ANNEX 1. Example of a technical report to a conservation agency

Diel activity patterns and refuge use by redfin minnows *Pseudobarbus afer* within the Groendal Wilderness Area

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Report to the Eastern Cape Parks and Tourism Agency on Research within the Groendal Wilderness Area (Permit R0151)

Summary

The endangered Eastern Cape redfin minnow *Pseudobarbus afer* is a conservation priority because it is threatened primarily by non-native piscivorous fish. In order to gain an understanding of the probable invasion consequences, this study examined the diel activity and refuge use patterns of redfin minnows within the Groendal Wilderness Area. These patterns were compared to those of chubbyhead barb *Barbus anoplus* that occurs within the Great Fish River system. This was achieved by using a combination of field observations and laboratory-based experiments. The main findings and conservation implications were:

- Redfin minnows exhibited high diurnal activity and shoaling behaviour in the wild. Although they showed high refuge use in the laboratory, they were diurnally active, a behaviour that was consistent with field observations.
- The diel activity patterns of redfin minnows were in contrast to those of chubbyhead barbs that were nocturnally active and occurred in low abundances.
- Diurnal activity behavioural patterns by redfin minnows may, in part, explain their susceptibility to high predation by visual non-native predators, such as largemouth and smallmouth bass. Conservation priority should therefore focus on limiting invasions by these piscivores.

Introduction

Non-native invasive species are major drivers of biodiversity loss. In freshwater ecosystems, impacts by non-native fish species on native biota have been observed to range from subtle, such as influencing behaviour, distribution and habitat use, to local extirpation and broad ecosystem impacts, including disruption of food webs (Strayer 2010). In the Eastern Cape, South Africa, many non-native fish species have established within many rivers, and there are serious concerns on the conservation of native species. Of particular conservation concern is the Eastern Cape redfin minnow *Pseudobarbus afer* that is cited on the IUCN Red-List as being endangered. This redfin minnow is primarily threatened by non-native piscivores, such as largemouth bass *Micropterus salmoides*, smallmouth bass *M. dolomieu* and sharptooth catfish *Clarias gariepinus* that have isolated them to headwater tributaries of the Swartkops and Sundays rivers near Port Elizabeth.

In order to understand the potential consequences of different non-native predators, this study examined the behavioural diel activity and refuge use patterns for the redfin minnows *P. afer* and compared them with chubbyhead barbs *Barbus anoplus.* Chubbyhead barbs are widespread within many rivers in the Eastern Cape and are not red listed. Anecdotal evidence, based on previous field observations, suggests that chubbyhead barbs occur in sympatry with non-native piscivores, such as largemouth and smallmouth bass and rainbow trout in certain habitats. By contrast, reports have shown local extirpation of the redfin minnows in habitats where they co-occur with these non-native piscivores. Diel activity patterns and refugia use for both species were therefore examined based on both field experiments within non-invaded streams and using laboratory experiments. The aim of this experimental research was to relate the findings of the observed responses for both *P. afer* and *B. anoplus* to the potential invasion of their habitats by different non-native predators.

Methods

Field observations

Redfin minnows were sampled in the Waterkloof River, a tributary of the Swartkops River within the Groendal Wilderness Area. Sampling was conducted from 28 July - 1 August 2013. Due to the endangered status of this species, fish abundance was estimated using non-destructive minnow traps (50 cm long by 25 cm diameter and 3 cm diameter opening, with 2 mm mesh) that were baited with trout pellets diets. The traps were randomly positioned in different habitats along the stream. The traps were deployed from 07:00 to 16:00 hrs and 18:00 to 06:00 hrs for diurnal and nocturnal observations, respectively. The sampled stream consisted of a series of pools and riffles (Figure 1). A total of 20 traps were used and sampling was conducted over three consecutive days and nights. The microhabitat around each trap was assessed based on water depth (cm), dominant substratum and the presence or absence of bank vegetation. Substratum composition was categorised based on a modified Wentworth scale as coarse gravel (< 6 cm), cobble (6 - 25 cm), boulder (25 cm - 100 cm) and bedrock (> 1 m). To determine the presence and diel patterns of predatory fish, six double-ended fyke nets were set randomly and monitored over the same three consecutive days and nights. The fyke nets were set between 07:00 to 16:00 hrs and 18:00 to 06:00 hrs for diurnal and nocturnal observations, respectively. Both minnow traps and fyke nets were observed between 16:00 to 18:00 hrs and 06:00 to 08:00 hrs for the day and night captures, respectively. All fish that were captured were identified, measured (standard length) and released back into the river alive. Raw data are in Appendix 1.

Figure 1: The habitats that were sampled using minnows traps in the Waterkloof River within the Groendal Wilderness Area

Laboratory experiments
A total of 60 redfin minnows were captured by seine netting and transported to the laboratory in oxygenated tanks. The batches for the two species were left to acclimatise in holding tanks (90 cm × 32 cm × 40 cm in length, width and height, respectively) in the laboratory for a period of at least four weeks. The laboratory was designed to simulate a 12 hour day and night photoperiod using timer-controlled fluorescent lights. The day period was therefore illuminated from 00:00 to 12:00 hrs, whereas the night period commenced from 12:00 to 00:00 hrs. This illumination cycle allowed for the observations of both diurnal and nocturnal activity during the course of a working day. We maintained the fish in clean filtered water. Water temperature was maintained at 20°C and dissolved oxygen was kept at saturation level. The fish were fed daily on standard commercial aquarium fish flakes. Diel activity patterns of the fish were monitored using experimental tanks measuring 30 cm × 23 cm × 24 cm in length, width and height, respectively. Each tank, which contained an undergravel bed with an air-lift oxygenation system, was divided into three equal areas; pipe refuge, “grass” refuge, and open water. A factorial experiment was conducted to test the effects of size class and conspecifics (Kadye and Booth 2014).

Results and Discussion

A total of 1931 redfin minnows were collected during the field experiments (Table 1). Redfin minnows exhibited a shoaling behaviour, and showed high diurnal activity pattern whereby 73% (n = 1407) of the fish were captured during the day compared to 27% (n = 524) during the night. The highest abundances were recorded in habitats with boulders and cobble substratum, whereas gravel substratum had the lowest abundances (Table 2). In addition to redfin minnows, Cape kurper *Sandelia capensis* were captured using the minnow traps while seven longfin eels, *Anguilla mossambica* were captured using fyke nets. All fish were released alive and there were no mortalities recorded from any sampling gear.

In the laboratory, redfin minnows showed high refuge use during day compared to night for both individual and grouped fish (Figure 2). Specifically, redfin minnows utilised pipe and grass refugia, whereas open water was least utilised. Nevertheless, these minnows were active during day compared to night (Figure 2).

Table 1: Summary of the total number of redfin minnows that were captured using minnow traps in Waterkloof River within the Groendal Wilderness Area. Sampling was conducted over three days and nights.

	Photoperiod		
Sampling Day	Day	Night	Grand Total
1	598	179	777
2	398	200	598
3	411	145	556
Grand Total	1407	524	1931

Table 2: Redfin minnows abundance in relation to different substratum categories in Waterkloof River within the Groendal Wilderness Area.

	Photoperiod		
Substratum	Day	Night	Grand Total
boulder	846	313	1159
cobble	512	169	681
gravel	49	42	91
Grand Total	1407	524	1931

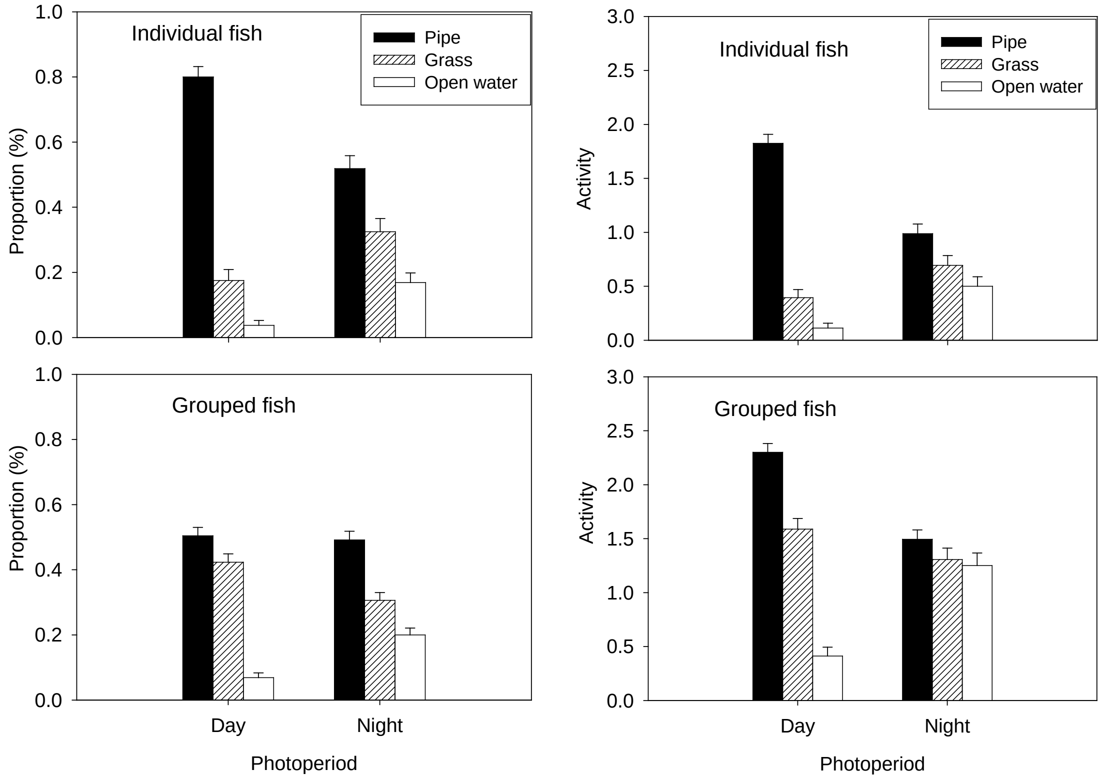

Figure 2: Refuge use and activity patterns for redfin minnows based on laboratory experiments.

The results on redfin minnows were in contrast to those observed for chubbyhead barbs. Based on the field experiments, we found that chubbyhead barbs were nocturnal, whereas redfin minnows showed high diurnal activity. For chubbyhead barbs, their nocturnal behaviour suggests a response to the costs associated with daytime activity. Because nocturnal activity is considered to be inefficient for visual foraging (Fraser et al. 1997) particularly for stream-dwelling minnows (Reebs et al. 1995), and due to the absence of fish predators within its habitat, the nocturnal habit of chubbyhead barbs suggests a response to visual terrestrial predators, such as diving and wading birds. In comparison, diurnal activity by redfin minnows suggests a response to the relative costs associated with nocturnal activity. Although the cost of nocturnal activity could be inferred from the presence of longfin eel *A. mossambica* that was active at night in habitats with the minnows, visual terrestrial predators, such as birds, could potentially be associated with the cost of diurnal activity for this species. Furthermore, the sympatric occurrence of redfin minnows with *S. capensis* suggests potential for resource competition. However, studies show that when animals are faced with different costs associated with satisfying minimum energy requirements, due to either predation or competition, they often learn to discriminate between these costs and individual risks. Based on the field experiments, the redfin minnow's diel activity patterns suggest three probable adaptive mechanisms to both direct and indirect costs, such as predation and competition, respectively. First, by being active during the day, minnows could potentially capitalise on feeding efficiency that would be conferred by the light hours for both prey detection and capture while avoiding predation from nocturnal predators, such as longfin eel. Second, the shoaling behaviour by minnows that was observed in this study may suggest an adaptive mechanism to visual terrestrial predators. Shoaling behaviour in fishes has been observed to be an important anti-predator strategy in streams that are subject to high predation risk. Third, our results indicated stronger depth-dependence for redfin abundance during the day compared to night. This suggests that although the redfin minnow was diurnal, it was more active in deeper pools, which could potentially curtail the risk of visual terrestrial predators, particularly shallow diving and wading birds.

Implications on biological invasions

Non-native piscivorous fish are considered to be the major threat to both populations for these minnows (Tweedle et al. 2009). Although most populations for both species occur in headwater streams, periodic incursions by the non-native fishes into these sections have been reported (Ellender et al. 2009). The probable invasion pathways include deliberate illegal introductions by anglers, especially for largemouth and smallmouth bass, and through movement from mainstem sections into headwater streams when habitats become connected during periods of high flow (particularly for sharptooth catfish).

Previous studies on predation impact have shown local extirpations of redfin minnows in habitats invaded by largemouth and smallmouth bass and trout that are known to be visual predators (Lowe et al. 2008; Russel 2011). By comparison, chubbyhead barbs occur in sympatry with trout and bass in certain habitats where they have been deliberately stocked as fodder fish (Booth pers. obs). The nocturnal habits of chubbyhead barbs suggest a pre-adaptive response to potential predation by diurnal visual predators. This nocturnal behaviour may explain, in part, the co-occurrence of the chubbyhead barbs with non-native predators, such as bass and trout. Nevertheless, it is unclear whether such co-occurrences are associated with both predation and non-consumptive costs. In addition, potential invasion by sharptooth catfish, which is now dominant in the mainstem sections of many rivers in the region (Kadye & Booth 2013), may offset this prior advantage as it is known to have nocturnal habits (Bruton 1979).

The diurnal activity of redfin minnows may, also in part, explain its vulnerability to visual predators. Although we observed its shoaling behaviour as a potential anti-predator mechanism, such behaviour may have also evolved in response to a known visual predator, such as birds, and may therefore be an inappropriate behaviour if exposed to a novel aquatic predator. This shoaling behaviour may increase vulnerability to visual predators, such as bass and trout, as observed in predation impact studies (Lowe et al. 2008). Some studies suggest that when prey species learn to recognise novel predators, they respond by either altering their diel activity (Bool et al. 2011) or they shift their habitat use by moving to shallow habitats to avoid predation by non-native piscivores (Schlosser 1987). Behavioural modifications by native prey species in response to the presence of non-native predators may nonetheless be associated with non-consumptive effects, such as use of suboptimal habitats and limited foraging time that would have an effect on population fitness (Harvid et al. 2013), whereas, shifting habitat use could expose these fishes to terrestrial prey (Loppnow et al. 2013). The diurnal activity and shoaling behaviour of redfin minnows may explain why this species has experienced severe localised extirpations in river sections that have been invaded by visual predators, particularly *Micropterus* spp. that are known to be active during day time periods. Conservation efforts should therefore continue to prioritise protection of redfin minnows habitats from invasions by these piscivores.

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Appendix 1 – Data collected on redfin minnows, *Pseudobarbus afer*, in the Groendal Wilderness Area using baited minnow traps.

Date	Trap	Latitude	Longitude	Photo	Depth	Substrate	Vegetation	Fish caught
28 July 2013	1	33°43'01.17"	25°17'01.29"	Night	42	cobble	present	9
28 July 2013	2	33°43'01.05"	25°17'00.95"	Night	29	cobble	present	0
28 July 2013	3	33°43'01.00"	25°17'00.75"	Night	22	cobble	present	4
28 July 2013	4	33°43'00.90"	25°17'00.46"	Night	15	cobble	present	11
28 July 2013	5	33°43'00.69"	25°17'00.20"	Night	52	cobble	absent	0
28 July 2013	6	33°43'00.64"	25°16'59.91"	Night	40	gravel	absent	0
28 July 2013	7	33°43'00.59"	25°16'58.47"	Night	12	cobble	present	0
28 July 2013	8	33°43'00.54"	25°16'58.54"	Night	22	boulder	absent	9
28 July 2013	9	33°43'00.39"	25°16'57.37"	Night	60	boulder	absent	25
28 July 2013	10	33°43'00.27"	25°16'56.81"	Night	55	boulder	present	31
28 July 2013	11	33°43'00.32"	25°16'56.53"	Night	15	gravel	present	8
28 July 2013	12	33°43'00.27"	25°16'56.33"	Night	58	gravel	present	5
28 July 2013	13	33°43'00.24"	25°16'56.03"	Night	28	boulder	present	3
28 July 2013	14	33°43'00.27"	25°16'55.76"	Night	12	cobble	absent	0
28 July 2013	15	33°43'00.24"	25°16'55.53"	Night	36	cobble	present	14
28 July 2013	16	33°43'00.17"	25°16'55.29"	Night	28	boulder	present	11
28 July 2013	17	33°43'00.18"	25°16'55.12"	Night	42	boulder	present	18
28 July 2013	18	33°43'00.01"	25°16'54.86"	Night	48	boulder	present	12
28 July 2013	19	33°43'59.92"	25°16'54.74"	Night	35	boulder	present	5
28 July 2013	20	33°43'59.77"	25°16'54.54"	Night	48	cobble	present	14
29 July 2013	1	33°43'01.17"	25°17'01.29"	Day	42	cobble	present	13
29 July 2013	2	33°43'01.05"	25°17'00.95"	Day	29	cobble	present	0
29 July 2013	3	33°43'01.00"	25°17'00.75"	Day	22	cobble	present	12
29 July 2013	4	33°43'00.90"	25°17'00.46"	Day	15	cobble	present	15
29 July 2013	5	33°43'00.69"	25°17'00.20"	Day	52	cobble	absent	84
29 July 2013	6	33°43'00.64"	25°16'59.91"	Day	40	gravel	absent	0
29 July 2013	7	33°43'00.59"	25°16'58.47"	Day	12	cobble	present	10
29 July 2013	8	33°43'00.54"	25°16'58.54"	Day	22	boulder	absent	13
29 July 2013	9	33°43'00.39"	25°16'57.37"	Day	60	boulder	absent	74
29 July 2013	10	33°43'00.27"	25°16'56.81"	Day	55	boulder	present	56
29 July 2013	11	33°43'00.32"	25°16'56.53"	Day	15	gravel	present	2
29 July 2013	12	33°43'00.27"	25°16'56.33"	Day	58	gravel	present	12
29 July 2013	13	33°43'00.24"	25°16'56.03"	Day	28	boulder	present	40
29 July 2013	14	33°43'00.27"	25°16'55.76"	Day	12	cobble	absent	2
29 July 2013	15	33°43'00.24"	25°16'55.53"	Day	36	cobble	present	27
29 July 2013	16	33°43'00.17"	25°16'55.29"	Day	28	boulder	present	21
29 July 2013	17	33°43'00.18"	25°16'55.12"	Day	42	boulder	present	55
29 July 2013	18	33°43'00.01"	25°16'54.86"	Day	48	boulder	present	37
29 July 2013	19	33°43'59.92"	25°16'54.74"	Day	35	boulder	present	30
29 July 2013	20	33°43'59.77"	25°16'54.54"	Day	48	cobble	present	95
29 July 2013	1	33°42'59.37"	25°16'53.76"	Night	45	cobble	present	5
29 July 2013	2	33°42'59.09"	25°16'53.45"	Night	35	cobble	present	0

29 July 2013	3	33°42'58.91"	25°16'53.19"	Night	18	cobble	present	6
29 July 2013	4	33°42'58.64"	25°16'52.93"	Night	12	cobble	absent	3
29 July 2013	5	33°42'58.52"	25°16'52.61"	Night	45	cobble	absent	12
29 July 2013	6	33°42'58.34"	25°16'52.24"	Night	30	gravel	absent	0
29 July 2013	7	33°42'58.22"	25°16'52.01"	Night	15	gravel	present	5
29 July 2013	8	33°42'58.03"	25°16'51.66"	Night	18	boulder	absent	13
29 July 2013	9	33°42'58.17"	25°16'51.50"	Night	66	boulder	absent	27
29 July 2013	10	33°42'58.33"	25°16'51.27"	Night	51	boulder	present	29
29 July 2013	11	33°42'58.51"	25°16'51.10"	Night	12	gravel	present	0
29 July 2013	12	33°42'58.72"	25°16'50.88"	Night	40	gravel	present	5
29 July 2013	13	33°42'58.88"	25°16'50.75"	Night	19	cobble	present	5
29 July 2013	14	33°42'58.96"	25°16'50.71"	Night	15	cobble	absent	7
29 July 2013	15	33°42'59.10"	25°16'50.68"	Night	42	cobble	present	9
29 July 2013	16	33°42'59.20"	25°16'50.57"	Night	25	boulder	absent	14
29 July 2013	17	33°42'59.32"	25°16'50.44"	Night	44	boulder	present	24
29 July 2013	18	33°42'59.52"	25°16'50.14"	Night	40	boulder	absent	15
29 July 2013	19	33°42'59.93"	25°16'49.47"	Night	38	boulder	present	11
29 July 2013	20	33°42'00.01"	25°16'49.17"	Night	36	cobble	present	10
30 July 2013	1	33°42'59.37"	25°16'53.76"	Day	45	cobble	present	7
30 July 2013	2	33°42'59.09"	25°16'53.45"	Day	35	cobble	present	6
30 July 2013	3	33°42'58.91"	25°16'53.19"	Day	18	cobble	present	0
30 July 2013	4	33°42'58.64"	25°16'52.93"	Day	12	cobble	absent	0
30 July 2013	5	33°42'58.52"	25°16'52.61"	Day	45	cobble	absent	21
30 July 2013	6	33°42'58.34"	25°16'52.24"	Day	30	gravel	absent	1
30 July 2013	7	33°42'58.22"	25°16'52.01"	Day	15	gravel	present	5
30 July 2013	8	33°42'58.03"	25°16'51.66"	Day	18	boulder	absent	6
30 July 2013	9	33°42'58.17"	25°16'51.50"	Day	66	boulder	absent	96
30 July 2013	10	33°42'58.33"	25°16'51.27"	Day	51	boulder	present	67
30 July 2013	11	33°42'58.51"	25°16'51.10"	Day	12	gravel	present	0
30 July 2013	12	33°42'58.72"	25°16'50.88"	Day	40	gravel	present	4
30 July 2013	13	33°42'58.88"	25°16'50.75"	Day	19	cobble	present	5
30 July 2013	14	33°42'58.96"	25°16'50.71"	Day	15	cobble	absent	5
30 July 2013	15	33°42'59.10"	25°16'50.68"	Day	42	cobble	present	20
30 July 2013	16	33°42'59.20"	25°16'50.57"	Day	25	boulder	absent	23
30 July 2013	17	33°42'59.32"	25°16'50.44"	Day	44	boulder	present	65
30 July 2013	18	33°42'59.52"	25°16'50.14"	Day	40	boulder	absent	18
30 July 2013	19	33°42'59.93"	25°16'49.47"	Day	38	boulder	present	24
30 July 2013	20	33°42'00.01"	25°16'49.17"	Day	36	cobble	present	25
30 July 2013	1	33°42'59.08"	25°16'48.62"	Night	32	cobble	present	5
30 July 2013	2	33°42'58.78"	25°16'48.59"	Night	25	cobble	present	7
30 July 2013	3	33°42'58.49"	25°16'48.54"	Night	36	cobble	present	8
30 July 2013	4	33°42'57.99"	25°16'48.54"	Night	16	cobble	absent	3
30 July 2013	5	33°42'57.34"	25°16'48.40"	Night	49	cobble	absent	8
30 July 2013	6	33°42'56.71"	25°16'48.32"	Night	40	gravel	absent	6
30 July 2013	7	33°42'56.05"	25°16'48.21"	Night	18	gravel	present	6
30 July 2013	8	33°42'55.60"	25°16'48.10"	Night	38	boulder	absent	10

30 July 2013	9	33°42'54.77"	25°16'47.49"	Night	62	boulder	absent	22
30 July 2013	10	33°42'54.01"	25°16'46.86"	Night	35	cobble	absent	1
30 July 2013	11	33°42'53.90"	25°16'46.41"	Night	25	gravel	absent	0
30 July 2013	12	33°42'54.02"	25°16'45.89"	Night	55	gravel	absent	7
30 July 2013	13	33°42'54.41"	25°16'44.97"	Night	28	boulder	present	8
30 July 2013	14	33°42'54.82"	25°16'44.68"	Night	13	cobble	present	4
30 July 2013	15	33°42'54.90"	25°16'44.33"	Night	39	cobble	present	8
30 July 2013	16	33°42'55.57"	25°16'43.87"	Night	30	boulder	absent	3
30 July 2013	17	33°42'55.84"	25°16'43.43"	Night	40	boulder	present	15
30 July 2013	18	33°42'56.03"	25°16'42.87"	Night	42	boulder	absent	8
30 July 2013	19	33°42'56.00"	25°16'42.54"	Night	30	cobble	present	4
30 July 2013	20	33°42'53.83"	25°16'42.18"	Night	38	cobble	present	12
31 July 2013	1	33°42'59.08"	25°16'48.62"	Day	32	cobble	present	12
31 July 2013	2	33°42'58.78"	25°16'48.59"	Day	25	cobble	present	4
31 July 2013	3	33°42'58.49"	25°16'48.54"	Day	36	cobble	present	12
31 July 2013	4	33°42'57.99"	25°16'48.54"	Day	16	cobble	absent	0
31 July 2013	5	33°42'57.34"	25°16'48.40"	Day	49	cobble	absent	20
31 July 2013	6	33°42'56.71"	25°16'48.32"	Day	40	gravel	absent	7
31 July 2013	7	33°42'56.05"	25°16'48.21"	Day	18	gravel	present	0
31 July 2013	8	33°42'55.60"	25°16'48.10"	Day	38	boulder	absent	39
31 July 2013	9	33°42'54.77"	25°16'47.49"	Day	62	boulder	absent	67
31 July 2013	10	33°42'54.01"	25°16'46.86"	Day	35	cobble	absent	38
31 July 2013	11	33°42'53.90"	25°16'46.41"	Day	25	gravel	absent	0
31 July 2013	12	33°42'54.02"	25°16'45.89"	Day	55	gravel	absent	18
31 July 2013	13	33°42'54.41"	25°16'44.97"	Day	28	boulder	present	11
31 July 2013	14	33°42'54.82"	25°16'44.68"	Day	13	cobble	present	0
31 July 2013	15	33°42'54.90"	25°16'44.33"	Day	39	cobble	present	26
31 July 2013	16	33°42'55.57"	25°16'43.87"	Day	30	boulder	absent	11
31 July 2013	17	33°42'55.84"	25°16'43.43"	Day	40	boulder	present	41
31 July 2013	18	33°42'56.03"	25°16'42.87"	Day	42	boulder	absent	52
31 July 2013	19	33°42'56.00"	25°16'42.54"	Day	30	cobble	present	22
31 July 2013	20	33°42'53.83"	25°16'42.18"	Day	38	cobble	present	31