During 1998 a protracted blue-green algal bloom in the Lagoon meant that water could not be drained out when River levels were low. In addition, extensive rainfall occurred in the local catchment area in late summer. Consequently, Croppers Lagoon did not undergo a dry phase in 1998/99 and drying of the Lagoon was postponed until the following summer, 1999/2000.

In 1999, Croppers Lagoon and Lake Moodemere were sampled on one occasion, during January (Table 3). Over the Summer of 1999/2000 water levels in Croppers Lagoon dropped significantly, however it did not completely dry out prior to re-flooding. Samples were taken from Croppers Lagoon 1 day prior to re-flooding which occurred on 5 September 2000 and both Croppers Lagoon and Lake Moodemere were sampled 1 day, 1 week, 1 month and 3 months after re-flooding commenced (Table 3; Figures 8 and 9). In addition, samples were taken from the River Murray just upstream of the two wetlands, 1 month and 3 months after re-flooding commenced.

Table 3: Croppers Lagoon and Lake Moodemere, dates sampled. (● = sampled; ○ = not sampled; ▭ = post re-flooding).

Sampling Date	Croppers Lagoon	Lake Moodemere
27/2/1998	●	●
3/8/1998	●	●
3/11/1998	●	●
26/1/1999	●	●
4/9/2000 **1 day prior to re-flooding**	●	○
6/9/2000 **1 day after re-flooding**	●	●
12/9/2000 **1 week after re-flooding**	●	●
3/10/2000 **1 month after re-flooding**	●	●
28/11/2000 **3 months after re-flooding**	●	●

Figure 4: (a) Big Mussel Lagoon prior to re-flooding (18 December 1999), (b) Little Mussel Lagoon (left) and Big Mussel Lagoon (right), approximately 24 hours after re-flooding commenced (20 December 1999), and (c) Big Mussel Lagoon post re-flooding (16 December 1998).

(a) Big Mussel Lagoon: Prior to re-flooding

(b) Little Mussel Lagoon (left) and Big Mussel Lagoon (right): 24 hours after re-flooding

(c) Big Mussel Lagoon: post re-flooding

Figure 5: Chambers Creek wetland (16 December 1998).

Figure 6: Moira Lake: (a) prior to re-flooding (24 February 1998), and (b) post re-flooding (5 November 1998).

(a) Prior to re-flooding

(b) Post re-flooding

Figure 7: Barmah Lake (5 November 1998).

Figure 8: Croppers Lagoon: (a) prior to re-flooding (taken by Paul Lloyd, 4 September 2000), and (b) post re-flooding (28 November 2000).

(a) Prior to re-flooding

(b) Post re-flooding

Figure 9: Lake Moodemere (28 November 2000).

2.2.4 Parameters

A total of ten sites in each wetland, on each sampling date, were sampled for the parameters outlined below. Within each wetland five sample sites were located around the riparian zone, and five around the centre of the wetland.

2.2.4.1 Water depth

Water depths were measured at the designated sample sites in treatment and control wetlands on each sampling date. A meter ruler was used to measure water depth in shallow areas and where water depth exceeded 1 meter, a weighted rope was used to estimate water depth.

2.2.4.2 Water quality

Water quality measurements were taken from the water column of the designated sample sites in each wetland using an HORIBA U-10 Water Quality Checker. Measurements taken include pH, conductivity (mS/cm), turbidity (NTU), Dissolved Oxygen (mg/L) and Temperature (ºC).

2.2.4.3 Nutrient availability

Samples collected from the water column of the designated sample sites in each wetland were analysed for total nutrient concentrations (Total Carbon, Total Nitrogen and Total Phosphorus) and dissolved nutrient concentrations (Dissolved Organic Carbon, Nitrate + Nitrite, Ammonia and ortho-phosphate).

All samples were collected in LDPE plastic bottles that had been soaked overnight in Neutracon wash (to remove all nutrients), rinsed thoroughly in Reverse Osmosis (RO) water and also rinsed with a sample of the water to be collected. Samples were placed on ice in an esky immediately following collection. Those samples to be analysed for total nutrient concentration were placed in a freezer as soon as possible (within several days of collection). Samples to be analysed for dissolved nutrient concentration were filtered using 0.45µm filter papers (Gelman 0.45µm pore size, cellulose acetate filter paper) within a week of collection (stored at <4°C) and then placed in a freezer. All samples were stored frozen until they were defrosted and analysed.

Samples were analysed for total and dissolved carbon concentrations using an O·I·Analytical Model 1010 Wet Oxidation TOC Analyser. For analysis of total nitrogen and total phosphorus, a digest reagent was added to samples which were then autoclaved, decanted and run through a LACHAT Flow Injection Analyser. The LACHAT Flow Injection Analyser was also used to detect concentrations of ammonia, nitrate + nitrite and ortho-phosphate in samples which had previously been filtered.

It should be noted that water samples collected for nutrient analysis from Croppers Lagoon and Lake Moodemere, just prior to and following re-flooding of Croppers Lagoon (September 2000), were stored frozen and then defrosted in preparation for analysis. However, analysis could not take place immediately and these samples were then stored in the refrigerator (< 4ºC) for a significant length of time before they could be analysed. The accuracy of these nutrient analyses is uncertain.

2.2.4.4 Phytoplankton productivity

Samples were collected from the water column of the designated sample sites in each wetland and analysed for chlorophyll *a* concentration, to provide an estimate of algal biomass (see Steinman and Lamberti, 1996). Algal biomass in a wetland is dependent upon levels of primary production by the algal community, however it is also affected by other factors including the abundance and grazing activity of invertebrates (Udy *et al*., 2001). In this experiment, chlorophyll *a* concentrations are used to provide an indication of the level of phytoplankton productivity occurring in the water column of the wetland.

All water samples for chlorophyll *a* analysis were collected in LDPE plastic bottles that had been washed (as for samples collected for nutrient analysis, see above). Samples were placed on ice in an esky immediately following collection, and then in a refrigerator (< 4ºC) as soon as possible. A volume of each sample was filtered using GF/C filter papers as soon as possible after collection (no longer than one week after sampling). The filter paper (with particulate matter) was placed into a glass tube with a known volume of 90% ethanol and heated in a water-bath at 80ºC for 5 minutes. After cooling, the extract was then re-filtered (using 0.45µm filter paper) and analysed for chlorophyll *a* using a UV-1201 Shimadzu Spectrophotometer.

2.2.5 Data analysis

The experiment was designed to be analysed using a two-way, replicated ANOVA (factors: treatment and sampling date) that took into account the serial connectedness of the data resulting from repeated measures taken in each wetland over time. However, this analysis is not applicable to the data collected, as the three pairs of wetlands cannot be considered to be replicates of each other. The three treatment wetlands were exposed to markedly different levels of drying and desiccation, and they were re-flooded at different times of the year, in different years. Furthermore, one of the control wetlands (Barmah Lake) did not remain permanently inundated for the duration of the experiment. On this basis, it is believed that the most meaningful representation of the data will be made simply by graphing results for each parameter and observing patterns between treatment and control wetlands over time. Calculations of 2 x standard error (least significant difference) are used as a guide to indicate where noteworthy differences may exist.[1]

[1] Advice on data analysis provided by A/Prof. Ross Cunningham, Research Fellow – Biostatistics, Australian National University

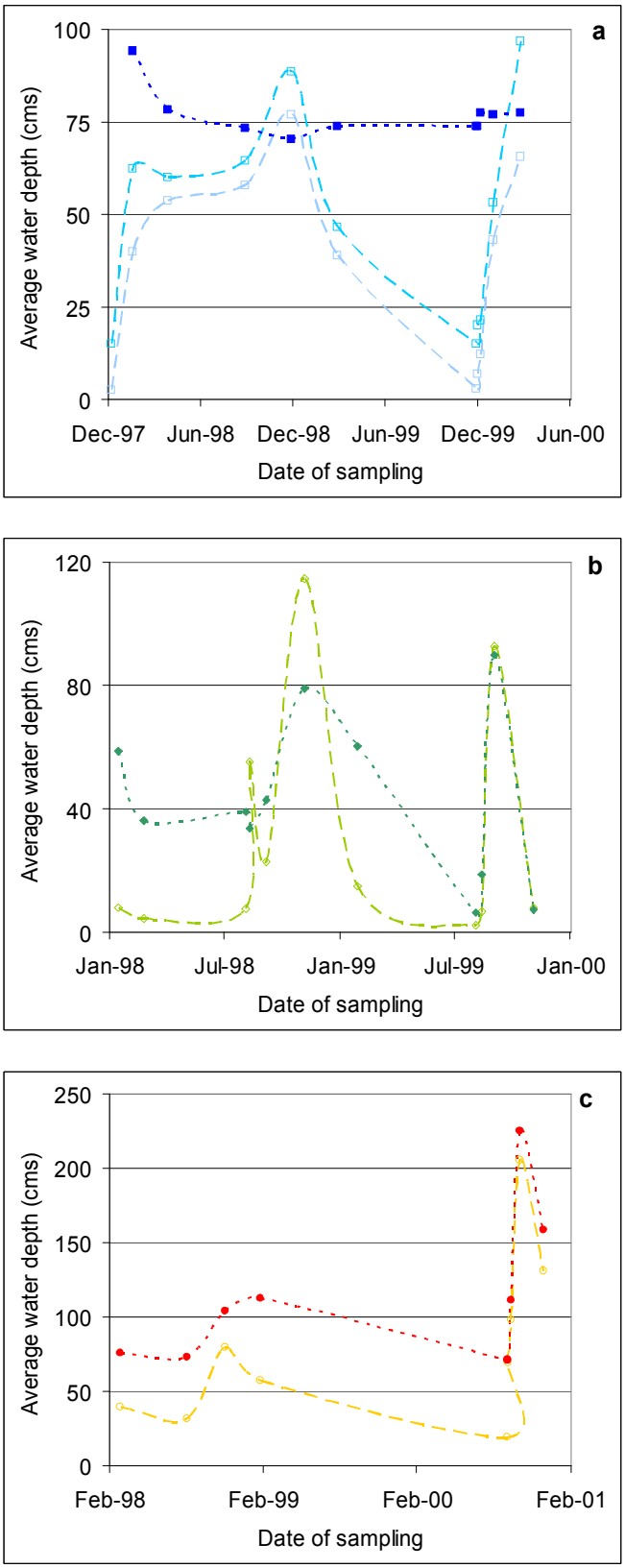
2.3 Results

The water level in Mussel Lagoons dropped considerably over spring and summer months during 1997/98 and again during 1999/2000, however complete desiccation did not occur on either occasion. It is worth noting that the Lagoons dried to a similar extent in both 1997 and 1999. Just prior to re-flooding in 1999 the shallower Big Mussel Lagoon was almost completely dried with just a small pool of water (~5 cm deep) remaining in the centre and extensive sediment cracking around the remainder of the Lagoon. Little Mussel Lagoon is deeper and although the riparian edges were exposed and dried, it still contained a considerable amount of water (~30 cm deep). Water levels in Mussel Lagoons gradually increased after re-flooding in December 1999. Chambers Creek Wetland remained permanently inundated throughout the experimental period. Water levels recorded in this pair of wetlands on each sampling date are illustrated in Figure 10(a).

A dry phase was imposed on Moira Lake over the summer of 1997/98 and again the following summer (1998/99). During both dry phases Moira Lake underwent complete desiccation, with extensive sediment cracking observed across the lake bed. Re-flooding of Moira Lake took place in August in both 1998 and 1999 and water levels in the Lake varied after re-flooding depending on flows in the River Murray. It is understood that the proposed control wetland, Barmah Lake, remained inundated throughout 1997-1998. However, during 1999 water levels in Barmah Lake dropped substantially and on two occasions (1 day and 3 months after re-flooding) water levels were so low that the riparian zone was exposed for a short period of time. Water levels recorded in this pair of wetlands on each sampling date are illustrated in Figure 10(b).

Attempts were made on numerous occasions to dry Croppers Lagoon, initially over the summer of 1997/98 and then again in 1999/2000. Whilst the water levels dropped substantially over the summer of 1999/2000, exposing the riparian zone and resulting in sediment cracking in some areas of the wetland, there were still large pools of water present in the deeper parts of the Lagoon. Croppers Lagoon gradually re-filled after the regulator was opened in September 2000. Lake Moodemere remained completely inundated throughout the experimental period. Water levels recorded in this pair of wetlands on each sampling date are illustrated in Figure 10(c).

Figure 10: Average water depth (cms) on each sampling date at (a) Big (—□—) and Little (—□—) Mussel Lagoons and Chambers Creek Wetland (---■---), (b) Moira (—◇—) and Barmah (---◆---) Lakes and (c) Croppers Lagoon (—○—) and Lake Moodemere (---●---).



2.3.1 Permanent inundation

In contrast to the significant changes in nutrient availability and phytoplankton productivity recorded in the treatment wetlands over the drying and re-flooding cycle (see below), carbon, nitrogen and phosphorus concentrations in all three control wetlands generally remained low and showed little variation between sampling dates for the duration of the experiment. Possibly as a result of the limited availability of nutrients in these wetlands, chlorophyll *a* concentrations recorded in the three control wetlands also remained low and varied little throughout the experimental period. The patterns illustrated in Figure 11 (a – h), comparing Barmah and Moira Lakes, are representative of those patterns recorded in the other control wetlands relative to their corresponding treatment wetland.

2.3.2 The dry phase

As the dry phase progressed in treatment wetlands, the water column became increasingly warmer, more turbid and higher in conductivity in comparison to the corresponding control wetlands. Dissolved oxygen (DO) concentration and pH also increased in a number of the treatment wetlands as drying progressed (Little Mussel Lagoon and Moira Lake), probably influenced by numerous factors including water level (increasing the effect of wind action on shallow water column) and increased rates of phytoplankton and microbial production (consuming CO_2 and producing O_2, altering the CO_2 / HCO_3^- / CO_3^{2-} equilibrium and hence increasing pH).

Evaporation of the water column and the associated 'concentration effect' also resulted in increased concentrations of total nutrient pools [total carbon (TC), total nitrogen (TN) and total phosphorus (TP)] in treatment wetlands as they dried (Moira Lake and Mussel Lagoons) (Figures 11 and 12, respectively). Dissolved organic carbon (DOC) concentration, and the proportion of the TC pool it represented, also increased dramatically in the water column of wetlands during the dry phase (Moira Lake and Mussel Lagoons) (Figures 11 and 12). Despite the evaporation and concentration processes, little to no increase in ammonia (NH_4^+) concentration was recorded in the water column of any of the treatment wetlands during drying (Figures 11 – 13).

The effects of the drying phase on other bio-available nutrients varied according to the treatment wetland considered. For example, the drying process had little effect on the nitrate+nitrite (NO_X^-) concentration recorded in Mussel Lagoons, where concentrations did not differ significantly to those recorded in the corresponding control wetland (Figure 12). In contrast, NO_X^- concentrations increased significantly in Moira Lake during both drying phases (e.g. 1998 and 1999), indicating that microbial activity responsible for the conversion of NO_X^- (e.g. denitrification and dissimilative nitrate reduction) was limited in this wetland, allowing its accumulation in the water column (Figure 11). The NO_X^- concentrations recorded in Moira Lake during both drying phases were orders of magnitude (e.g. > x100) greater than would be expected by concentration processes alone (using increases in conductivity as a guide).

In general, the concentration of ortho-phosphate (PO_4^{3-}) in the water column of treatment wetlands remained very low, and represented a low proportion of the TP pool, during the drying process (Figures 11 – 13). However, relatively and substantially higher PO_4^{3-} concentrations were recorded in Little Mussel Lagoon and Moira Lake (during the first dry phase, 1998) towards the very end of the dry phase in each of these wetlands (Figures 12 and 11, respectively). The increased availability of PO_4^{3-} in the water column observed during the latter stages of the dry phase may be related to the significant increases in pH observed at the same time in these wetlands. High pH levels (> pH 9) are known to promote the desorption of phosphate ions from Fe(III) hydroxides, causing a release of phosphate from wetland sediments into the water column (e.g. Fabre, 1988; Boers, 1991).

Higher chlorophyll *a* concentrations were recorded in all treatment wetlands during the dry phase, relative to concentrations recorded in these wetlands on other sampling dates and compared to concentrations recorded in corresponding control wetlands (Figures 11 – 13). The degree to which chlorophyll *a* concentrations increased however, differed between treatment wetlands. For example, chlorophyll *a* concentration in Mussel Lagoons increased to a lesser degree than would have been expected by the 'concentration effect' (estimated by the change in conductivity) while concentrations in Moira Lake (in both 1998 and 1999) increased to a far greater degree than would have been expected by the concentration effect alone. This suggests that in Moira Lake, the phytoplankton population was

actually growing during the dry phase, despite the extreme conditions present in the wetland at this time (e.g. very high turbidity, pH and water temperature).

While Croppers Lagoon did not dry completely, water levels in the Lagoon did drop substantially (Figure 10), with some of the riparian zone undergoing desiccation and sediment cracking prior to the initiation of re-flooding in September 2000. Nutrient concentrations in Croppers Lagoon demonstrated significant variation between sampling dates in the period prior to re-flooding. As such, there were no clear patterns that may have suggested an association between nutrient concentrations and changes in water level (and specifically the drying process) as was possible with data collected from the other treatment wetlands. The extended period of isolation from the River Murray, together with the lengthy blue-green algal bloom in the wetland, may have influenced this variation.

Nonetheless, when Croppers Lagoon was sampled one day prior to re-flooding the concentrations of TC, TN, TP, NO_X^- and chlorophyll *a* in the water column were significantly higher than those recorded in Lake Moodemere, the corresponding control wetland (sampled 2 days later) (Figure 13). Both NH_4^+ and PO_4^{3-} concentrations had increased slightly, although were not significantly higher than concentrations recorded in Lake Moodemere, at the end of the dry phase (Figure 13). These results are similar to those observed in the other treatment wetlands. Interestingly however, when sampled just prior to re-flooding the DOC concentration in Croppers Lagoon was very low (Figure 13), unlike the other treatment wetlands where DOC accumulated to relatively high concentrations during the drying phase. This may suggest that bio-available forms of carbon were limited in availability in Croppers Lagoon at the end of the dry phase, perhaps due to the extended period of isolation from the River and the protracted blue-green algal bloom.

Figure 11 (a – d): Changes in average concentrations of (a) TC, (b) DOC, (c) TN and (d) NH_4^+ in Moira (—◆—) and Barmah (---◆---) Lakes over two drying/re-flooding cycles. Error bars represent ± 2 x standard error and 🡻 indicates measurements taken during drying phases in 1998 and 1999. Because of the extremely high TC and DOC concentrations recorded in Moira Lake during the dry phases, the y axes on these graphs (a and b) are presented as Log values.

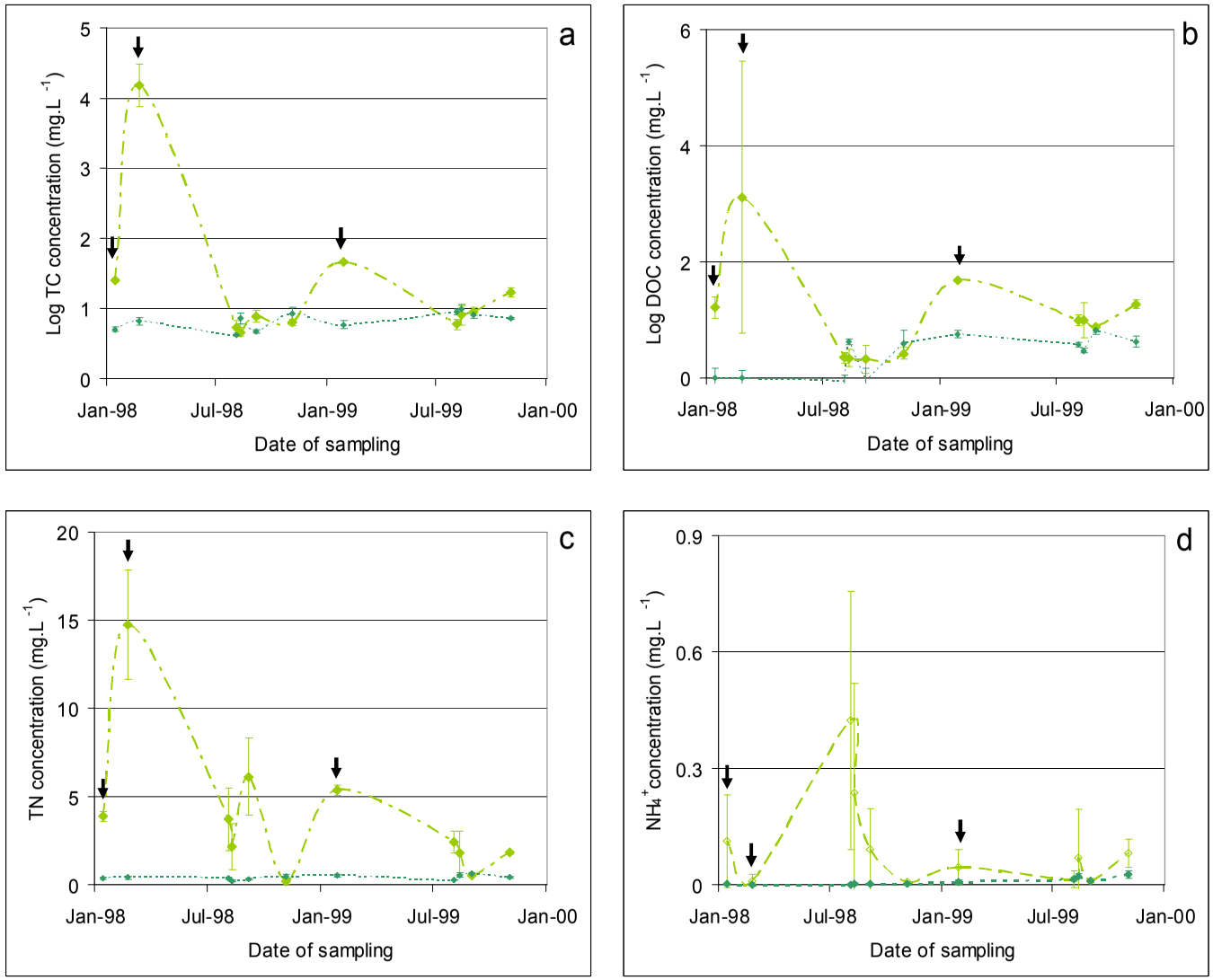
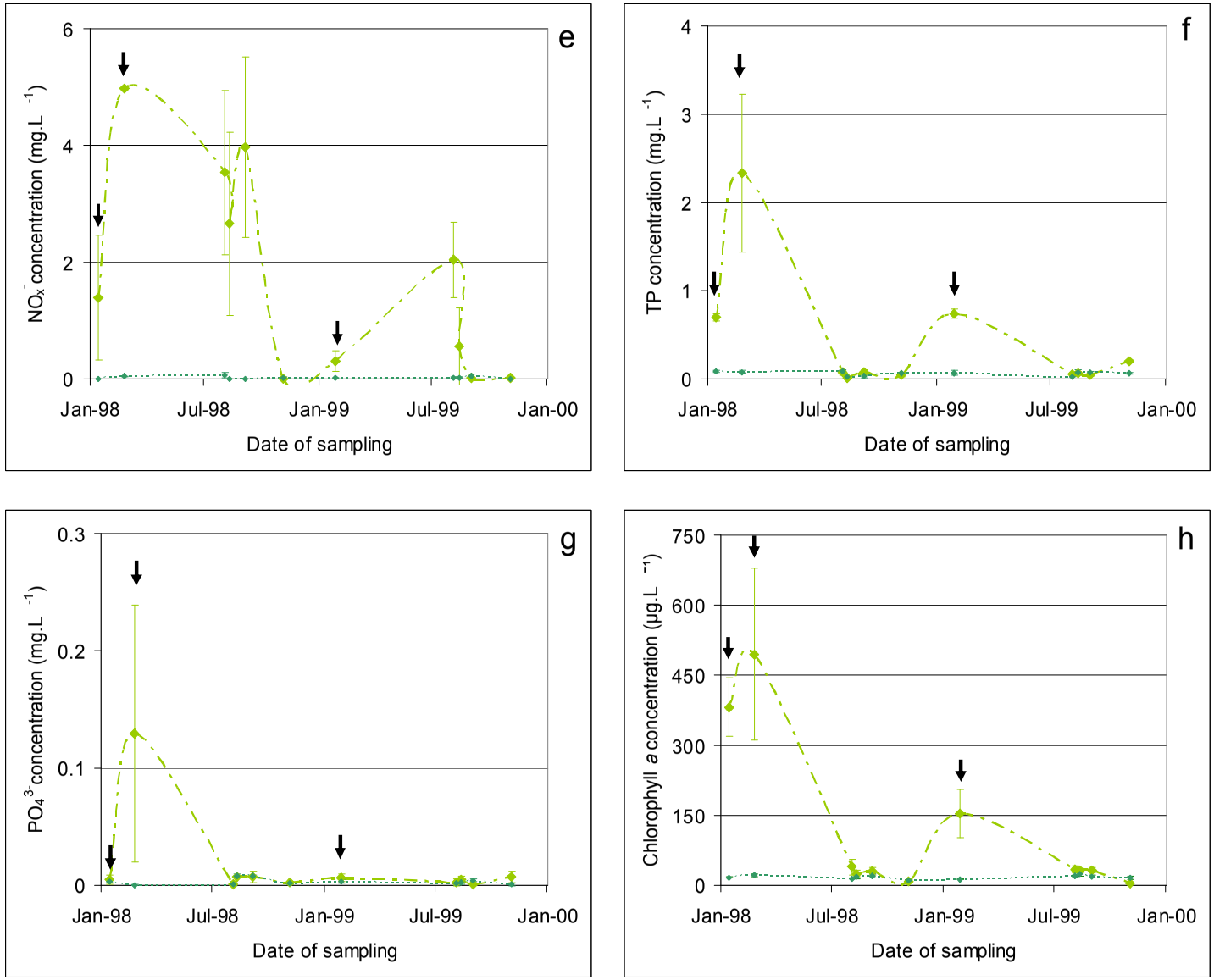


Figure 11 (e – h): Changes in average concentrations of (e) NO_x^-, (f) TP, (g) PO_4^{3-}, and (h) chlorophyll *a* in Moira (—◆—) and Barmah (---◆---) Lakes over two drying/re-flooding cycles. Error bars represent ± 2 x standard error and ⬇ indicates measurements taken during dry phases in 1998 and 1999.



2.3.3 The re-flooding phase

During the re-flooding phase, turbidity and conductivity levels in treatment wetlands were generally lower relative to those recorded during the dry phase, and were similar to measurements in the corresponding control wetlands during the same period. Conductivity levels in the South Australian wetlands (Mussel Lagoons and Chambers Creek Wetland) were orders of magnitude higher than in the wetlands further upstream, even during the re-flooding phase, probably due to contamination from the adjacent evaporation basin (Loveday Swamp) used for the disposal of saline irrigation drainage water (SAFGA, 1991).

Water temperatures after re-flooding were dependent upon the time of year that inundation commenced. For example, re-flooding of Mussel Lagoons was initiated in December (early summer) so water temperatures were relatively high (~ 25ºC) in the Lagoons in the three months following. Re-flooding commenced in Moira Lake during August (late Winter), and in Croppers Lagoon during September (early Spring), so water temperatures were initially low and then gradually warmed over the following three months (from ~13ºC up to ~22ºC).

The measurements of DO concentration and pH in Moira Lake and Croppers Lagoon taken during the re-flooding phase showed relatively little variation and closely reflected those measurements in the corresponding control wetlands. In contrast, significant changes in DO concentration and pH were measured in both Big Mussel (BM) and Little Mussel (LM) Lagoons after re-flooding. In the week after re-flooding commenced, both the DO concentration and pH in BM Lagoon had decreased dramatically while in LM the opposite occurred. After these initial changes, the DO concentration and pH in both Lagoons gradually converged over the remaining sampling dates: pH returned to around pH8.3 and DO concentration to 5 – 7 $mg.L^{-1}$. It is likely that in LM Lagoon, DO and pH were primarily influenced by phytoplankton activity while in BM Lagoon they were primarily influenced by sediment microbial activity (see below).

2.3.3.1 Mussel Lagoons

Flooding in BM Lagoon resulted in a large increase in NO_X^- concentration and a small but significant increase in TP concentration in the water column in the first week, despite the diluting effect of inflowing river water (with low nutrient concentrations) (Figure 12 e, f). The increased availability of NO_X^- and TP persisted for less than one month following the initiation of re-flooding (Figure 12 e, f). Re-flooding did not produce any significant increase in the availability of TC, DOC, TN, NH_4^+ or PO_4^{3-} in the water column of the wetland in the 24 hours – 1 week after re-flooding (Figure 12 a-d, g). While re-flooding did not produce an immediate increase in DOC or PO_4^{3-} concentrations, in following months the concentrations of both of these nutrients, as well as TP, increased in the water column of BM Lagoon. When sampled three months after re-flooding, DOC, PO_4^{3-} and TP concentrations in BM Lagoon were significantly greater than in the corresponding control wetland (Figure 12 b,g). The increased concentrations of some nutrients in BM Lagoon following re-flooding (compared to the control wetland) were not associated with an increase in phytoplankton biomass. Post re-flooding chlorophyll *a* concentrations recorded in this part of the Lagoons were low, and did not differ significantly to those in the control wetland during this period (Figure 12h).

Little Mussel Lagoon dried to a lesser degree than BM Lagoon and still contained a substantial volume of water at the end of the dry phase. In LM Lagoon, despite the diluting effect of inflowing river water, re-flooding produced a dramatic increase in NH_4^+ and PO_4^{3-} concentrations in the water column during the first week of inundation (Figure 12 d, g) and a small but significant increase in TN concentration, while high TP concentrations were maintained (Figure 12 c, f). The high NH_4^+, PO_4^{3-} and TP concentrations in LM Lagoon persisted for less than one month while the relatively high TN concentrations persisted for at least one month after re-flooding, although were depleted when sampled three months after re-flooding. Re-flooding did not result in any significant increase in TC, DOC or NO_X^- concentrations in the water column of the wetland in the 24 hours – 1 week after re-flooding commenced (Figure 12 a, b, e). However, as was observed in BM Lagoon, the DOC concentration in LM Lagoon remained significantly higher than in the control wetland (despite the diluting effect of inflowing river water) throughout the three months following re-flooding (Figure 12b). Similarly, PO_4^{3-} and TP concentrations increased significantly between one and three months after re-

flooding (Figure 12g). In contrast to BM Lagoon however, the increased availability of nutrients in LM Lagoon after re-flooding was associated with a significant increase in the already high phytoplankton biomass (persisting at the end of the dry phase), which was maintained for at least one month after re-flooding (Figure 12h). Three months after re-flooding the chlorophyll *a* concentration in LM Lagoon was similar to that in the control wetland and BM Lagoon.

Figure 12 (a – d): Average concentrations of (a) TC, (b) DOC, (c) TN and (d) NH_4^+ in Big Mussel (—□—) and Little Mussel (—□—) Lagoons, Chambers Creek Wetland (---□---) and the River Murray (◆) 1 day prior to re-flooding (■) and 1 day, 1 week, 1 month and 3 months after re-flooding (□) of Mussel Lagoons. Error bars represent ± 2 x standard error.

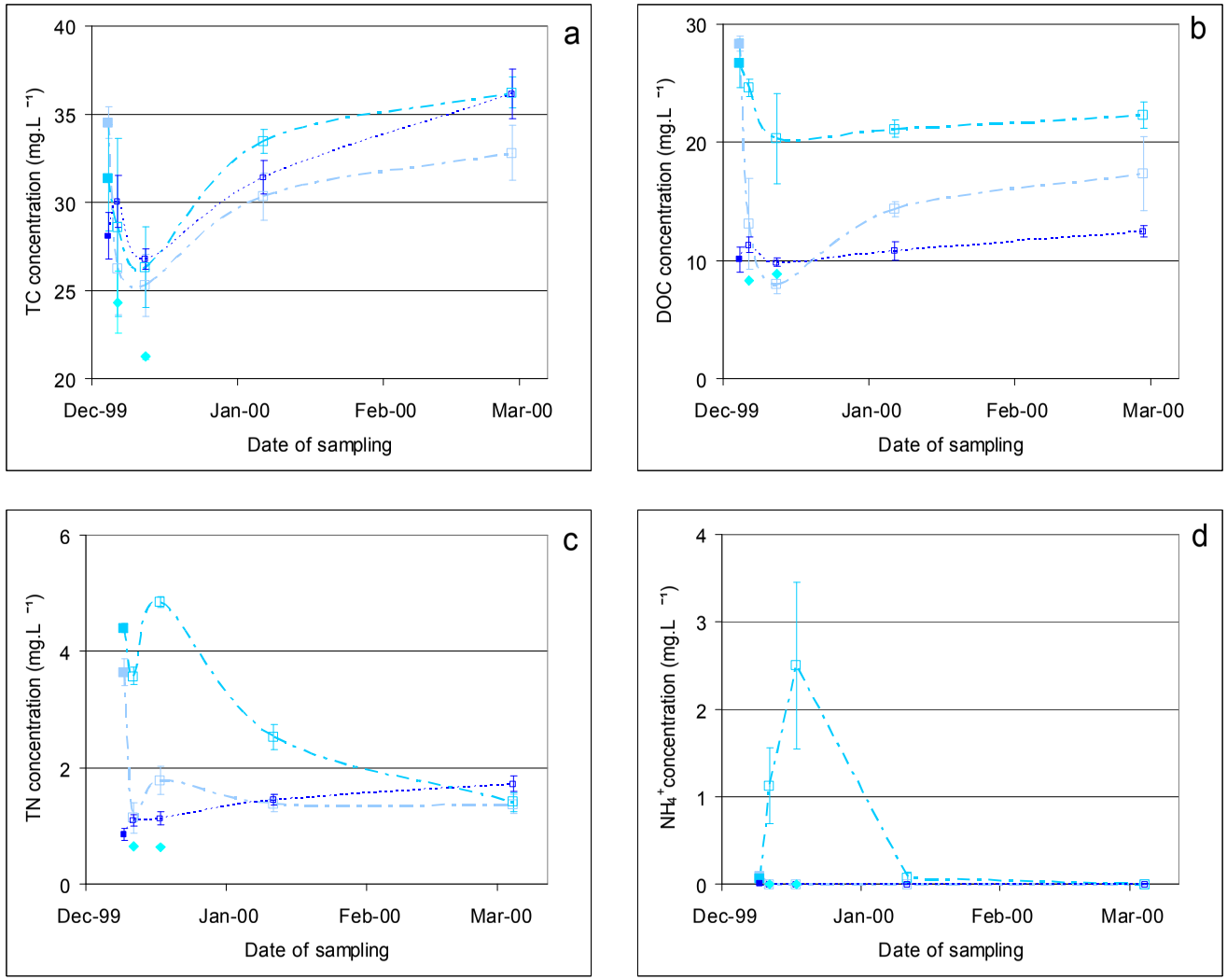
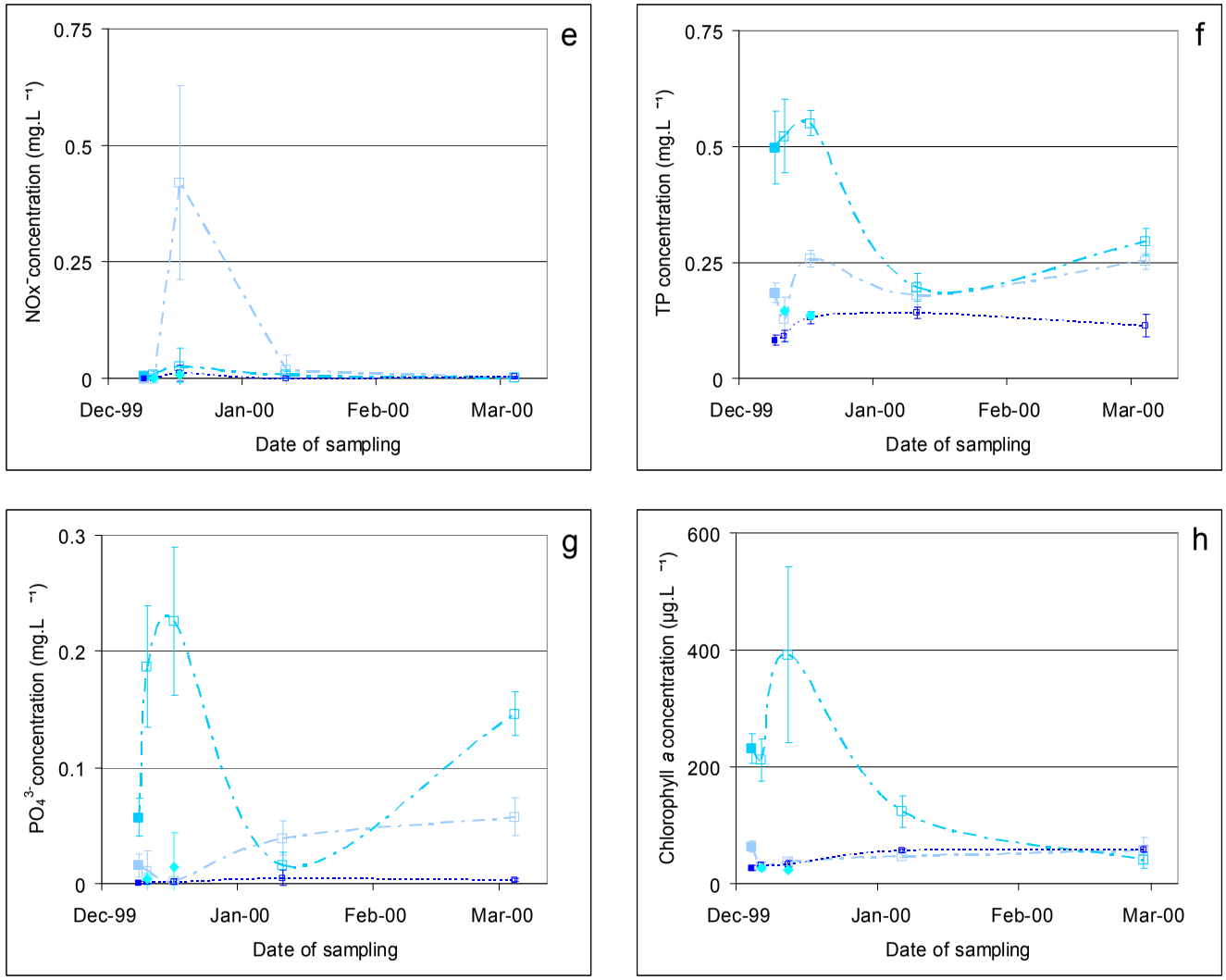


Figure 12 (e – h): Average concentrations of (e) NO_x^-, (f) TP, (g) PO_4^{3-} and (h) chlorophyll *a* in Big Mussel (—□—) and Little Mussel (—□—) Lagoons, Chambers Creek Wetland (---□---) and the River Murray (◆) 1 day prior to re-flooding (■) and 1 day, 1 week, 1 month and 3 months after re-flooding (□) of Mussel Lagoons. Error bars represent ± 2 x standard error.



2.3.3.2 Moira Lake

Moira Lake underwent two drying/re-flooding cycles within the duration of this experiment. The first re-flooding phase produced a significant increase in NH_4^+, NO_X^- and TN concentrations in the water column of Moira Lake in the first week of inundation (Figure 11 c-e). NH_4^+ concentration in the water column of Moira Lake gradually decreased over the next three months, while NO_X^- and TN concentrations remained high for at least the first month of re-flooding. Three months after re-flooding, concentrations of NH_4^+, NO_X^- and TN had decreased and were similar to those recorded in Barmah Lake (Figure 11 c-e). Concentrations of TC, DOC, TP and PO_4^{3-} did not increase in Moira Lake after re-flooding, with low concentrations recorded that were similar to those in Barmah Lake over the same period (Figure 11 a-b,f-g). Unlike Mussel Lagoons, there was no delayed increase in the concentration of DOC, PO_4^{3-} or any other nutrient in the three months after re-flooding. In Moira Lake the post-flood increase in nitrogen availability was associated with a small (but significant) peak in phytoplankton abundance in the 24 hours following inundation (Figure 11h). However, this was very short-lived as chlorophyll *a* concentrations in the water column of Moira Lake were similar to those in Barmah Lake on all remaining post-flood sampling dates (during the first re-flooding event).

The second re-flooding phase in Moira Lake was notably different from the first, with a much slower rate of re-filling compared to the first re-flooding event, due to low flows in the River Murray. Water levels in both Moira and Barmah Lakes had also dropped significantly when sampled three months after re-flooding, again due to low flows in the River. Nonetheless, increased NH_4^+, NO_X^- and TN concentrations were also recorded in the first week of the second re-flooding phase (as in the first re-flooding phase) (Figure 11 c-e). However, unlike the first re-flooding phase, the concentrations of NO_X^- and TN decreased rapidly and were not significantly different to those concentrations in the control wetland after the first week of re-flooding. A small but significant increase in the DOC concentration also occurred in the first week of re-flooding, however was depleted when Moira Lake was sampled one month after re-flooding (Figure 11b). Low concentrations of TC, PO_4^{3-} and TP were recorded in the water column of Moira Lake during first week of the second re-flooding event, which were not significantly different from the concentrations in Barmah Lake during this period (Figure 11 a,f-g).

Between one and three months after the second re-flooding phase commenced, a significant increase in DOC, TC, NH_4^+, TN, PO_4^{3-} and TP concentrations were recorded in the water column of Moira Lake (Figure 11 a-d, f-g). This increase may have been related to the substantial drop in water level which occurred in the lake between these sampling dates. However, the drop in water level occurred in early November (resulting from low river levels causing water to flow out of the wetland), a time of year when potential for evaporation is low. As such, it is unlikely that the 'concentration effect' associated with the evaporation process was responsible for the increased nutrient concentrations at this time. Furthermore, despite an equally large drop in water level in the corresponding control wetland over the same period, no such increase in nutrient availability was recorded in Barmah Lake (Figure 10b).

Despite the increased availability of nutrients in Moira Lake during the second re-flooding phase, there was only a small (but significant) increase in phytoplankton abundance in the first 24 hours (Figure 11h). After one week the chlorophyll *a* concentration in Moira Lake was not significantly different to the concentration in Barmah Lake and remained low for the rest of this re-flooding phase.

2.3.3.3 Croppers Lagoon

A substantial volume of water still remained in Croppers Lagoon at the end of the dry phase (September 2000), although there was some sediment desiccation and cracking in the riparian zone. After re-flooding commenced the DOC concentration in Croppers Lagoon increased significantly, to levels similar to those recorded in the corresponding control wetland (Figure 13b). It is possible that bio-available carbon had become limiting in the Lagoon as a result of the extended period of isolation from the river. Following this initial increase, DOC concentrations in Croppers Lagoon on all remaining sampling dates were not significantly different from concentrations in either the control wetland, Lake Moodemere, or the inflowing river water (Figure 13b). All other nutrient concentrations, including TC, NH_4^+, NO_X^-, TN, PO_4^{3-} and TP, decreased after re-flooding, to concentrations similar to those in the corresponding control wetland (Figure 13 a, c-g). Following this initial decrease in phosphorus concentrations, the concentration of both PO_4^{3-} and TP in the water column of Croppers Lagoon increased and when sampled three months after re-flooding concentrations (in Croppers Lagoon) were still significantly higher than in Lake Moodemere and the inflowing river water (Figure 13 f,g).

Re-flooding did not result in an increase in phytoplankton abundance, in fact chlorophyll *a* concentration was diluted substantially by inflowing river water in the first 24 hours of re-flooding and remained low on all other sampling dates in re-flooding phase (Figure 13h).

Figure 13 (a – d): Average concentrations of (a) TC, (b) DOC, (c) TN and (d) NH_4^+ in Croppers Lagoon (—○—), Lake Moodemere (---●---) and the River Murray (◆) 1 day prior to re-flooding (●) and 1 day, 1 week, 1 month and 3 months after re-flooding (○) of Croppers Lagoon. Error bars represent ± 2 x standard error.

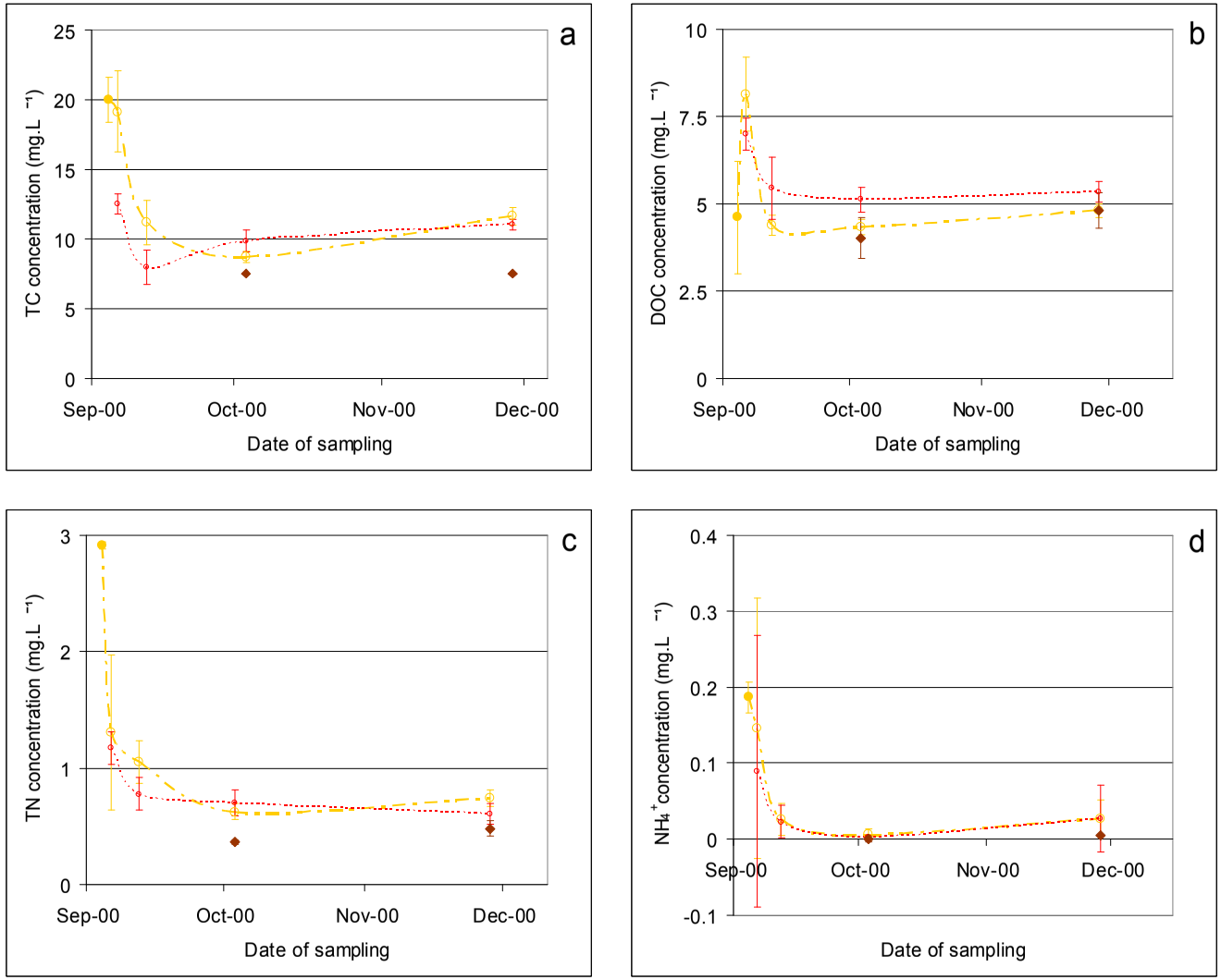
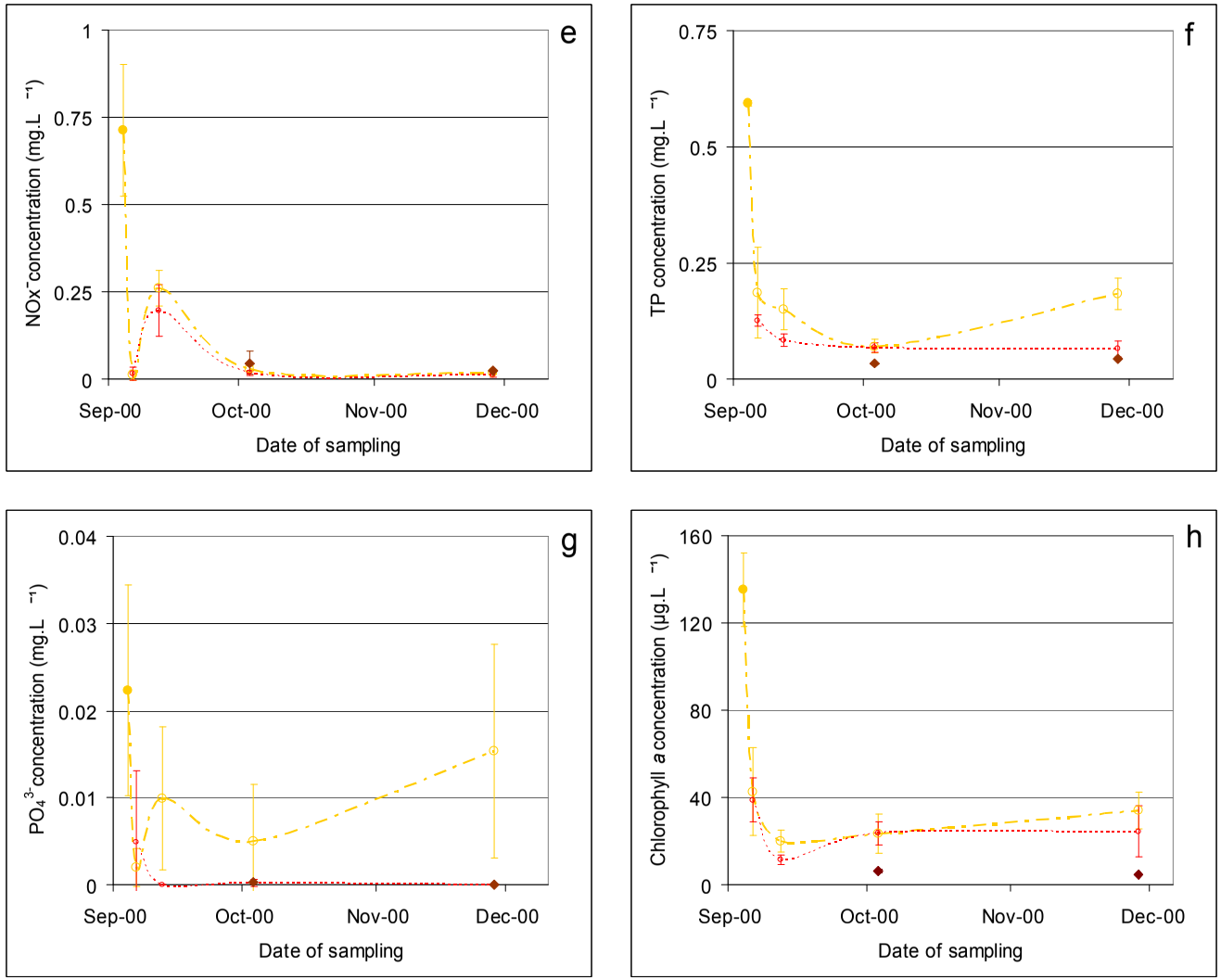


Figure 13 (e – h): Average concentrations of (e) NO_x^-, (f) TP, (g) PO_4^{3-} and (h) chlorophyll *a* in Croppers Lagoon (—○—), Lake Moodemere (---●---) and the River Murray (◆) 1 day prior to re-flooding (●) and 1 day, 1 week, 1 month and 3 months after re-flooding (○) of Croppers Lagoon. Error bars represent ± 2 x standard error.



2.4 Discussion

2.4.1 The flood pulse

The drying and re-flooding cycle, typical of floodplain wetland hydrology in dryland regions, increased nutrient availability in the water column of treatment wetlands in this experiment. The increased nutrient concentrations recorded during the re-flooding phase were significantly greater than in the corresponding control (permanently inundated) wetlands and the inflowing river water (when sampled). Therefore, those nutrients that were more available during the first week of re-flooding were most likely released from the wetland sediments. This is consistent with a number of *in situ* (e.g. Briggs *et al*., 1985; Scholz *et al*., 2002) and laboratory-based (Briggs *et al*., 1985; Fabre, 1988; Qiu and McComb, 1994; Qiu and McComb, 1996; Turner and Haygarth, 2001) studies which have also demonstrated that re-flooding previously dried wetland sediments can result in a large release of nitrogen and phosphorus into the water column.

Increased nutrient availability resulting from significant releases from wetland sediments upon re-flooding was however, relatively short-lived. High nutrient concentrations were generally depleted between 1 week – 1 month after re-flooding, depending on the nutrient considered. Previous studies have focused on the release, or not, of nutrients from dried wetland sediments upon re-flooding, rather than the length of time increased nutrient availability persists. However, based on data presented in the literature it can be estimated that in previous studies high nutrient concentrations following drying and re-flooding of wetland sediments have persisted for a similar length of time, between ~ 1 week and 2 months after the commencement of re-flooding (Briggs *et al*., 1985; Qiu and McComb; 1994, 1996; Scholz *et al*.,2002).

Significant variation in the type of nutrients released from sediments upon re-flooding was recorded between ephemeral wetlands in this experiment. For example, even the two adjacent wetlands produced different results, with re-flooding in Big Mussel Lagoon increasing the availability of NO_X^- and TP while in Little Mussel Lagoon re-flooding increased the availability of NH_4^+, TN, PO_4^{3-} and TP. Similar variation has also been reported in previous research. For example, a large release of NH_4^+ from dried North Lake (Perth, WA) sediments occurred upon re-flooding (Qiu and McComb, 1996),

while no flush of NH_4^+ or NO_X^- occurred following re-flooding of desiccated sediments collected from Lake Hume (Albury, NSW) (Mitchell and Baldwin, 1999). Furthermore, Qiu and McComb (1994) sampled sediments from six wetlands located around Perth (WA) and reported significant variation in the effect of drying/re-flooding on phosphate release from these wetland sediments. Significant amounts of phosphate was released from sediments of three of the six wetlands following drying/re-flooding, however one wetland showed no significant release of phosphate and in the remaining two wetlands drying/re-flooding actually resulted in a reduction in phosphate concentrations in the overlying water column (compared to permanently inundated sediments). Sediment characteristics, including the Fe and Ca content and the proportion of humic substances and silt, were proposed as possible sources of the variation recorded (Qiu and McComb, 1994). A decrease in the potential for P release observed by Mitchell and Baldwin (1998) following drying/re-flooding of sediments collected from Chaffey Reservoir (NSW) was related not only to sediment mineral content, but also to a shift in the resident microbial community and carbon limitation, both of which resulted from the drying process.

Possible causes of the variation in nutrient availability, observed between the ephemeral wetlands following drying/re-flooding in this experiment, may include differences in sediment character and the resident microbial consortia, as concluded by previous researchers (e.g. Qiu and McComb, 1994; Mitchell and Baldwin, 1998). However, the results of this experiment indicate that factors such as the degree of drying and length of isolation during the dry phase, the rate of re-filling, timing of re-flooding and the number of drying/re-flooding cycles may also play a role in producing the variation observed. The potential influence of each of the factors, or a combination of these factors, needs to be investigated with further study.

In addition to the initial increase in nutrient availability recorded shortly after re-flooding, another increase in the availability PO_4^{3-} and TP (and a number of other nutrients) was also observed in all of the ephemeral wetlands in this experiment between one and three months after the commencement of re-flooding. It is proposed that this may be related to repeated drying/re-flooding cycles in these wetlands. For example, no such increase in the concentration of any nutrient was recorded in Moira Lake during the first drying/re-flooding cycle, however following the second drying/re-flooding cycle a

significant increase in the concentration of every nutrient tested except NO_X^- was recorded in the lake on the final sampling date. Similar patterns were recorded in both BM and LM Lagoons, in which numerous drying/re-flooding cycles had taken place, and in Croppers Lagoon where a substantial drop in water levels occurred during the earlier attempt to dry the wetland. While this particular phenomenon has not been identified or reported on in the literature, the effects of repeated drying/re-flooding cycles have previously been considered (Baldwin *et al*., 2000) and shown to influence wetland sediment release and sorption of ortho-phosphate as well as adaptations in the resident microbial community (where SRB developed resistance to the drying process) (Baldwin *et al*., 2000). The underlying mechanisms producing the above phenomenon cannot be determined from the data collected and warrants further investigation.

The flood pulse, as described by the FPC, comprises a chain of events where inundation of the floodplain increases nutrient availability, promoting increased phytoplankton productivity, which in turn supports significant increases in animal biomass up the food-chain (Junk *et al*., 1989). Despite the increased availability of nutrients after flooding of the ephemeral wetlands in this experiment, re-flooding was generally characterized by low phytoplankton abundance, similar to that reported by Briggs *et al*. (1993) in their study of two ephemeral wetlands on the floodplain of the Lachlan River (NSW). While increased nutrient availability did not result in an increase in phytoplankton abundance, the nutrients released from wetland sediments upon re-flooding were nonetheless depleted within a relatively short period of time (between ~ 1 week and 1 month after re-flooding). Therefore, these nutrients were most likely assimilated by other biota present in the wetlands (e.g. macrophytes, sediment microbial community).

Together with the results of the study by Briggs *et al*. (1985, 1993) and Briggs and Maher (1985), this may suggest that the 'flood pulse' in some ephemeral floodplain wetlands may take a different trophic pathway to that outlined by the FPC for tropical river-floodplain systems (Junk *et al*., 1989). For example, inundation of the two ephemeral floodplain wetlands in the Murray-Darling Basin (mentioned above) increased the availability of nitrate and phosphate in the water column (Briggs *et al*., 1985) as predicted by the FPC, however the re-flooding phase was characterized by the lowest levels of phytoplankton productivity recorded throughout the study (Briggs *et al*., 1985). In contrast, the highest

levels of macrophyte production were measured during this period, peaking approximately three months after re-flooding (Briggs and Maher, 1985). This may suggest that competition by the macrophyte community for the large pool of available nutrients after inundation could limit any increase in phytoplankton productivity and subsequently animal biomass, as predicted by the FPC, with flooding resulting in a significant increase in vegetation biomass instead.

An exception to these observations was the sustained, significant increase in phytoplankton biomass recorded in Little Mussel Lagoon after re-flooding. A number of factors may explain why increased phytoplankton productivity was recorded in LM Lagoon but not in any of the other ephemeral wetlands studied after re-flooding. Firstly, Little Mussel (LM) Lagoon did not completely dry, retaining substantially more water at the end of the dry phase compared to Big Mussel (BM) Lagoon and Moira Lake. Furthermore, the water remaining in LM Lagoon at the end of the dry phase supported a large residual phytoplankton community. In addition, re-flooding of LM Lagoon took place in summer compared to the winter-spring re-flooding events in Moira Lake and Croppers Lagoon. The higher water temperatures during the re-flooding phase may have stimulated biological processes including primary production (e.g. Søndergaard, 1988; Anderson and Beardall, 1991; Rae and Vincent, 1998; Udy *et al*., 2001). Finally, LM Lagoon was the only wetland in this experiment where a significant increase in bio-available phosphate (PO_4^{3-}) was recorded shortly after re-flooding. Therefore, factors that may be important in determining whether or not an increase in phytoplankton biomass occurs after re-flooding, as per the FPC, include: (a) the extent of drying, (b) phytoplankton abundance at the time of re-flooding, (c) the timing of re-flooding, and (d) the release of phosphate from sediments following re-flooding.

In conclusion, the 'flood pulse' as described by the FPC can occur in ephemeral wetlands in dryland river-floodplain systems (e.g. LM Lagoon). However, considerable variation in the observed patterns exists among wetlands. As such, it is not possible to reject either the null or alternative hypotheses outlined under Aim 1 (page 15) on a generalized basis, results are specific to the wetland considered. The hydrological variability that characterizes wetlands in dryland regions may play a role in influencing the variability in the patterns observed. In this experiment, factors such as the degree of drying and length of isolation during the dry phase, the rate of re-filling, timing of re-flooding and the

number of drying/re-flooding cycles are believed to be potentially important in producing the variation observed. So, while the FPC provides an effective conceptual basis to investigate patterns and processes in dryland wetlands, drying and re-flooding in these wetlands may stimulate trophic pathways not described by the FPC. Future investigation may allow identification of these alternative trophic pathways, and quantify rate and/or biomass changes in associated biota following re-flooding.

2.4.2 The effects of permanent inundation

In this experiment, both the drying and re-flooding phases were associated with significant increases in nutrient availability and, in some cases, phytoplankton productivity. In fact equally, if not more, dramatic changes were recorded in the ephemeral wetlands during the dry phase as during the re-flooding phase. For example, the DOC concentration measured in the last remaining pool of water during the first dry phase in Moira Lake (>15 000 $mg.L^{-1}$) was far in excess of any value reported previously in the literature (e.g. 0.5 – 50 $mg.L^{-1}$ in aquatic ecosystems worldwide; Mullholland *et al*., 1990 as cited in Kortelainen, 1993).

Also, phytoplankton biomass increased to concentrations in excess of 150 µg chl *a*.L^{-1} in all ephemeral wetlands during all dry phases (except BM Lagoon during the 1999 dry phase), with the highest concentration recorded in Moira Lake just prior to complete desiccation in the first dry phase (average: 495 µg chl *a*.L^{-1}). While not reaching the high concentrations recorded in other ephemeral wetlands in Australia (e.g. 1 370 µg chl *a*.L^{-1} in Ryans 2 Billabong, River Murray floodplain and 2 383 µg chl *a*.L^{-1} in Thomsons Lake, Perth; Boon, 1990; Balla and Davis, 1995 respectively), the phytoplankton biomass recorded during the dry phase in these ephemeral wetlands was high relative to concentrations recorded on other sampling dates and compared to concentrations recorded in the corresponding control wetlands. In fact, data suggested that in Moira Lake the phytoplankton community was actually growing during the dry phase, despite the harsh conditions present in the wetland at this time (e.g. very high turbidity, pH and water temperature). In a backwater pond on the Salado River floodplain (Argentina) Gabellone *et al*. (2001) also found that some species of phytoplankton (e.g. euglenophytes) were adapted to, and in fact increased in abundance, during the dry phase. The dramatic changes recorded during the dry phase in this study, support the emerging view being expressed in the literature: that changes during the dry phase are of equal importance to

those that follow re-flooding (e.g. Humphries and Baldwin, 2003; McMahon and Finlayson, 2003; Kingsford *et al*., 2004).

Permanent inundation of ephemeral wetlands effectively removed these periods of peak nutrient availability and phytoplankton productivity, resulting in continuously low levels (of nutrient availability and phytoplankton productivity). Consequently, the alternative hypothesis proposed under Aim 2 (page 14) was supported. There are no examples in the literature of ecosystem-scale studies which have compared nutrient availability and/or phytoplankton productivity in ephemeral *vs* permanently inundated wetlands. However, the results of a laboratory experiment conducted by Qiu and McComb (1994, 1996), using intact sediment cores collected from North Lake (Perth, WA), also indicated that dried/re-flooded sediment cores had higher nutrient availability (NH_4^+, NO_X^- and PO_4^{3-}) than those which had remained permanently inundated.

The limited availability of nutrients and reduced primary productivity resulting from permanent inundation could potentially impact upon the entire wetland food-web. There are examples in the literature to support this suggestion. For example, on the River Murray floodplain, Boulton and Lloyd (1991) found that invertebrates in an ephemeral wetland were not only more diverse, but were also significantly more abundant than the community in nearby permanently inundated wetlands. Kingsford *et al*. (2004), using waterbird data (number of species and density) collected over almost two decades on six floodplain systems in arid eastern Australia, also reported that permanent inundation of wetlands (resulting from river regulation) significantly reduced both the diversity and density of associated waterbirds in comparison to ephemeral wetlands, and proposed that this was most likely due to a reduction in the species on which the waterbirds feed (invertebrates, aquatic macrophytes and fish).

In combination, the results of this experiment together with previous research support the hypothesis that permanent inundation of ephemeral wetlands, by altering the naturally variable hydrological cycle, can significantly reduce nutrient availability, primary production and hence secondary production. Therefore, alteration of the natural hydrological cycle in this way essentially changes the structure and function, the ecology, of these wetlands. Equally, the results of this experiment indicate that some of

the changes resulting from river regulation and permanent inundation can be somewhat reversed, within a relatively short period of time, given re-instatement of a more natural hydrological regime.

Degree of drying: impacts on nutrient availability and community metabolism after re-flooding, a rnesocosm experiment

3.1 Introduction

A 'pulse' in nutrient availability and phytoplankton productivity, similar to that described by the FPC, can occur in ephemeral wetlands of the River Murray following a drying/re-flooding cycle (see Chapter 2). However, considerable variation in pulse characteristics were observed and in some cases differed markedly from the predictions of the FPC (see Chapter 2). Particular features of the hydrological cycle in each wetland, including variations in the degree of drying and length of isolation during the dry phase, the timing and rate of re-flooding and drying/re-flooding history, may explain deviations from the FPC model. In the ecosystem-scale experiment described in Chapter 2, the extent to which a wetland dried during a dry phase appeared to be a factor influencing both the release of nutrients from wetland sediments into the water column, as well as a pulse in phytoplankton productivity, after re-flooding. This chapter examines the influence of the dry phase, specifically the effect of the degree of wetland drying, on patterns of nutrient availability and primary productivity comprising the 'flood pulse'.

Sediment microbial consortia play a central role in mediating the cycling of nutrients in wetland systems (e.g. Baldwin and Mitchell, 2000). The extent of wetland drying affects oxygen availability, moisture stress, water temperature and other biochemical factors that influence wetland microbial communities: stimulating the activity of some microbes (e.g. aerobic heterotrophs), limiting the activity

of others (e.g. anaerobes) and possibly inducing resting stages or causing death (Boyd and Pippopinyo, 1994; Qiu and McComb, 1995; Baldwin and Mitchell, 2000; van Oorschot *et al*., 2000). Consequently, extent of wetland drying will influence nutrient and organic matter transformations that are mediated by the microbial community. For example, changes to the microbial consortia resulting from different levels of wetland drying are known to alter the rates of organic matter mineralization, nitrification and denitrification and the assimilation of bio-available nutrients from the water column by microbes (De Groot and Van Wijck , 1993; Boyd and Pippopinyo, 1994; Qiu and McComb, 1995; van Oorschot *et al*., 2000).

In addition, the extent of wetland drying may also influence abiotic processes related to the cycling of nutrients in ephemeral wetlands. For example, the capacity of wetland sediments to adsorb P from the surrounding water can increase significantly as the drying phase progresses (De Groot and Van Wijck, 1993). This phenomenon is attributed to the oxidation of iron sulphide (FeS) present in anoxic sediments, forming Fe(OOH) and increasing the capacity for further P sorption (De Groot and Van Wijck, 1993). Therefore, wetlands dried to a greater extent, having the greatest capacity for sediment P sorption, may have a substantial proportion of the bio-available P 'locked-up' in the sediments at the end of the dry phase.

A number of studies have also considered the effects of different levels of drying on various elements of the flood pulse (e.g. Qiu and McComb, 1996; Boon *et al*., 1997). For example, the extent to which wetland sediments are desiccated can affect the potential for microbial activity and hence nutrient cycling, after re-flooding (Qiu and McComb, 1996; Boon *et al*., 1997). Small reductions in sediment moisture content (e.g. after 4 weeks of air drying) had little affect on the activity of methanogens, responsible for converting organic matter into methane, after one week of re-flooding under anaerobic conditions (compared to permanently inundated sediments), however in sediments dried to a greater extent (e.g. after 9 weeks of air drying) methanogen activity was markedly reduced (Boon *et al*., 1997). In contrast, nitrifying bacteria responsible for the conversion of ammonium into nitrate/nitrite, are able to rapidly recover even after complete sediment desiccation, with a lag in measurable activity of <5 days following re-flooding (Qiu and McComb, 1996). These laboratory experiments indicate that the availability of particular nutrients following re-flooding may vary in response to different levels of drying

depending on the capacity of the particular microbes with which they are associated to survive and recover after the dry phase.

The influence of the degree of wetland drying on the potential for a pulse in carbon, nitrogen and phosphorus availability after re-flooding has received some attention, yet results remain inconclusive. For example, James *et al*. (2004) demonstrated that in comparison to saturated floodplain sediments, partial drying (20% and 60% desiccation) substantially reduced the amount of NH_4^+ and NO_X^- released from the sediment into the water column following re-flooding, while complete desiccation significantly increased the release of mineral-N after re-flooding (James *et al*., 2004). However, the dried sediment cores used in this experiment were re-flooded for a period of 2 weeks prior to draining, re-filling and the commencement of the sampling regime. Given that evidence to date suggests the greatest changes in N availability can occur during the first two weeks of re-flooding (e.g. Briggs *et al*., 1985; Qiu and McComb, 1996), the experimental design may have biased the results obtained. In addition, data collected by James *et al*. (2004) contrast considerably to those by Mitchell and Baldwin (1999) who reported little to no impact of the degree of sediment desiccation on the release of mineral N from the sediment into the water phase, or rates of nitrification and denitrification, after reservoir sediments dried to varying degrees *in situ* were re-flooded.

The effects of the extent of wetland drying on the P cycle are complex with both biotic and abiotic processes, and interactions between the two, playing an important role in determining P availability upon re-flooding. The removal of phosphate from the water column during the dry phase is known to be positively correlated with rates of bacterial respiration (Qiu and McComb, 1995). Death and cell lysis of the microbial consortia as a result of drying contributes to the release of phosphate from sediments into the water column upon re-flooding and, the greater the uptake of P by microbes during the dry phase (prior to complete desiccation) the larger the release of P upon re-flooding (Qiu and McComb, 1995).

In addition, the extent to which sediments have been desiccated is known to affect the ability of sediments to sequester P once they are re-flooded (Baldwin, 1996). Oxidation, followed by desiccation, progressively reduces the capacity of sediments for P-sorption after re-flooding and

Baldwin (1996) also attributed this to changes in the iron cycle. This reduction was proposed to be the product of 'aging' of amorphous iron phases in the sediment, with a high affinity for P, to form crystalline iron phases with a low affinity for P (Baldwin, 1996).

Therefore, the overall effect of the degree of wetland drying on P availability after re-flooding is not known. Although, it will depend on the relative importance of microbial consumption of available P *vs* adsorption by sediment-mineral complexes during the dry phase, as well as the proportion of the microbial population killed and the extent of sediment mineral aging at the end of the dry phase. Together these factors will determine whether P is 'locked-up' in the wetland sediments or is released into the water column for biotic consumption when re-flooding takes place. In fact Watts (2000), investigating P release from sediments that were dried to varying degrees *in situ* and re-flooded, suggested interactions between biotic and abiotic factors explained the variation in patterns of P release from sediments dried to different degrees.

While numerous studies have suggested that carbon limitation of biological processes following re-flooding may result from increased rates of organic matter decomposition during the dry phase (Briggs *et al*., 1993; Bianchi *et al*., 1996; Mitchell and Baldwin, 1998), there has been no attempt to examine the effects of different levels of drying on carbon availability after re-flooding. Likewise, the effect of the extent of wetland drying on the potential for a pulse in primary productivity after re-flooding also remains unknown, although was implicated as one of a number of factors that may influence the occurrence, or not, of a pulse in phytoplankton production after re-flooding in the field experiment described in Chapter 2.

The aim of this research was to examine the role of the dry phase in determining patterns of nutrient availability and primary productivity following re-flooding in ephemeral floodplain wetlands. Specifically, the effects of different levels of drying were compared using a mesocosm experiment, in an attempt to provide insight into the variation in the 'flood pulse' observed between wetlands at the ecosystem-scale. Therefore, the following hypotheses were tested:

1. **H_O**: Following re-flooding, the availability of carbon, nitrogen and phosphorus in the water column of wetland mesocosms exposed to different levels of drying are not significantly different.

   H_A: Following re-flooding, the availability of carbon, nitrogen and phosphorus in the water column of wetland mesocosms exposed to different levels of drying are significantly different.

2. **H_O**: Following re-flooding, the community metabolism (incorporating primary production and respiration) of wetland mesocosms exposed to different levels of drying are not significantly different.

   H_A: Following re-flooding, the community metabolism (incorporating primary production and respiration) of wetland mesocosms exposed to different levels of drying are significantly different.

3.2 Materials and methods

3.2.1 Experimental design

The experiment, conducted on the grounds of the Murray-Darling Freshwater Research Centre in Mildura (Victoria), used mesocosms consisting of round plastic tubs, 45cm high, 60cm in diameter with a 115L capacity. Tubs were scrubbed with neutracon and rinsed thoroughly with Reverse Osmosis (RO) water prior to use. Sediment, collected from an inundated area of Barmah Lake (see Chapter 2) and kept inundated with lake water during transport and storage, was placed in each tub to a depth of 10cm. Water collected from the River Murray (Mildura) was then used to fill each tub. A watering system, consisting of a series of small irrigation pipes across the bottom of the tub, was required to direct water to the bottom layer of sediment in treatment 3 tubs during the drying phase. These watering systems were fitted to all tubs. To allow aeration of the water column an air bubbler was connected to each tub, thus completing the experimental unit or 'mesocosm'. The mesocosms were then left to settle for 3 weeks prior to beginning the experiment. Water levels in the mesocosms were maintained by the regular addition of RO water.

A total of 15 experimental units were randomly assigned to one of 5 treatments, therefore each treatment was replicated three times. The five treatments were designed to mimic the range of conditions observed in the field experiment (Chapter 2), and were as follows:

- TREATMENT 1: water level in the experimental unit was allowed to evaporate from 30cms (maximum capacity) to 5 cms above the sediment (a drop in water level of approximately 85%).
- TREATMENT 2: water level left to evaporate to the surface of the sediment, so that the sediment was not covered by water but did remain wet.
- TREATMENT 3: surface water left to completely evaporate and then the top half of the sediment dried while maintaining the bottom half under damp conditions. The aim of this treatment was to produce some desiccation and oxidation of the sediment surface while providing a refuge for the microbial community.
- TREATMENT 4: water in the experimental unit was allowed to evaporate until the sediment was completely desiccated with extensive sediment cracking.

- CONTROL: water level in the experimental unit was maintained throughout the experiment at full capacity (30cms above the sediment).

3.2.2 Sampling regime

The experiment, excluding the settling period, ran for almost nine months between 26 October 1999 and 20 July 2000. Following an initial 3 week settling period, all 15 mesocosms were kept completely inundated for a further three weeks during which time samples were collected on three occasions.

On 16 November 1999 the 'dry phase' was initiated. This involved allowing the water level in tubs allocated to treatments 1 – 4 to decrease naturally over time by evaporation, to mimic the natural drying process occurring in ephemeral floodplain wetlands. Once the water in each tub had reached the required level of drying for its assigned treatment, water levels were maintained through the addition of RO water at least weekly, and more frequently during summer. Control tubs were kept inundated to full capacity throughout the experiment in the same manner. RO water was used to maintain water levels, rather than river water, to avoid the cumulative concentration of nutrients over the treatment period. Water quality measurements and water samples for nutrient analysis were taken regularly throughout the dry phase.

By early April 2000 all tubs had reached the required level of drying as per the allocated treatments. However, before re-flooding of the mesocosms could be initiated torrential rainfall was experienced in the region during the week 10 – 16 of April 2000. Although the tubs were temporarily covered during this period, tubs 2, 3, 4 and 6 took on a small amount of rainwater. To reduce water levels in these tubs to that required by their respective treatments, tubs 4 and 6 were allowed to naturally evaporate while small electric fans were used to accelerate evaporation in tubs that had taken on more water (tubs 2 and 3, treatments 3 and 4 respectively). By 25 April 2000 all tubs were dried as required by the allocated treatments. Figure 14 provides an illustration of each of the treatments at the end of the dry phase.

On the morning of 27 April 2000 the 'treatment' mesocosms were re-flooded with water collected (and sampled) from the River Murray (Mildura). The 'control' mesocosms were also topped-up with the water collected from the River Murray (water levels in these tubs had been allowed to drop slightly in the week prior to re-flooding). The tubs were re-filled slowly by hand using a small bucket in order to minimize disturbance of the sediment surface.

Following re-flooding, water quality measurements and water samples for nutrient analysis were taken 12 hours, 24 hours, 48 hours, 72 hours, 1 week, 2 weeks, 4 weeks, 8 weeks and 12 weeks after re-flooding. Measurements of community metabolism were taken 24 hours, 1 week, 2 weeks, 4 weeks, 8 weeks and 12 weeks after re-flooding.

3.2.3 Parameters

3.2.3.1 Water quality

Water quality was monitored throughout the experiment using an HORIBA U-10 Water Quality Checker. Measurements taken include pH, conductivity ($mS.cm^{-1}$), turbidity (NTU), Dissolved Oxygen ($mg.L^{-1}$) and Temperature (ºC).

3.2.3.2 Nutrient availability

Samples collected from the water column of the mesocosms were analysed for total carbon (TC), total nitrogen (TN), total phosphorus (TP), dissolved organic carbon (DOC), ammonia (NH_4^+), nitrate + nitrite (NO_X^-) and ortho-phosphate (PO_4^{3-}) concentrations.

All samples were collected in LDPE plastic bottles that had been soaked overnight in Neutracon wash (to remove all nutrients) and rinsed thoroughly in RO water. Samples to be analysed for total nutrient concentration were placed in a freezer immediately following collection. Samples to be analysed for dissolved nutrient concentration were filtered using 0.45µm filter papers (Gelman 0.45µm pore size, cellulose acetate filter paper) within a week of collection (stored at 4°C) and then placed in a freezer. All samples were stored frozen until they were defrosted and analysed.

Figure 14: Treatments 1 – 4 and the control at the end of the 'dry phase': (a) treatment 1 (tub 8); (b) treatment 2 (tub 12); (c) treatment 3 (tub 10); (d) treatment 4 (tub 13); (e) control (tub 9).

a) Treatment 1

b) Treatment 2

c) Treatment 3

d) Treatment 4

e) Control

Samples were analysed for TC and DOC concentrations using an O·I·Analytical Model 1010 Wet Oxidation TOC Analyser. For analysis of TN and TP, a digest reagent was added to samples which were then autoclaved, decanted and run through a LACHAT Flow Injection Analyser. The LACHAT Flow Injection Analyser was also used to detect concentrations of NH_4^+, NO_x^- and PO_4^{3-} in samples which had previously been filtered.

3.2.3.3 Community metabolism

Community metabolism encompasses primary production and respiration processes occurring within a defined boundary (Udy *et al*., 2001). Methods for measuring community metabolism and primary productivity are based on the photosynthesis/respiration equation (Anderson and Beardall, 1991):

$$6CO_2 + 6H_2O + \text{Energy} \underset{\text{Respiration}}{\overset{\text{Photosynthesis}}{\rightleftharpoons}} C_6H_{12}O_6 + 6O_2$$

In this experiment, measurements of community metabolism were based on changes in oxygen concentration (Udy *et al*., 2001). Previous field-based studies measuring community metabolism in streams and rivers have generally used a benthic dome system, which is partially inserted into sediment on the river bed (e.g. Udy *et al*., 2001). Given the repeated measures of community metabolism taken throughout this experiment, the benthic domes were likely to result in considerable disturbance of sediment in the tubs. Such disturbance could have impacted on measures of water quality, nutrient concentrations and primary productivity. As such, a variation of the benthic dome was designed for use in this experiment and is described below (also refer to Figure 15).

At the required sampling times (24h, 1w, 2w, 4w, 8w and 12w after re-flooding) each mesocosm was converted into a closed system so that the rate of change in oxygen concentration could be measured. In order to create a closed system, the air bubblers were removed and a circular sheet of clear perspex, edged with a thin layer of foam, was carefully fitted just below the surface of the water in each tub. The foam edging ensured that the exchange of oxygen between the atmosphere and the water column in each mesocosm was negligible. Each perspex 'lid' contained a rubber bung inserted with a temperature and dissolved oxygen (DO) probe and attached data loggers (Tinytalk II Temperature Sensor and Logger; American Marine Pinpoint DO Probe and and Tinytalk II Voltage

Logger). The DO probes were calibrated (at the ambient water temperature) immediately prior to each sampling time. Each lid also had two small hoses, inserted either side of the DO probe, that were attached to a small bilge pump (991 12-volt in-line "Whale" bilge pump with a motor speed controller) to allow a gentle circulation of water within the tub and across the DO probe membrane. In order to estimate community metabolism at each sampling time, changes in the oxygen concentration and temperature were measured within the closed systems every two minutes over a period of at least 24 hours.

Figure 15: The closed system designed to measure community metabolism in this experiment.

During warm weather oxygen bubbles would often form under the perspex lid as a result of increased rates of photosynthesis. When this occurred, the lids on all tubs would be removed and air bubblers replaced for approximately one hour. After this 'flushing' time, the lids would be replaced and measurements continued. These flushing periods were recorded, and were not included in subsequent calculations.

Measurements taken from the DO probe were recorded by the data logger in voltage units. These were converted to DO concentration (mg $O_2.L^{-1}$) according to barometric pressure (at height above sea level) and average temperature recorded during the 24 hour sampling period. Changes in DO

concentration over time (mg $O_2.L^{-1}.minute^{-1}$) were then multiplied by the volume of water in the tub and divided by the surface area of sediment in the tub, in order to convert values into the units of mg $O_2.m^{-2}.minute^{-1}$. Data was then used to calculate:

- **Community respiration over 24 hours** (CR_{24}) – determined from night-time changes in DO concentration. This rate is assumed to remain constant throughout the daylight hours, and therefore is extrapolated over 24 hours to obtain CR_{24} (Bott, 1996; Udy *et al*., 2001).
- **Gross Primary Productivity (GPP)** – the changes in DO concentration recorded during day-light hours represents both the oxygen produced as a result of photosynthesis and that consumed by community respiration. GPP is the **total** production of oxygen resulting from photosynthesis (e.g. $GPP = NPP + R_{day}$) (Bott, 1996).
- **Productivity : Respiration (P:R)** – the P:R ratio indicates if the system is a net producer of organic carbon (eg. accumulating biomass, "autotrophic") or a net consumer of organic carbon (e.g. "heterotrophic") and is calculated using the equation: $P:R = GPP/CR_{24}$ (Bott, 1996; Udy *et al*., 2001).

3.2.3.4 Phytoplankton biomass

In addition to measuring overall community metabolism, I was also interested in investigating changes in phytoplankton vs macrophyte production in the mesocosms following re-flooding. Attempts to measure chlorophyll *a* concentration were made (using method described in Chapter 2, Section 2.2.4.4) as part of the sampling regime and were initiated with the commencement of the experiment. However, phytoplankton biomass in the water column of the mesocosms was so low that the sample volume required to reach the limits of detection on the spectrophotometer was too large, and would have required a substantial proportion of the water column to be replaced following each sampling date. This was likely to have a large impact on the other measurements being taken during the experiment and hence, it was decided that changes in phytoplankton biomass would not be measured in this experiment.

3.2.3.5 Macrophyte biomass

At the end of the experiment the macrophyte and algal communities that had established in most of the mesocosms were identified and then harvested. The plants harvested from each mesocosm were weighed, dried at 50ºC for 3 days and then at 105ºC for 2 days and weighed again to quantify the biomass present in each mesocosm.

3.2.4 Data analysis

Data collected during the dry phase of the experiment was graphed and patterns observed between treatment and control wetlands over time. The post re-flooding data set was analysed using a general linear mixed model technique[2] (see McCullogh and Searle, 2001) and had two factors: (1) treatment (treatments 1-4 and the controls) and (2) time (date of sampling). This technique is based on a standard analysis of variance (ANOVA), however also accounts for the possible serial dependence (e.g. non-independence) of the data, produced by the use of repeated measures. The assumptions that must be met by the data set in order to use this technique with confidence are the same as those required by a standard ANOVA, namely normality, linearity and constant variance. All of these assumptions were met following natural log transformation of the data set. Statistical computations were carried out using the GENSTAT 7.1 program.

As with an ANOVA, this test was able to indicate whether or not a significant difference between treatments and/or sampling dates existed for a particular parameter, however was unable to provide information on how the treatments and/or sampling dates differed. When a significant difference was indicated by the test, the Least Significant Difference (LSD = 2 x standard error) was used as a post-hoc guide to determine which of the treatments/sampling dates differed significantly to the other treatments/sampling dates. All graphs presented in this chapter were produced in Microsoft Excel (XP). Macrophyte biomass data, collected upon completion of the experiment, was not included in the analysis described for other post-re-flooding data. Macrophyte data was graphed, and the LSD used to indicate significant differences between treatments.

[2] Data were analysed by A/Prof Ross Cunningham, Research Fellow - Biostatistics, Australian National University

3.3 Results

3.3.1 The dry phase

As the water column in treatments 1 – 4 ('treatments') evaporated, conductivity progressively increased, reaching levels approximately 2.5 times that recorded in the permanently inundated (control) mesocosms. Both pH and DO concentration were also generally higher in the treatment mesocosms than the controls during the drying phase while there was little difference in the water temperatures recorded in treatment and control mesocosms during this period. Water temperature gradually increased in all mesocosms following commencement of the experiment, peaking in mid-February, and then decreasing after the onset of Autumn. High turbidity levels (~150 NTU) were recorded in all mesocosms during the first month of the experiment and following this initial period, suspended material progressively settled out of the water column in treatment mesocosms reducing turbidity levels (to ~50 NTU). In contrast, turbidity in two of the control mesocosms increased significantly over the duration of the dry phase (to >500 NTU).

An increase in total nutrient pools (TC, TN and TP) in treatment and control mesocosms was recorded during the dry phase (Figure 16 b,e,g). However, evaporation of the water column and the associated 'concentration effect' resulted in greater increases in TC and TN concentrations in treatment mesocosms compared to the increases recorded in permanently inundated mesocosms (Figure 16 b,e). DOC concentration, and the proportion of the TC pool it represented, also increased dramatically in the water column of treatment mesocosms during the dry phase, while remaining relatively constant in controls (Figure 16 a). Although TP concentration in the treatment mesocosms increased over the dry phase, concentrations were consistently lower than those recorded in the permanently inundated mesocosms (Figure 16 g).

Generally, the dissolved inorganic nitrogen (DIN = $NH_4^+ + NO_X^-$) concentration and the proportion of the TN pool it represented (< 5%) was low in all mesocosms during the dry phase. NH_4^+ was rapidly removed from the water column after mesocosms were established and despite evaporation and concentration processes, little to no increase in NH_4^+ and NO_X^- concentrations was recorded in the water column of any of the treatment mesocosms during drying (Figure 16 c-d). A large increase in

NO_X^- concentration was recorded in several of the control mesocosms toward the end of the dry phase, however had been depleted by the time of re-flooding (Figure 16 d).

Throughout the dry phase the PO_4^{3-} concentration in treatment mesocosms closely mirrored concentrations recorded in the controls (Figure 16 f). Unlike the pattern observed for nitrogen however, PO_4^{3-} concentration and the proportion of the TP pool it represented was considerably higher during the earlier part of the dry phase compared to values recorded after re-flooding. However, after mid-January PO_4^{3-} clearly became depleted in those mesocosms still containing water, as indicated by the dramatic drop in PO_4^{3-} concentration in the water column of treatment 1 and control mesocosms toward the end of the dry phase (Figure 16 f).

Figure 16 (a – d): Average concentrations of (a) DOC, (b) TC, (c) NH_4^+ and (d) NO_X^- recorded in treatment 1 (—■—), treatment 2 (—▲—), treatment 3(—◆—), treatment 4(—●—), and control (---□---) mesocosms during the dry phase. ⬇ indicates the commencement of the dry phase.

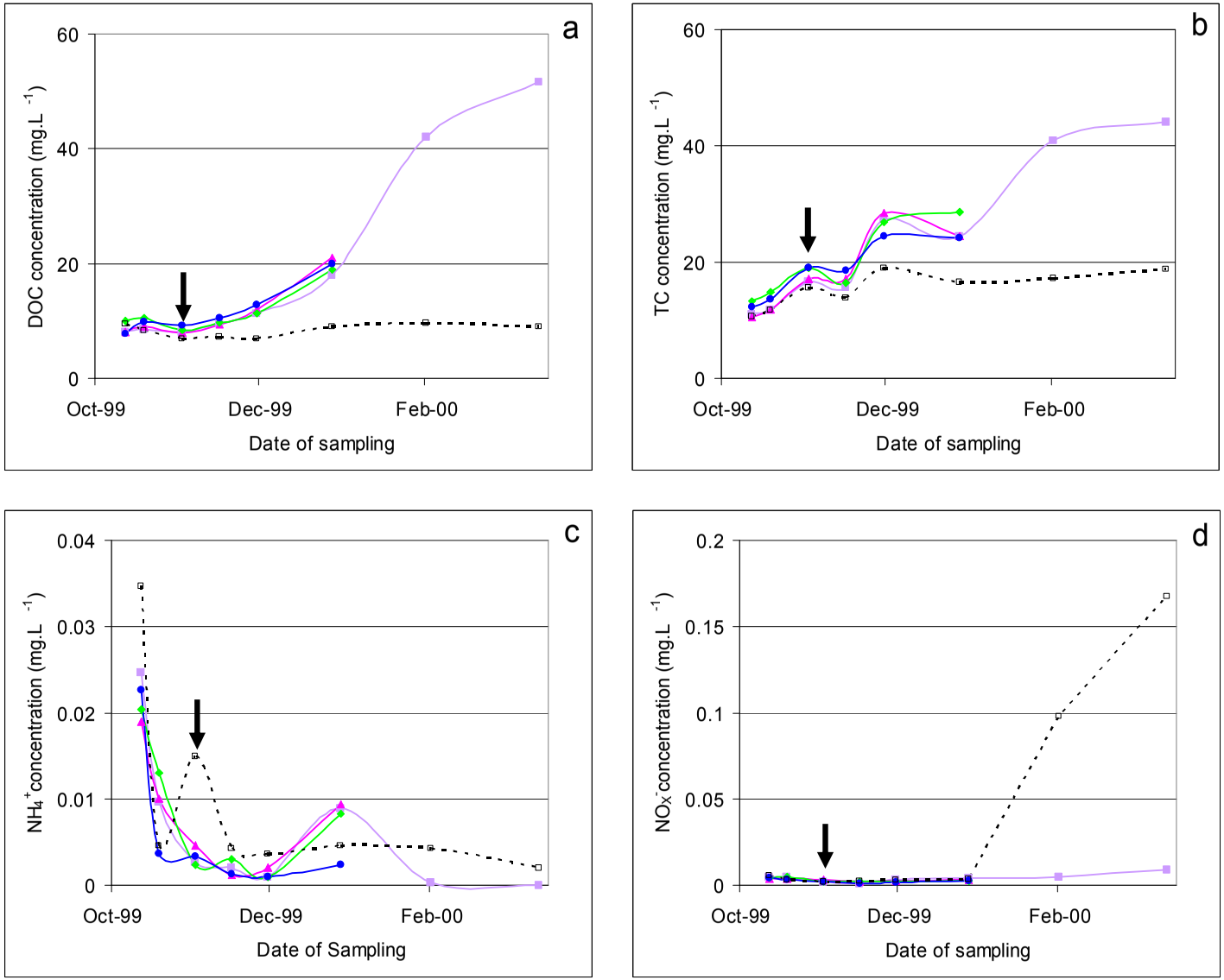
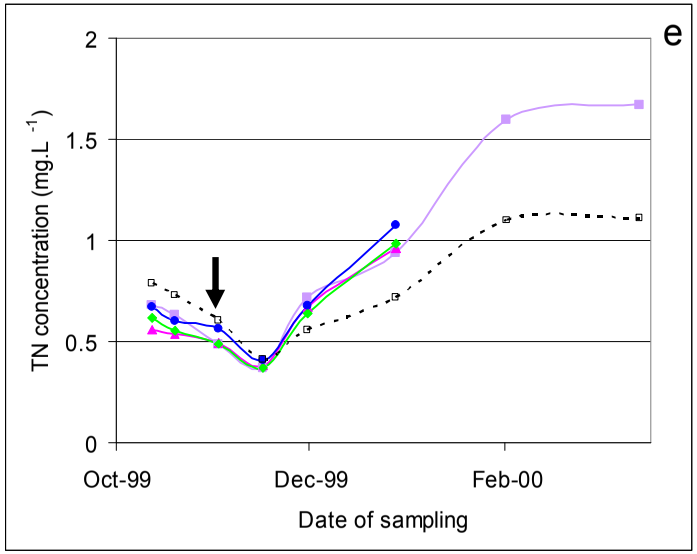
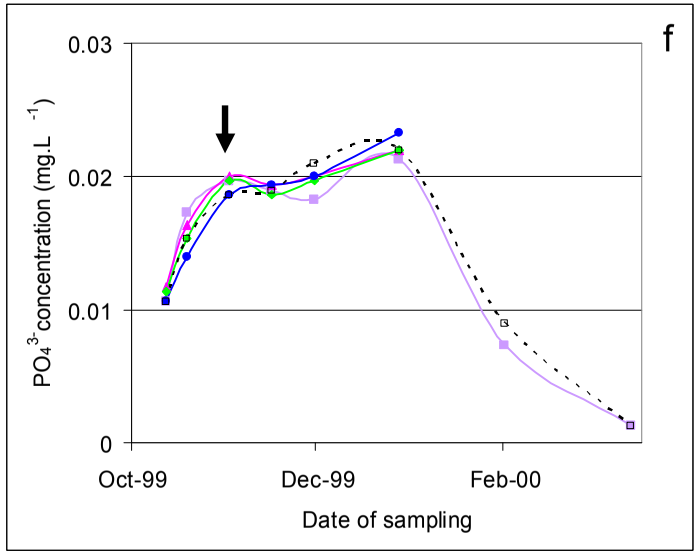
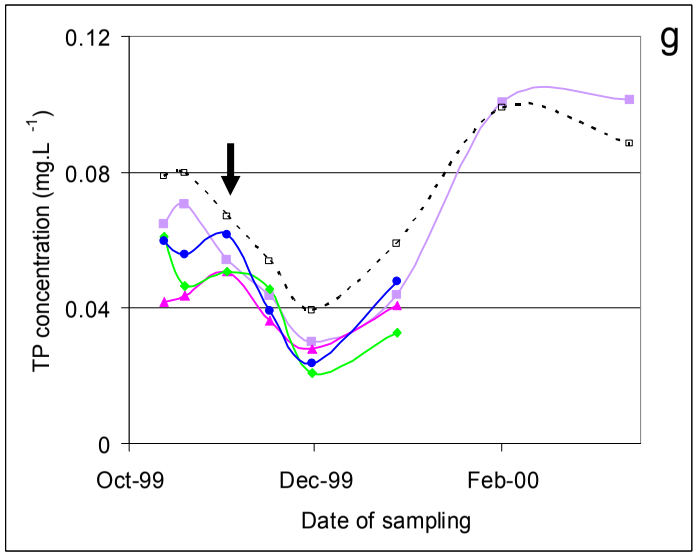


Figure 16 (e – g): Average concentrations of (e) TN, (f) PO_4^{3-}, and (g) TP recorded in treatment 1 (—■—), treatment 2 (—▲—), treatment 3(—◆—), treatment 4 (—●—), and control (---□---) mesocosms during the dry phase. 🡻 indicates the commencement of the dry phase.







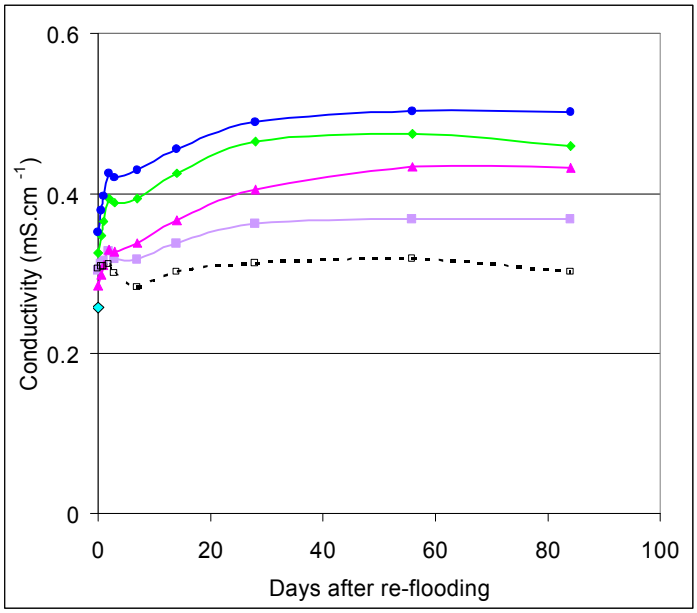
3.3.2 The re-flooding phase

Following re-flooding on the 27 April 2000, the water quality of permanently inundated mesocosms (controls) differed to that in mesocosms exposed to various degrees of drying (treatments). The water column of control mesocosms generally had a higher pH, was more turbid and had lower conductivity levels compared to treatment mesocosms during the re-flooding phase, although there was little difference in the water temperature and DO concentration in treatment and control mesocosms after re-flooding.

Water temperature gradually decreased in all mesocosms following re-flooding, from ~16ºC at the time of re-flooding down to ~8ºC at the end of the experiment. Following a distinct drop in DO concentration in all treatment and control mesocosms during the first 48 hours after re-flooding, the DO concentration in all mesocosms returned to, and was subsequently maintained at values similar to that at the time of re-flooding (~ 6 $mg.L^{-1}$). While the pH of the water column was considerably higher in control mesocosms compared to treatments, a marked drop in pH was also recorded in all mesocosms (including controls) in the 48 hours after re-flooding commenced.

However, the degree of drying that treatment mesocosms were exposed to during the dry phase appeared to have little effect on water quality after re-flooding, with the exception of conductivity levels. Conductivity remained relatively constant throughout the experiment in the permanently inundated mesocosms, in contrast re-flooding resulted in a marked increase in conductivity in all treatment (treatment 1 – 4) mesocosms, with the greatest increases recorded in the first 72h of re-flooding but with further increases recorded throughout the first month after re-flooding. Increasing degrees of drying/desiccation resulted in progressively higher conductivities in the water column after re-flooding (e.g. T4 > T3> T2> T1> C) (Figure 17). In contrast, degree of drying had little effect on water temperature, pH, turbidity and dissolved oxygen concentration after re-flooding.

Figure 17: Average conductivity (mS.cm^{-1}) recorded in treatment 1 (—■—), treatment 2 (—▲—), treatment 3(—◆—), treatment 4(—●—), and control (---□---) mesocosms over the re-flooding phase.



3.3.2.1 Carbon

The general linear mixed model analysis indicated that a significant difference between treatments and between sampling dates existed for both carbon parameters (Table 4). A significant interaction between the two factors (treatment and sampling date) also affected TC concentrations.

Table 4: Results of analysis of dissolved organic carbon (DOC) and total carbon (TC) concentrations following re-flooding of mesocosms. (***** = significant difference).

		DOC	TC
Source of Variation	**Degrees of Freedom**	**Chi-squared probability**[3]	**Chi-squared probability**
Treatment	4	< 0.001*	0.011*
Sampling Date	8	< 0.001*	< 0.001*
Interaction: Treatment and Sampling Date	32	0.896	0.050*

[3] Chi-squared probabilities are based on a comparison of the Wald Test Statistic with the chi-squared distribution, using appropriate degrees of freedom

Partial drying reduced DOC and TC concentrations after re-flooding compared to permanent inundation (controls) and complete sediment desiccation (treatment 4). However, the reduction was only significant in mesocosms where water level was reduced to the sediment surface (treatment 2) (Figure 18 a – b).

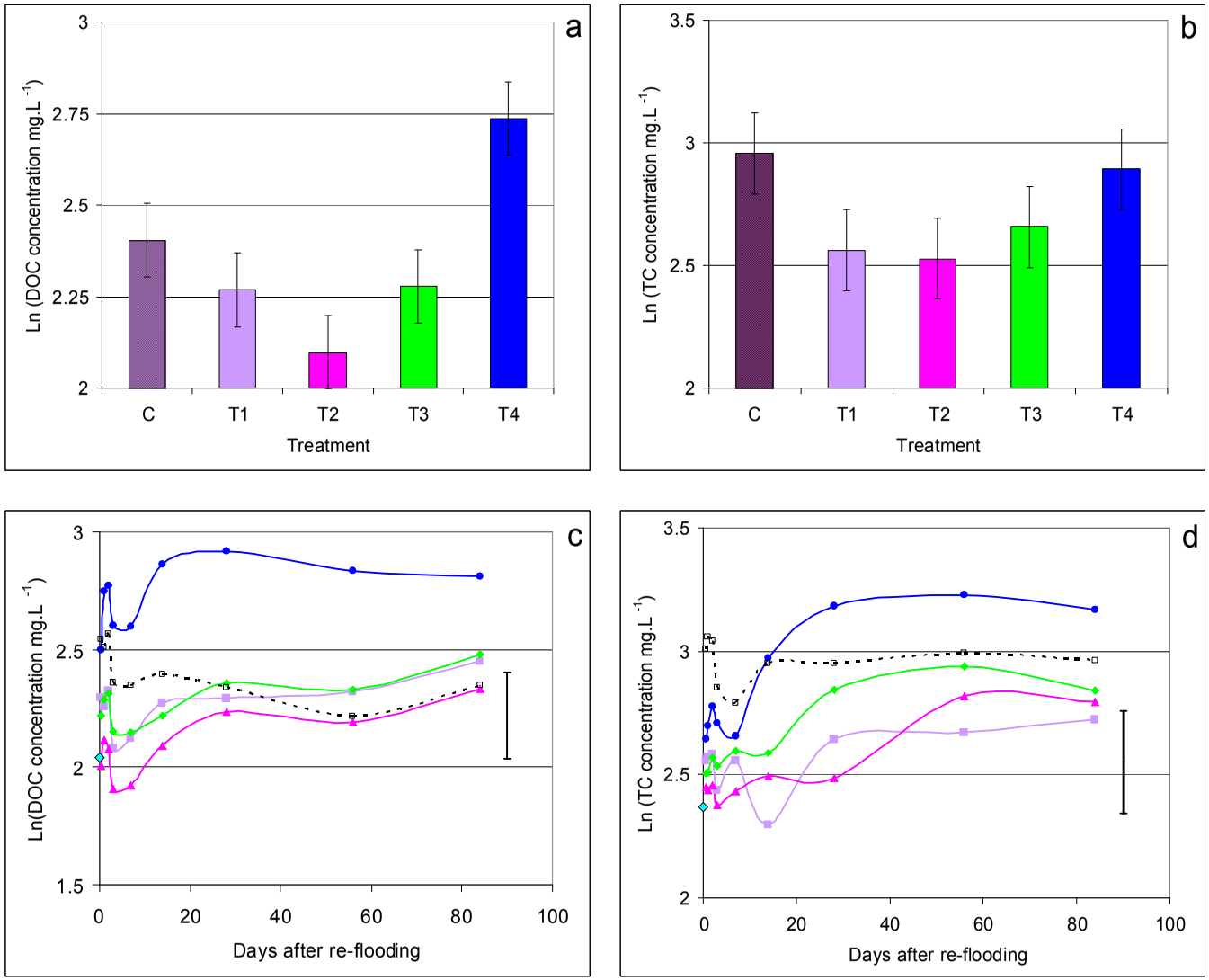
While there was no difference in the average TC concentrations in the permanently inundated and completely desiccated mesocosms over the three month re-flooding phase (Figure 18 b), the DOC concentration and the proportion of the TC pool it represented was significantly higher in completely desiccated mesocosms (86 $\pm$ 12%). Furthermore, DOC represented the lowest proportion of the TC pool in the permanently inundated mesocosms (58% $\pm$ 6%) and intermediate proportions in the partially dried mesocosms (treatment 1, 2 and 3: 76 $\pm$ 9%, 66 $\pm$ 8% and 69 $\pm$ 8%, respectively).

The significantly higher DOC concentration recorded in completely desiccated mesocosms after re-flooding (Figure 18 a) was primarily due to the large release of DOC from sediments into the water column during the first 12 - 48 hours of re-flooding (Figure 18 c). Similar increases in DOC concentration were also recorded in the other treatments, but to a much lesser extent (Figure 18 c). Following this initial increase in DOC concentration upon re-flooding, no significant changes in DOC concentration occurred in any of the treatments for the remainder of the experiment (Figure 18 c).

Results of the analysis suggest that an interaction between the two factors, treatment and sampling date, occurred in the TC data. This was due to a large increase in TC concentration in the water column of treatment 4 mesocosms between 1 week and 1 month after re-flooding, a pattern not observed in any of the other treatments (Figure 18 d).

While DOC and TC concentrations in the permanently inundated mesocosms showed little variation over the entire experimental period, including the period after re-flooding, the effects of complete sediment desiccation persisted until the end of the experiment, such that treatment 4 mesocosms had the highest concentrations of DOC and TC in the water column when measured three months after re-flooding (Figure 18 c – d).

Figure 18: For the three month period following re-flooding, (a, b) average DOC and TC concentrations (respectively) for each treatment; and (c, d) changes in average DOC and TC concentrations (respectively) over time for treatment 1 (—■—), treatment 2 (—▲—), treatment 3(—◆—), treatment 4(—●—), and control (---□---) mesocosms. ◆ = river water used to re-flood mesocosms. Data were natural log transformed. Error bars represent the LSD (2 x standard error).



3.3.2.2 Nitrogen

The analysis indicated that treatment had a significant effect on all three nitrogen parameters (Table 5). There was also a significant effect of sampling date, and a significant interaction between the two factors, on NH_4^+ and NO_X^- concentrations but not TN concentrations.

Table 5: Results of the general linear mixed model analysis of ammonia (NH_4^+), nitrate + nitrite (NO_X^-) and total nitrogen (TN) concentrations following re-flooding of mesocosms. (***** = significant difference).

		NH_4^+	**NO_X^-**	**TN**
Source of Variation	**Degrees of Freedom**	**Chi-squared probability**	**Chi-squared probability**	**Chi-squared probability**
Treatment	4	< 0.001*	< 0.001*	0.043*
Sampling Date	8	< 0.001*	< 0.001*	0.858
Interaction: Treatment and Sampling Date	32	0.008*	0.036*	0.961

Partial drying (treatments 1, 2 and 3) had little effect on NH_4^+ concentration after re-flooding. Average NH_4^+ concentrations recorded in treatments 1, 2 and 3 over the three month period after re-flooding were not significantly different to each other, or to the concentrations recorded in the permanently inundated control mesocosms (Figure 19 a).

In contrast, complete sediment desiccation significantly increased NH_4^+ concentrations in the water column after re-flooding (Figure 19 a). This was due to the very large increase in NH_4^+ concentration in treatment 4 mesocosms during the first week of re-flooding (Figure 19 d). Following this initial increase, the NH_4^+ concentration in treatment 4 mesocosms then decreased and by one month after re-flooding was not significantly different to the concentrations recorded in the other treatments/control (Figure 19 d). This pattern was only observed in treatment 4 mesocosms and hence the significant interaction between treatment and sampling date highlighted by the data analysis for this parameter. In all other treatments and the control, NH_4^+ concentration did not change significantly on any of the sampling dates during the three month period after re-flooding (Figure 19 d).

While not affecting NH_4^+ concentrations, the degree of sediment desiccation did influence NO_X^- and TN concentrations following re-flooding. In those mesocosms where sediment moisture levels were maintained (treatments 1 and 2), NO_X^- and TN concentrations were low, did not change significantly in the three month sampling period following re-flooding (Figure 19 e – f), and were not significantly different to each other (Figure 19 b – c). In contrast, partial (treatment 3) and complete (treatment 4) sediment desiccation increased both NO_X^- and TN concentrations following re-flooding.

Partial sediment desiccation (treatment 3) produced significantly higher NO_X^- concentrations after re-flooding compared to the treatments that experienced a drop in water level (treatments 1 and 2) and the permanently inundated controls (Figure 19 b). Complete sediment desiccation increased NO_X^- availability even further after re-flooding, producing NO_X^- concentrations that were significantly higher than those in treatment 3 mesocosms (Figure 19 b).

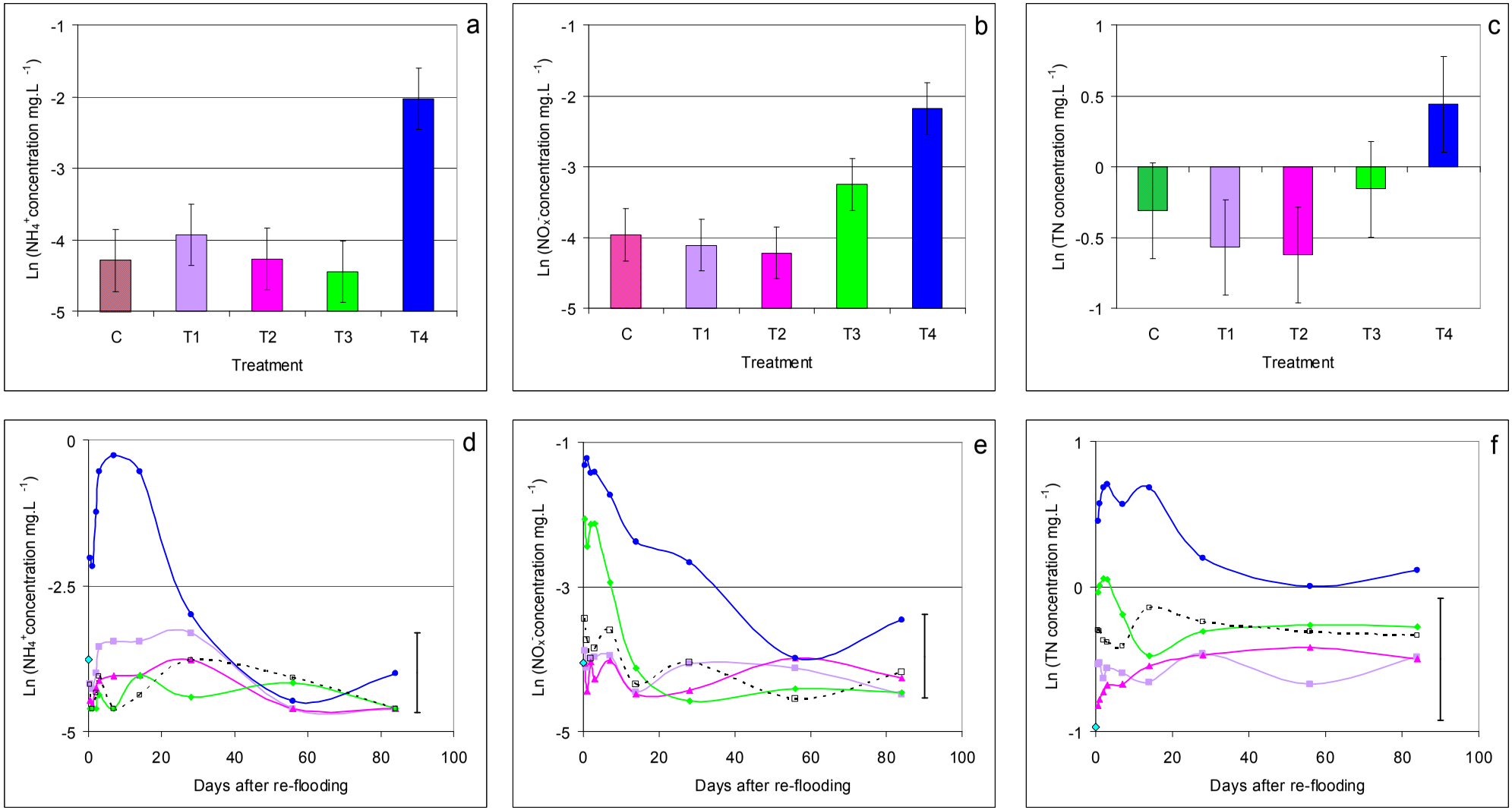
In addition, the changes in NO_X^- concentration recorded over time following re-flooding in treatments 3 and 4 were significantly different to each other and to the pattern observed in the other treatments/control (Figure 19 e). A large release of NO_X^- from sediments into the water column occurred in both treatment 3 and 4 mesocosms upon re-flooding, resulting in increased concentrations for the first 72 hours of flooding. However, the increased NO_X^- concentration in treatment 3 was depleted much more rapidly than in treatment 4 mesocosms (Figure 19 e). As such, the NO_X^- concentration in treatment 3 mesocosms was not significantly different to that in the control, treatment 1 and 2 mesocosms when sampled one week after re-flooding, whereas increased NO_X^- concentrations in treatment 4 mesocosms did not become depleted until 1 – 2 months after re-flooding was initiated.

Complete sediment desiccation significantly increased TN concentrations in the water column after re-flooding compared to permanently inundated, treatment 1 and 2 mesocosms (Figure 19 c). Partial sediment desiccation (treatment 3) also increased TN concentrations following re-flooding, however represented an intermediate between these two groups (not significantly different to either) (Figure 19 c). An increase in TN concentration, due to a release of TN from sediments into the water

column, was recorded in the first 12 hours of re-flooding in both treatment 3 and 4, more so in treatment 4 (Figure 19 f). However, on all sampling dates for the remainder of the experiment TN concentration in these two treatments, as in all other treatments, did not change significantly (Figure 19 f).

In the three months after re-flooding, the proportion of TN available as DIN ($NH_4^+ + NO_X^-$) was highest in treatment 4 (29 $\pm$ 18%), increasing in the first week of re-flooding up to ~ 55% and then decreasing over the following months (~15% and 1% recorded 1 and 2 months after re-flooding, respectively). In treatment 3, DIN represented on average 10% ($\pm$ 9%) of the TN pool, increasing during the first week to 15 – 25% of TN but then decreased markedly after that. Treatments 1, 2 and the control had the lowest proportions of DIN which never increased above 10% on any one sampling date (4 $\pm$ 3%, 2 $\pm$ 1% and 2 $\pm$ 1% respectively). The effects of complete and partial sediment desiccation on the availability of nitrogen in the water column of mesocosms was no longer evident at the end of the experiment, three months after re-flooding was initiated (Figure 19 d – f).

Figure 19: For the three month period after re-flooding, (a, b, c) average NH_4^+, NO_X^- and TN concentrations (respectively) for each treatment; and (d, e, f) changes in average NH_4^+, NO_X^- and TN concentrations (respectively) over time for treatment 1 (-■-), treatment 2 (-▲-), treatment 3(-◆-), treatment 4(-●-), and control (--□--) mesocosms. ◆ = river water used to re-flood mesocosms. Data were natural log transformed. Error bars represent the LSD (2 x standard error).



3.3.2.3 Phosphorus

The analysis indicates that treatment did not affect either of the phosphorus parameters, although there were significant differences between sampling dates after re-flooding (Table 6). There was no significant interaction between the two factors, treatment and sampling date, for both PO_4^{3-} and TP data.

Table 6: Results of the general linear mixed model analysis of ortho-phosphate (PO_4^{3-}) and total phosphorus (TP) concentrations following re-flooding of mesocosms. (***** = significant difference).

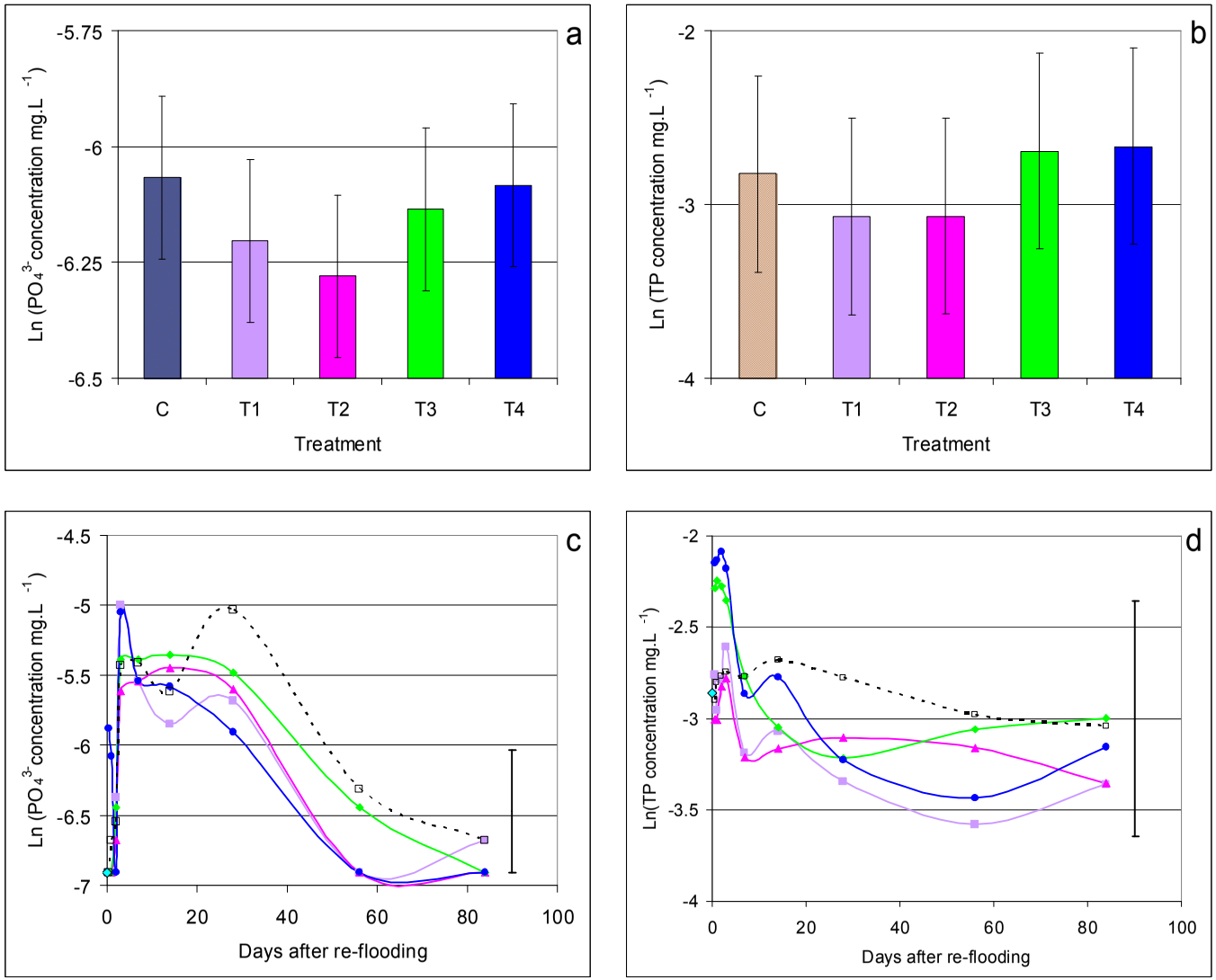
		PO_4^{3-}	**TP**
Source of Variation	**Degrees of Freedom**	**Chi-squared probability**	**Chi-squared probability**
Treatment	4	0.661	0.924
Sampling Date	8	< 0.001*	< 0.001*
Interaction: Treatment and Sampling Date	32	0.638	0.831

Degree of drying had no effect on phosphorus concentrations after re-flooding. The average PO_4^{3-} and TP concentrations in mesocosms dried to varying degrees (treatments 1 – 4) were not significantly different to each other, or to the concentrations recorded in the permanently inundated mesocosms following re-flooding (Figure 20 a, b). In general, PO_4^{3-} concentrations after re-flooding were relatively low (concentrations averaged by treatment and time period were < 0.007 $mg.L^{-1}$) compared to those concentrations recorded early in the dry phase (e.g. November 99 – January 00, see Figure 16 f). An initial release of PO_4^{3-} from sediments in treatment 4 mesocosms was recorded within the first 12 hours of re-flooding, however this was rapidly assimilated/adsorbed and was no longer evident after 24 hours (Figure 20 c). On all remaining sampling dates the PO_4^{3-} concentration in treatment 4 mesocosms did not differ significantly to concentrations recorded in any other treatment (Figure 20 c).

The PO_4^{3-} concentration in each of the treatments and the control increased significantly between 48 and 72 hours after re-flooding, then remained relatively constant until between 1 and 2 months after re-flooding when concentrations decreased significantly (Figure 20 c). The TP concentration in all treatments and the control progressively decreased between 72 hours and 2 months after re-flooding, with a significant decrease occurring between 72 hours and 1 week after re-flooding (Figure 20 d). Closer inspection of the data indicates that a large drop in TP concentration occurred in treatments 3 and 4 between these sampling dates, with smaller decreases occurring in treatments 1 and 2 and the control (Figure 20 d).

Generally, in the three months after re-flooding PO_4^{3-} represented a relatively small proportion of the total phosphorus pool available in the water column of the mesocosms (e.g. treatment averages were < 10%). Notable increases in the percentage of available phosphorus occurred in all treatments and the control between 48 hours – 1 month after re-flooding (up to ~25%), with percentages on other sampling dates being virtually nil.

Figure 20: For the three month period after re-flooding, (a, b) average PO_4^{3-} and TP concentrations (respectively) for each treatment; and (c, d) changes in average PO_4^{3-} and TP concentrations (respectively) over time for treatment 1 (-■-), treatment 2 (-▲-), treatment 3(-◆-), treatment 4(-●-), and control (--□--) mesocosms. ◆ = river water used to re-flood mesocosms. Data were natural log transformed. Error bars represent the LSD (2 x standard error).



3.3.2.4 Community metabolism

The analysis indicated that treatment only had a significant effect on CR_{24} data, while sampling date significantly affected all three community metabolism parameters (Table 7). An interaction between the two factors, treatment and sampling date, was not detected for any of the parameters.

Table 7: Results of the general linear mixed model analysis of Community Respiration over 24 hours (CR_{24}), Gross Primary Productivity (GPP) and Productivity:Respiration ratio (P:R) following re-flooding of mesocosms. (***** = significant difference).

		CR_{24}	**GPP**	**P:R**
Source of Variation	**Degrees of Freedom**	**Chi-squared probability**	**Chi-squared probability**	**Chi-squared probability**
Treatment	4	0.002*	0.124	0.741
Sampling Date	8	0.017*	< 0.001*	0.004*
Interaction: Treatment and Sampling Date	32	0.192	0.273	0.993

Partial drying resulted in reduced community respiration after re-flooding, compared to permanent inundation (controls) and complete desiccation (treatment 4) (Figure 21 a). However, only treatment 2 mesocosms had significantly lower CR_{24} rates than those in control and treatment 4 mesocosms after re-flooding (Figure 21 a). The changes in CR_{24} over time after re-flooding were similar in all treatments and the control mesocosms, with CR_{24} rates decreasing significantly between 1 and 2 months after re-flooding and then increasing between 2 and 3 months after re-flooding (Figure 21 d). The increase in CR_{24} between the last two sampling dates was largest in control and treatment 1 mesocosms (Figure 21 d).

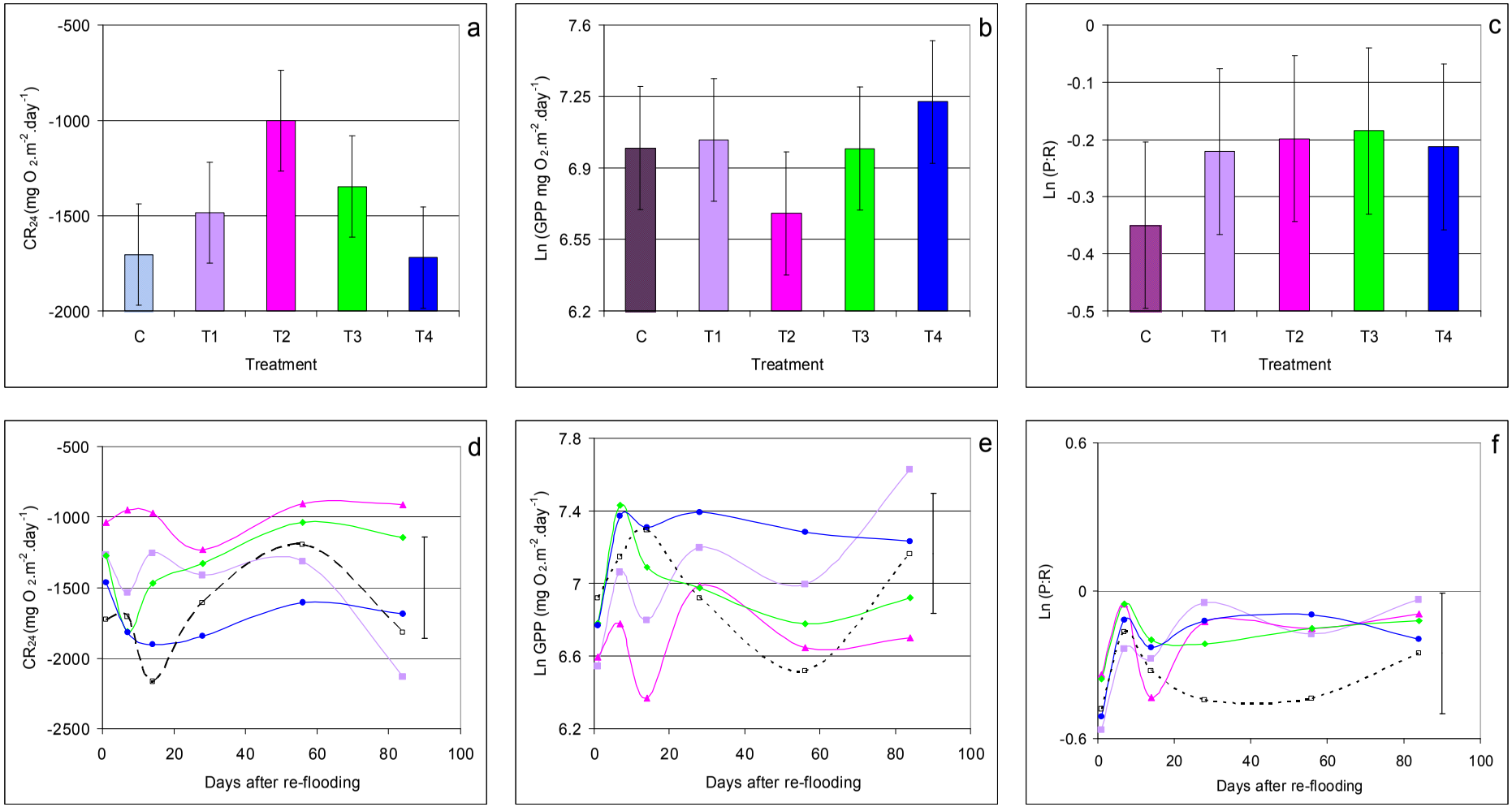
Although not significantly different to the other treatments/control mesocosms, the lowest GPP values following re-flooding were also recorded in treatment 2 mesocosms (Figure 21 b). GPP values in all treatments and the control mesocosms increased significantly between 24 hours and 1 week after re-flooding (Figure 21 e). As observed for CR_{24}, GPP values also decreased significantly between 1 and

2 months, then increased significantly between 2 and 3 months, after re-flooding (Figure 21 e). The increase in GPP was also greatest in control and treatment 1 mesocosms.

When sampled just after re-flooding (24 hours after re-flooding), no significant difference in CR_{24} or GPP existed between any of the treatments and the control (Figure 21 d, e). After three months CR_{24} and GPP measurements were lowest in treatment 2 mesocosms and highest in treatment 1 mesocosms and the difference between these two treatments was significant (Figure 21 d, e). Treatments 3 and 4 and the permanently inundated mesocosms had intermediate values of CR_{24} and GPP at the end of the experiment.

For the entire period following re-flooding the P:R ratio in all treatments and the control mesocosms was less than 1. Despite a significant increase in the P:R ratio in all treatments/control mesocosms between 24 hours and 1 week after re-flooding (Figure 21 f) the P:R ratio still remained less than 1. Degree of drying appeared to have little affect on P:R values for the period after re-flooding (Figure 21 c). The permanently inundated (control) mesocosms had the lowest P:R ratio following re-flooding, although values in the controls were not significantly different to those recorded in the other treatments (Figure 21 c).

Figure 21: For the three month period after re-flooding, (a, b, c) average CR_{24}, GPP and P:R (respectively) for each treatment; and (d, e, f) changes in average CR_{24}, GPP and P:R (respectively) over time for treatment 1 (-■-), treatment 2 (-▲-), treatment 3(-◆-), treatment 4(-●-), and control (--□--) mesocosms. Error bars represent the LSD (2 x standard error). GPP and P:R data were natural log transformed. Note that as respiration rates increase, CR_{24} values become more negative.

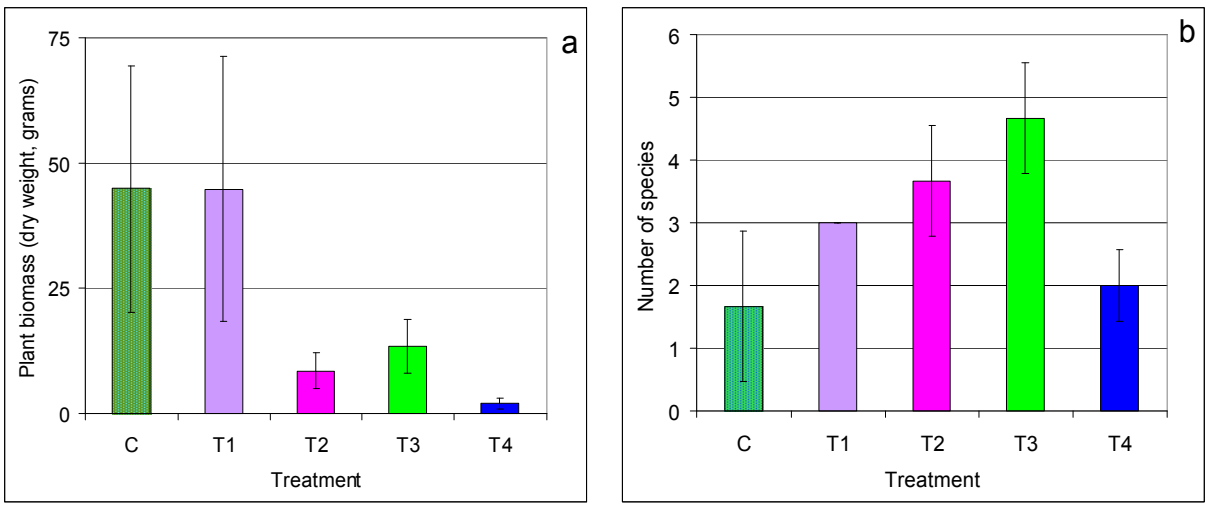


3.3.2.5 Macrophyte community

Degree of drying clearly influenced both the plant biomass that accumulated (Figure 22 a), as well as the species diversity that established (Figure 22b), in mesocosms after re-flooding. In those treatments where the water column was maintained throughout the experiment, namely the control and treatment 1 mesocosms, a substantially greater plant biomass had accumulated in comparison to those mesocosms in which the sediment was exposed to the atmosphere (treatments 2, 3 and 4) (Figure 22 a).

It also appears that the extent of drying and species richness may be positively associated (Figure 22 b). As the extent of drying increased in mesocosms, the number of aquatic plant species supported also increased, except when the mesocosm underwent complete sediment desiccation (Figure 22 b). The data suggest that complete sediment desiccation substantially reduced both plant biomass and species richness (Figure 22 a, b).

Figure 22: (a) Biomass and (b) species richness of the aquatic fauna harvested from mesocosms at the end of the experiment, averaged for each treatment. Error bars represent the LSD (2 x standard error).



3.4 Discussion

In the ecosystem-scale experiment described previously (Chapter 2), the extent of drying in an ephemeral wetland appeared to play a role in influencing both the release of nutrients from wetland sediments into the water column, as well as a pulse in phytoplankton productivity, after re-flooding. It was proposed that the degree of wetland drying may contribute to the variation from the FPC model that was observed amongst ephemeral floodplain wetlands in the study. The mesocosm experiment described in this chapter investigated and confirmed the hypothesis that degree of wetland drying influences the nature of the flood pulse after re-flooding. Specifically, the two alternative hypotheses proposed at the start of the chapter (section 3.1) were supported.

3.4.1 Degree of drying and the flood pulse – Nutrient availability

Complete sediment desiccation had no measurable effect on TC concentration, however significantly increased DOC concentration in the water column following re-flooding. While previous research has suggested that drying can result in carbon limitation after re-flooding (e.g. Mitchell and Baldwin, 1998, 1999; Baldwin and Mitchell, 2000), these results indicate that complete sediment desiccation can return a proportion of the organic carbon pool to the water column upon re-flooding, probably released as a result of the death and partial decomposition of microbial, plant and animal biomass during the drying process.

Corresponding with the findings of numerous other *in situ* (Briggs *et al*., 1985; Scholz *et al*., 2002) and laboratory based studies (Briggs *et al*., 1985; Qiu and McComb, 1996; James *et al*., 2004), complete desiccation of Barmah Lake sediments also resulted in substantial increases in the availability of NH_4^+, NO_X^- and TN in the water column after re-flooding. This flush of nitrogen upon re-flooding is believed to be due to a release of nutrients accumulated in the sediment during the drying process, largely due to microbial death and cell lysis which occurs during the dry phase (Qiu and McComb, 1995, 1996).

Complete sediment desiccation however, had little effect on phosphorus concentrations after re-flooding in this mesocosm experiment. Apart from a small release of PO_4^{3-} from sediments into the water column upon re-flooding, which was rapidly adsorbed by sediments or assimilated by biota, the

PO_4^{3-} and TP concentrations in these mesocosms did not differ significantly to the concentrations in any other treatment for the remainder of the experiment. The absence of a pulse in available phosphorus after re-flooding of completely desiccated sediments is consistent with the patterns observed in Moira Lake, studied as part of the ecosystem-scale experiment described in Chapter 2. In addition, studies of Australian ephemeral wetlands have also found that sediment desiccation can have little effect on, or actually decrease, P availability after re-flooding (Qiu and McComb, 1994; Mitchell and Baldwin, 1998; Scholz *et al*., 2002). Factors proposed to explain the reduced P concentrations after re-flooding in these studies included differences in sediment characteristics, such as iron and calcium content and the proportion of humic substances and silt (Qiu and McComb, 1994), as well as shifts in the resident microbial community and carbon limitation (Mitchell and Baldwin, 1998) resulting from the drying process. It is possible that any, or all, of these factors play a role in explaining the lack of a significant increase in phosphorus availability after re-flooding in this mesocosm experiment.

In a wetland in the Camargue (France) De Groot and Van Wijck (1993) demonstrated that the capacity of wetland sediments to adsorb P from the surrounding water phase can increase significantly as the drying phase progresses, attributing this phenomenon to the oxidation of iron sulphide (FeS) present in anoxic sediments, forming Fe(OOH) and increasing the capacity for further P sorption. Given the large release of DOC and DIN following re-flooding of the completely desiccated treatments, it is likely that a substantial proportion of the microbial consortia present in these mesocosms were killed during the drying process. In fact, it is known that sediment desiccation can result in the death of up to 75% of the resident microbial consortia (Qiu and McComb, 1995). In this mesocosm experiment, it is possible that the phosphate released into sediments following microbial death and cell lysis during the dry phase, was adsorbed by sediment-mineral complexes (via the mechanism described by De Groot and Van Wijck (1993)), thus preventing a significant pulse release of P from sediments after re-flooding. Hence, the low phosphate concentration recorded in treatment 4 mesocosms after re-flooding may have been due to a substantial proportion of the phosphate pool being “locked-up” in the sediments.

Partial drying of the mesocosms affected both carbon and nitrogen dynamics, but as in those mesocosms that were completely desiccated, had little effect on phosphorus availability after re-flooding. However, in contrast to complete sediment desiccation, partial drying reduced DOC and TC concentrations after re-flooding to levels that were significantly lower than in permanently inundated mesocosms (the controls). This may be related to the loss of bio-available carbon reported to occur following drying and re-flooding (e.g. Mitchell and Baldwin, 1998, 1999; Baldwin and Mitchell, 2000), due to increased rates of organic matter decomposition during the drying phase (De Groot and Van Wijck, 1993; Boyd and Pippopinyo, 1994; van Oorschot *et al*., 2000). The microbial consortia in partially dried mesocosms (treatment 2) did not experience moisture stress, so while increased oxygen availability may have resulted in the death of obligate anaerobes, the majority of the resident microbial consortia would have survived the dry phase. Consequently there was no return of organic carbon to the water column following re-flooding, as was the case in mesocosms exposed to complete sediment desiccation.

Partial drying had little effect on NH_4^+ availability after re-flooding relative to the permanently inundated and completely desiccated mesocosms, although partial sediment desiccation (treatment 3) could increase NO_X^- and TN availability after re-flooding. These results differ to those reported by James *et al*. (2004), where sediment de-watering by 20% and 60% was found to significantly reduce the availability of NH_4^+ and NO_X^- after re-flooding, although these differences are most likely a product of the design differences in the two experiments (see Section 3.1).

The amount of NO_X^- and TN released from partially desiccated sediments (treatment 3) into the water column 12 hours after re-flooding (although less) was not significantly different to the amount released from completely desiccated sediments (treatment 4). As such, death and cell lysis of a considerable proportion of the microbial consortia in the partially desiccated sediments may also be the source of the increased NO_X^- and TN concentrations recorded in treatment 3 mesocosms after re-flooding.

However, there is some evidence to suggest that a proportion of the microbial consortia in treatment 3 mesocosms may have survived the dry phase, influencing the nature of the 'flood pulse' in these mesocosms. First, the NO_X^- and TN concentrations in treatment 3 mesocosms upon re-flooding,

although not significantly different, were consistently lower than those recorded in treatment 4 mesocosms. If the amount of nutrients released into the water column upon re-flooding is proportional to the extent of sediment microbial mortality during the dry phase, an association demonstrated for phosphorus release from a number of wetland sediments (Qiu and McComb, 1995; Turner and Haygarth, 2001), then this may indicate that a smaller proportion of the microbial consortia were killed (and hence some of the microbial consortia survived) during the drying phase in partially dried mesocosms.

Second, the degree of drying also affected the rate at which NO_X^- in the water column was depleted after re-flooding. In partially desiccated mesocosms (treatment 3) the accumulated NO_X^- was depleted in the water column ~ 1 week after re-flooding, compared to the completely desiccated mesocosms (treatment 4) where NO_X^- did not become depleted until 1 – 2 months after re-flooding. The 'refuge' provided by the lower sediment layer in treatment 3 mesocosms may have allowed populations of denitrifyers and/or dissimilative nitrate reducers, responsible for the assimilation of NO_X^- (e.g. Qiu and McComb, 1996), to survive the dry phase and become active more rapidly after re-flooding, hence resulting in the more rapid depletion of NO_X^- from the water column. Although the 'refuge' sediment layer in treatment 3 mesocosms would have been predominately oxic, there is some evidence from previous research that suggests even strictly anaerobic microbes can exist and be active in mostly oxic sediments (Boon *et al*., 1997; Baldwin *et al*., 2000). Boon *et al*. (1997) proposed that methanogenesis was occurring in anaerobic micro-sites present within the mostly oxic sediments, while Baldwin *et al*. (2000) proposed that sulfate-reducing bacteria had developed tolerance after being exposed to repeated periods of oxidation/desiccation.

Nutrient concentrations in the water column of permanently inundated mesocosms in the post re-flooding period were generally intermediate between the reduced concentrations recorded in partially dried mesocosms and the increased concentrations recorded in completely desiccated mesocosms. In particular, permanent inundation produced significantly reduced concentrations of DOC, NH_4^+, NO_X^- and TN compared to concentrations recorded in the completely desiccated/re-flooded mesocosms. Similar patterns have been reported by *in situ* and laboratory based experiments which have

compared permanently inundated and dried/re-flooded wetland sediments (e.g. Chapter 2, Qiu and McComb, 1996; James *et al*., 2004).

3.4.2 Degree of drying and the flood pulse – Community metabolism

The community metabolism values, GPP and CR_{24}, recorded in the mesocosms in this experiment ranged between 106 – 915 mg $C.m^{-2}.day^{-1}$ and (-)227 – (-)1173 mg $C.m^{-2}.day^{-1}$, respectively. In a comparable study by Bunn *et al*. (2003), where benthic chambers were used to measure community metabolism in a number of waterholes on the Cooper Creek floodplain (Australia), GPP values ranged between 10 – 4015 mg $C.m^{-2}.day^{-1}$ and CR_{24} values ranged between (-)22 – (-)2462 mg $C.m^{-2}.day^{-1}$. The rates of production and respiration in the mesocosms in this experiment were generally higher than those recorded by Bunn *et al*. (2003) in the deeper areas of the Cooper Creek waterholes, but significantly lower than the values recorded in the highly productive shallow littoral zones of the waterholes (which were regularly in excess of 1000 mg $C.m^{-2}.day^{-1}$). However, unlike most of the waterholes which were found to be net producers of carbon (e.g. P:R > 1), the mesocosms after re-flooding were in general, 'heterotrophic' or net consumers of carbon (e.g. P:R < 1), with the permanently inundated controls recording the lowest average P:R values (although not significantly lower) for the period after re-flooding.

Degree of drying influenced community metabolism after re-flooding, with partial drying (treatment 2) significantly reducing rates of community respiration compared to rates recorded in permanently inundated and completely desiccated mesocosms. Given that autotrophic respiration is a secondary mechanism of energy production involving the decomposition of organic carbon (Anderson and Beardall, 1991), it is likely that the reduced concentrations of DOC in the water column of these mesocosms after re-flooding is associated with the limited rates of community respiration recorded. While a number of previous studies have investigated rates of community respiration at different stages of the drying processes (e.g. Boyd and Pippopinyo, 1994; van Oorschot *et al*., 2000), no studies to date have examined community metabolism following drying and re-flooding, against which the results of this study can be compared. However, these results are consistent with the proposal of Mitchell and Baldwin (1998, 1999) and Baldwin and Mitchell (2000) that, as a result of increased rates

of organic matter decomposition during the dry phase (De Groot and Van Wijck, 1993; Boyd and Pippopinyo, 1994; van Oorschot *et al*., 2000), drying in some cases may cause carbon limitation of biological processes (such as community respiration) after re-flooding.

While re-flooding of the completely desiccated sediments significantly increased concentrations of carbon and nitrogen in the overlying water column, the increased availability of these nutrients was not translated into an increase in community metabolism (rates of primary production and/or respiration) after re-flooding. On the basis of these results I propose that, in ephemeral floodplain wetlands of the River Murray, the availability of inorganic P plays an important role in determining the occurrence (or not) of the classical 'flood pulse' response outlined by the FPC, where flooding is associated with increased nutrient availability which supports significant increases in primary, and subsequently secondary, production (Junk *et al*., 1985). Any potential increase in primary productivity in ephemeral floodplain wetlands of the River Murray seems to be limited by the availability of phosphate. In fact, while a number of environmental factors such as light availability, temperature and nutrient availability, are known to influence rates of primary production (e.g. Rae and Vincent, 1998; Udy *et al*., 2001), phosphate is considered to be the natural growth limiting nutrient in fresh water-bodies (e.g. Schindler, 1977, 1987; Boers, 1991b).

This hypothesis is also consistent with observations made in the ecosystem-scale experiment (Chapter 2), where Little Mussel (LM) Lagoon was the only ephemeral wetland to demonstrate the classical 'flood pulse', (as per the FPC). In LM Lagoon an increase in phytoplankton abundance was recorded after re-flooding and this coincided with an increase in the availability of phosphate, also not recorded in any other wetland in the experiment. Additionally, inundation of two ephemeral wetlands on the floodplain of the Lachlan River (Murray-Darling Basin) increased the availability of both nitrate and phosphate in the water column (Briggs *et al*., 1985), and while the re-flooding phase was characterized by the lowest levels of phytoplankton productivity recorded throughout the study (Briggs *et al*., 1985), the highest levels of macrophyte production were measured during this period (Briggs and Maher, 1985).

3.4.3 Degree of drying and the flood pulse – Macrophyte community

Degree of drying also impacted on the macrophyte community which established in the period following re-flooding. A drop in water level, where the water column was reduced but maintained throughout the dry phase (treatment 1), had no measurable effect on macrophyte biomass when measured three months after re-flooding: biomass in treatment 1 mesocosms was equal to that in the permanently inundated mesocosms at the end of the experiment. However, a significant reduction in plant biomass was recorded when water levels were dropped to at or below the sediment surface (e.g. treatments 2 and 3). While the macrophyte community was able to re-establish in these mesocosms after re-flooding, the biomass after 3 months was still significantly less than in treatment 1 and the control. Complete sediment desiccation decreased plant growth after re-flooding even further, with a significantly lower biomass recorded in these mesocosms compared to all other treatments at the end of the experiment.

Interestingly, these results are consistent with the findings of one previous study (Hunter *et al*., 2000), but contradict another (Casanova and Brock, 2000). For example, growth (both root and stem production) of the macrophyte *Scirpus validus* (softstem bulrush) was found to be significantly reduced in mesocosms exposed to water level drawdown (below sediment surface) and re-flooding compared to permanently inundated mesocosms (Hunter *et al*., 2000), as was found in this mesocosm experiment. In contrast, in a complex experiment investigating the effects of water depth, flooding duration and flooding frequency on wetland plant communities, Casanova and Brock (2000) reported the lowest plant biomass in continuously flooded mesocosms, the highest biomass in pots where the sediment was kept moist but never flooded, and intermediate plant biomass in those mesocosms experiencing fluctuating water levels (e.g. various regimes of drying and re-flooding). The contradictory results may be related to the difference in the duration of the two experiments, this mesocosm experiment ran for greater than double the duration of the experiment by Casanova and Brock (2000) (4 and 9 months, respectively).

It is noted that the significantly reduced plant biomass in the completely desiccated and partially dried mesocosms at the end of this experiment may have been exaggerated by the date of re-flooding. The mesocosms were re-flooded in autumn (rather than in spring as would occur in River Murray wetlands

under natural conditions) and this may have reduced the potential for plant growth in these mesocosms during the post-re-flooding phase. Timing of re-flooding has been shown to effect both biomass production and species diversity in wetland plant communities (e.g. Nielsen and Chick, 1997; Warwick and Brock, 2003). For example, in a mesocosm experiment Warwick and Brock (2003) demonstrated that biomass production of aquatic plants grown during autumn was significantly reduced in comparison to those grown during summer.

While the permanently inundated mesocosms supported the highest plant biomass at the end of the experiment (along with treatment 1 mesocosms), permanent inundation appears to reduce species diversity of macrophytes established in the mesocosms. Similar results were reported by Casanova and Brock (2000) who found that the number of wetland plant species established was lowest in those pots that remained permanently inundated throughout the experiment (described above). Nielsen and Chick (1997) also reported that long-term inundation led to a significant decrease in the number of taxa found in the permanent billabongs (mesocosms) compared with all other treatments (which had fluctuating water levels).

3.4.4 In summary

On the basis of the results collected in this experiment it can be concluded that degree of drying did influence the nature of the flood pulse in these mesocosms. Compared to permanent inundation, re-flooding of completely desiccated sediments increased carbon (C) and nitrogen (N) availability while partial drying generally decreased, or had little effect on, C and N availability after re-flooding. However, degree of drying had little effect on phosphorus availability or rates of primary production measured after re-flooding, and it is possible that these two factors are related. Partial drying reduced rates of community respiration after re-flooding, possibly a reflection of the reduced carbon concentrations measured in these mesocosms in this phase of the experiment. Degree of drying also influenced the macrophyte community (measured after 3 months of flooding), with plant biomass generally decreasing and species diversity increasing as the degree of drying increased (with the exception of complete sediment desiccation which had lasting negative effects on both macrophyte biomass and species diversity). Therefore, assuming that results of this mesocosm experiment are representative of patterns and processes occurring at the wetland-scale, degree of wetland drying

may explain some of the variation from the classical ‘flood pulse’ (as per the FPC) that was observed in the ephemeral wetlands that were part of the ecosystem-scale experiment described in Chapter 2.

4

The effect of experimental scale on nutrient availability following drying and re-flooding of floodplain wetland sediments

4.1 Introduction

It is generally accepted that the scale at which an ecological experiment is conducted should be relative to the organism being studied and take into consideration the objectives of the research (e.g. Wiens, 1989; Cooper *et al.*, 1998; MacNally and Quinn, 1998). Despite this, the explicit consideration of scale in experimental design is not common in ecological research (e.g. Hoekstra et *al.*, 1991; Levin, 1992; Wagener *et al.*, 1998; Skelly, 2002; also see Section 1.3). Experiments are regularly conducted at scales that are chosen arbitrarily or based on tradition (e.g. Allen and Starr, 1982; Wiens, 1989; Sugihara and May, 1990; MacNally and Quinn, 1998; Hobbs, 2003). However, patterns and/or processes identified by an experiment cannot be extrapolated to different scales, as inferences are constrained by the *extent* (spatial and/or temporal boundary of the experiment) and ***grain*** (spatial and temporal size and/or intensity of the individual units of observation) of the experiment (Wiens, 1989). As highlighted by Wiens (1989, p387):

> "... we cannot generalize beyond the extent without accepting the assumption of scale-independent uniformitarianism of patterns and processes (which we know to be false), and we cannot detect any elements of patterns below the grain."

Therefore, while experiments conducted at smaller scales are easier to replicate, control and manipulate and are usually cheaper and quicker to perform than larger-scale experiments (Carpenter, 1996; Culp *et al.,* 2000; Kampichler *et al.*, 2001; Skelly and Kiesecker, 2001; Skelly 2002), whether or not the results from these studies can be used to infer patterns and processes at larger scales is contingent upon the scale-dependent structure of the ecological system being studied (e.g. Wiens, 1989; Bergström and Englund, 2002). Hypotheses describing the potential structure of environmental systems include: (a) those systems that are structured as a set of scale 'domains', where each domain is characterized by the patterns of a particular phenomenon which do not change over the scale spectrum within that domain (e.g. hierarchy theory proposes one type of structure consisting of domains, "a system of systems within systems" King, 1997); (b) self-similar structures, where the same pattern occurs regardless of the scale at which it is observed (e.g. a system which has fractal properties Gleick, 1987; Scheuring and Riedi 1994); or (c) continuous scale-dependency, where different patterns and processes are apparent regardless of the scale at which observations are made (Wiens, 1989).

Our ability to extrapolate results from smaller scaled experiments to represent patterns and processes occurring in natural systems is further complicated by artifacts inherent in the 'containerization' process. By using mesocosms (or microcosms), we may disrupt processes and organisms of interest during the establishment process, alter dimensional relationships, modify biotic and abiotic conditions and, in general, as scale decreases so to does spatial heterogeneity (e.g. Stephenson *et al.,* 1984; Schindler, 1987; Wiens, 1989; Carpenter, 1996; Sarnelle, 1997; Cooper *et al.*, 1998; Kampichler *et al.*, 2001). Due to these artifacts, environmental conditions in small-scale experiments will often diverge from those in natural conditions (Cooper *et al.,* 1998; Berg *et al.,* 1999).

So, it is essential that ecologists determine the minimum scale at which an experiment can be conducted and still produce results that are representative of patterns and processes occurring in the natural systems in which we are interested (Sarnelle, 1997; Culp *et al.*, 2000). To date, the discussion of the role of scale in ecological research appears to have been driven by the fields of population, community and landscape ecology, research interested in the distribution, abundance and interactions of plants and animals and their environment. With most researchers agreeing, the larger the organism

being studied, the more it moves around, and the longer it lives, the larger the experimental scale needs to be (see Hoekstra *et al.*, 1991; Cooper et *al.*, 1998).

This prompted me to consider how experimental scale influences the results of investigations into the effects of dryinglre-flooding on nutrient availability and cycling, in which microbes play a central role. Based on the general rule outlined above relating organism size, movement and life-span to appropriate experimental scaling, it would follow that the effects of dryinglre-flooding on wetland nutrient cycles can be realistically mimicked in small-scale experiments (both in time and space) because of the characteristics of the microscopic organisms that are central to the nutrient cycling process (e.g. Wagener *et al.*, 1998).

Indeed, small-scale laboratory experiments have formed the basis of many advances in our understanding of bacterial dynamics in both marine and freshwater systems (Sanford *et al.*, 2001). Similarly, most of our knowledge to date on the effects of dryinglre-flooding on nutrient availability and primary productivity in river-floodplain systems has been derived from the use of micro- and meso-cosm experiments (–65%; see Table 8). This follows a general trend in the wider field of ecology toward small-scale experimentation (e.g. refer to Section 1.3). For example, the review written by Baldwin and Mitchell (2000) synthesizing knowledge to date on the effects of drying/re-flooding on nutrient dynamics of lowland river-floodplain systems, highlighted that "surprisingly little [of this] research has been conducted on the effect of in *situ* drying on N and P cycles in either sediments or soils. With some exceptions ..., almost all of our knowledge of the effect of drying is derived from the soil literature and most of that is concerned with the effects of laboratory drying on determining analyte concentrations".

Prompted by the prevalent use of small-scale experiments in ecology, a number of researchers have suggested that a rnulti-scale approach to ecological investigations should be a priority for future ecological research (e.g. Levin, 1992; Cooper *et al.,* 1998; MacNally and Quinn, 1998; Drenner and Mazumder, 1999; Culp *et al.,* 2000; Kampichler *et al.*, 2001). Such an approach combines ecosystem scale experiments with smaller-scale experiments, and should allow us to determine the most appropriate experimental scales for particular questions (e.g. Walker *et al.*, 1995; Cooper *et al.*, 1998;

Mac Nally and Quinn, 1998). Cooper *et al.* (1998) go even further to specify that the "execution of multi-scale experiments should be conducted where the same manipulations are conducted at a variety of spatial and temporal scales".

Over the last ten years or so, a relatively small number of ecological studies have incorporated such a multi-scale approach and the results of these studies have further emphasized the potential impact that experimental scale can have on the conclusions reached by ecologists (e.g. see Section 1.3; 'Quinn and Keough, 1993; Petersen et *al.*, 1997; Sarnelle; 1997; Ahn and Mitsch, 2002). For example, De Groot and Van Wijck (1993) conducted a laboratory experiment along with field observations as part of their investigation into the impacts of desiccation on a freshwater marsh in the Camargue (France). The laboratory-based experiment produced results that were inconsistent with patterns observed in the field, and they concluded that this was due to experimental artifacts which affected the drying process in the microcosm.

However, from the small pool of multi-scale ecological studies which have incorporated various sized mesocosm experiments in conjunction with field observations, there is some evidence to suggest larger mesocosm experiments may be better able to reproduce natural conditions (as in the ecosystem) compared with smaller meso- and micro-cosms. A frequently cited example is the multi-scaled bio-manipulation experiments conducted by Sarnelle (1997), where results from the small-scaled experiment (15 L enclosures) were unable to mimic whole-lake responses, while the larger-scaled experiment (10 000 L enclosures) recreated the responses observed in the lake. Similarly, Schindler (1977, 1987) found that a larger mesocosm experiment produced results that were similar to those from the whole-lake experiment, namely phytoplankton growth in the lake was limited by phosphorus availability, while the small microcosm experiment incorrectly suggested that growth of phytoplankton in the lake was carbon-limited.

As such, the objective of the study outlined in this chapter was to provide a critical assessment of the use of small-scale laboratory experimentation, our primary source of knowledge to date, in the field of nutrient dynamics in ephemeral wetlands. Specifically, I aimed to establish if the changes in nutrient availability resulting from drying and re-flooding and permanent inundation at the 'wetland' scale can

be isolated and reproduced in smaller mesocosm experiments. Hence, a multi-scaled approach incorporating three different sized mesocosm experiments along with an ecosystem-scale experiment, was used to test the following hypotheses:

H_O: The flood pulse, specifically the changes in nitrogen and phosphorus availability in the water column of the meso-, mini- and/or micro-cosm experiments following drying and re-flooding replicates that recorded in the ecosystem-scale experiment.

H_A: The flood pulse, specifically the changes in nitrogen and phosphorus availability in the water column, in the meso-, mini- and/or micro-cosm experiments following drying and re-flooding does not replicate that recorded in the ecosystem-scale experiment.

Table 8: A summary of the experimental scales utilized in studies concerning nutrient dynamics and/or phytoplankton productivity in wetland systems exposed to drying and re-flooding.

	Experimental scale		
Reference	**Micro-cosm***	**Mini- and Meso-cosms#**	**Ecosystem■**
Briggs et *al.*, 1985.		✓	✓
Fabre, 1988.		✓	
Boon, 1990.			✓
Briggs *et al.*, 1993.			✓
De Groot and Van Wijck, 1993.		✓	✓
Boyd and Pippopinyo, 1994.		✓	
Qiu and McComb, 1994.	✓	✓	
Qiu and McComb, 1995.	✓		
Baldwin, 1996.	✓		
Bianchi *et al.*, 1996.			✓
Qiu and McComb, 1996.		✓	
Boon et *al.*, 1997.	✓	✓	✓
Mitchell and Baldwin, 1998.	✓		
Mitchell and Baldwin, 1999.	✓		
Baldwin et *al.*, 2000.	✓		
van Oorschot *et al.*, 2000.		✓	
Watts, 2000.		✓	
Gabellone *et al.*, 2001.			✓
Sánchez-Carrillo and Alvarez-Cobelas, 2001.			✓
Turner and Haygarth, 2001.	✓		
Scholz *et al.*, 2002.			✓
James *et al.*, 2004.		✓	
TOTAL number of studies (Percentage)	8 (–30%)	10 (–37%)	9 (–33%)

*Microcosm experiments included those studies conducted in a laboratory using a small mass of sediment (e.g. between 1 – 30 grams dry weight of sediment).

#Mini- and meso-cosm experiments included those studies generally conducted on intact sediment cores or with larger masses of sediment, either in the laboratory or in a non-controlled environment.

■Ecosystem experiments included those studies where wetlands were directly sampled during dry and/or re-flooding phases.

4.2 Materials and methods

This experiment was designed to investigate whether or not the patterns of nutrient availability observed in response to wetland drying and re-flooding could be reproduced at smaller experimental scales. Accordingly, the same experiment was repeated at four different scales:

- the ecosystem scale (Figure 23 a),
- the mesocosm scale (Figure 23 b),
- the minicosm scale (Figure 23 c) and
- the microcosm scale (Figure 23 d).

The experiment at each scale comprised a 'treatment' in which sediment was desiccated and re-flooded as well as a permanently inundated 'control'. The four components of the experiment are described in detail below.

Figure 23: Experimental units for the (a) ecosystem experiment (Barmah Lake), (b) mesocosm experiment, (c) minicosm experiment and (d) microcosm experiment.

(a) Ecosystem experiment – Barmah Lake

(b) Mesocosm experiment

(c) Minicosm experiment

(d) Microcosm experiment

4.2.1 Ecosystem experiment

For the purpose of this experiment, results collected from Moira and Barmah Lakes as part of the field experiment described in detail in Chapter 2, represented the treatment and control respectively. Only nitrogen and phosphorus data collected during the first re-flooding phase (August - November 1998) were considered in this experiment. The experimental units (e.g. wetlands) were un-replicated and both wetlands were sampled repeatedly over time. Data were natural log transformed to allow direct comparison to the data collected at the other three scales (also natural log transformed).

4.2.2 Mesocosm experiment

For the purpose of this experiment, results collected from treatment 4 and control mesocosms as part of the experiment described in detail in Chapter 3, represented the treatment and control respectively. Only nitrogen and phosphorus data collected during the re-flooding phase was included in this experiment. The treatment and control were each replicated 3 times and were sampled repeatedly over-time.

4.2.3 Minicosm experiment

Minicosms were contained in a 1.2 L plastic (HDPE) bucket, 12.5 crns high and ~12.5 crns in diameter, previously cleaned with neutracon and rinsed with distilled water. A layer of sediment (~2.5 crns high) collected from Barmah Lake was placed in the bottom of each bucket (wet weight 377 ± 41 grams, n=80) and then Reverse Osmosis (RO) water was slowly added (disturbing the sediment as little as possible) until each bucket was full.

A total of 80 minicosms were randomly allocated to one of two treatments and to one of eight sampling dates and were destructively sampled. Treatments included those minicosms to remain permanently inundated throughout the experiment (controls) and those to be dried and re-flooded (treatments) and samples were taken 12 hours, 24 hours, 48 hours, 72 hours, 1 week, 2 weeks, 3 weeks and 4 weeks after re-flooding. As such, each treatment and each sampling date was replicated 5 times. Buckets were placed in a controlled temperature room (CTR) with a 12 hour:12 hour light-dark cycle and were not aerated during the experiment.

The experiment ran between 8 August and the 29 September 2000. After all buckets had been filled with RO water on the 8 August, they were allowed to settle in the CTR at 25°C for 1 week. The temperature was then increased to 40°C (±2°C) until all of the water in the treatment minicosms had evaporated and the sediment was completely desiccated. This took approximately 2½ weeks. Throughout this time, to prevent control buckets from losing a significant volume of water by evaporation, RO water was added on a daily basis to maintain water levels at full capacity. On 1 September RO water was used to re-flood the sediment in the treatment minicosms. The temperature in the CTR was dropped to 18°C (±1°C) for the remainder of the experiment.

The appropriate minicosms were destructively sampled at 12 hours, 24 hours, 48 hours, 72 hours, 1 week, 2 weeks, 3 weeks and 4 weeks after re-flooding. The 'water column' in each minicosm identified for sampling was decanted and one sub-sample was taken for analysis of total nitrogen and phosphorus concentrations and immediately frozen, while a second sub-sample taken for analysis of dissolved inorganic nitrogen and phosphorus concentrations was immediately filtered (0.45µm filter paper) and then frozen. Samples for nutrient analysis were also taken of the RO water used to re-flood the treatment mesocosms on 1 September. Water samples for nutrient analysis were stored in LDPE sample bottles which had been previously washed with neutracon and rinsed in Milli-Q (MQ) water. Nutrient samples were analysed as per description in Chapter 2 (Section 2.2).

4.2.4 Microcosm experiment

Microcosms were contained in 36 ml glass test-tubes, 15 crns high and 2 crns in diameter, previously cleaned with neutracon and rinsed with MQ water. A layer of sediment (~ 4 crns high) collected from Barmah Lake was placed in the bottom of each test-tube (wet weight 13.4 ± 1.5 grams, n=120) and then Reverse Osmosis (RO) water was slowly added (disturbing the sediment as little as possible) until each test-tube was full.

Each test-tube could only provide enough volume for either dissolved nutrient analysis or total nutrient analysis. Therefore, a total of 120 test-tubes were randomly allocated to one of two treatments, to one of six sampling dates and to either total or dissolved nutrient analysis, and were destructively sampled. Treatments included those microcosms to remain permanently inundated throughout the experiment

(controls) and those to be dried and re-flooded (treatments) and samples were taken 12 hours, 24 hours, 72 hours, 1 week, 1½ weeks and 2 weeks after re-flooding. As such, each treatment and each sampling date was replicated 5 times. Microcosms were placed in a controlled temperature cabinet (CTC) with a 12 hour: 12 hour light-dark cycle.

The experiment ran between 15 August and the 25 December 2000. After all test-tubes had been filled with RO water on the 15 August, they were allowed to settle in the CTC at 25°C for 1 week. The temperature was then increased to 40°C (±2°C) until all of the water in the treatment microcosms had evaporated and the sediment was completely desiccated. This took approximately 3½ months. Throughout this time, control microcosms also lost water by evaporation and RO water was added regularly to maintain water levels at full capacity. To prevent the control microcosms from becoming stagnant they were aerated periodically, for short periods of time (~12 – 24 hours), during this drying process. The last period of aeration was completed 24 hours before the treatment microcosms were re-flooded. On 11 December RO water was used to re-flood the sediment in the treatment test-tubes. The temperature in the CTC was dropped to 18°C (±1°C) for the remainder of the experiment.

The appropriate microcosms were destructively sampled at 12 hours, 24 hours, 72 hours, 1 week, 1½ weeks and 2 weeks after re-flooding. The water phase in each test-tube was decanted into a sample bottle and those for analysis of total nitrogen and phosphorus concentrations were immediately frozen, while those taken for analysis of dissolved inorganic nitrogen and phosphorus concentrations were filtered (0.45µm filter paper) and then frozen. Samples for nutrient analysis were also taken of the RO water used to re-flood the treatment test-tubes on 11 December. Water samples for nutrient analysis were stored in LDPE sample bottles which had been previously washed with neutracon and rinsed in MQ water. Nutrient samples were analysed as per description in Chapter 2 (Section 2.2).

4.2.5 Data analysis

Although the experiments conducted at each of the four scales were designed to be as similar as possible, there were some inherent differences between the four experiments. For example, the experiments conducted at the ecosystem and mesocosm scales had to be sampled repeatedly throughout the experiment and they were exposed to seasonal changes in temperature, whereas the

experiments conducted at the minicosm and microcosm scales had to be destructively sampled and were exposed to constant temperatures after re-flooding. Furthermore, the ecosystem scale experiment could not be replicated, whereas replication could be increased as the experimental scale was reduced.

Consequently, it was not possible to directly compare the effect of experimental scale in a statistical test. Rather, the results collected at each experimental scale were analysed using statistical tests where appropriate, to indicate changes occurring in nutrient availability in response to treatment and time. For example, the minicosm experiment and the microcosm experiment were each analysed using two-way replicated ANOVAs (following natural log transformation of data to meet the assumption of normality), while the mesocosm experiment was analysed using a general linear mixed model technique (see Chapter 3) and the data collected for the ecosystem-scale experiment was not analysed by any statistical test (see Chapter 2). Using the results of each individual statistical test (where performed) as a guide, the patterns in nutrient availability observed at each scale were simply compared to highlight the similarities and differences. A natural log transformation of data from the ecosystem-scale experiment allowed direct comparison with data of the other three experiments.

It is also noted that the ecosystem-scale experiment compared the effects of dryinglre-flooding in Moira Lake with the effects of permanent inundation in Barmah Lake while the meso-, mini- and microcosm experiments studied the effects of dryinglre-flooding and permanent inundation using sediments collected from Barmah Lake only. Sediments from Moira Lake were not collected for use in the smaller-scale experiments as they had been exposed to complete desiccation on two previous occasions, which may have influenced results (e.g. Baldwin *et al.*, 2000). Therefore, in comparing the results of the four different experiments, I am making an underlying assumption that dryinglre-flooding of Barmah Lake sediment in the smaller-scaled experiments produces the same response as would be recorded if Moira Lake sediment was used in these experiments.

4.3 Results

A brief summary of the relevant results collected in the Ecosystem and Mesocosm experiments are outlined below, along with more comprehensive reviews of the results collected in the Minicosm and Microcosm experiments. A more detailed presentation and discussion of results from the Ecosystem and Mesocosm experiments are provided in Chapters 2 and 3, respectively. Finally, a comparison of results collected from each of the four experimental scales is undertaken.

4.3.1 Ecosystem experiment

Re-flooding of Moira Lake in 1998 resulted in a large release of NH_4^+, NO_X^- and TN from the previously desiccated sediments, as indicated by an increase in the respective concentrations in the water column in the first 24 hours after re-flooding compared to Barmah Lake (Figure 24 a-c). No such increase in PO_4^{3-} or TP concentrations was recorded in the 'treatment' wetland upon re-flooding (Figure 25 a-b).

While NH_4^+ concentration in Moira Lake gradually decreased over the three months following re-flooding, both NO_X^- and TN concentrations remained high for approximately the first month but were depleted when the lake was sampled three months after re-flooding (Figure 24 a-c). The DIN pool in the treatment wetland primarily consisted of NO_X^- in the three months after re-flooding. During this period, the N concentrations in the control wetland, Barmah Lake, remained low and varied little (Figure 24 a-c).

In contrast to the changes that occurred to the N-cycle as a result of the drying and re-flooding process, in general, PO_4^{3-} and TP concentrations recorded in Moira Lake did not differ from those recorded in Barmah Lake on most sampling dates after re-flooding (Figure 25 a-b). Three months after re-flooding commenced the concentrations of NH_4^+, NO_X^-, TN, PO_4^{3-} and TP in Moira Lake were not significantly different to those in Barmah Lake.

4.3.2 Mesocosm experiment

Re-flooding of treatment mesocosms resulted in a significant release of NH_4^+, NO_X^-, TN and PO_4^{3-} from the previously desiccated sediments, as indicated by an increase in the respective concentrations in the water column of these mesocosms in the first 12 hours of re-flooding compared to concentrations in the water used to re-flood the mesocosms (Figure 24 d-f, Figure 25 c). A small, but not significant, increase in TP concentration was also recorded in the treatment upon re-flooding (Figure 25 d).

Following the initial release of nutrients from the dried sediments in treatment mesocosms after inundation, the NO_X^- concentration gradually decreased, the NH_4^+ concentration increased further during the first week and was then rapidly depleted, and the TN concentration remained high for approximately two weeks after re-flooding and then decreased (Figure 24 d-f). On most sampling dates after re-flooding the DIN pool in the treatment mesocosms consisted of roughly equal proportions of NH_4^+ and NO_X^-. During this period, the N concentrations in the control mesocosms remained low and varied little (Figure 24 d-f).

In contrast to the changes that occurred to the N-cycle as a result of the drying and re-flooding process, in general the PO_4^{3-} and TP concentrations recorded in the treatment did not differ from those recorded in the control on most sampling dates after re-flooding (Figure 25 c-d). Three months after inundation took place the concentrations of NH_4^+, NO_X^-, TN, PO_4^{3-} and TP in the treatment were not significantly different to those in the control.

4.3.3 Minicosm experiment

The results of the two-way ANOVA indicated that a significant difference between treatments and between sampling dates existed for all nitrogen and phosphorus parameters measured (Table 9). Furthermore, a significant interaction between the two factors (treatment and sampling date) affected all of the nutrient concentrations recorded, except for total phosphorus.

Table 9: Results of two-way ANOVA (with replication) for NH_4^+, NO_X^-, TN, PO_4^{3-} and TP concentrations following re-flooding of minicosms. (* = significant difference).

		P – value				
Source of Variation	**Degrees of Freedom**	**NH_4^+**	**NO_x^-**	**TN**	**PO_4^{3-}**	**TP**
Treatment	1	<.001*	<.001*	<.001*	<.001*	<.001*
Sampling time	7	0.036*	<.001*	<.001*	<.001*	0.026*
TreatmentxTime Interaction	7	<.001*	<.001*	<.001*	<.001*	0.714

Drying and re-flooding of Barmah Lake sediments resulted in a significant release of NH_4^+, TN and TP into the water column of treatment minicosms, as indicated by an increase in the respective concentrations in the first 12 hours of re-flooding compared to the concentrations in the water used to re-flood the minicosms (Figure 24 g and I, Figure 25 f). No such increase in NO_X^- or PO_4^{3-} concentrations was recorded in the treatment minicosms upon re-flooding (Figure 24 h and Figure 25 e).

Following the initial release of nutrients in the treatment minicosms upon re-flooding, the data suggest that both TN and NH_4^+ continued to be released from the sediment throughout the 30 days following re-flooding, most rapidly in the first 72h and at a slower rate after that (Figure 24 g and i). NO_X^- remained low during the first two weeks after re-flooding and then increased significantly over the remaining 2 weeks of the experiment as NH_4^+ was converted into NO_X^- via nitrification (%DIN remained between 55 – 70%, % NO_X^- increased from < 5% to 30% and % NH_4^+ decreased from ~65% to 30%) (Figure 24 g, h). There was no evidence to suggest that denitrification was occurring in the treatment minicosms during the 30 days following re-flooding. On most sampling dates after re-flooding, the DIN pool in treatment minicosms consisted primarily of NH_4^+.

In contrast to the treatment minicosms, NH_4^+ concentration in the control was depleted just 72 hours after inundation and remained low for the duration of the experiment (Figure 24 g). NO_X^- and TN concentration, and %DIN, progressively (and significantly) decreased in the control following the first

week of sampling (Figure 24 h-i), hence the significant interaction between treatment and sampling date highlighted by the ANOVA for these parameters (Table 9). On most sampling dates after re-flooding, the DIN pool in the controls consisted primarily of NO_X^-.

While re-flooding dried sediments did not promote an initial release of PO_4^{3-} into the water column of treatment minicosms, a significant increase in the PO_4^{3-} concentration was recorded in the treatment between 72 hours and 2 weeks after re-flooding (Figure 25 e). During this period, PO_4^{3-} represented an increasing proportion of the TP pool, from less than 5% up to ~80%. Therefore, it is most likely that the increased PO_4^{3-} concentration was due to mineralization of a large proportion of the organic phosphorus pool (rather than a release from the sediments). In addition, the TP concentration decreased significantly during this period (Figure 25 9, suggesting that a proportion of the PO_4^{3-} being produced via mineralization was then being adsorbed by the sediments or utilized by benthic microbes.

The TP concentration in the control minicosms was significantly higher than in the treatment, however PO_4^{3-} represented a much smaller proportion of the TP pool in the control (e.g control vs treatment: up to ~25% vs ~80% respectively) (Figure 25 e-f). While there was evidence that mineralization of the TP pool was also occurring in the control minicosms in the 30 days after re-flooding, the data suggest that only a small proportion of the TP pool was being mineralized compared to that in the treatments.

At the end of the experiment, 30 days after re-flooding of treatment minicosms took place, NH_4^+, NO_X^- and TN concentrations were significantly higher in the treatment than in the control (Figure 24 g-i). The PO_4^{3-} concentration in the treatment was not significantly different to the control at the end of the experiment while the TP concentration in the control was significantly higher than in the treatment (Figure 25 e-f).

4.3.4 Microcosm experiment

The results of the two-way ANOVA indicated that a significant difference between treatments existed for NH_4^+ and TN concentrations and a significant difference between sampling dates existed for all nutrient parameters measured except PO_4^{3-} (Table 10). Furthermore, a significant interaction between the two factors (treatment and sampling date) affected NH_4^+, TN and PO_4^{3-} concentrations.

Table 10: Results of two-way ANOVA (with replication) for NH_4^+, NO_X^-, TN, PO_4^{3-} and TP concentrations following re-flooding of the treatment microcosms. (* = significant difference).

		P – value				
Source of Variation	**Degrees of Freedom**	**NH_4^+**	**NO_x^-**	**TN**	**PO_4^{3-}**	**TP**
Treatment	1	<.001*	0.681	<.001*	0.217	0.068
Sampling time	5	<.001*	0.028*	<.001*	0.373	<.001*
TreatmentxTime Interaction	5	<.001*	0.087	<.001*	0.038*	0.659

Re-flooding of treatment microcosms resulted in a significant release of TN, PO_4^{3-} and TP from the previously desiccated sediments, as indicated by an increase in the respective concentrations in the water column in the first 12 hours of re-flooding compared to concentrations in the water used to re-flood the test-tubes (Figure 24 l and Figure 25 g-h). No initial release of NH_4^+ or NO_X^- from dried sediments was recorded upon re-flooding in the treatment microcosms (Figure 24 j-k).

A significant increase in NH_4^+ concentration was recorded in the water column of treatment test-tubes between 24 hours – 1 week after re-flooding (Figure 24 j) and this corresponded with a large increase in %DIN, from < 2% up to ~50%. This suggests the increase may have been due to either a delayed release from sediments or due to mineralization of the TN pool, but clearly the increased availability of NH_4^+ did not stimulate the activity of bacteria responsible for nitrification (as was observed in the minicosm experiment). Nonetheless, the NH_4^+ was depleted between 1 – 1½ weeks after re-flooding (Figure 24 j), most probably assimilated by the benthic microbial community. In the control, NH_4^+ concentration did not change significantly during the first week of sampling, however a significant

decrease in NH_4^+ concentration was also recorded in the control between 1 – 1½ weeks and similarly, there was no evidence to suggest that the NH_4^+ was being converted to NO_X^- via nitrification in these microcosms (Figure 24 j-k).

On average, the NO_X^- concentration in treatment microcosms was not significantly different to that in the control microcosms during the 2 week sampling period (Figure 24 k). In fact, NO_X^- concentration was very low in the water column of both treatment and control test-tubes on most sampling dates, but increased significantly on the last sampling date, 2 weeks after re-flooding of the treatment microcosms (Figure 24 k). The reason for this increase in NO_X^- concentration is unclear.

Following the initial release of TN following re-flooding of dried Barmah Lake sediments, TN concentration continued to increase significantly in the water column of treatment microcosms during the first 72 hours of re-flooding (Figure 24 l). TN concentration in the treatment did not change significantly between 72 hours and 1½ weeks after re-flooding, despite the large decrease in NH_4^+ concentration in treatment microcosms (–0.7 $mg.L^{-1}$) during this period (Figure 24 j, l). Given that NO_X^- concentrations did not change significantly during this period, these results suggest that TN continued to be released from the sediments, supplementing the TN pool and maintaining the concentration in the water column. In fact, between 1½ – 2 weeks after re-flooding TN concentration in the treatment microcosms increased significantly, possibly supporting the suggestion that TN was still being released from the sediments in these microcosms (Figure 24 l). In the control, changes in TN concentration during the two week sampling period were different to those recorded in the treatment (Figure 24 l), hence the significant interaction highlighted by the ANOVA. TN concentration did not change significantly in the control during the first 72 hours of sampling, then decreased significantly between 72 hours – 1 week and proceeded to increase significantly over the remaining two sampling dates (Figure 24 l).

While both PO_4^{3-} and TP were released from the dried sediments upon re-flooding (e.g. during the first 12 hours), the TP concentration recorded in the treatment did not differ significantly from those in the control for the remainder of the experiment (Figure 25, g-h). In both treatment and control microcosms the TP concentration decreased significantly between 72 hours and 1 week after re-flooding, and then

progressively increased over the remaining sampling dates (1½ – 2 weeks after re-flooding) (Figure 25 h). Similarly, on average over the duration of the sampling period, PO_4^{3-} concentrations in the treatment were not significantly different to the control, and did not change significantly over time (Figure 25 g). However, an increase in the PO_4^{3-} concentration in the control between 24 -72 hours and a simultaneous decrease in the treatment, and vice versa between 72 hours and ▮ week, resulted in significantly higher PO_4^{3-} concentrations in the control (compared to the treatment) when sampled 72 hours after re-flooding, hence the significant interaction factor indicated by the ANOVA.

Interestingly, the decrease in TP concentration recorded in both treatment and control microcosms between 72 hours and 1 week after re-flooding corresponded with a significant increase in PO_4^{3-} concentration in treatment microcosms, but a significant decrease in PO_4^{3-} concentration in control microcosms (Figure 25 g-h). In addition, the proportion of the TP pool represented by PO_4^{3-} increased greatly in the treatment from –5% up to **-85%** and also in the control from –25% up to –75% between these sampling dates. This may indicate that mineralization of the TP pool was occurring in both the treatment and control microcosms, although was being more rapidly assimilated by microbes and/or adsorbed in the control microcosms.

Figure 24 (a – f): Average NH_4^+, NO_X^- and TN concentrations (natural log transformed) recorded in Treatments (—●—) and Controls (---□---) after re-flooding in the (a – c) ecosystem (error bars = ± 2 x standard error) and (d – f) mesocosm experiments (error bars = 2 x standard error). ● = water used to re-flood experimental units.

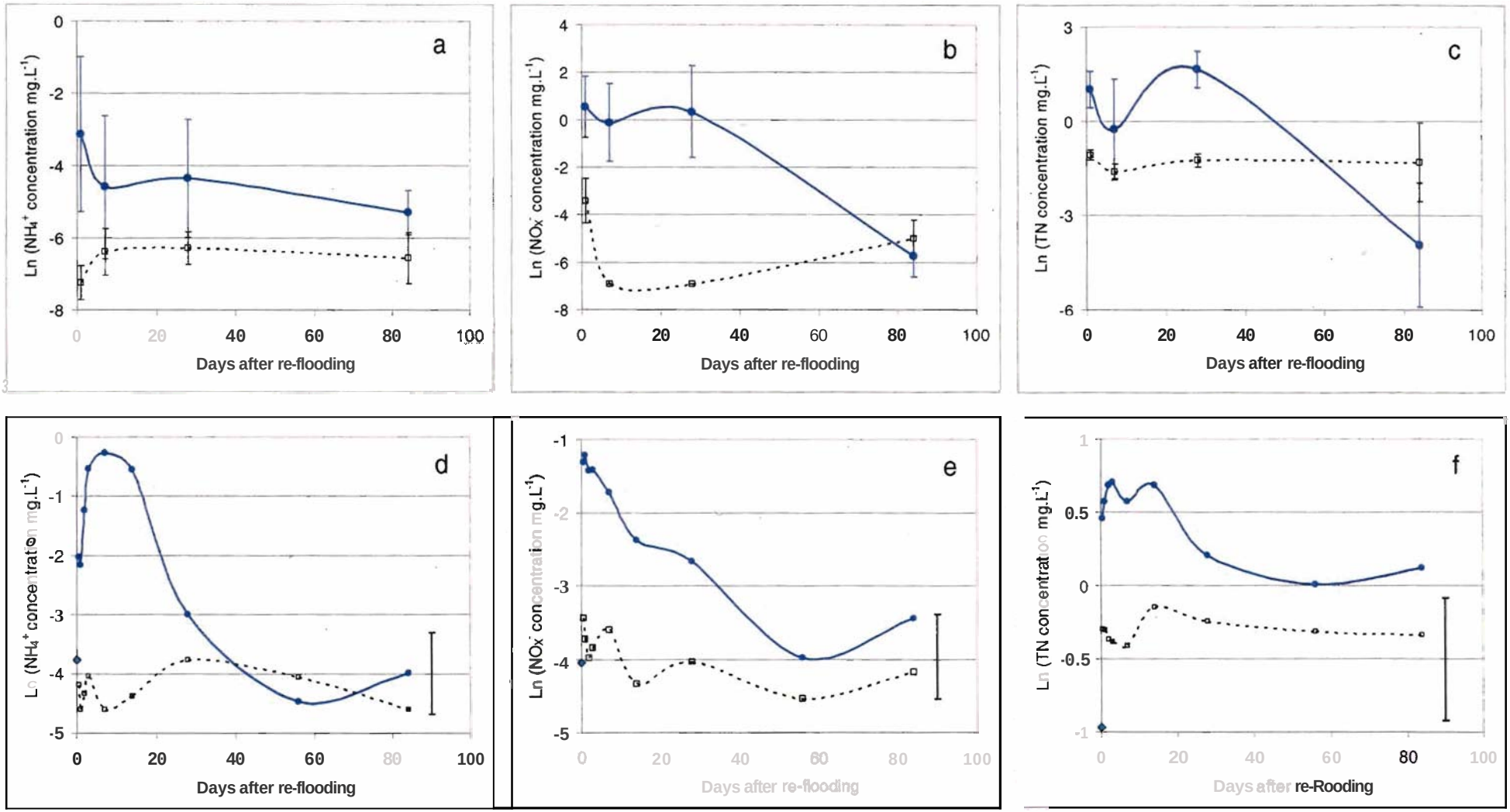


Figure 24 (g – l): Average NH_4^+, NO_X^- and TN concentrations (natural log transformed) recorded in Treatments (—●—) and Controls (---□---) after re-flooding in the (g – i) minicosm and (j – l) microcosm experiments. ◆ = water used to re-flood experimental units. Error bars represent 2 x standard error.

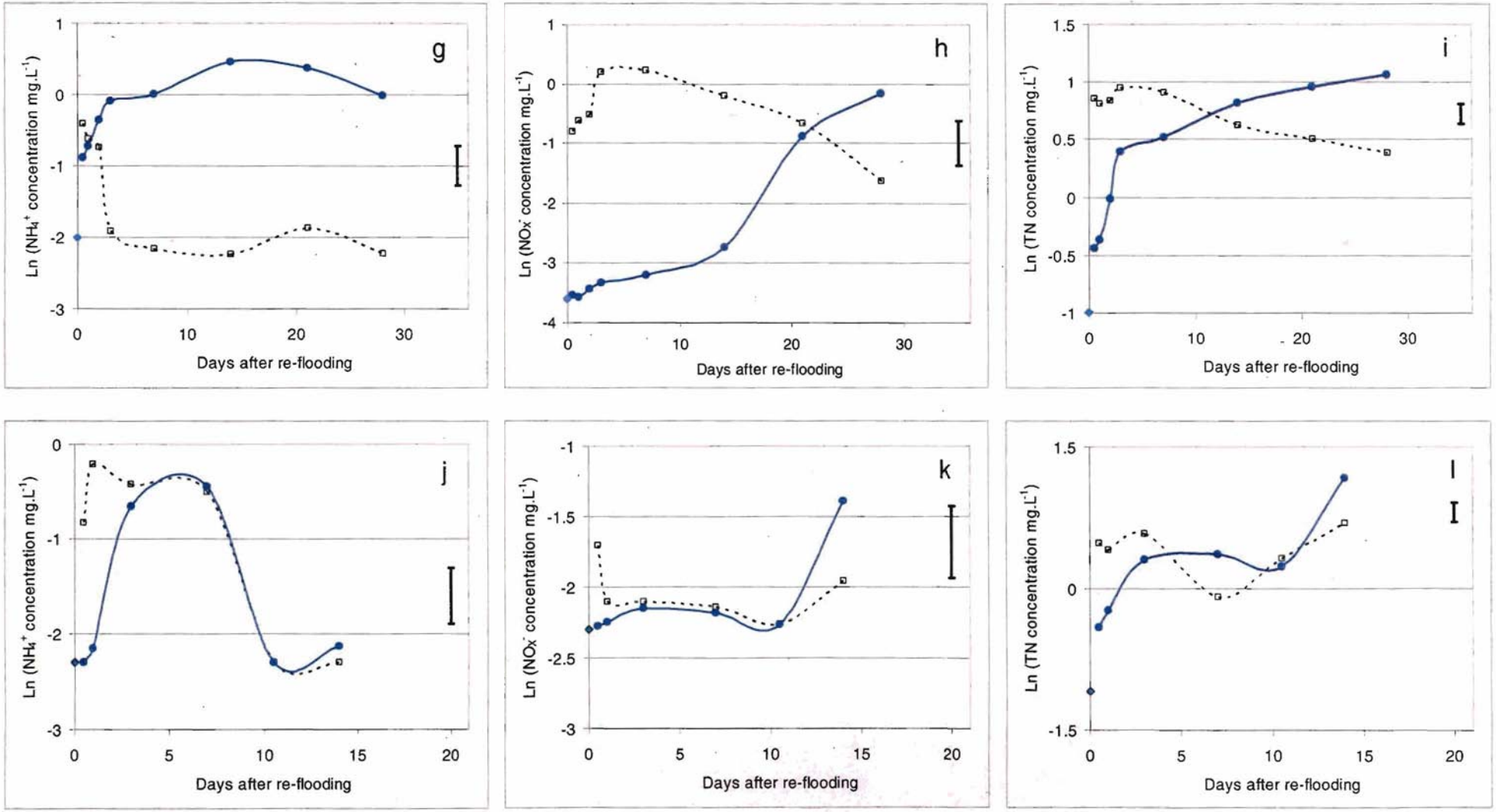
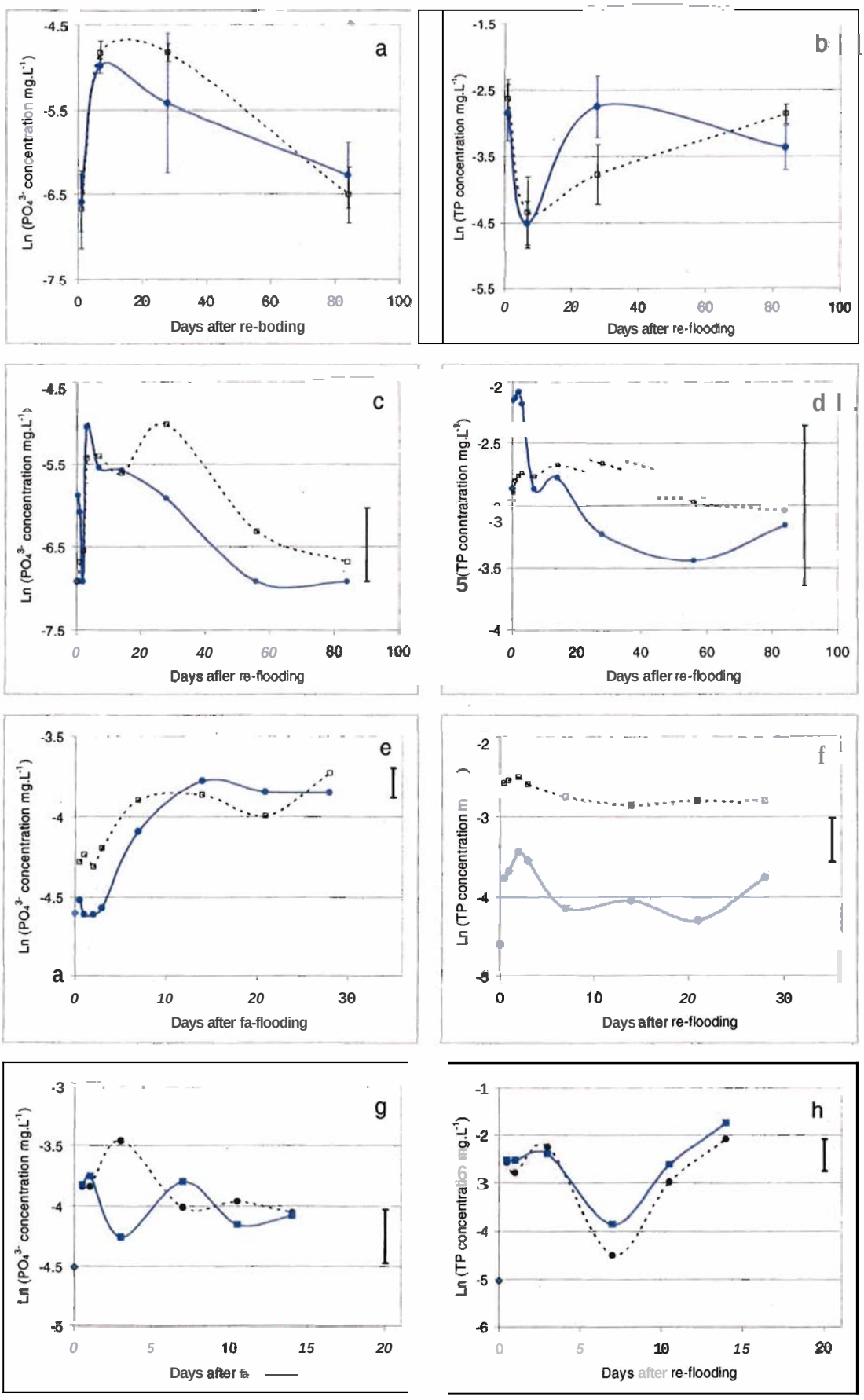


Figure 25 (a – h): Average PO_4^{3-} and TP concentrations (natural log transformed) recorded in Treatments (-●-) and Controls (--□--) after re-flooding in the (a – b) ecosystem, (c – d) mesocosm, (e – f) minicosm and (g – h) microcosm experiments. ● = water used to re-flood experimental units. Error bars represent 2 x standard error (except on graphs a and b where error bars represent ± 2 x standard error).



4.3.5 Comparison between experimental scales

The patterns and processes of interest, including the release of nutrients from sediments following drying and re-flooding, changes in nutrient concentrations after re-flooding and the processes driving these changes, and differences between treatments and controls were identified and compared amongst the four experimental scales. The results are summariied in Table 11.

Table 11: A comparison of patterns and processes observed in experiments conducted at the ecosystem, mesocosm, minicosm and microcosm scales. (T = treatment, C = control; ✓ = yes, - = no or data not available).

		Experimental Scale			
Parameter		**Ecosystem**	**Mesocosm**	**Minicosm**	**Microcosm**
Significant release from dried sediments upon re-flooding (e.g. within 12-24h)	NH_4^+	✓	✓	✓	-
	NO_X^-	✓	✓	-	-
	TN	✓	✓	✓	✓
	PO_4^{3-}	-	✓	-	✓
	TP	-	-	✓	✓
Range of concentrations during experiment ($mg.L^{-1}$) (incl. T's and C's)	NH_4^+	0 – 1.37	0 – 1.09	0 – 2.16	0 – 1.16
	NO_X^-	0 – 6.00	0 – 0.51	0. 23– 1.87	0 – 0.89
	TN	0 – 8.67	0.2 – 2.58	0.58 – 3.52	0.55–3.25
	PO_4^{3-}	0–0.019	0–0.021	0–0.018	0–0.082
	TP	0.003–0.19	0–0.18	0.01–0.16	0.008–0.2
After re-flooding: Treatment *vs* Control	NH_4^+	T>C	T>C	T>C	C>T
	NO_X^-	T>C	T>C	C>T	T=C
	TN	T>C	T>C	C>T	C>T
	PO_4^{3-}	T=C	T=C	T=C	T=C
	TP	T=C	T=C	C>T	T=C
DIN pool in Treatment	$NH_4^+ > NO_X^-$			✓	✓
	$NH_4^+ = NO_X^-$		✓		
	$NO_X^- > NH_4^+$	✓			
After re-flooding: evidence of process in T	Nitrification			✓	
	N-mineralization		✓		
	P-mineralization			✓	✓

Table 11 continued.

Parameter		Experimental Scale			
		Ecosystem	Mesocosm	Minicosm	Microcosm
After re-flooding: time taken for T to converge on C	NH_4^+	~1 mo	~1 mo	-	~72 h
	NO_X^-	1–3 mo	~2 mo	~3 wk	0
	TN	1–3 mo	~1 mo	~2 wk	~72 h
	PO_4^{3-}	< 24 h	~48 h	~1 wk	~1 w
	TP	< 24 h	0	-	~12 h
Coefficient of Variation – Treatments *	NH_4^+	-	99 ± 57	17 ± 6	69 ± 79
	NO_X^-	-	84 ± 35	31 ± 24	66 ± 37
	TN	-	20 ± 7	11 ± 3	16 ± 9
	PO_4^{3-}	-	73 ± 68	47 ± 63	68 ± 25
	TP	-	31 ± 17	43 ± 26	49 ± 30
Coefficient of Variation – Controls *	NH_4^+	-	92 ± 76	107 ± 62	130 ± 108
	NO_X^-	-	100 ± 49	50 ± 34	77 ± 87
	TN	-	69 ± 6	15 ± 5	15 ± 10
	PO_4^{3-}	-	98 ± 73	40 ± 24	46 ± 33
	TP	-	90 ± 16	36 ± 13	45 ± 33
Plant community established during experiment	Treatment	✓	✓	-	-
	Control	✓	✓	-	-

* Average ± standard deviation of CV calculated for replicates of each parameter on each sampling date. Note that CV could not be calculated for the ecosystem scale as the treatment and control were not replicated at this scale.

Red highlight = lowest CV ; Blue Highlight = highest CV

4.4 Discussion

4.4.1 Ecosystem vs smaller-scale experiments

Drying and re-flooding of Moira Lake resulted in a post-flood pulse in N availability, in contrast to the consistently low N concentrations recorded in the permanently inundated Barmah Lake. The high NH_4^+ concentrations recorded after re-flooding were gradually depleted, while high NO_X^- and TN concentrations were sustained for at least one month following re-flooding before becoming depleted Data collected in the ecosystem experiment was not detailed enough to allow identification of underlying processes such as mineralization and nitrification (possibly influencing the patterns observed), a commonly reported constraint associated with large-scale experiments that highlights one of the primary reasons for using meso- and micro-cosms in ecological research (e.g. Carpenter, 1996).

No such pulse in P availability was recorded after re-flooding in Moira Lake, in fact PO_4^{3-} and TP concentrations did not vary greatly to those recorded in the permanently inundated wetland, where P concentrations were also consistently low. This may be a reflection of the relatively low, possibly limiting, concentrations of bio-available P in this system. While TP concentrations recorded in Barmah Lake would suggest the wetland may be classified as mesotrophic (e.g. with moderate nutrient concentrations and supporting moderate levels of productivity) (e.g. http://www.nrm.gov.au/monitoring/indicators/wetlands/nutrients.html, 2005), the concentrations of PO_4^{3-} recorded in the water column of Barmah Lake were extremely low ($-3 \pm 3\ \mu g.L^{-1}$). When compared with measurements of bio-available phosphorus reported for other billabongs and wetlands on the River Murray floodplain, it is clear that the concentrations of bio-available P recorded in Barmah Lake were indeed very low and most probably limiting to biotic processes in the wetland (Table 12).

Table 12: PO_4^{3-} and **TP** concentrations measured in the study site (Barmah Lake) and reported for other wetlands located on the River Murray floodplain.

Reference	Wetland Location	PO_4^{3-} conc ($mg.L^{-1}$)	TP conc ($mg.L^{-1}$)
Chapter 2	Barmah Lake, MDB	0.003 ± 0.003 (range: 0 – 0.013)	0.061 ± 0.036 (range: 0.003 – 0.189)
Briggs *et al.* (1985)	2 wetlands, MDB	0.11 -0.13 (averages)	
Boon (1990)	1 billabong, MDB	<0.005 – 0.158	0.14 – 1.73
Boon *et al.* (1990) 'The Murray' Ch 11.	5 billabongs, MDB	<0.005 – 0.73	0.015 – 2.61

While there are no examples in the literature of ecosystem-scale studies which have compared **N** and P availability in ephemeral vs permanently inundated wetlands, a number of ephemeral wetlands have been monitored over a drying/re-flooding cycle (Briggs *et al.*, 1985; Scholz et *al.*, 2002). These surveys recorded similar, although not identical, changes in nutrient availability following drying and re-flooding to those recorded in Moira Lake. For example, Briggs *et al.* (1985) reported higher nitrate loads during the re-flooding phase (compared to the dry phase) in two ephemeral wetlands, however also recorded higher phosphate concentrations following re-flooding. In their survey of the Menindee Lakes, a wetland complex on the floodplain of the Darling River in western New South Wales, Scholz et *al.* (2002) reported that the post-re-flooding phosphorus concentrations in Lakes Malta and Balaka (the only two lakes to undergo complete drying) closely reflected that of the inflowing flood waters, with no apparent release of P from the previously exposed sediments into the water column. However, they also found that **N** concentrations in the two lakes after re-flooding did not increase above that in the inflowing flood waters. As concluded in the field experiment described in Chapter 2, considerable variation in the 'flood pulse' may be a feature of ephemeral floodplain wetlands.

As observed in Moira Lake, the P concentrations recorded after drying and re-flooding in treatment **mesocosms** did not differ significantly to the concentrations in mesocosms that were kept permanently inundated. Increased NH_4^+, NO_X^- and TN concentrations were also recorded in the mesocosms after drying and re-flooding, and the increased NH_4^+ concentration was depleted more rapidly (btw 2w – 1m) than the increased NO_X^- concentrations (btw 1m – 2m), as in Moira Lake. However, in contrast to the patterns observed in Moira Lake after drying and re-flooding, the increased

TN pool in the treatment mesocosms was depleted more rapidly (btw I w – 2w) than the increased concentrations of NH_4^+ and NO_X^-, and the range of NO_X^- and TN concentrations recorded in the treatment mesocosms (0 – 0.51 and 0.2 – 2.58 $mg.L^{-1}$ respectively) were markedly lower than those recorded in Moira Lake after re-flooding (0 – 6.00 and 0 – 8.67 $mg.L^{-1}$).

The only example of a comparable mesocosm experiment published in the literature, using similar sized experimental units and run over a similar duration, is that reported by Briggs et *al.* (1985). Their experiment monitored changes in NO_X^- and PO_4^{3-} concentrations following the re-flooding of sediments that had been dried in ***situ*** in two ephemeral floodplain wetlands, but did not incorporate a permanently inundated control. Patterns in both mesocosm experiments were consistent when changes in the relevant parameters, at corresponding sampling intervals, were compared. Namely, NO_X^- and PO_4^{3-} concentrations increased considerably in the 3-4 days after re-flooding and then progressively decreased, compared to concentrations recorded in the water used to re-flood the mesocosms.

The results of the **minicosm** experiment also demonstrated consistencies with similarly scaled experiments. For example, re-flooding of dried wetland sediments resulted in a large increase in NH_4^+ concentration in both the minicosm experiment described in this chapter and the experiment conducted by Qiu and McComb (1996). In both experiments the increased availability of NH_4^+ in the water column also stimulated nitrification (e.g. the consumption of NH_4^+ and production of NO_X^-) following an initial lag phase, although the lag phase was shorter in the experiment conducted by Qiu and McComb (1996) (i.e. ~4 days) compared to this minicosm experiment (~14 days).

Notably, of the four experimental scales incorporated in this study, the minicosm experiment produced the least variable data set (Table 11, Section 4.3.5). This is consistent with claims made in the scientific literature, that by enabling greater replication and control of relevant variables, small-scale laboratory experiments are able to reduce variability of data, .increasing precision and in turn improving statistical power – seemingly generating a greater insight and understanding of the processes underlying the particular patterns of interest at larger scales (e.g. Wiens, 1989; Carpenter, 1996; Petersen *et al.*, 1997; Culp *et al.*, 2000; Ahn and Mitsch, 2002). If then, the aim of conducting a small

scale experiment is to decrease variability in the data collected, to produce patterns and processes that are easily identified and interpreted, and to improve the power of statistical tests applied to the data – the results from this multi-scale study would suggest that the minicosm experiment was most suitable for this purpose.

However, while the minicosm experiment produced relatively precise data, easily identifiable patterns and underlying processes (particularly for N parameters), and results that were consistent with those of similarly scaled studies, changes in nutrient availability after re-flooding in the minicosms differed markedly to the changes observed in the ecosystem-scale experiment. While P concentrations in Moira Lake after drying and re-flooding did not differ significantly to the concentrations in the permanently inundated Barmah Lake, PO_4^{3-} and TP concentrations following dryinglre-flooding in treatment minicosms were significantly reduced in comparison to concentrations recorded in the permanently inundated minicosms. Furthermore, changes in NH_4^+, NO_X^- and TN availability in response to drying/re-flooding and permanent inundation in the minicosm experiment were essentially the reverse of the patterns observed in the ecosystem-scale experiment.

The **microcosm** experiment was also unable to reproduce the patterns observed at the ecosystem-scale. In contrast to the observations in Moira Lake, drying and re-flooding in the microcosms did not result in any initial release of NH_4^+ or NO_X^- from the sediments into the water column. In a similar laboratory-based investigation into the effects of sediment desiccation on the N-cycle, Mitchell and Baldwin (1999) also reported that a 'flush' of mineral N was absent upon rewetting of desiccated sediments, in contrast to previously reports in the literature (e.g. Briggs et *al.*, 1985; Qiu and McComb, 1996). Furthermore, changes in N concentration recorded in the treatment microcosms after drying and re-flooding differed greatly to those changes recorded in Moira Lake. Also, N concentrations in the water column of Moira Lake were considerably higher than in the permanently inundated Barmah Lake (during the first month of the re-flooding phase), while in the microcosm experiment N concentrations in the treatments were generally either equal to, or less than, the concentrations in the controls.

As in the meso- and mini-cosm experiments, the N concentrations recorded in the microcosm experiment were substantially lower than those recorded in the ecosystem-scale experiment (see Table 11, Section 4.3.5). It is possible that the elevated N concentration recorded in Moira Lake after re-flooding (compared to the container experiments) was related to the large biomass of carp present in the Lake during the drying process. An estimated 5 000 tonne of carp (*Cyprinus carpio*) were trapped in the wetland (by the regulators) as it dried over the summer of 1997198 (Johnson, 1998) and the death of these fish may have represented a large N-source, potentially returned to the water column upon re-flooding. Clearly, no such accumulation of organic biomass (and potential N-source) occurred in the small-scale experiments during the dry phase, possibly reducing the quantity of N released into the water column after re-flooding.

While no flush of mineral N occurred after re-flooding in the treatment microcosms, sediment desiccation did result in a significant release of both PO_4^{3-} and TP into the water column (compared to concentrations in the water used to re-flood the microcosms) upon re-flooding. Other similarly scaled experiments have produced variable results, some consistent and others inconsistent with the results of the microcosm experiment described in this chapter. For example, in a study of 29 lowland permanent grassland soils (England and Wales), Turner and Haygarth (2001) also reported that rewetting of desiccated sediments increased the P released by between 185 - 1,900%. Conversely, dried sediment collected from Chaffey Reservoir (NSW, Australia) showed no release of reactive-P upon re-flooding, even under anoxic conditions (Mitchell and Baldwin, 1998). However, the changes in P availability recorded in the microcosm experiment were actually similar to those recorded in the ecosystem experiment, in that P concentrations in the treatments did not differ significantly to those in the controls in both experiments.

It is noted that the decrease in experimental scale, from minicosm to microcosm, did not further reduce the variability of the data. Skelly and Kiesecker (2001) tested the hypothesis that small-scale laboratory experiments, by allowing greater replication and control of relevant variables, can reduce the variability of data (increasing precision and therefore improving statistical power). In their review of 52 studies, comparing field, mesocosm and laboratory experiments on the growth performance of larval anurans they found that the reduction of experimental scale yielded just a small increase in the

number of replicates used per treatment, and that the reduction of experimental scale did not result in increased precision of data collected.

4.4.2 In summary

None of the smaller-scaled experiments included in this investigation were able to replicate the specific response to drying/re-flooding recorded at the ecosystem scale, therefore the alternative hypothesis proposed in section 4.1 was supported. However, the mesocosm experiment did produce results that were more similar to those at the ecosystem scale than those produced by the micro- and mini-cosm experiments. This supports previous findings by a number of other multi-scale ecological experiments, where larger sized mesocosm experiments were able to mimic whole-lake responses while small laboratory-based experiments were not (e.g. Schindler, 1977, 1987; Sarnelle, 1997).

Samples taken at the ecosystem scale measured not only the direct effect of the manipulation (e.g. drying/re-flooding vs permanent inundation) on nutrient availability, but also incorporated the indirect effects on nutrient availability of the manipulation on all other biotic and abiotic components of the wetland. Samples taken at the smaller experimental scales incorporated varying degrees of this complexity, and the differences in the degree of complexity reproduced at each scale may account for the inability of the smaller scale experiments to replicate the patterns observed in the wetland ecosystem.

Similar proposals have been made in population and community ecology where it is believed that reductions in spatial heterogeneity, an artifact of the containerization process, is one of the reasons why experiments conducted over small spatial extents produce results that are inconsistent with natural phenomena (e.g. Cooper *et al.*, 1998). Here, it is not just the presence or absence of a complex of habitat types within the experimental boundary, but the presence or absence of a complex set of interactions that may alter the response to drying/re-flooding and permanent inundation (measured as changes in nutrient availability) at different scales.

For example, in a study investigating the effects of submerged macrophytes on the N cycle, Eriksson and Weisner (1999) found that rates of nitrification in mesocosms containing macrophytes was several times higher than those without macrophytes (sediment and water only), resulting in a greater production of NO_X^- in the presence of macrophytes compared to a net consumption of NO_X^- in their absence. It was proposed by the authors that the macrophytes provided a surface area for the establishment of epiphytic communities, including the bacteria responsible for nitrification. Therefore, the presence of macrophytes and the range of interactions with which they are associated in the ecosystem- and mesocosm experiments, and their absence in the two smaller experiments, may have contributed to the differences in the results collected in each of the experiments assessed.

It follows then, that an experiment conducted at a spatial extent larger than the mesocosms that were utilized in this series of experiments, incorporating greater degrees of complexity, may have been able to more accurately reproduce the patterns observed at the ecosystem scale. In ecotoxicological research, where the manipulations of interest often cannot be conducted in aquatic ecosystems for ethical reasons, the use of artificial streams (elaborate outdoor complexes) has been growing in popularity because they incorporate greater degrees of complexity than are possible in traditional laboratory experiments and field biomonitoring techniques, and have been shown to produce results that can correspond with in-river trends (see Culp *et al.*, 2000). Although, there are a number of examples in the literature suggesting that even large mesocosms cannot reproduce natural ecosystem-scale phenomena (Boon *et al.*, 1997; Ahn and Mitsch, 2002).

Therefore, it can be concluded that in the wetland system examined, the role of smaller scale experiments in providing insight into the mechanisms underlying the patterns we observe at the ecosystem scale, is questionable – extrapolation of results from small-scale experiments should be undertaken with considerable caution. The results of this study therefore confirm that a multi-scale approach to ecological research is wise, where large-scale field experimentation and/or monitoring provides a check on the accuracy, and hence relevance, of conclusions reached via mesocosm experiments.

5

General Summary

The primary aim of this research was to investigate, using a multi-scale experimental approach, the impact of river regulation and in particular permanent inundation on ephemeral floodplain wetlands of the River Murray. The Flood Pulse Concept (FPC) was used to generate hypotheses relating to patterns and processes believed to be fundamental in driving structure and function in these wetlands, in order to better understand changes occurring during drying and re-flooding cycles and subsequently, the effects of permanent inundation on these wetlands. The findings of this research can be summarized under three major headings:

1. The potential for patterns and processes in floodplain wetlands of the River Murray to be reproduced at smaller experimental scales;

2. Changes in nutrient availability and phytoplankton productivity during drying and following re-flooding in ephemeral floodplain wetlands of the River Murray, including application of the Flood Pulse Concept to dryland river-floodplain systems; and

3. The effects of permanent inundation on the ‘flood pulse’ in ephemeral floodplain wetlands of the River Murray.

5.1 The potential for patterns and processes in floodplain wetlands of the River Murray to be reproduced at smaller experimental scales

The first outcome of this research acknowledges a trend in ecological research toward the use of small-scale laboratory based experimentation to develop understanding of large-scale natural phenomena (e.g. DeMelo *et al*., 1992; Duarte *et al*., 1997; Kampichler *et al*, 2001), and addresses the limited scientific validation of this approach to date (section 4.1). Specifically, the same experiment was conducted at four different scales (ecosystem-scale experiment *vs* meso-, mini- and micro-cosm experiments) and the results compared (Chapter 4). This demonstrated that experimental scale does influence results collected and consequently, conclusions drawn (section 4.4).

Data collected in the ecosystem-scale experiment indicated that both the drying and re-flooding phases in ephemeral wetlands can be associated with significant increases in nutrient availability and phytoplankton productivity, although variable responses were recorded amongst wetlands (section 2.3). While the ecosystem-scale experiment was able to highlight patterns in response to drying/re-flooding and permanent inundation, the variation in responses and the coarse temporal grain of the data collected, prevented identification of the underlying processes which would explain these patterns. This is a regularly reported constraint associated with large-scale experiments that highlights one of the common drivers for use of meso- and micro-cosms in ecological research (e.g. Carpenter, 1996).

The mesocosm experiment produced results that were consistent with those at the ecosystem scale in some respects, although the extent of increased N availability and the rate of N depletion after re-flooding in the mesocosm experiment were considerably different to that recorded in the ecosystem-scale experiment (section 4.4). However, of the three smaller scale experiments (meso-, mini- and micro-cosm experiments) the mesocosm experiment produced results that were most similar to those collected in the ecosystem-scale experiment (section 4.4).

The minicosm experiment produced the least variable data set (table 11), supporting claims in the scientific literature that small-scale laboratory experiments, by enabling greater replication and control of relevant variables, are able to reduce variability of data, increasing precision and in turn improving statistical power – seemingly generating a greater insight and understanding of the processes underlying the particular patterns of interest at larger scales (e.g. Wiens, 1989; Carpenter, 1996; Petersen *et al*., 1997; Culp *et al*., 2000; Ahn and Mitsch, 2002). Whilst the minicosm experiment produced relatively precise data, easily identifiable patterns and underlying processes (particularly for N parameters), and results that were consistent with those of similarly scaled studies, changes in nutrient availability after re-flooding in the minicosms were not consistent with the changes observed in the ecosystem-scale experiment (section 4.3, 4.4).

The microcosm experiment was also unable to reproduce the patterns observed at the ecosystem-scale (section 4.3, 4.4). In addition, the decrease in experimental scale from minicosm to microcosm, did not result in further reduction of the variability of the data collected (table 11). This has also been reported in other fields of ecology which have utilized experiments over a range of spatial scales (see Skelly and Kiesecker, 2001).

Notably, patterns observed in the smaller scale experiments not only differed (often markedly) from those recorded in the ecosystem scale experiment, but also differed considerably to each other in the patterns and processes they produced. Even the two smallest scale experiments, which incorporated similar levels of structural complexity/heterogeneity, produced very different results (section 4.3). For example, in the minicosm experiment a significant amount of NH_4^+ was released from dried sediments into the water column immediately upon re-flooding, with nitrification clearly resulting in the subsequent utilization of available NH_4^+ and a concurrent increase in the concentration of NO_X^- in the water column. In contrast, there was no immediate release of NH_4^+ upon re-flooding of dried sediments, and nitrification was not apparent, in treatment microcosms. Furthermore, changes in nitrogen concentrations recorded in treatment minicosms were significantly different to those in the permanently inundated controls, whereas nitrogen concentrations in the dried/re-flooded microcosms were generally not different to those in the permanently inundated microcosms.

Consequently, in this multi-scale investigation, the results indicate it is unlikely that the small scale experiments (mini- and micro-cosm experiments) were able to isolate and reproduce processes occurring in the wetland of interest. The 'containerization effect', where small scale experiments conducted in the laboratory produce systems and results that diverge considerably from the natural system and phenomena they seek to replicate, provides a likely explanation for the results collected (e.g. Cooper *et al*., 1998).

If it is not possible to identify particular processes underlying and influencing the patterns we observe at the ecosystem scale, and if there is concern regarding the capacity for small scale laboratory-based experiments to accurately replicate the natural patterns and processes of interest - how do we investigate these processes to improve our understanding of the structure and function of the ecosystem of interest?

I propose a number of approaches to address this issue. Firstly, to measure underlying processes, such as nutrient release from sediments or the activity of nitrifying bacteria following drying/re-flooding, consideration should be given to measuring actual rate changes of the parameter of interest (e.g. as for estimations of community metabolism, where changes in O_2 concentration over time are used to determine primary production and respiration) *in situ* as part of ecosystem based experiments. Secondly, on the basis of the results collected in this series of experiments, there was some evidence to suggest that an experiment conducted at a spatial extent larger than the mesocosms used, incorporating greater degrees of complexity, may have been able to more accurately reproduce the patterns and underlying processes occurring at the ecosystem-scale (section 4.4). As such, it may be possible to investigate natural phenomena in larger containers while still maintaining the ability to manipulate and control factors of interest. Finally, the results of this study confirm that a multi-scale approach to ecological research is wise, where large-scale field experimentation provides a check on the accuracy, and hence relevance, of conclusions reached via small-scale experiments. The combined use of large and small-scale experiments will also highlight areas of research where ecosystems can be investigated successfully at small-scales, providing opportunities for the most efficient use of resources.

5.2 Changes in nutrient availability and primary productivity during drying and following re-flooding in ephemeral floodplain wetlands of the River Murray, including application of the Flood Pulse Concept to dryland river-floodplain systems

The second set of outcomes from this research (described in detail in Chapters 2 and 3) has built on the knowledge base developed by previous research into the effects of drying and re-flooding cycles in ephemeral floodplain wetlands. In particular, this study has provided a quantitative, experimentally based examination of changes in nutrient availability and primary productivity at the ecosystem scale, and consequently has increased our understanding of the ecology of such wetlands.

In the ephemeral wetlands studied, both the drying and re-flooding phases were associated with significant changes in nutrient availability and phytoplankton productivity. In general, drying resulted in marked increases in temperature, turbidity, conductivity, total nutrient and DOC pools, and phytoplankton biomass in the evaporating water column (section 2.3.2). In some wetlands increases in DO, pH, NO_X^- and PO_4^{3-} concentrations were also recorded (section 2.3.2). The dramatic changes recorded during this phase support the emerging view being expressed in the literature: that changes during the dry phase are likely to be equally important (to wetland structure and function) as those that follow re-flooding (e.g. Humphries and Baldwin, 2003; McMahon and Finlayson, 2003; Kingsford *et al*., 2004).

The research in this thesis also demonstrated that a pulse in nutrient availability and phytoplankton productivity following floodplain inundation, as per the Flood Pulse Concept (FPC), can occur in ephemeral floodplain wetlands of the River Murray. In Little Mussel Lagoon (Murray-Darling Basin, Australia), relatively high NH_4^+, PO_4^{3-}, DOC, TN and TP concentrations were recorded after re-flooding, in turn supporting a sustained increase in phytoplankton biomass (section 2.3.3.1).

However, considerable variation in flood pulse characteristics (nutrient availability and primary productivity) were recorded amongst the wetlands studied, with some re-flooding events deviating substantially from the FPC model. Specifically, the re-flooding phase was generally characterized by

low phytoplankton abundance even when the availability of nutrients was increased (section 2.3). Together with the results of Briggs *et al*. (1985, 1993) and Briggs and Maher (1985), these results suggest that drying and re-flooding in ephemeral wetlands may stimulate trophic pathways other than that described by the FPC for tropical river-floodplain systems (Junk *et al*., 1989). For example, while the 'pulse' in tropical systems is transferred up the food-chain resulting in significant increases in animal biomass on the floodplain, in dryland systems competition by aquatic macrophytes, and possibly the sediment microbial community, for available nutrients may limit production of phytoplankton and algae. This may alter the trophic pathway of the pulse and result in, for example, increased vegetation rather than animal biomass. Future investigation could identify these alternative trophic pathways, and quantify rate and/or biomass changes in associated biota following re-flooding.

It is proposed that the hydrological variability characteristic of ephemeral wetlands in dryland regions may play a role in influencing the variable nature of the flood pulse observed, with factors such as degree of drying and length of isolation during the dry phase, the rate of re-filling, timing of re-flooding and the repeated drying/re-flooding cycles being potentially important. Together with observations arising from the ecosystem experiment, the mesocosm experiment (Chapter 3) investigated and supported the hypothesis that degree of wetland drying influences the nature of the flood pulse.

In general, complete sediment desiccation increased nutrient availability in the water column following re-flooding in comparison to permanent inundation, while partial drying of wetland sediments was found to reduce nutrient concentrations after re-flooding (section 3.3.2). However, phosphorus availability was the exception, remaining unaffected by level of drying (section 3.3.2). Death and partial decomposition of microbial, plant and animal biomass in completely desiccated mesocosms allowed a proportion of the available nutrients to be returned to the water column upon re-flooding, hence increasing nutrient concentrations. In contrast, a drop in water level (without sediment desiccation occurring) seemed to allow a greater proportion of the microbial community to survive the dry phase, which would have affected nutrient availability in a number of ways. Firstly, minimal moisture stress and increased oxygen availability would have provided favourable conditions for microbial mineralization of organic nutrients throughout the dry phase, resulting in the depletion of organic nutrient pools in these mesocosms (e.g. De Groot and Van Wijck, 1993). In addition, little to

no microbial mortality at the end of the dry phase would have resulted in minimal return of available nutrients to the water column upon re-flooding (e.g. Qiu and McComb, 1995), and would have also allowed more rapid assimilation of available nutrients following re-flooding. Together, it is likely that these factors explain the greatly reduced nutrient concentrations that were recorded after re-flooding of partially dried mesocosms.

Level of drying had little impact on phosphorus availability following re-flooding, probably because a substantial proportion of available phosphorus was adsorbed by, and essentially locked-up in, sediments during the dry phase (section 3.4.1) – preventing a release of available phosphorus upon re-flooding as observed for carbon and nitrogen (section 3.3.2).

Few studies have attempted to link changes in nutrient availability with changes in rates of primary productivity in ephemeral wetlands over a drying/re-flooding cycle (e.g. Briggs and Maher, 1985; Briggs *et al*., 1985, 1993). None have directly measured changes in community metabolism after re-flooding. Results of the mesocosm experiment indicate that degree of drying does influence community metabolism after re-flooding, with partial drying significantly reducing rates of community respiration compared to permanent inundation and complete desiccation (section 3.3.2). It is likely that the reduced concentrations of DOC in the water column of these mesocosms after re-flooding is associated with the limited rates of community respiration recorded (section 3.3.2).

In addition, while re-flooding of the completely desiccated sediments significantly increased concentrations of carbon and nitrogen in the overlying water column, the increased availability of these nutrients was not translated into an increase in community metabolism (rates of primary production and/or respiration) after re-flooding (section 3.3.2). On the basis of results from the ecosystem and mesocosm experiments, it appears that in ephemeral floodplain wetlands of the River Murray the availability of inorganic P plays an important role in determining the occurrence (or not) of the classical ‘flood pulse’ outlined by the FPC (Junk *et al*., 1989). Future research could confirm this association and investigate the set of environmental conditions which result in increased phosphorus availability following re-flooding (as was recorded in Little Mussel Lagoon).

Degree of drying not only influenced nutrient availability and community metabolism after re-flooding of the mesocosms, but also impacted on the macrophyte community. A significant reduction in plant biomass was recorded when water levels were dropped to at or below the sediment surface (section 3.3.2). While the macrophyte community was able to re-establish in these mesocosms after re-flooding, the biomass after 3 months was still significantly less than in mesocosms where the water column was maintained throughout the experiment. Complete sediment desiccation decreased plant growth even further, with a significantly lower biomass recorded in these mesocosms (compared to all other treatments) at the end of the experiment (section 3.3.2). While permanently inundated mesocosms supported the highest plant biomass at the end of the experiment (along with treatment 1 mesocosms), permanent inundation appears to reduce species diversity of macrophytes established in the mesocosms (section 3.3.2).

In summary, the results of the ecosystem and mesocosm experiments established that drying and re-flooding in ephemeral wetlands of the River Murray could be associated with dramatic changes in nutrient availability and, in some cases, patterns of primary productivity. The FPC provides a good framework within which patterns and processes in dryland wetlands can be investigated, although significant variation in the nature of the flood pulse was recorded amongst these wetlands, in part due to the degree of drying experienced during the dry phase. This information provided the foundation against which the effects of permanent inundation of these wetlands could be assessed.

5.3 The effects of permanent inundation on the 'flood pulse' in ephemeral floodplain wetlands of the River Murray

Finally, this research demonstrates that permanent inundation effectively removes periods of peak nutrient availability and phytoplankton productivity associated with the drying and/or re-flooding phases in ephemeral floodplain wetlands of the River Murray, resulting in continuously low levels of nutrient availability and phytoplankton productivity (Chapters 2 and 3).

When considered in conjunction with the results of previous research, where the negative effect of permanent inundation on higher trophic levels in ephemeral floodplain wetlands has also been demonstrated, including reduced invertebrate and waterbird abundances (e.g. Boulton and Lloyd, 1991; Kingsford *et al*., 2004), there is strong evidence to suggest that permanent inundation of ephemeral floodplain wetlands can significantly reduce nutrient availability, primary and secondary production. Therefore, alteration of the natural hydrological cycle in this way essentially changes the structure and function, the ecology, of these wetlands.

Without regulation and extraction, flows in the River Murray were highly variable with annual flows ranging between 2,500 Gl and 40,000 Gl (Eastburn, 1990): Walker *et al*. (1995) calculated that a 50-year hydrological record is not long enough to represent the River Murray's natural flow regime. Given that flows from the River Murray represent the primary water source for most of the associated floodplain wetlands (rather than local run-off), particularly in semi-arid parts of the catchment (Reid and Brooks, 1998), the natural hydrology of these wetlands would have mirrored this variation (Pressey, 1986; Boon *et al*., 1990). Under natural conditions the 7,000-odd wetlands on the River Murray floodplain formed a mosaic, with wetlands in the mosaic at any one point in time representing a range of hydrological states (Pressey, 1986; Boon *et al*., 1990): some inundated for significant periods of time, some partly or completely dried and others recently re-flooded, depending on their location, morphology, connections with the river and local climate. Based on the results of this study, we also now know that this mosaic of wetlands representing a range of hydrological regimes probably also supported:

- a range of productive states – some highly productive (e.g. 'autotrophic', supporting large increases in plant and/or animal biomass) and others less productive (e.g. 'heterotrophic'); and
- a range of biodiversity – some wetlands supporting highly diverse communities of flora and fauna (e.g. those which had recently undergone partial drying and re-flooding), others not so (e.g. those which had been inundated for longer periods of time and those recently undergone complete desiccation and re-flooding).

It is probably this diversity (in hydrology, productivity and biodiversity) that is most important to the structure and function of individual wetlands and of the river-floodplain system as a whole. Individually and in concert, wetlands in the mosaic would contribute significantly to the integrity of the River Murray system.

The deterioration of wetlands has been listed as one of the 10 key threats to the sustainability of Australia's inland waters (State of the Environment Advisory Council, 1996), and modification of natural water regimes was identified the as the highest priority issue for wetland management in Australia (Bunn and Schofield, 1997), with ephemeral wetlands considered the most impacted upon by altered hydrology (Moore and Lloyd, 1996). It has been acknowledged that: (a) the impact of dams and weirs and corresponding changes to river-floodplain connectivity, on the food webs and other ecological processes of floodplain wetlands is poorly understood (Kingsford, 2000a), and (b) "our patchy ecological understanding of the responses of systems to water regime change continues to impede our ability to manage wetlands effectively" (Brock *et al*., 1999). By contributing to this knowledge gap, the conclusions arising from this thesis will provide valuable information to those involved in management and rehabilitation of river-floodplain systems in dryland regions, particularly of floodplain wetlands in the Murray-Darling Basin.

For example, the Murray-Darling Basin Commission (MDBC) is responsible for *The Living Murray* program which incorporates a range of initiatives to restore the River Murray to a 'healthy working river'. The MDBC recently announced that included in these is an environmental water allocation (EWA) of 500 GL/year (MDBMC, 2003). While this EWA is expected to benefit the whole of the River Murray ecosystem, it will specifically be utilized to improve the state of six significant ecological assets along the River (MDBMC, 2003). In recognition of the importance of floodplain wetlands, and the crucial role they play in maintaining the integrity of the river system, all of the six sites chosen as significant ecological assets are wetland complexes present on the floodplain of the Murray (MDBMC, 2003). Importantly, results presented in this thesis indicate that the changes resulting from river regulation and permanent inundation can be somewhat reversed, within a relatively short period of time, given re-instatement of a more natural hydrological regime. In addition, these results also suggest that it is important that rehabilitation of the River Murray system, and of its floodplain wetlands

in particular, focus not only on flooding events but equally on drying phases. Furthermore, the greatest potential benefit to the system as a whole may be achieved through the re-creation of a wetland mosaic, representing a range of hydrological and therefore biological states, as would have existed under natural conditions.

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