



# Optimising a Sampling Design for Endangered Wetland Plant Communities: Another Call for Adaptive Management in Monitoring

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Received: 18 November 2013 / Accepted: 30 October 2014 / Published online: 12 November 2014  
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**Abstract** Effective monitoring requires clear questions and a well-designed sampling regime. However, objectives often evolve over time which can render the initial sampling design ineffective. Using a vegetation monitoring program employed in Newnes Plateau Shrub Swamps, Australia, as a case study, we examine a sampling design based on small numbers of 400 m<sup>2</sup> plots to assess if it can meet the stated monitoring objectives of detecting significant changes in number and abundance of species per wetland. To determine this, we intensively sampled four monitored wetlands using randomly distributed 4 m<sup>2</sup> plots to obtain representative estimates of species composition and abundance. The 400 m<sup>2</sup> plots captured 91 % of the common species and a similar proportional distribution of life-forms as found in the 4 m<sup>2</sup> plots, but missed 62 % of the sparse species found in 4 m<sup>2</sup> plots. Insufficient replication of 400 m<sup>2</sup> plots made detection of statistically significant changes at the swamp scale difficult or impossible. Our review showed the weak sampling design was contributed to by 1) an initial lack of clearly stated management triggers and 2) changes in monitoring objectives and triggers over time, without revising the sampling design. We highlight the need for an adaptive approach to monitoring.

**Keywords** Sampling effort · Species-area curve · Wetland vegetation · Spatial scale · Adaptive management

## Introduction

Effective and robust monitoring requires an adaptive and data driven approach (Lindenmayer and Likens 2009). However, there is strong tendency to develop non-intensive and rapid-assessment methods at the expense of statistical rigour in monitoring ecological systems (Lee et al. 2005). Often in an attempt to increase efficiency and decrease expense, a smaller number of larger plots are used. This can lead to higher sampling error, e.g. not detecting a species which is present or miss-classifying the species abundance, and lower power to reject the null hypothesis. High (or unknown) sampling error in a monitoring program can lead to missing or miss-identifying impacts such as changes in presence or abundance of rare or weedy species, with flow on effects for implementation of management actions. Here, we review an existing wetland monitoring program, to examine if the low-intensity, rapid-assessment sampling method used can achieve the stated objectives of detecting a statistically significant change in species diversity and abundance due to anthropogenic impacts. We then discuss the limitations of the current monitoring program and suggest several modifications that could improve the existing sampling design.

Often, the overarching aim of a monitoring program is to detect adverse environmental impacts early, before catastrophic damage occurs. To achieve this aim, it is necessary to collect data for a set of relevant ecological indicators, with sufficient statistical power to detect changes of concern. The indicators chosen need to reflect the type and magnitude of predicted changes and impacts of concern, e.g. increased depth to water table would be predicted to reduce hydrophyte abundance, and hence hydrophyte abundance could be an indicator. Common ecological indicators include changes in soil nutrient levels and litter accumulation (Tongway and Hindley 2004) or changes in species density, or number of functional groups or life-form groups present (Philippi et al. 1998; Stevens et al.

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2004; Ramsay et al. 2006). To detect whether environmental impacts have occurred, it is necessary to accurately and efficiently capture a change in a chosen indicator, such as species abundance, which requires careful consideration of sampling methods, e.g. plot size, replication and spatial arrangement (Gitay et al. 1991; Hellmann and Fowler 1999; Gotelli and Colwell 2001; Scheiner et al. 2011). For example increasing the size of a plot has been shown to decrease the accuracy of both the abundance estimates and detection of plant species (Jalonen et al. 1998; Klimes et al. 2001; Archaux et al. 2007). Modifying the sampling extent changes the probability of encountering rare species or ‘hot spots’ that support a high number of species (Palmer and White 1994). A change in sample size (replication) has a direct impact on statistical power (Downs et al. 2002; Legg and Nagy 2006). The sampling methodology employed should be focused on efficiently detecting unacceptable changes that are attributable to the perceived threats. Nevertheless, sampling methodologies are often based on (i) ‘rules-of-thumb’, (ii) funding availability or (iii) human convenience rather than statistical rigour and ecological principles.

Here we review the Newnes Plateau wetland flora monitoring program where the principle of increased plot size and reduced replication has been applied in heterogeneous wetlands. The monitoring program is focused on the wetland vegetation type ‘Newnes Plateau Shrub Swamp’ (NPSS) (Benson and Baird 2012). The potential anthropogenic impacts on the hydrology of these wetland plant communities include landform deformation and/or cracking of aquitards due to subsidence from underground long-wall mines (Benson and Baird 2012); it is not known whether the hydrological impacts will be evident across an entire wetland or confined to portion(s) of a wetland. The stated objective of the monitoring is to determine whether plant species diversity, composition or abundance have been negatively impacted by changes in the water table due to underground mining. The stated triggers of the monitoring program are a) at the species level, a “significant decline in condition, health, or decline in population numbers compared to baseline”, b) “major dieback of flora compared to baseline” c) an “increase in weed species” and d) a “significant change in species diversity” within a wetland (Blick et al. 2013). The program has evolved from using visual assessments to determine ecologically significant changes to a requirement that a Before-After, Impact-Control approach (with associated statistical tests) be used to determine if statistically significant changes have occurred. In addition, the program has grown from monitoring eight wetlands to 26 individual NPSS wetlands. These 26 wetlands are monitored seasonally using between one and six 400 m<sup>2</sup> plots per wetland, placed using expert opinion (53 plots in total), with wetlands ranging in size from less than 0.5 ha to more than 5 ha (Blick et al. 2013). A review of this program was initiated when the plot data failed to detect major vegetation

die-back in a wetland. Here, we focus on reviewing the sampling design employed, and ask whether it has the potential to detect a significant change in species abundance and diversity (management triggers ‘a’ and ‘d’ above) within a wetland.

To address this question, we intensively sampled four wetlands in the current monitoring program using large numbers of randomly distributed 4 m<sup>2</sup> plots to obtain a representative estimate of species composition and abundance. We compare the species diversity, life-form diversity, and abundance from the established sampling design (400 m<sup>2</sup> plots) with those data obtained from the intensive survey and discuss how well data from the 400 m<sup>2</sup> plots meet the requirements of the monitoring program. Specifically, we ask three questions:

- 1) What proportion of the total species, common species and sparse species found in the 4 m<sup>2</sup> plots are recorded in the 400 m<sup>2</sup> plots in each wetland?
- 2) Do the 400 m<sup>2</sup> plots capture the same proportional distribution of life-form groups as the larger number of small plots distributed across each wetland?
- 3) Are estimates of species abundance obtained in the established 400 m<sup>2</sup> plots similar to those obtained using a larger number of small plots distributed across each wetland?

## Methods

### Study Site

Newnes Plateau is a state forest reserve situated approximately 20 km northeast of Lithgow in the Blue Mountains, Australia (33° 23 S, 150° 12 E). It covers close to 400 km<sup>2</sup>, with elevations ranging between 950 and 1300 m ASL. The plateau is floristically diverse with approximately 600 native plant species (Royal Botanical Gardens and Domain Trust, <http://www.rbgsyd.nsw.gov.au>, accessed 14, March 2013) in a mosaic of forest, woodland, heath and shrub wetlands over Triassic sandstone. The endangered ecological communities ‘Newnes Plateau Shrub Swamp’ (NPSS) generally grow on a peat substrate overlaying sandstone along drainage lines and are characterised by a shrub canopy and sedge or rush (Cyperaceae, Juncaceae, Restionaceae) understorey, with occasional emergent eucalypt trees (Benson and Baird 2012).

The wetlands were selected a priori for this study because they contained at least one 400 m<sup>2</sup> plot (i.e. were included in the current monitoring program), covered a range of sizes and hydrology types (from periodically to permanently wet), and were all located in different catchments (Table 1). Three wetlands (Clarence West, Bungleboori North, and Carne Central) each contained one 400 m<sup>2</sup> plot, while West Wolgan

**Table 1** Wetland area and number of plots for the four wetlands

Wetland	Area (m <sup>2</sup> )	Number of plots	
		400 m <sup>2</sup> (location)	4 m <sup>2</sup>
Bungleboori North	5,343	1 (core)	10
Carne Central	50,867	1 (core)	84
Clarence West	11,936	1 (core)	12
West Wolgan	42,074	6 (3 core, 3 edge)	75

contained six 400 m<sup>2</sup> plots. The 400 m<sup>2</sup> plots were located in the centre of the wetland in ‘representative areas’ using expert opinion (Blick et al. 2013). In some wetlands additional plots were placed at the wetland edge to capture changes occurring at higher elevations.

#### Data Collection: Species Composition and Abundance

In November 2012 (late spring) we sampled the current plots (400 m<sup>2</sup> plots;  $n=9$ ) using the established method. Two researchers used the Braun-Blanquet method, with modified cover/abundance classes (1 = <5 % of plot, rare; 2 = <5 % of plot, uncommon; 3 = <5 % of plot, common; 4 = 5–20 %; 5 = 20–50 %; 6 = 50–75 %; 7 = >75 %), with 45 min spent recording the presence and cover/abundance classes of all species found in each plot.

To obtain an estimate of species presence and abundance in each wetland we implemented a stratified random design and used species-area curves to estimate the number of species in each wetland. For the stratified random design, each wetland was divided into 25 m × 25 m grid cells and a single randomly located point was placed in each cell to locate the plot using Geospatial Modelling Environment (Beyer 2009) tools in ArcGIS 10.1 (ESRI, CA, USA). Each point located inside the mapped wetland boundary was used as the centre of a 4 m<sup>2</sup> (2 m × 2 m) plot (Fig. 1). The plots were located in the field using a consumer-grade handheld GPS device (Juno SB, Trimble) with real-time positional accuracy of 2–5 m in good conditions. GPS accuracy varied throughout the day according to forest and cloud cover. To minimise positional bias, we considered the plot to be located when the GPS distance was less than 3 m from each waypoint, rather than attempting to take several readings over a short period which may replicate errors over time (see Dodd (2011)). As we were not trying to resample these waypoints we considered a consumer-grade GPS sufficient for locating randomly distributed plots. The number of plots in Clarence West Swamp was reduced to 12 to minimise impact on the dense fragile vegetation. All plots were divided into four contiguous 1 m<sup>2</sup> quadrats, within which the cover of each species (defined as shoot presence) was visually estimated to the nearest 1 %. The plot size was chosen as we could be reasonably sure of detecting all species in the

1 m<sup>2</sup> plots (Klimes et al. 2001) and of the accuracy of percentage cover estimates within plots.

#### Data Analysis

We used species-accumulation curves to compare the number of species recorded using 4 m<sup>2</sup> plots with that of the 400 m<sup>2</sup> plots. Species-accumulation curves were generated using the *speccaccum* function with the *random* algorithm from the *vegan* package in R (Oksanen et al. 2012; R Core Team 2012). Patterns of species density were then interpolated and mapped, using data derived from 4 m<sup>2</sup> plots, by applying ordinary kriging (spherical model, with a 50 m search radius, and a minimum of four points) in ArcGIS 10.1.

We tested whether the proportional species recorded across six life-forms (tree, shrub, forb, grass, rush/sedge, exotic forb) within each wetland, differed according to sampling design, using chi-squared tests. All analyses were carried out using untransformed data and evaluated using the *core stats* package in R.

## Results

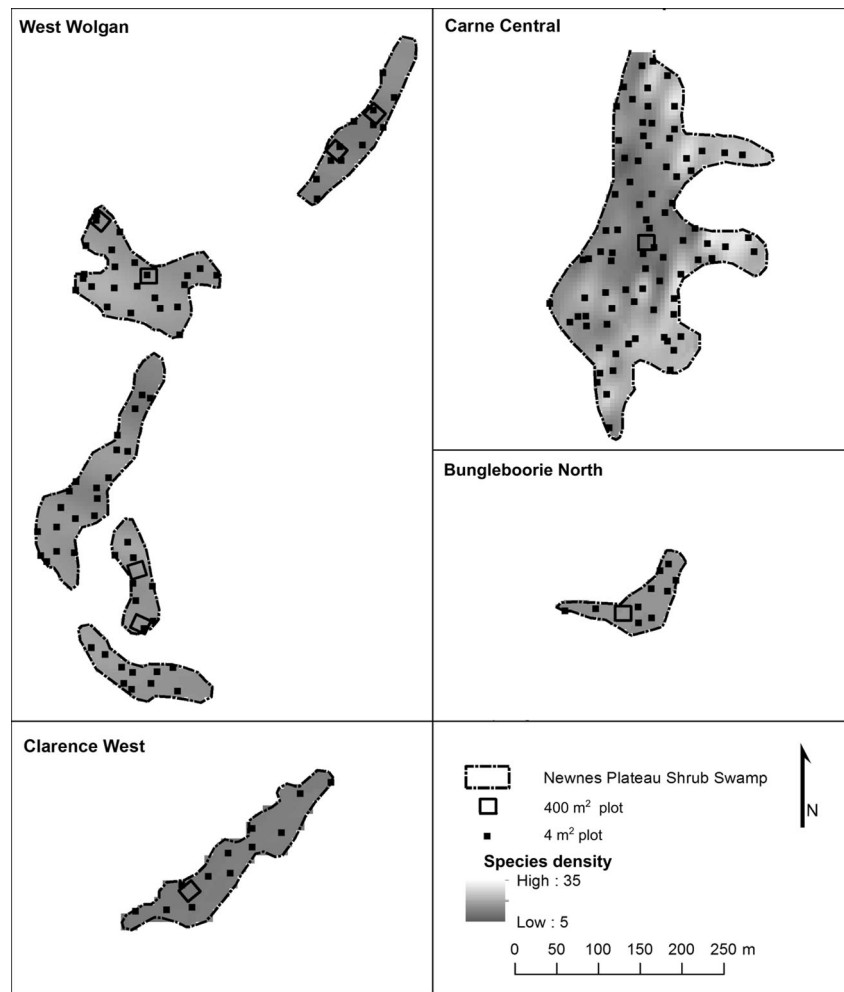
### Species Density

The total number of species found in each wetland using 4 m<sup>2</sup> plots ranged between 41 (Clarence West) and 135 (Carne Central; Fig. 2). These numbers were 1.6 to 5 times greater than those found in the 400 m<sup>2</sup> plots, with 400 m<sup>2</sup> plots capturing a higher proportion of total species in the smaller wetlands than the larger wetlands (Fig. 2). To acquire a comparable number of species in the smaller plots as the larger plots, much less area needed to be surveyed. For example, in Carne Central, just two 4 m<sup>2</sup> plots were required, a total sampling area of 8 m<sup>2</sup>. While in Bungleboori North Swamp, just four 4 m<sup>2</sup> plots were required; a total of 16 m<sup>2</sup> or 4 % of the area in a single 400 m<sup>2</sup> plot.

The proportional distribution of species across life-forms in each wetland did not differ significantly between methods, though the total number of species representing each life-form varied between methods (Fig. 3). For each life-form, the greatest number of species were recorded in 4 m<sup>2</sup> plots, except for the rush/sedge group in Clarence West, where two additional species were recorded in the 400 m<sup>2</sup> plot (Fig. 3).

Spatial interpolation of the 4 m<sup>2</sup> plot data showed considerable variation in species density across the wetlands (mean species = 16.6 ± 4.8 SD per 4 m<sup>2</sup>), with larger wetlands tending to show greater variation than smaller wetlands. The highest species density was recorded on the eastern edge of Carne Central, where typical wetland and forest species were intermixed, and the lowest species density recorded in areas

**Fig. 1** Species density as modelled by kriging from the 4 m<sup>2</sup> plot data in each wetland. Pixels = 25 m<sup>2</sup>, open squares = location of 400 m<sup>2</sup> plot (symbol to scale); closed square = location of 4 m<sup>2</sup> plots (symbol not to scale, plot size = 1/6 of a pixel). Examination of the variance raster associated with the kriging layer showed that the estimates were least reliable on the lobes of wetlands; where there were fewer 4 m<sup>2</sup> plots to train the interpolation, and thus the kriged species density should be interpreted cautiously, especially at the northern and south-eastern tips of West Wolgan



of dense rush or sedge, e.g. areas dominated by *Empodisma minus*, in Carne Central and West Wolgan (Fig. 1).

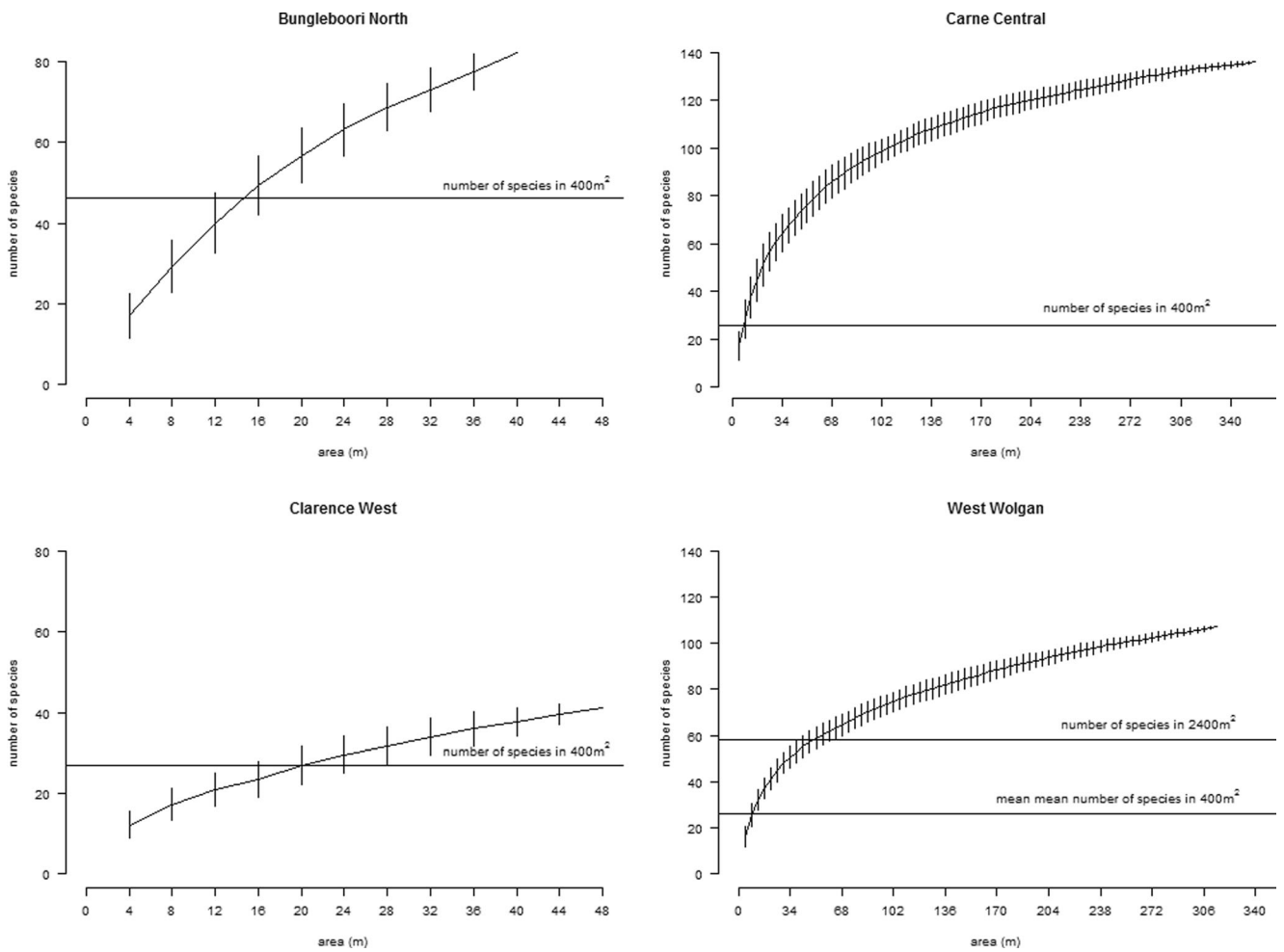
### Species Abundance

All common species (mean cover >15 % in the 4 m<sup>2</sup> plots) were recorded in the 400 m<sup>2</sup> plots (Fig. 4). In comparison, the 400 m<sup>2</sup> plots missed 232 sparse species (mean cover <15 % in the 4 m<sup>2</sup> plots) found in the 4 m<sup>2</sup> plots across all four wetlands. Species with higher Braun-Blanquet cover classes (5, 6 and 7) in the 400 m<sup>2</sup> plots tended to have higher percentage cover across the wetland. For Braun-Blanquet cover classes 1 to 4 there was little correspondence between percentage cover across the wetland recorded in 4 m<sup>2</sup> plots and Braun-Blanquet scores recorded in 400 m<sup>2</sup> plots (Fig. 4).

### Discussion

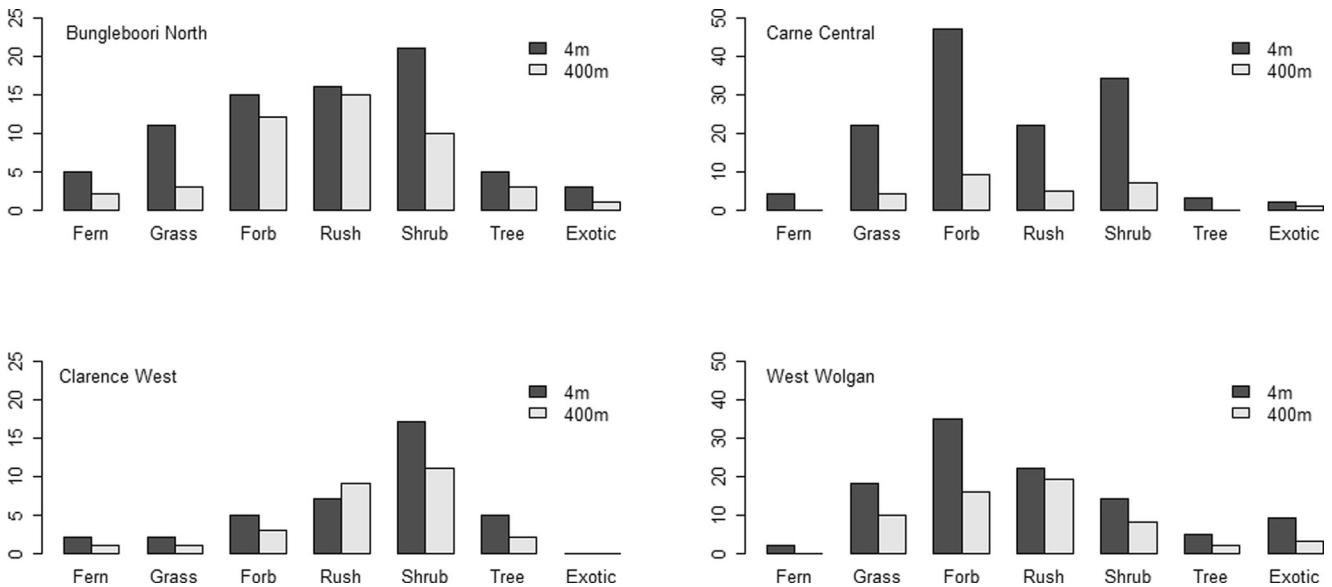
Here we examined a sampling method employed to monitor vegetation in Newnes Plateau Shrub Swamps to see whether it can achieve the stated objectives of detecting a change in

species diversity, composition or abundance due to altered hydrology. We conducted detailed studies of four of the monitored wetlands to better understand whether the natural spatial variation across the wetlands is recorded by the established sampling method. These studies demonstrate that the established sampling method using 400 m<sup>2</sup> plots does record both the common species and the life-forms present; though it lacks ability to detect statistically significant changes in vegetation over time at the wetland scale due to an absence of spatial replication within wetlands. Clearly the choices made in the design stage of a monitoring program will determine its effectiveness (Lindenmayer and Likens 2010). Sampling choices should depend on a) the reason for sampling, b) the predicted changes that might occur and c) the trigger values (i.e. the level of unacceptable change) set. Our study demonstrates a case where a lack of detail in the original objectives, subsequent changes in objectives, and introduction of trigger values defined as statistically significant changes in the indicator variables, has led to an inadequate sampling design. Below we discuss how the monitoring objectives and design could be improved based on our current knowledge of the wetland system.



**Fig. 2** Species area curves for each wetland calculated using 4 m<sup>2</sup> plots. The vertical bars indicate the 90 % confidence interval. The horizontal line shows the number of species recorded with the 400 m<sup>2</sup> plots for each

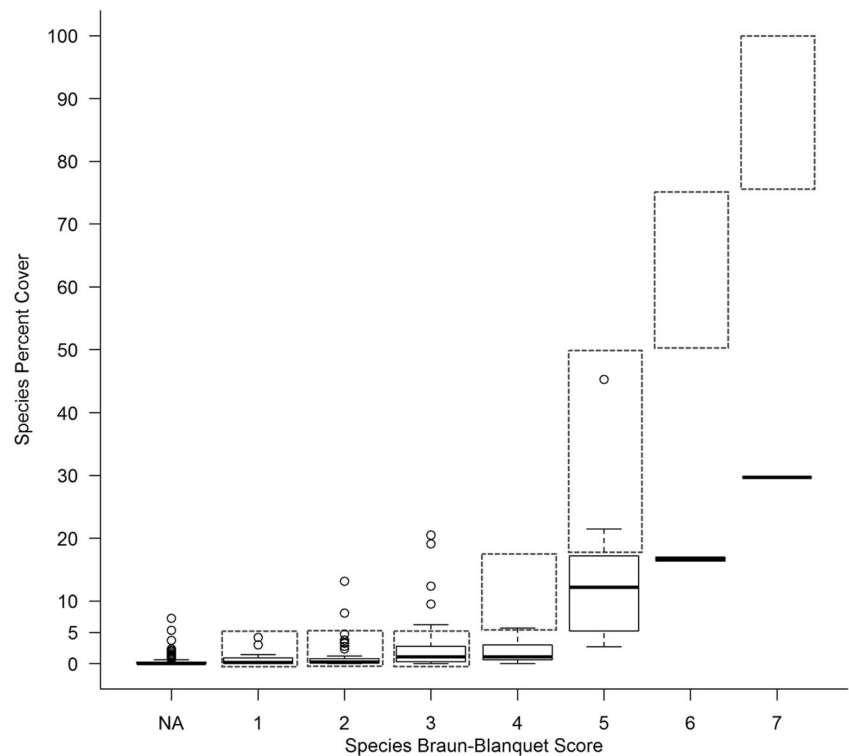
wetland except West Wolgan where the mean number of species per plot and the total found in all six plots are shown



**Fig. 3** Total number of species recorded in each life-form group by each design per wetland. *Note:* the y-axes differ between graphs; “Forb” includes both annual and perennial forbs; “Rush” is the rush/sedge group

and includes Cyperaceae, Juncaceae, Restionaceae species; “Exotic” includes all non-native species (which happen to be all forbs). See Table 1 for number of replicates per design for each wetland

**Fig. 4** Species Braun-Blanquet cover class in the 400 m<sup>2</sup> plots across all wetlands versus mean percentage cover estimate in 4 m<sup>2</sup> plots for that wetland; NA = not found in a 400 m<sup>2</sup> plot, dashed boxes show the range for each cover class. Number of species in each cover class: NA=232; 1=23; 2=37; 3=38; 4=17; 5=9; 6=2; 7=1



It is well known that as area sampled increases, so too does the number of species that will be recorded. The spatial distributions of species, along with the size (grain) and distribution of plots (extent) all influence the species accumulation curve (Hurlbert 1990; Scheiner et al. 2011). Though the species accumulation curves presented here do not reach an asymptote, they provide the lower bound estimates of species richness for these four wetlands (Gotelli and Colwell 2001). The species accumulation curves clearly illustrate that a small number of 400 m<sup>2</sup> purposively-located plots capture less than a third of species present in these heterogeneous wetlands. This may be because most plots are located in central, wetter, areas of the wetland which also tend to be less diverse. Most of the species missed by 400 m<sup>2</sup> plots were sparse species. This bias towards the more abundant species is a common problem with plot-based methods (Gaston 1999). The implications here are that the current sampling method of a small number of 400 m<sup>2</sup> plots will likely miss changes in the widely dispersed sparse species and the uncommon clumped species, such as herbaceous weeds, which are listed as one of the indicator groups (Blick et al. 2013).

In the current sampling design it is evident that replication is not related to the geographic extent of these wetland plant communities. For example, the current sampling design has one plot in Bungleboori North (area = 0.5 ha) and one plot Carne Central (area = 5 ha) which is an order of magnitude difference in area. The haphazard nature of the current sampling design is exemplified in West Wolgan (area = 5 ha) which has six plots, instead of one. The difference in sampling

intensity between Carne Central (reference wetland, not undermined) and West Wolgan (undermined) is largely determined by compliance and regulatory frameworks rather than biological or statistical reasons. Due to the different sizes of the wetlands, the 400 m<sup>2</sup> plot represents a different proportion of each wetland and hence capture a different proportion of species in each wetland; making comparisons of changes between ‘impact’ and ‘control’ wetlands difficult. As we found a greater variance in species density within larger wetlands (perhaps indicative of a wider range of habitats present), we recommend that the current monitoring design could be improved by distributing sampling effort across the wetland and be scalable with wetland size.

There are many ways the current sampling design could be made more effective. One such method is the stratified random method used here to characterise the wetlands but small randomly distributed plots are difficult to relocate accurately, potentially leading to reduced precision (i.e. repeatability) of cover estimates; in addition they are inefficient to sample due to the amount of time required to locate a large number of individual plots. In order to capture the range of habitats present in each wetland, it would be ideal to sample across the environmental gradients. Specifically across the hydrological gradient as the main impact from undermining is predicted to be changes in flow patterns of groundwater. Given that detailed hydrological information is not available for each wetland, nor are there clear hydrological zones present in all wetlands from which to sample [some wetlands range from dry to damp while others range from dry to standing surface

water (Brownstein et al. unpublished data], transects could be an effective method of capturing the variation present in a wetland. We suggest a method that would use replicate transects in each wetland that would be more statistically robust in detecting ecologically relevant changes. The proposed method would use a sub-sampling regime with smaller plots or point intercept samples along transects spanning the width of the wetland (i.e. each transect spans the whole soil moisture/water depth gradient in each wetland), with the number of replicate transects scaled to wetland size, addressing the main sampling issues. Smaller plots within the transects would have the advantage that they can be searched quickly and accurately (Klimes et al. 2001). In addition placing small sub-plots along transects allows plots to be relocated with greater speed and accuracy, compared to relocating individual plots randomly distributed across the wetland (such as the sampling method used here to characterise the wetlands). A second, more novel approach, would be to use remote sensing (Chabot and Bird 2013). Aerial imagery with a spatial resolution of 0.15 m or less would provide detailed coverage over the whole of the wetland and allow quantification of factors such as vegetation die-back and changes in canopy species composition, canopy height and extent of disturbed or bare ground. Using aerial imagery would overcome problems associated with on-the-ground plot based sampling such as: restricted spatial scales (Booth and Cox 2008; Baldeck et al. 2014) and disturbance to fragile vegetation (Fletcher and Erskine 2012). Either a transect based or remote sensing based method would be an improvement on the current 400 m<sup>2</sup> plot based method as they would both be more effective for detecting changes in vegetation within a wetland.

Due to the lack of replication here (14 of the 26 wetlands in current monitoring program contain only a single plot) many simple and common statistics, e.g. means and confidence intervals, cannot be calculated for a wetland. In addition, the plot locations were chosen subjectively, which invalidates a basic assumption of many statistical tests. A statistically sound sampling design is required for testing hypotheses and determining whether triggers have been exceeded (Barrett and Gray 2011). In all four wetlands, the 400 m<sup>2</sup> plots were located in areas of relatively low species density (Fig. 1); this may be due in part to an unintentional bias toward choosing areas that are easy to access, or an intentional bias towards choosing the wettest areas (which are also the least diverse). Removing any unintentional bias in sampling locations and sampling across equivalent extents in each wetland will improve this sampling design.

The lack of replication also raises an issue of plant cover estimates; as others have found poor repeatability (between workers or time points) for plant cover estimates in a single quadrat, regardless of quadrat size (Jalonen et al. 1998; Archaux et al. 2007). In addition, there was little correspondence between estimates of percentage cover in the 4 m<sup>2</sup> plots,

especially for the species in cover classes 1 to 4 in the 400 m<sup>2</sup> plot (the vast majority of the species recorded). Using multiple plots within a wetland allows i) internal variability and ii) the precision of the estimates of species abundances to be assessed (Jennings et al. 2009). One way to increase spatial replication without spending additional money is to reduce the frequency of wetland monitoring. The current program requires seasonal monitoring, though given that many of the species present are long-lived, it is possible that impacts to vegetation will occur over years, not months. In this case, resources may be better spent sampling only once per year (instead of the current four times) and increasing the replication per wetland. Visiting the sites less often would also reduce trampling damage to the fragile wetland vegetation, which could lead to a cumulative impact over time. Results from other studies suggest that fragile communities such as subalpine button grass can sustain prolonged damage with as few as 100 passes (Whinam and Chilcott 2003). Ultimately the effect of track damage could increase species richness and alter species composition (Scott and Kirkpatrick 1994; Gremmen et al. 2003), decreasing the sensitivity and reliability of the un-replicated sampling design.

Resource and time limitations often provide the main constraints in the design and implementation of monitoring programs. The choice of which scale to measure is often arbitrary and based on funding or convenience rather than knowledge (Harris 2003). The 400 m<sup>2</sup> plot with Braun-Banquet cover/abundance scores is quick to sample and is a standard sampling protocol used for vegetation mapping in New South Wales (see Schultz et al. 2014) but we would argue that it does not suit the requirements here. This large plot size is difficult to accurately search in dense shrubby wetland vegetation and due to the small size of some wetlands there is limited space for replicate plots. In addition the cover/abundance scale with uneven classes makes setting trigger values more complex, e.g. a change in cover/abundance score from 1 to 2 is potentially not the same as a change in cover/abundance score from 3 to 4. While the single 400 m<sup>2</sup> plot design is an informative way to record both the common species and calculate the proportion of species across life-forms in a wetland; this type of design seems better suited for broad landscape-scale vegetation mapping as it not effective for detecting change over time in vegetation.

In order to sample most efficiently, a clear statement of monitoring objectives and trigger values (i.e. level of unacceptable change) for management action is needed. Now that more is known about Newnes Plateau shrub swamp wetland systems, it is time to re-evaluate the monitoring objectives and develop detailed objectives and trigger values accordingly. For example, the current objective of detecting ‘a change in species diversity and abundance’ should be made specific to reflect the expected impact of reduced water availability, e.g. trigger values should be set related to a reduction in

hydrophyte abundance or increases in terrestrial species abundance within a wetland. Using plant functional groups rather than individual species can make it easier to interpret trends in abundance (Casanova 2011; Campbell et al. 2014). In addition, trigger values for total live vegetation cover and bare ground would help determine whether vegetation dieback is occurring within a wetland, which when combined with the hydrological data will make it possible to relate negative changes in vegetation to undermining. Once these detailed hypotheses and triggers have been established, a rigorous sampling design could be developed with the statistical power to detect unacceptable changes in wetland vegetation.

When implementing monitoring programs, it seems that the basic tenets of the scientific method and sampling design are often overlooked. There are many examples of monitoring programs with inefficient or ineffectual sampling designs (e.g. Legg and Nagy 2006; Martin et al. 2007), and here we present yet another example. We join other authors (e.g. Downs et al. 2002; Reid and Quinn 2004), to reiterate that the definition of clear monitoring objectives is an often overlooked step that has the potential to save time, money and reduce environmental impacts. Effective monitoring requires periodic review of objectives and revision of methods to ensure that the most efficient method for detecting unacceptable changes is being utilised.

**Acknowledgments** Thank you to Cameron Kilgour and Vanessa Glenn for their assistance with fieldwork and plant identification. This work was supported by funds from Centennial Coal Ltd.

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