Seasonal changes in water quality and macrophytes and the impact of cattle on tropical floodplain waterholes

N.E. Pettit¹, T.D. Jardine², S. K. Hamilton³, V. Sinnamon⁴, D. Valdez², P.M. Davies¹, M.M. Douglas⁵ and S.E. Bunn²

¹*Centre of Excellence in Natural Resource Management, Albany, University of Western Australia, Australia,* ²*Australian Rivers Institute, Griffith University, Nathan, Qld., Australia,* ³*Kellogg Biological Station and Department of Zoology, Michigan State University, Michigan, USA,* ⁴*Kowanyama Aboriginal Land and Natural Resource Management Office, Kowanyama, Qld., Australia,* ⁵*Charles Darwin University.*

Abstract

This study indicates the critical role of hydrologic connectivity in floodplain waterholes in the wet-dry tropics of northern Australia. These waterbodies provide dry season refugia for plants and animals, are a hotspot of productivity, and are a critical part in the subsistence economy of many remote Aboriginal communities. We examined seasonal changes in water quality and aquatic plant cover of floodplain waterholes, and related changes to variation of waterhole depth and visitation by livestock. The waterholes showed declining water quality through the dry season which was exacerbated by more frequent cattle usage as conditions become progressively drier, which also increased turbidity and nutrient concentrations. Aquatic macrophyte biomass was highest in the early dry season, but biomass declined as the dry season progressed. Remaining macrophytes were flushed out by the first wet season flows although they quickly re-establish later during the wet season. Waterholes of greater depth were more resistant to the effects of cattle disturbance, and seasonal flushing of the waterholes with wet season flooding homogenized the water quality and increased plant cover of previously disparate waterholes. Therefore, maintaining high levels of connectivity between the river and its floodplain is vital for the persistence of these waterholes.

Keywords: macrophytes, water quality, seasonal waterbodies, lacustrine, floodplain

Introduction

Freshwater bodies in seasonally inundated floodplains that retain water throughout the year in all but the driest years (relatively permanent) are highly productive ecosystems that function as important refugia for aquatic plants and animals during the dry season (Junk 1997; Bunn *et al.* 2005; Sheldon *et al.* 2010; Pettit *et al.* 2011a). Seasonal inundation and subsequent isolation are the primary drivers of ecological processes within these systems (Junk and Furch 1993; Finlayson 2005; Tockner *et al.* 2010; Warfe *et al.* 2011). In northern Australia, floodplain waterbodies are often called waterholes or billabongs, and can be the only fresh water that persists on the landscape over protracted dry seasons (Finlayson 2005). About 45 % of Australia's freshwater resources lie in tropical Australia and there is now an escalating focus on the management and development of river floodplain ecosystems in this region (Cresswell *et al.* 2009). Throughout this region waterholes are highly valued for ecosystem services ranging from the provision of plants and animals (e.g., fishes, turtles) to livestock water supply, to ecotourism (Jackson *et al.* 2005).

Ecological resilience is measured as the magnitude of disturbance that a system can absorb before it is restructured into another state often with different controlling variables and processes (Gunderson and Holling, 2002; Scheffer *et al.*, 2003). Resilience therefore means that a system can undergo change after a disturbance to revert back to more or less the same stable state (Forman and Godron, 1981; Walker and Salt, 2006). Biological thresholds can be considered a 'tipping point' where the magnitude of the disturbance overcomes system resilience and it changes to an alternate state. Alternate states in shallow freshwater ecosystems have been widely reported in Europe and North America and occur for example, when a waterbody passes a threshold in water clarity that determines whether macrophytes or phytoplankton are competitively advantaged (Breukers *et al.*, 1997; Scheffer *et al.*, 2001; Scheffer and Carpenter, 2003; Scheffer and Jeppesen, 2007; Davis *et al.*, 2010). In many floodplain landscapes, waterholes often change seasonally from a clear water phase dominated by submerged macrophytes to turbid systems dominated by phytoplankton, sometimes with lower oxygen and diminished biodiversity (Mitchell and Rogers 1985; Breukers *et al.* 1997; Scheffer and van Nes 2007; Ward *et al.* 2012).

Tropical floodplain rivers are found across northern Australia and support a diverse array of plants and animals (Finlayson, 2005). These floodplain rivers have a distinct seasonal flow

regime, with most rainfall occurring between December and February (Leigh and Sheldon 2009) and most rivers retain their natural hydrological regime (Douglas *et al.* 2005). These rivers thus have strong connectivity with their floodplains (Jardine *et al.* 2012) and during high flows, water spreads across the floodplain inundating lacustrine and palustrine waterbodies (Ward *et al.* 2012), with variability of flows contributing to habitat diversity of over time (Warfe *et al.* 2011). Whilst there is good information on the distribution patterns of the many permanent and semi-permanent waterholes and wetlands scattered across the region (Finlayson *et al.* 2006), very little is known of the ecology of these ecosystems, including the adaptations of the biota to seasonal fluctuations of wetting and drying as well as the importance of connectivity to the river.

The Mitchell River in tropical north Queensland, Australia, which contains a large number of permanent and ephemeral floodplain waterholes, across an area of 7150 km² of the alluvial megafan (EPA 2005). Within the Mitchell River floodplain water quality and biodiversity of waterholes are under threat from cattle as well as feral pigs and horses (Kennard 2010). Many of these waterholes harbour locally threatened native plant species such as lotus (*Nelumbo nucifera*) and common reed (*Phragmites australis*) that have cultural values to Aboriginal inhabitants. These waterholes also provide important habitat for fishes, reptiles, waterbirds and other wildlife. Knowledge of the attributes of waterholes that confer stability and resilience is critically important in managing them, particularly in the face of possible combined stressors of invasive animals, invasive alien plants, water resource development, and climate change (Vorosmarty *et al.* 2010).

The aims of this study were to understand the effects of the seasonal hydrological cycle on water quality and aquatic plants in a set of waterholes on the Mitchell River floodplain in tropical northern Australia. We also examined the usage and effect of non-native large mammals on water quality and aquatic plant communities. We hypothesised that seasonal flooding, as well as particular physical attributes of the waterholes will provide some resilience to the interplay of seasonal drying and exogenous disturbances.

Methods

Study site

This study was conducted on perennial palustrine waterholes on the floodplain of the Mitchell River in northern Queensland, Australia (Fig. 1). The climate in this region is tropical with a distinct wet season (November to May) and dry season, with long term average annual rainfall of about 970 mm (CV = 26%) of which 95% falls in the wet season, and a mean annual potential evapotranspiration of 1905 mm (Creswell *et al.* 2009). Rainfall during the study period (August 2009 to June 2010) indicated generally average conditions for the wet and dry seasons with a lack of any rain in October 2009 and a wetter than average January and April for the 2009/10 wet season (Fig. 2).

The Mitchell River is perennial (Kennard *et al.* 2010) and arises from the western slopes of the Great Dividing Range in eastern Queensland, and flows west for around 500 km to discharge into the Gulf of Carpentaria, 30km north-east of the community of Kowanyama (Fig. 1). Wet season high flows inundate at least some of the floodplain in most years but floods have a short residence time of around 1-3 months (Ward *et al.* 2012). The study area is part of the 7152 km² Mitchell River fan aggregation which is listed in the directory of important wetlands in Australia (EPA 2005). This alluvial fan contains over 9,000 waterbodies ranging in size from many hectares to small shallow depressions and includes deeply incised stream lines with permanent waterholes, seasonally flooded back plains and numerous perennial and ephemeral depressions (CSIRO 2009). Soils are alluvial sands, silts and clays and vegetation is a grassy woodland savanna characterised by an overstorey including *Eucalyptus microtheca* and a ground-layer of grasses including *Dichanthium* spp. The waterholes are generally fringed with an area of riparian vegetation of varying density, including an overstorey of *E. microtheca*, *Melaleuca* spp., *Excoecaria parvifolia*, *Corypha utan*, with *Barringtonia acutangula* occurring on the wetter fringes, with a grass (e.g. *Dichanthium* spp.) and sedge (e.g. *Cyperus* spp., *Eleocharis* sp.) understorey. The Mitchell river catchment is very sparsely populated (~1 person per 13km²) and the predominant land-use, cattle grazing on unimproved native pasture, occurs throughout the region (CSIRO 2009; Warfe *et al.* 2011). Large populations of feral pigs as well as some horses are common throughout the region (Mitchell River Watershed Management Group 2001).

Sampling procedure

Nine floodplain waterholes were selected for this study (Fig.1) and ranged in size from 0.7 to 5.7 ha. Sampling was sequential, following a dry season to wet season cycle (Fig. 2), with initial sampling in the mid-dry season (August 2009), further sampling done at the end of the

dry season (October 2009) and a third sampling after the 2009/10 wet season, early in the following dry season (June 2010). To assess animal use at each waterhole a 100-m shoreline transect parallel to the water's edge (as it was at the time of sampling) was selected, with three 50-m perpendicular transects at 0, 50 and 100 m, extending into the fringing riparian vegetation. Animal prints (pugging) and waste (cowpats) were recorded along each of the four transects (within 1 m either side).

At the initial sampling time only (August 2009), riparian vegetation fringing each waterhole was surveyed along the three perpendicular transects at 0, 25 and 50 m within a 5 x 5 m quadrat. This gave a total of nine riparian quadrats at each site; three at the water's edge, three within the riparian zone, and three at or near the boundary with the terrestrial zone. All plant species within each quadrat were identified and a visual estimate made of projected foliage cover, of % litter and % bare earth. As an indicator of cattle usage of the riparian areas of the waterholes, the number of cow pats within each 5 x 5m quadrat, as well as along each transect, was recorded. Soil samples (top soil, 0 – 5 cm) were also taken from each plot and analysed for organic carbon (C), total nitrogen (N) and total phosphorus (P, NaOH extractable).

To measure aquatic vegetation within the waterhole, shorter 20 m long transects were extended out into the water at 0, 50 and 100 m along the shoreline transect. Aquatic macrophyte species composition and % cover were estimated within 1 x 1 m quadrats at 0, 5, 10, 15 and 20m into the water along these three aquatic transects.

Within the approximate centre of each waterhole, a vertical profile of water quality measurements was taken from a boat using a Quanta Water Quality Monitoring System (Hydrolab, Austin, Texas) and included temperature (°C), conductivity (mS cm⁻¹), pH, turbidity (NTU), and dissolved oxygen (%). Water samples were also stored on ice and frozen immediately to measure total N (mg L⁻¹) and total P (mgL⁻¹) as well as filtered samples to measure dissolved N (NO₃⁻ + NO₂⁻, hereafter referred to as NO_x as well as NH₄⁺ (mg L⁻¹)) and PO₄³⁻ (mg L⁻¹) with detection limits of 0.04 and 0.01 mg L⁻¹ for TN and TP, respectively, and 0.001 mg L⁻¹ for dissolved N &P. Particulate material in suspension (seston) was collected on three GF/F glass-fibre filters, kept in the dark, frozen, and analysed for chlorophyll *a* (µg L⁻¹). Nutrients and chlorophyll were analysed using standard colorimetric methods (APHA 1998).

The use of waterholes by cattle, pigs and horses was monitored by time-lapse cameras (Moultrie Game Spy, EBSCO Industries, Inc., Alabaster, AL), mounted on riparian trees and set to automatically take a photograph of the waterhole edge every hour. These cameras were installed in August 2009 and photos retrieved in October 2009 and June 2010. Photographs from the time lapse camera were also used to estimate general changes in cover of macrophytes at each waterhole, with three of the authors (TJ, SK, NP) independently estimating total macrophyte cover from the photographs, at each waterhole for particular dates and time. These three estimates were in broad agreement (all $r > 0.89$; $p < 0.001$) and also showed a good relationship with cover measured along the transects on the three sampling dates ($r = 0.83$; $p < 0.001$). This gave us some confidence that cover estimates made from camera data reflected reasonably well the aquatic macrophyte cover at the waterholes. These averaged estimates of macrophyte % cover taken from the waterhole photographs therefore complemented macrophyte cover measured along the transects at the three sampling times and provided a record of change in macrophyte cover throughout the nine months of the study that could not be achieved from measures taken only at the three sampling times.

Data analysis

All data used in statistical tests were tested for normality and transformed ($\log(x+1)$) where necessary. Plant community data for aquatic vegetation were analysed using non-parametric routines in PRIMER Version 6.1.5 (Clarke and Gorley 2006) and the PERMANOVA+ software addition (Anderson *et al.* 2008). Species diversity (Shannon-Wiener index (H)) and richness was measured using the DIVERSE routine in PRIMER. To test for differences in aquatic plant communities among waterholes and sampling times, the permutational multivariate analysis (PERMANOVA) technique was employed using Bray Curtis similarity. A similarity percentages (SIMPER) routine was performed to identify which plant species contributed most to the average dissimilarity among different waterholes and sampling times. To explore which group of environmental variables best explained the variation observed in aquatic plant community data, the distance based linear models (DistLM) routine in PERMANOVA (Legendre and Anderson 1999) was used. A Bray-Curtis similarity matrix of the plant community data was used for this analysis, and a step-wise selection of transformed ($\log(x+1)$) environmental variables (%litter, % bare ground, abundance cattle dung, soil total

N, total P and C, C:N and % canopy cover) was conducted using the adjusted R^2 selection criterion and a permutation test of significance (Legendre and Anderson 1999).

Overall water quality differences between the waterholes for the 11 environmental variables measured (Secchi depth, maximum depth, surface pH, conductivity, dissolved oxygen, turbidity, total N, total P, NH_4^+ , NO_x and PO_4^{3-}), was visually assessed using principal components analysis (PCA) after data were transformed ($\log(x+1)$) and normalised (subtract mean and divide by standard deviation, Clarke and Gorley 2006). Differences in water quality data between waterholes and seasons was analyzed using analysis of similarities (ANOSIM; Clarke and Gorley 2006). The relationship between maximum depth and turbidity and nutrients as well between nutrients and chlorophylla for the waterholes, over each sampling time was analysed using Pearson correlation coefficient (Clarke and Gorley 2006). Relationships were also examined between cattle numbers and waterhole water quality variables. Cattle density was evaluated using daily counts of cattle from the time-lapse cameras.

Results

Waterhole plants

There were no differences in aquatic plant community composition at the nine waterholes between sampling times (pseudo $F_{2,20} = 1.23$, $p = 0.277$). There was also no significant difference in species richness (pseudo $F_{2,20} = 1.81$, $p = 0.171$) or diversity (H) (pseudo $F_{2,20} = 1.16$, $p = 0.352$) of aquatic plants between sampling times. Rather, differences between sampling times were mainly related to changes in biomass (and discussed in the following section). There was some seasonal change in certain species with the water lilies *Nymphaea violacea* and *Nymphoides indica* only prominent at Crayfish, Turrum, Maggie Hole and Surprise Yards waterholes in the mid-dry (August 2009) and early-dry samplings (June 2010, Table 1). Fishhole Creek waterhole was dissimilar from the other waterholes with the dominance of the floating exotic *Eichhornia crassipes* year round. The submerged aquatic *Ceratophyllum demersum* was the most prominent macrophyte at waterholes with higher levels of water clarity including Pirral, Duckhole, Red Lily and Maggie Hole, while at the more turbid waterholes, Turrum and Magker, the best indicator was the semi-aquatic

riparian/emergent *Ludwigia ascendens* which grows on the waterhole margins and extends out into the water when levels are high.

Water quality variables that were significantly related to the aquatic macrophyte species composition were conductivity (pseudoF_{1, 21} = 3.86, p = 0.004) and total P (pseudoF_{1, 21} = 1.94, p = 0.04). Sites with generally high conductivity were Pirral and Duckhole in August and October where the most dominant plants were the submerged *C. demersum* and the floating fern *Azolla filiculoides*. At Fishhole creek where the floating exotic *E. crassipes* was dominant conductivity and total P was much lower than at the other sites (Tables 1 & 2).

Cover of aquatic macrophytes varied considerably among waterholes on a given sampling date as well as within waterholes throughout the year (Fig. 3). For example, at Duckhole macrophyte cover was 80-90% for most of the dry season, predominantly the floating fern *A. filiculoides* and the submerged *C. demersum* (Table 1), while there were virtually no macrophytes during the dry season at Turrum, Surprise Yards and Maguera waterholes (Table 1, Fig. 3). With the initial inundation of the waterholes in the early to mid-wet season, there was a dramatic reduction in cover at Duckhole of what is most likely to be the floating *A. filiculoides* as it is pushed or blown to the edges of the waterhole. Following the flood, there was a quick response of plants, with cover of the macrophytes increasing rapidly in most waterholes (Fig. 3).

There were large changes in the appearance and character of the waterholes as the season progressed (Fig. 4). In the mid-dry season (August 09), water levels were still reasonably high and there was aquatic vegetation visible across most of the waterholes (Figs. 4a, d). At the end of the dry season (October 09), water levels were very low and the water was usually highly turbid. There were large areas of exposed bank, aquatic vegetation was sparse and there were extensive areas of animal disturbance (cattle, horses and feral pigs) within and surrounding the waterhole (Fig. 4b, e). At the end of the wet season water levels were at their highest and water was less turbid, and there was generally a prolific growth of macrophytes within the waterholes (Fig. 4c, f). The riparian areas surrounding the waterholes were also affected by cattle as measured by the frequency of cow pats within plots. Higher cow pat numbers correlated with reduced total riparian plant cover ($r = -0.56$, $p < 0.01$) and an increase in soil %N ($r = 0.47$, $p < 0.02$) and %C ($r = 0.46$, $p < 0.05$).

Changes in water quality

Environmental conditions within the waterholes changed considerably over the three sampling times. There were significant differences in water quality between sampling times ($R = 0.187$, $p = 0.002$) with differences between August 09(early dry)- and October 2009 (late dry, $R = 0.359$, $p = 0.001$), and between June 10 (early dry) and October 09 (late dry, $R = 0.193$, $p = 0.024$), but not between June 10 (early dry) and August 09 (mid dry) sampling times ($R = 0.025$, $p = 0.257$). Differences in water quality between sites showed the waterholes Makger, Surprise Yards, Crayfish Hole and Turrum diverged strongly from the other sites late in the dry season but all sites were similar by the early dry season sampling in June 2010 (Table 2, Fig. 5). This was mostly related to water depth, conductivity and nutrients, along PC1 (explaining 56% of variation), while pH and dissolved oxygen were important for the separation of sites along PC2 (19.5% of the variation, Fig 5). The water quality variables which were most responsible for differences between waterholes in the mid- and early-dry sampling times were PO_4 , DO, pH and Secchi depth, while at the late-dry sampling time NO_x , NH_4 and pH were most important.

Drivers of water quality changes in waterholes

There was a wide range in water depth across the waterholes (Table 2) and some of the variation in water quality can be explained by differences in water depth (Fig. 6). Over the study period, maximum water depth decreased as the dry season progressed from the mid-dry season (August 2009) to the late dry season (October 09), while turbidity, conductivity, nutrients and sestonic chlorophyll *a* increased (Table 2). For all waterholes in the mid and late dry seasons, nutrients (total P and total N) and turbidity were negatively correlated with water depth (all $r > -0.31$, $p < 0.1$), while turbidity was positively correlated with nutrients (total P and total N) and total N was positively correlated with chlorophyll *a* (all $r > 0.63$, $p < 0.05$); Table 2, Fig. 6). These relationships were strongest in the late dry season (October 2009) but had broken down and were not significant by the next early dry season (June 2010) sampling, which followed the wet season flooding (all $r < 0.28$, $p > 0.1$, Table 2, Fig. 6).

Cattle numbers identified from cameras set up at each waterhole indicated that the number visiting the water increased substantially as the dry season progressed (Fig. 7a). Numbers decreased quickly once wet season rains began in early November (Fig. 7a). At the site level, the number of cattle utilizing waterholes was positively correlated with an increase in water total N ($r = 0.516$, $p < 0.02$) and total P ($r = 0.617$, $p < 0.005$) in both early and late dry

season samples (Fig. 7b). In the late dry season, cattle numbers were positively correlated with water column chlorophyll *a* levels ($r = 0.571$, $p < 0.05$, Fig. 7b) and negatively correlated with light penetration ($r = 0.819$, $p < 0.001$, Fig. 7d).

Discussion

The waterholes examined in this study are subject to seasonal inundation, which may last only a few weeks or longer and occur in one or several episodes (Ward *et al.* 2012). The waterholes subsequently experience a protracted dry season during the rest of the year, in which they often decrease considerably in depth and area, creating stressful conditions for aquatic biota (Pettit *et al.* 2011b; Warfe *et al.* 2011). Our results from these tropical Australian floodplain waterholes have parallels to those reported for shallow floodplain waterbodies elsewhere in Australia as well as in South America, with floods resetting waterbodies so that they become biophysically very similar by the end of the wet season but then diverging strongly during the dry season (Lewis *et al.* 2000; Thomaz *et al.* 2007; Roozen *et al.* 2008; Davies *et al.* 2008; Sheldon and Fellows 2010). Hydrologic connectivity has consistently been found to be a critical driver of water quality and aquatic community assemblages in floodplain waterholes, despite wide variation in climate (Mitchell and Rogers 1985; Gallerdo *et al.* 2009).

The waterholes surveyed in this study show some resilience to the seasonal cycle of drying and flooding, which sees them undergo significant changes throughout the year as they become more dissimilar under drying conditions (Fig. 8). With the onset of the wet season, after the initial inundation phase, water quality improves, turbidity decreases and macrophytes re-establish so that waterholes return to a similar functioning state at the end of this seasonal cycle (Fig. 8). The predictability in the timing and amplitude of seasonal flooding is important in the development of these adaptations in waterhole biota (Junk and Furch 1993). However some variability in flows is important in maintaining resilience by maintaining a range of biological and physical characteristics that interact with life history traits of the biota (Naiman *et al.* 2008; Davies 2010). This variation in the physical environment such as shape and depth of waterholes and the type and extent of fringing vegetation will also contribute to resilience in that there is a diversity of biophysical ecosystem functions (Colloff and Baldwin 2010).

Disturbances other than the natural flood regime are likely to test the resilience of these waterholes and possibly lead to a change of state (Fig. 8). This change may come about through stressors that are beyond the natural disturbance regime, such as when livestock grazing is introduced. However, given the natural variation of waterholes on floodplains, some are likely to be more resistant to these changes than others. For example, our study indicated the deeper waterholes were more resistant to the effects of increased disturbance from cattle and feral animal use, particularly in the late-dry season. Shallow waterholes are more susceptible to high turbidity due to wind-derived re-suspension of sediments which can be exacerbated by cattle disturbance which have greater access to these shallow waterholes. Therefore in shallow waterholes greater seasonal flushing is required to return the system to similar conditions to the pre-dry season state (Fig. 8). How an ecosystem re-establishes after disturbance is not assured (Holling and Gunderson 2002) and divergence from the previous state is most likely after a subsequent disturbance which may result in the establishment of a different but relatively stable state, such as one adapted to more xeric conditions and with greater livestock and feral animal disturbance (Fig. 8).

Cattle numbers visiting the waterholes increased significantly as the dry season progressed, but visits quickly diminished after significant rains made water readily available throughout the floodplain. Other studies have shown similar seasonal waterhole use by large herbivores (Armour *et al.* 1994; James *et al.* 1999; Thrash 2000) and pigs (Caley 1997; Bowman and McDonough 1991). Although we measured no direct effects, cattle grazing on aquatic macrophytes were observed, which given the large numbers by the end of the dry season, is likely to have a substantial effect on macrophyte biomass. Increasing cattle numbers may also explain the increase in total N and P levels, not only in the water but also the surrounding riparian zone. This increase in nutrient levels may lead to the eutrophication of the waterhole resulting in algal blooms and a reduction in water quality. . Cattle trampling in the littoral zone can also inhibit the re-establishment of macrophytes and destroy the band of benthic algae which can be a critical carbon source for the aquatic food webs in waterholes (Bunn *et al.* 2003; 2005).

Our results indicate that to some extent these effects of cattle and other feral animals on the waterholes are obscured by seasonal flooding, which effectively resets the system (Fig. 8). Similar conclusions were made for the effects of pigs on other waterholes in north-east

Queensland (Doupé *et al.* 2010). However cattle and other feral animals using waterholes may have more detrimental long-term effects such as eliminating susceptible plant species and providing enhanced dispersal opportunities for aquatic invasive plants by carrying propagules to and from waterholes in mud or faeces. Feral pigs may also have more direct impacts through consuming buried plant rhizomes and tubers as well as predating on turtles and mussels aestivating in the mud, which become accessible as the waterholes dry out at the end of the dry season (Fordham *et al.* 2008; V. Sinnamon pers. obs.).

After the initial inundation of the waterholes at the beginning of the wet season remaining macrophytes may be flushed out of the waterholes, but recovery is rapid as macrophytes resprout from tubers and rhizomes or seed as flow subsides and high, clear water conditions are conducive to aquatic plant growth. These seasonal flow dynamics are an important driver of waterhole macrophytes which has been reported also for shallow floodplain lakes in Europe (van Geest *et al.* 2007), South Africa (Mitchell and Rogers 1985) and South America (Welcomme 1985; Loverde-Oliveira *et al.* 2009). The magnitude and duration of flooding are also likely to be important as flows of sufficient size and duration are required to change water chemistry, replenish nutrients and allow germination or resprouting and growth of aquatic plants (Davies *et al.* 2008). Small magnitude, short duration floods were shown to be inadequate to stimulate macrophyte production in floodplain waterbodies on the Parana River in South America (Carignan and Neiff 1992).

Differences in aquatic plant composition seen among waterholes were related to particular conditions within waterholes. This is also indicated by the lack of seasonal differences in species richness or composition for waterholes, as the initial waterhole environment determines aquatic macrophyte composition (Rolon *et al.* 2008). In this study the amphibious plants that are well adapted to seasonal wetting and drying cycles such as the perennial grass *Pseudoraphis spinescens* and the perennial herbs *Ludwigia adscendens* and *Persicaria* spp. were associated with waterholes that undergo significant drying and increased turbidity. These life forms are likely to become more prevalent if there is a trend of increased drying for these waterholes. The biggest difference in macrophyte community composition in the present study was seen at Fishhole Creek waterhole, with the dominance of the floating exotic herb *Eichhornia crassipes*, which resulted in this waterhole having the lowest species richness and diversity. This plant is native to South American floodplains and grows and reproduces very rapidly, quickly occupying all available space (Neiff *et al.* 2008). . *E.*

crassipes is a weed of national significance in Australia and poses a major threat to wetlands throughout tropical northern Australia (<http://www.weeds.org.au/WoNS/waterhyacinth/>, accessed 24/7/12).

Floodplain waterholes in the wet-dry tropics of northern Australia play a crucial role in the river-floodplain ecosystem (Finlayson 2005; Pettit *et al.* 2011a), and our results show the critical role of hydrologic connectivity in their maintenance. Exogenous disturbances such as cattle and feral animals, as well as the invasion of exotic plants, are largely intractable and ongoing management problems that clearly have negative effects on the floodplain waterhole ecosystem. Alterations to the wet season hydrology of these floodplain rivers, either anthropogenic or climate-induced, may compound the effects of these exogenous disturbances and reduce the resilience of these ecosystems (Finlayson *et al.* 2005). Limiting cattle use of waterholes is generally not feasible nor, in economic terms, is it desirable, except in particular cases such as for protecting waterholes of cultural or ecological significance. Our study re-enforces the importance of maintaining flow connectivity between these floodplain waterholes and the river together with sufficient variability in the flow regime (Naiman *et al.* 2008, Opperman *et al.* 2010) to allow the seasonal resetting of conditions, to maintain waterhole resilience.

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Figure 1: Location of the nine waterholes on the Mitchell River floodplain in north Queensland, Australia.

Figure 2: Monthly rainfall for the period of the study compared to the long-term average rainfall at the Kowanyama, within 30km of the study sites. Sampling times were (1) the mid-dry season (August 2009), (2) the late-dry season (October 2009), and (3) the end of wet season (June 2010).

Figure 3: Changes in aquatic plant cover on eight waterholes over the study period, from the mid-dry season (August 2009) to mid-wet season (February 2010). Cover was estimated from time-lapse cameras. Arrows indicate approximate date when each waterhole filled with flood water during the wet season (Mag = Magker; CH = Crayfish Hole; FH = Fish Hole CK; SY = Surprise Yards; Tur = Turrum; RL = Red Lily; DH = Duckhole).

Figure 4: Change in appearance of two representative waterholes from the mid-dry season (August 2009) to late-dry season (October 2009) and mid-wet season (February 2010).

Figure 5: PCA ordination of waterhole environmental variables for the mid-dry season (August 2009), the late-dry season (October 2009), and the end of wet season (June 2010) sampling dates. Vector plot indicates environmental variables with $r^2 > 0.35$ for each axis. Site abbreviations are: Mak = Magker; SY = Surprise Yards; RL = Red Lily; CH = Crayfish Hole; FC = Fishhole Ck; MH = Maggie Hole; Pir = Pirrial; Tur = Turrum; DH = Duckhole. Environmental variables are: pH; DO = Dissolved oxygen; TN = water total nitrogen; TP = water total phosphorus; Turb = turbidity at the water surface; Cond = conductivity at the water surface; Depth = maximum depth; Secchi = Secchi depth.

Figure 6: Relationships for the nine waterholes in August 2009, October 2009 and June 2010 between maximum water depth and water total P, N and turbidity (a, b, c); turbidity and total P and N (d, e); and between total N and chlorophyll *a* (f). An r^2 value and trend line is only presented for significant ($p < 0.05$) relationships.

Figure 7: Association of cattle numbers with waterhole characteristics. a) increasing cattle numbers over the dry season (with 2 day moving average). Relationship between cattle numbers and b) water total N and P; c) sestonic chlorophyll *a*; d) Secchi depth. All relationships are significant at $p < 0.01$.

Figure 8: Conceptual model of waterhole resilience to seasonal changes and the crucial role of wet season inflows. Shallow waterholes undergo greater changes during dry season drought, which is exacerbated by cattle disturbance. However the resetting effects of wet season flooding restores the shallow waterholes by the end of the wet season to conditions similar to those in deeper waterholes.

Table 1: Changes in average vegetation cover (%) of the six most common aquatic macrophytes in waterholes over the study period, including 1 = mid-dry season (August 2009), 2 = late dry season (October 2009) and 3 = early dry season (June 2010).

Waterhole	Crayfish			Duckhole			Fishhole			Maggie			Magker			Pirrial			Red Lily			Surprise			Turrum		
Sampling date	1	2	3	1	2	3	1	2	3	1	2	3	1	3	3	1	2	3	1	2	3	1	2	3	1	2	3
Species																											
<i>Azolla filiculoides</i>				82	88	1.7				0.4	4.5	2.5	0.1			0.2		3.2	4.6							0.1	
<i>Pseudoraphis spinescens</i>			4.7			0.7				2.3		3.7	0.2		0.1				17	0.2	44			0.1	0.7		4
<i>Utricularia gibba</i>				1		2.7				19	1.7	4	2.4		21				0.2	0.4	6				15		8.7
<i>Ludwigia ascedens</i>				1.1	0.3	15				0.1	0.1	0.1	18	0.3	6.7			0.3	0.3		8				11		16
<i>Ceratophyllum demersum</i>				77	32	31				0.3	6.5	28				53	37	51	3	51	20						
<i>Persicaria attenuata</i>																			2.8	0.4	3						
<i>Nymphoides indica</i>	30		22							9	0.1	2.3													0.2		6.7
<i>Nymphoides aurantiaca</i>	6.3		7										1.1		0.9				6.1	1.9	3.4						
<i>Nymphaea violacea</i>			0.3	4.4	0.7	11						36			10	0.6					5			5			2
<i>Marsilea sp</i>										0.6		0.1															
<i>Aponogeton sp</i>	0.2																										
<i>Lemna aequinoctialis</i>						0.2					24		0.9					0.1							1.2		
<i>Eichhornia crassipes</i>							25	11	100																		
<i>Nitella sp</i>	29						3.9	0.1											5.6								
<i>Oryza sp</i>			2			0.3						5.3									2						1
<i>Caldesia acanthocarpa</i>																					1						2.7
<i>Cyperus sp</i>			2				0.1	0.5																			
<i>Cyanotis axillaris</i>												0.1			1.7												
<i>Bacopa floribunda</i>																								0.1			
Total Cover	66	0	39	166	121	63	29	11.5	100	32	37	82	23	0.3	40	54	37	55	40	54	92	0	0	5.1	28	0	41
Total Spp	4	0	7	4	4	8	3	3	1	7	6	10	6	1	6	3	1	4	8	5	9	0	0	3	6	0	7

Table 2: Changes in water quality and chlorophyll *a* in waterholes over the study period, including mid-dry season (August 2009), late dry season (October 2009) and early dry season (June 2010).

	Area	Max. Depth (cm)			Turbidity (NTU)			Cond (ms/cm)			Total P (mg/L)			Total N (mg/L)			Chlorophyll <i>a</i> (µg/L)		
waterhole	(ha)	Aug09	Oct09	Jun10	Aug09	Oct09	Jun10	Aug09	Oct09	Jun10	Aug09	Oct09	Jun10	Aug09	Oct09	Jun10	Aug09	Oct09	Jun10
Red Lilly	5.7	190	140	160	3.3	45	1.8	0.06	0.14	0.04	0.16	0.18	0.05	0.78	1.80	0.59	4.41	27.11	69.77
Pirrial	2.4	190	110	200	18.4	9	9.4	0.27	0.37	0.10	0.03	0.03	0.06	0.76	0.95	0.67	9.80	10.08	33.95
Maggie Hole	1.1	130	80	180	48.3	2000	6.6	0.07	0.11	0.05	0.09	1.50	0.15	0.74	5.30	0.44	7.51	26.14	2.94
Crayfish Hole	4.7	65	20	120	30.7	2000	23.4	0.12	0.29	0.04	0.08	3.60	0.07	0.81	16.00	0.46	9.02	166.62	5.58
Duckhole	0.7	110	70	170	19.2	241	4.2	0.08	0.36	0.09	0.11	0.43	0.08	0.85	3.80	0.67	9.80	113.69	15.41
Magker	2.1	110	25	110	1618	2000	25.2	0.05	0.14	0.06	1.30	2.60	0.15	4.10	9.00	0.93	7.35	78.41	23.63
Surprise Yards	1.3	80	9	130	150	971	28.1	0.09	0.34	0.06	0.36	1.10	0.06	3.60	21.00	0.75	137.40	520.84	22.11
Fishhole Ck	1.6	180	100	230	15.9	20	265	0.02	0.02	0.04	0.03	0.05	0.08	0.45	0.69	0.75	4.32	17.25	8.82
Turrun	2.5	100	50	170	300	2000	8.8	0.05	0.11	0.07	0.40	2.70	0.13	2.30	10.00	0.13	69.83	84.94	15.80
median	2.1	110	70	170	30.7	971	9.4	0.07	0.14	0.06	0.11	1.10	0.07	0.81	5.30	0.67	9.02	78.41	15.80

Figure 1:

Figure 2:

Figure 3:

Figure 4:

Figure 5:

Figure 6:

Figure7:

Figure 8: