Vegetation mounds as over-winter Habitat for Green and Golden Bell frogs *Litoria aurea*

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ABSTRACT

Long-term refugia or "over-winter" habitats are often overlooked in habitat restoration for the endangered Green and Golden Bell frog *Litoria aurea*. Studies identifying the occupation of this habitat or materials suitable to re-create it are lacking. Vegetation mounds were trialled and monitored for 26 months to determine if they could provide shelter conditions for Green and Golden Bell frogs. Covered and uncovered mounds were monitored at two sites (Arncliffe and Woonona) and both types of mounds were utilised by Bell frogs. Most frogs using the mounds for shelter remained active while inside the mounds, a few became torpid while in the mounds. The use of the mounds was influenced by ambient weather conditions. Vegetation mounds have a management advantage over other types of over-winter habitat in that they are portable, cheap and easy to maintain and easy to monitor. In addition, they provide a thermal and humidity gradient and allow frogs to move within the mound to select the preferred microhabitat conditions. As mounds temperatures are above ambient temperatures during winter, they may also assist in reducing the susceptibility of over-wintering frogs to chytrid infection. More detailed studies are needed to determine the optimal size, composition and best management use of the mounds.

Key Words: Green and Golden Bell frog, refugia, over-winter habitat, frog habitat, vegetation mounds.

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Introduction

Green and Golden Bell frogs *Litoria aurea* are threatened frogs [endangered in NSW but listed as vulnerable Nationally] that have been the focus of several translocation attempts (White and Pyke, 2008). The gross habitat requirements of Bell frogs are well described (Pyke and White, 1996; Pyke *et al.*, 2002) and, in general, habitat components comprise breeding habitat, foraging areas, diurnal shelter sites and long-term refugia (over-winter habitat: Pyke and White, 1996; DECC, 2008; Hamer *et al.* 2008).

Most studies on frogs concentrate on times when frogs are most active and detectable, such as during the breeding season. Very few studies have targeted frogs during the less active times of the year (eg. winter) when frogs are more difficult to locate (Hamer *et al.* 2003). Over-winter refugia may be used at any time of the year although it is most often used during winter. Over-winter refugia are used as shelter sites when ambient conditions are unsuitable for activities such as foraging and breeding, during overly hot or cold periods, or overly dry times. Over-winter habitat must provide a secure environment that is safe from predators and have thermal and moisture characteristics that are not stressful to sheltering frogs. Green and Golden Bell frogs that have been found in over-winter habitat are often inactive, adopting a body posture where the head is tucked down and limbs are drawn in tight against the flanks of the body (A. White pers. obs.).

Examples of over-winter habitat are quite varied and range from natural structures such as rocks and logs (Pyke and White, 2001) to artificial structures such as sheets of iron, cement blocks and bricks, dumped cars and household goods and green waste piles (Pyke and White, 1996; DECC, 2008). The variety of materials that can serve as over-winter habitat probably highlights the role of this type of habitat rather than its composition: over-winter habitat must protect the frog from predators and hostile weather conditions.

To date, there have been no controlled studies into the utilisation of various over-winter habitat materials. This is a knowledge gap that may have thwarted translocation attempts in the past. Translocations of Green and Golden Bells frogs at Botany (White, 1998) and Long Reef (Pyke *et al.*, 2008) failed because there were high frog mortalities during the winter period inferring that no adequate over-winter habitat was available at the time or it may reflect chytrid infections. Rock piles were provided at both sites in an attempt to provide winter refugia. Inadequate over-winter habitat may exacerbate stress in frogs making them more vulnerable to disease (Alford *et al.* 2007).

In this study, vegetation mounds were established and monitored at two sites where Green and Golden Bell frogs occur. Vegetation mounds comprising recently harvested green waste were selected as the material for over-winter habitat because field observations (L. Jurd pers. comm.) indicated that Green and Golden Bell frogs utilise vegetation mounds in frost-prone areas in western Sydney and are able to survive freezing conditions inside the mounds. The aim of the present study was to determine if Green and Golden Bell frogs would utilise vegetation mounds during adverse weather conditions, and whether the mounds could serve as a useful habitat component in managed sites where these frogs occur.

Methods

Study Sites

Study sites were established at Arncliffe in the southern suburbs of Sydney, and Woonona, about 6 kilometres north of Wollongong in the Illawarra district. These two populations were chosen as they have been monitored for 10 or more years. Both sites have relatively modest Green and Golden Bell frog populations: the population estimates for Arncliffe in 2014 was 36 adult frogs (Biosphere 2014a) and 12 for Woonona (Biosphere 2014b). The Arncliffe and Woonona sites are re-created habitat areas and contain over-winter habitat, although quite different in nature. At Arncliffe, large boulder fields (Figure 1) were established close to two Bell frog breeding ponds established in 1998 whereas at Woonona, smaller, vegetated rock piles were created as over-winter habitat (Figure 2; White, 2002). The Woonona frog ponds and habitat area was established in 2002.

The Arncliffe site contains two artificially constructed frog ponds, each 20 m long and 10 m wide, 80 cm deep. The ponds have a fringing cover of tall emergent vegetation and there is an open water area in the centre of each pond. Immediately adjoining the site is a golf course containing smaller ponds; the golf course is regularly used as a foraging site by the Green and Golden Bell frogs but breeding rarely occurs there (Biosphere, 2013).

The Woonona site is centred on six artificially constructed ponds; the sizes of ponds varies between 10-20 m long and 5-10 m wide. Each pond has a stand of tall, emergent reeds (mainly *Typha orientalis* and *Eleocharis sphaeculata*) and there is an area of open water in each pond. Excess emergent vegetation is removed from the ponds each year to retain the open water area. The area between the ponds consists of exotic grassland, or native woodland.

Green and Golden Bell frogs at Woonona and Arncliffe are micro-chipped and a long-term population data files exists for both sites.

Types of Mounds

Covered and uncovered vegetation mounds were established at Arncliffe and Woonona in late November 2012. Mounds consisted of 1.5 m^3 of recently cut plant material (grass and leaves) poured over a supporting framework of interwoven branches and sticks intended to keep the mounds from collapsing on themselves (Figure 3). Three covered mounds were established at Woonona (at ponds 2, 3 and 6) while one covered mound was established at Arncliffe (pond 1). Two uncovered mounds were established at Woonona (at ponds 1 and 5) while two uncovered mounds were established at Arncliffe (pond 2). All mounds were located in exposed sites where over-shadowing by trees would be minimal. All mounds were located within 5 m of a frog pond.

Covered mounds were overlain by a sheet of black plastic (builders' water-proofing membrane) which was weighed down in the corners with small rocks. The edges of the plastic were not pegged down and animals could easily crawl under the plastic sheeting to reach the vegetation mound beneath.

Figure 1. Boulder areas established as over-winter habitat, Arncliffe 2002. Photo A. White.

Figure 2. Vegetated rock areas established as over-winter habitat, Woonona 2005. Photo A. White.

Figure 3. Covered and Uncovered vegetation mounds at Arncliffe, 2013. Photo A. White.

Monitoring of the Mounds

The mounds were monitored on the same day once a month between December 2012 and February 2015, between 10 am and 4 pm. Mound temperatures were taken at three positions in each mound using a digital thermometer: the eastern and western sides of the mound were measured to account for any local heating effect that might occur during the transit of the sun during the day, and a measurement of the centre of the mound was also taken. The eastern and western measurement points were 10 cm below the surface of the mound and 10 cm in from the edge of the mound. The central measurement point was 30 cm below the surface and approximately 50 cm from the edges of the mound. Humidity measurements were also taken at the same sites on the same day using a HydroSensor probe. Care was taken not to remove the cover from the covered mounds while the temperature and humidity measurements were being taken. Ambient temperatures and humidity were also measured on the same day that fauna counts took place.

The vegetation in the mound was gently lifted away in sections to expose any animals that may have been sheltering inside. The animals were not handled and all efforts were made to minimise the disturbance to the animals. The vegetation was quickly replaced and the mound restored to its original condition once the count of animals had been completed.

Results

Temperature and Humidity in Covered and Uncovered Mounds

In spring and summer, air temperatures were higher than the mound temperatures in both covered and uncovered mounds (Figures 4 and 5). In the cooler months of the year, mound temperatures were higher than ambient air temperatures.

Core mound temperatures in covered mounds were consistently greater than the core temperatures in the uncovered mounds. In summer, the covered mounds were 2.1° warmer and 3.3⁰ warmer in winter. Covered core temperatures varied between 13.2° and 19.5° C whereas the uncovered core varied from about 11.1° to 20.0° degrees despite air temperatures varying between 6.6° and 37.8° during the course of this study (Bureau of Meteorology weather station data for Sydney Airport and Corrimal). The core of the mounds were thermally much more stable than the flanks of the mounds: flank temperatures varied between 7.5° and 25.5° C with the western flanks of both covered and uncovered mounds being slightly warmer on average than the eastern sides of the mounds (the mean difference between the eastern and western flanks of the mounds was 0.8° C).

Humidity levels inside the mounds did not vary as greatly as the external air humidity (Figures 6 and 7). Humidity levels in the core of the covered mounds between 46.8% and 77.6 % while the core humidity in the uncovered mounds varied between 30.5% and 66.4 %. For most months of the year, core humidity levels were higher than ambient levels; ambient levels only exceeded the core humidity during times of repeated rainfall (in this study high ambient humidity conditions occurred in June 2013 and January 2014; Figures 6 and 7). Humidity levels at the flanks of the covered mounds were higher than humidity levels at the flanks of uncovered mounds but both were lower than the core humidity levels. The humidity of the flanks of the mounds approximated the air humidity levels to within 5%.

Animal Use of Mounds

Covered and uncovered mounds were used as shelter sites by reptiles and frogs in this study (Table 1). Frogs utilised both types of mounds throughout the year but more frogs were found under the covered mounds in the hotter months of the year. Reptiles were more commonly found under the uncovered mounds, except during wet weather periods.

There was little variation between the use of mounds at Arncliffe and Woonona. Fewer Green and Golden Bell frogs were found in uncovered mounds at both sites but the time of year when they were found varied. Most of the Green and Golden Bell frog sightings were made in both mound types during the winter months of 2013 and 2014 (Table 1) however both sites had occasional mound use by Bell frogs at other times of the year.

Green and Golden Bell frogs that were aestivating were found on four occasions (in May 2013 in a covered mound

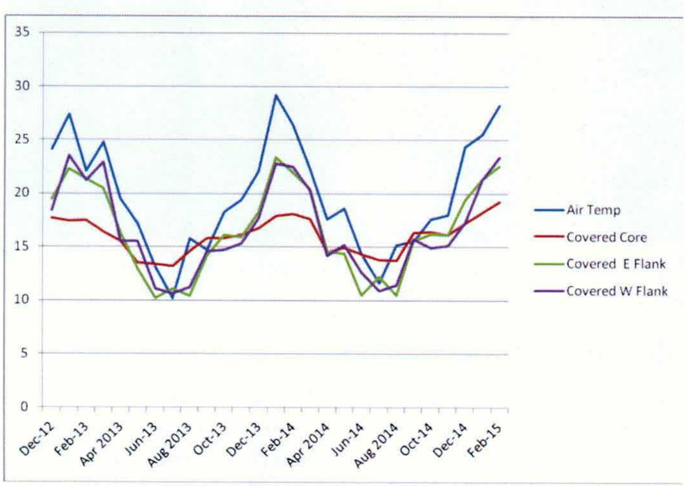

Figure 4. Changes in mound and air temperatures of covered mounds

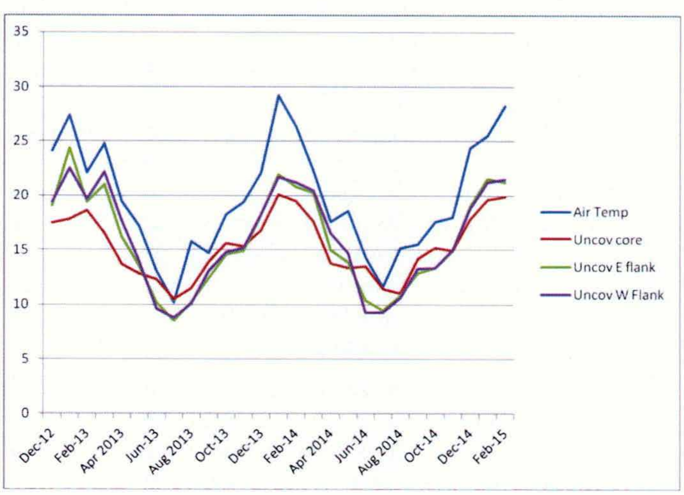

Figure 5. Changes in mound and air temperatures of uncovered mounds.

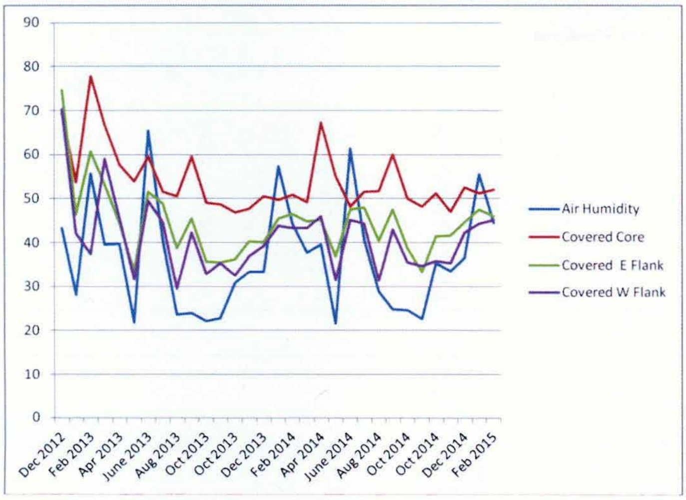

Figure 6. Changes in mound and air humidity of covered mounds.

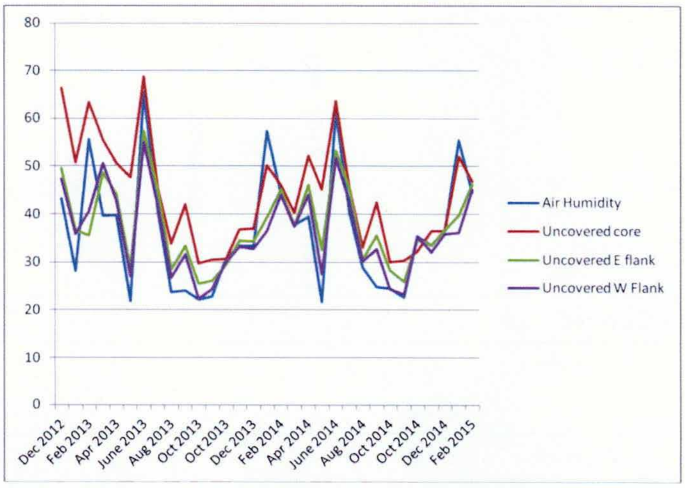

Figure 7. Changes in mound and air humidity of uncovered mounds.

at Woonona, June 2013 covered mound Arncliffe, July 2014 covered Mound Arncliffe and August 2014 covered mound Arncliffe). All other Bell frogs found under mounds were alert and not in an over-winter torpid-like state, although often very dark in colour (Figure 8).

Two frogs were found in mounds over successive months. It is not known if these frogs remained inside the mound for this entire time duration or had returned to the same mound after a period away from the mound. No frogs were found to inhabit the mound for three successive months.

Discussion

Thermal and Humidity Characteristics of Vegetation Mounds

Vegetation mounds appear to provide an insulated environment that has lower temperature and humidity fluctuations than outside conditions. Vegetation mounds also provide thermal (Figures 4 and 5) and humidity gradients (Figures 6 and 7) from the core to the outside of the mounds. These characteristics allow frogs (and other animals) to use vegetation mounds as temporary shelter sites or as over-winter habitat. There was relatively little difference in the thermal characteristics of covered compared to uncovered mounds but the covered core temperatures were greater in winter and cooler in summer. This implies that most of the thermal insulation in the mound is achieved by the bulk of the plant material in the mound and the plastic covering contributed little to the thermal properties of the mound.

In contrast, there was a distinct humidity difference between covered and uncovered mounds. Covered mounds had a consistently higher humidity level than uncovered mounds. The humidity level in the uncovered mounds was affected by the volume of plant material, the sides of the mounds were consistently drier than the cores of the mounds (Figures 6 and 7).

Hamer *et al.* (2003) trialled two refugia substrates (brick piles and aquatic vegetation trays) to see if Green and Golden Bell frogs were selective in their choice of refugia. They concluded that these frogs were not selective and the choice of refugia depended on the microhabitat (especially temperature) that was provided by the refugia.

Use of Mounds by Green and Golden Bell frogs

Green and Golden Bell frogs utilised vegetation mounds throughout the year (Table 1) at very low levels. Bell frogs using mounds in the warmer months of the year were more commonly found in covered mounds, despite the fact that the temperatures of covered and uncovered mounds were very similar. Humidity levels inside the covered mounds were higher during the spring and summer and so it is implied that Bell frogs selected covered mounds to take advantage of the greater humidity in them. In autumn and winter, Bell frogs were found in both types of mounds and so it appears that the humidity requirement may be less important in the cooler months of the year.

The mean number of Green and Golden Bell frogs found in covered mounds ($\bar{x}$ = 11.0, n=4) was double the number found in uncovered mounds ($\bar{x}$= 5.0, n = 3). There was a high variation in use of mounds with Woonona 3 (covered site) and Woonona 5 (uncovered) only having one Bell frog found in them over the entire monitoring period. In contrast, Arncliffe 1 and Woonona 2 (n=both covered sites) had 25 and 16 Bell frog records. The reason for such variation is not known but may be related to the proximity of the mound to frog movement corridors.

Green and Golden Bell frogs used mounds as temporary shelter sites or as long-term refugia (over-winter habitat). Two Bell frogs were found inside the same mounds over two consecutive monitoring periods; both frogs were inactive on both occasions and it is highly likely that the frogs had been under the mound for the period between surveys, however, this cannot be verified. Sheltering frogs were often found in a crouching position when unearthed but were quick to resume an alert posture and hop off if disturbed. Only four Bell frogs have been found in a torpid state. All four frogs were found in this condition between May and July (when ambient temperatures were lowest). These animals had a typical body pose whereby the arms and legs are drawn in tight under the body and the head was tucked down. These frogs were also very dark

Table 1. Frogs and reptiles found under covered and uncovered mounds.

		Dec 2012 – Feb 2013	March-May 2013	June-August 2013	Sept - Nov 2013	Dec 2013- Feb 2014	March-May 2014	June-August 2014	Sept - Nov 2013	Dec 2013- Feb 2014
Covered										
Arncliffe 1	GGBF	2	1	3	3		4	6	2	4
	Other		1 SMF				2 SMF	8 SMF	2 SMF	1 SMF
Woonona 2	GGBF	2		3	3	1	2	3	1	1
	Other					1MS	1 MS			
Woonona 3	GGBF							1		
	Other	1 RBBS		2 SMF	1 EWS	1 RBBS	1 SMF		1 RBBS	
Woonona 6	GGBF		2							
	Other	1 EWS				1 EWS	2 EWS	3 EWS		2 EWS
Uncovered										
Arncliffe 2	GGBF		3	3			2	2		1
	Other	1 SMF 1BTL			1 ESW		2 SMF	6 SMF		
Woonona 1	GGBF					1		1	1	
	Other	1 EWD	1 EWS	1 ESW 1MS	1 SMF		3 SMF	1 SMF	1MS	
Woonona 5	GGBF								1	
	Other	1 EWS	1 EWS 1 RBBS 1 SMF		3 EWS	2 ESW	4 EWS	2 EWS 1RBBS	2 SMF	3SMF

GGBF = Green and Golden Bell frog *Litoria aurea*, SMF= Striped Marsh Frog *Limnodysastes peronii*, EWS = Eastern Water Skink *Eulamprus quoyii*, EWD = Eastern Water Dragon *Physignathus lesueuri*, MS = Marsh Snake *Hemiaspis signata*, RBBS = Red Bellied Black Snake *Pseudechis porphryiacus*, BTL = Blue-tongue Lizard *Tiliqua scincoides*

in colour. Other Bell frogs were found in winter under mounds but these frogs were alert although slow to move.

Many of the Green and Golden Bell frogs found under the mounds were dark in colour (Figure 8). Green and Golden Bell frogs are a basking species and have been observed to rapidly brighten (and increased their green pigmentation) when exposed to sunlight (DECC, 2008). It appears that sheltering frogs are not responding to ambient light and greatly reduce the amount of green pigmentation across the body surface. The increase in dark pigmentation may be an attempt to absorb heat from the surrounding substrates.

Mounds may provide more moderate temperature and humidity environments for frogs and this may be important at times of adverse weather conditions. Older mounds (not tested in this trial) that have undergone further decomposition may provide additional heat to the core of the mound and so may be an even more useful refuge for ectothermic animals during prolonged cold spells. Heard *et al.* (2008) found that humidity was

Figure 8. Green and Golden Bell frogs in uncovered mound, Arncliffe May 2013. Photo A. White.

a significant factor in the choice of diurnal shelter sites for the related Growling Grass Frog *Litoria raniformis*.

In this trial, mounds were kept at a moderate size (1.5 m^3) and so the impact of covering the mound may have been more pronounced than for a more massive mound (with a greater thermal inertia). Larger mounds may be a better option as a management tool as they should provide a wider gradient of temperature and humidity for ectotherms to choose from. The use of black plastic covers may also be unnecessary with large mounds. Black plastic was chosen as it was suspected that it might have a bigger heat trapping capacity that other more reflective plastics. Adding heat to the mound may be critical during particularly sudden or prolonged cold spells and the use of plastic covers may still be required in frost-prone areas. It appears that the plastic covering moderates the core temperature of covered mounds but replicate data are needed to demonstrate this.

Temperature and humidity were found to be the most significant factors in shelter selection for tropical Cane Toads *Rhinella marinus* (Schwarzkopf and Alford 1996). In this species, microhabitat selection was determined by body temperature and dehydration rates (Seebacher and Alford 2002). In other toad species, the physical structure of the refugia was found to be of little importance whereas the relative humidity and temperature of the refugia were critical (Long and Prepas 2012).

For temperate frog species, like the Green and Golden Bell Frog, overheating and dehydration may only be a threat during summer, but in winter, lowered body temperatures may make these frogs more susceptible to disease, such as chytridiomycosis (Berger *et al.* 2004). The provision of relatively warmer over-winter habitat may allow Bell frogs to thermally resist the impact of frog chytrid disease during the winter months. Gantz and Sheafor (2012) reported that behavioural thermoregulation in various frog species can reduce the level of infection and incidences of mortality due to chytrid disease. Decomposing vegetation mounds are relatively warmer than other over-winter substrates, such as timber and rock, and may be provide an adequate thermal range to allow Bell frogs to thermally maximise their chances of resisting the chytrid fungus during the colder and drier times of the year.

The external cover placed over a mound has one obvious advantage; it helps reduce moisture loss from the mound. For moderate-sized mounds, such as were tested here, the addition of a cover had a measureable effect on the moisture content of the mound. For large mounds, this effect is likely to be reduced or negated altogether by the mass insulation of the plant material in the mound.

Drier mounds, such as uncovered mounds in the spring and summer months, were mostly inhabited by reptiles. Wetter mounds, such as covered mounds in the autumn and winter months, were rarely used by reptiles but were used by sheltering frogs.

Advantages of mounds as a habitat enhancement tool

Habitat creation is used increasingly as a conservation strategy (Pickett *et al.*, 2012). In many instances, the traditional habitat areas of a species have been lost or usurped for commercial or residential purposes. Off-set areas are used in an attempt to ameliorate the loss of this habitat by relocating the species to a new area where habitat will be recreated according to the understanding of the needs of that species (eg. Green and Golden Bell frog: White, 2002). For the Green and Golden Bell frog, the understanding of the gross habitat requirements of the species are well documented (Pyke and White, 1996, Pyke *et al.*, 2002) but the more subtle micro-environmental factors are still poorly known (Pyke and White, 2001). Over-winter habitat is one of the gross habitat features that has been identified as an essential habitat requirement yet we do not fully understand how best to provide this habitat.

The presence of over-winter habitat for Green and Golden Bell Frogs in created habitat areas is receiving greater attention (DECC 2008). It is believed that at least two translocation attempts of the Green and Golden Bell frogs have failed because of the lack (or inappropriateness) of over-winter habitat. The translocation of Bell frogs to Botany and to Long Reef both failed because of high frog mortalities over winter (White, 1998: Pyke *et al.*, 2008; White and Pyke 2008). It is noteworthy that in translocation attempts (Arncliffe and Woonona) where considerable attention was given to the provision of over-winter habitat, some (but not all) Green and Golden Bell frogs survived the winter period. At Arncliffe, extensive rock fields were established alongside and between the frog ponds (White, 1998) while at Woonona compost mounds were created close to each frog pond (White, 2002). It needs to be added that Arncliffe was a population-augmentation location (see White and Pyke 2008) rather than a translocation-only location and the presence of an existing population may add to the number of winter surviving frogs at Arncliffe.

Vegetation mounds have a considerable management advantage over other forms of over-winter habitat (such as metal or timber sheets or rocks). Mounds are portable, easily added to or modified, cheap to maintain and accessible for monitoring. Because mounds can be added to or be easily modified, they are ideal for field manipulation trails. Additional field trials are required to determine the optimal structural design of mounds so that they provide the required range of temperatures and humidity, as well as providing physical protection for the elements and predators. The best use of mounds also needs to be determined eg. how often should new plant material be added to the mound, should the mounds be watered, how do you stop unwanted species from occupying the mounds and how should the mounds be altered in response to sudden changes in weather conditions? Despite the lack of detailed knowledge of mound use, mounds appear to be able to provide a sufficient range of temperatures and humidities to make them a useful habitat feature.

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