



The status and impacts of Yellow Crazy ant (*Anoplolepis gracilipes*) on Nu'utele, Aleipata islands, Samoa: Final Report

Dr Ben Hoffmann

CSIRO Ecosystem Sciences, Darwin, Northern Territory

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Executive summary

This report describes research conducted in October 2010 and May 2011 on the island of Nu'utele, Samoa with the following aims:

1. To determine yellow crazy ant, *A. gracilipes* distribution and quantify rate of spread from the historic distribution;
2. To quantify the reproductive phenology of *A. gracilipes*;
3. To quantify the annual abundance cycle of *A. gracilipes*;
4. To quantify the annual nest density cycle of *A. gracilipes*;
5. To quantify *A. gracilipes* impacts on co-existing fauna
6. To identify *A. gracilipes* interactions with phytophagous insects and extrafloral nectar
7. To provide management recommendations

Where possible, results from Nu'utele are compared with identical measurements from Christmas Island, Indian Ocean and throughout Arnhem Land, Australia, where *A. gracilipes* is well studied and is also subject to management actions.

Distribution and rate of spread

Yellow crazy ant was found occurring in three populations: Nu'utele beach (0.37 ha); Vini beach (> 2.6 ha); and the western ridge top (1.36 ha), and also as two isolated detections on the central ridge. This distribution contrasts greatly to the results of the 2003 survey, where only a single population was found covering approximately 8 ha on Nu'utele beach. The reason for the great reduction in population size at Nu'utele beach is unclear. The maximum rate of spread was 20 m over seven months. This distance is consistent with the expected expansion rate of a population approximately six years old.

Reproductive phenology

Male reproduction patterns in Samoa appear to be consistent with places elsewhere globally, but this is not so for queen reproduction because only a single queen pupa was collected from a nest excavated in May, which is outside of the known reproductive period for this species.

Annual abundance cycle

Worker counts on cards and tuna lures confirmed the expectation that worker abundance would be greatest in the May sample. Abundance from card counts was on average 30 ants in October, compared to 83 in May. The average abundance score from tuna lures was 4 (ranging from 11-20 ants) in October and over 7 (> 100 ants) in May. The *A. gracilipes* population levels on Nu'utele during their times of high abundance are as great as those seen on Christmas Island, but fall below this critical level during the time of low abundance. From pupal samples it is clear that the abundance levels from Samoa are much greater than those from Arnhem Land, during comparable time periods, and it appears likely that there is also a difference in the period of greatest ant abundance, with pupal abundance increasing earlier in Samoa than in Arnhem Land.

Annual nest density cycle

Seasonal variation in nest densities conformed to expectations, being greater in May (one nest per 2.2 m²) when population levels were also greater, than in October (one per 4.4 m²). The nest density on Nu'utele is among the highest recorded anywhere in the world.

Impacts

A total of 24 ant species from 15 genera were collected within pitfall traps. The most abundant species (excluding *A. gracilipes*) were *Pheidole umbonata* (46.2% of total abundance), the exotic tramp *Paratrechina longicornis* (18.6%), and *Odontomachus simillimus* (17.4%). *Anoplolepis gracilipes* abundance within the infested plots was always much greater than the abundance of all other ants combined in either plot, being 7.6 and 5.9 times greater than native ant abundance within the infested and uninfested plots respectively in the 2010 sample, and 2.7 and 3.5 times greater respectively in 2011.

Other ant abundance was not statistically different between infested and uninfested plots in both sample times. However, other ant abundance was dominated by a single species, *Pheidole umbonata* (51% and 44% in the 2010 and 2011 samples respectively), and with this species excluded, other ant abundance was significantly

lower within the infested plots (average 5 ants per plot) compared to the uninfested plots (11 ants) within the 2010 sample, but not in the 2011 sample.

Ant species richness per plot was consistently greater within the infested site, and this difference was statistically significant in the 2011 sample, having an average of six species per plot in the infested site vs three in the uninfested site. The greater species richness in the infested plot in the 2011 sample was predominantly due to other native ant species rather than other exotic species (8 species vs 4 respectively).

Nine ant species from seven genera were collected within foliage beats. Excluding *A. gracilipes*, four exotic tramps comprised 86% of total abundance within both samples combined, being *Tapinoma melanocephalum* (39%), *Paratrechina longicornis* (23%), *Monomorium floricola* (19%) and *Tetramorium bicarinatum* (5%). Within the infested site the abundance of other ants was 2.6 and 1.8 times greater than that of *A. gracilipes* in the 2010 and 2011 samples respectively, but these differences were not statistically significant. Similarly, other ant abundance within the uninfested site was not statistically different from *A. gracilipes* abundance in the infested site in the 2010 sample, but was statistically greater in the 2011 sample. There was no significant difference between the abundance and species richness of other ants between the infested and uninfested sites in both sample times.

Other macro-invertebrates from 11 orders were collected in pitfall traps. Flies were the predominate group collected (46% of all samples combined). There was no difference in the overall abundance or ordinal richness of other macro-invertebrates between the infested and uninfested sites within any of the two sample times. There was a clear trend of fewer spiders within the infested site (5 vs 18 individuals in 2010 and 2 vs 16 in 2011), but this was not statistically significant.

Other macro-invertebrates from eight orders were collected in foliage beats. Spiders were the predominate group collected (37% of all samples combined). Just as for other macro-invertebrate data from pitfall traps, there was no difference in overall abundance or ordinal richness between the infested and uninfested sites within either of the two sample times. Spiders had fewer individuals within the infested site in both sample times, statistically significantly so in the 2011 sample.

There was a clear difference in total hermit crab abundance between infested and uninfested sites in both sample times. In the 2010 sample, when *A. gracilipes* abundance was lowest, the infested site had approximately one quarter of the crabs per plot of the uninfested site, being greatly statistically different. This statistical result was driven by large crabs. Only seven small crabs were found in the infested site compared to 28 in the uninfested site, but the proportion of small crabs to the total count was consistent between the two sites (27% and 26% respectively), indicating that any factor affecting hermit crab abundance applied equally to both size classes. The difference in crab abundance between the infested and uninfested sites were even more pronounced in the 2011 sample when *A. gracilipes* abundance was greater, with only four large crabs being found in the infested site, compared to an average of 2.7 crabs per sample in the uninfested site. Naturally this difference was highly statistically significant.

Interactions with phytophagous insects and extrafloral nectar

Multiple unidentified species of scale and at least one mealy bug species were found on six tree species. The only interaction noticed between *A. gracilipes* and these insects was with scales on Indian Mulberry (Nonu) *Morinda citrifolia*, but all of the insect species were found within the infested site. Six plant species were found to have extra floral nectaries or carbohydrate sources accessible to ants, but *A. gracilipes* was found attending these sources only on the Indian Mulberry. The infested site had approximately double the number of trees with EFNs (41%) compared to the uninfested site (26%). Similarly, phytophagous insects were found on 29% of assessable trees within the infested site compared to only 4% within the uninfested site. It is not possible to state whether the current distribution of *A. gracilipes* on Vini beach is a consequence of the vegetation composition (and hence EFN availability), or merely by chance, or to what extent vegetation composition on Nu'utele could potentially limit the distribution of *A. gracilipes*. Similarly, it is unclear whether the greater phytophagous insect density within the infested site is a cause or consequence of the *A. gracilipes* distribution.

Management recommendations

I do not recommend eradication from the island as a management goal. However suppression of the Vini beach population and local eradication of the Nu'utele beach

and western ridge populations is feasible. Regardless of management action or not, the distribution of the ant should be monitored annually to bi-annually. Research should also be continued to fill the knowledge gaps of the biology of the ant, especially the reproductive timing of queens.

Research recommendations

Monthly sampling of crazy ant nest contents and nest density should be continued to fill the knowledge gaps of the biology of the ant, especially to determine the timing of queens reproduction. Such information is critical for effective management, and should be known prior to any broad-scale management operation, because treatments should be timed around the queen reproductive phase. The distribution of the ant should be monitored annually to bi-annually to either ensure that management actions are achieving their goals or to re-assess its status and risk on the island. Additional research should be instigated to address the apparent relationship found between *A. gracilipes* distribution and the supply of carbohydrate resources from both plants and phytophagous insects. Such a deterministic relationship has never been demonstrated before between invasive ants and vegetation composition, and would allow the distribution and impacts of *A. gracilipes* within any area to be predicted based on vegetation composition. This research would require comparative work to be conducted on Nu'ulua, where *A. gracilipes* seems to be well-established island-wide.

1. Introduction

Many ant species that have been accidentally spread throughout the world have significant economic, environmental and social impacts in areas that they now infest. One of the most notable invasive ants is the Yellow crazy ant, *A. gracilipes*, and this species is present in Samoa, including on the Aleipata islands. The Aleipata islands are considered to be of great regional conservation significance because they are uninhabited, relatively pristine, contain many species threatened throughout greater Samoa, and lack many exotic species present within greater Samoa. The presence of *A. gracilipes* on these islands is therefore of great conservation concern.

Prior work among the Aleipata islands has shown that *A. gracilipes* is well distributed over the island of Nuulua (Vanderwoude et al. 2006), but is restricted to one side of the island of Nu'utele (Abbott 2006). The incomplete distribution of this ant over Nu'utele provides the greatest opportunity to investigate its spread and impact. Such information is an important component of any risk analysis underlying management options for invasive species. Similarly, *in situ* knowledge of the biology and ecology of a species, is vital to create effective management protocols. This is particularly important for *A. gracilipes* because globally there is great variation in its abundance, impacts and seasonal phenology, and its reproductive strategy is particularly problematic and unresolved (Drescher et al. 2007; Gruber et al. in press).

This report describes research investigating the distribution, biology and impacts of *A. gracilipes* on Nu'utele, conducted in October 2010 and May 2011.

The aims of the project were:

1. To determine *A. gracilipes* distribution and quantify rate of spread
2. To quantify the reproductive phenology of *A. gracilipes*;
3. To quantify the annual abundance cycle of *A. gracilipes*;
4. To quantify the annual nest density cycle of *A. gracilipes*;
5. To quantify *A. gracilipes* impacts on indigenous fauna
6. To identify *A. gracilipes* interactions with phytophagous insects and extrafloral nectar
7. To provide management recommendations



Plate 1. Worker and queen Yellow crazy ant, *Anoplolepis gracilipes*. Photo courtesy of Phil Lester, Victoria University of Wellington.

2. Methods

2.1 Fieldwork timing and data comparisons

Two field trips were conducted to obtain repeated measures, the first in October 2010 and the second in May 2011. These dates were chosen because research on *A. gracilipes* biology elsewhere has shown that these months are approximately the times of the extremes of the variation within the *A. gracilipes* reproductive and abundance cycles. It was anticipated that such trends are consistent within Samoan populations of *A. gracilipes*, with reproduction of sexuals and lowest worker abundance occurring within October, and no reproduction of sexuals coupled with greater worker abundance occurring in May. Whenever possible, results were compared with identical assessments from Arnhem Land and Christmas Island, where *A. gracilipes* is subject to control or eradication measures.

The taxonomy and biogeographic origin of some Samoan ants remains problematic. Ant species were classified as either “native” or “exotic” based on the most recent revision of Samoan ants (Wetterer & Vargo 2003) and my personal opinion. Species considered to be Indo-Pacific natives were considered to be native to Samoa, as were

other species (e.g. *Hypoponera punctatissima*) which are likely to be different species requiring taxonomic revision, and which are also not known to have adverse environmental impacts where they occur.

2.2 Distribution and rate of spread

The presence/absence of *A. gracilipes* was assessed at all locations accessed on the island, being the gently sloped lowland areas (<20 m elevation) around Nu'utele beach (south east) and the fales on Vini beach (north and northwest), as well as the walking trail linking the north and south of the island, the far western portion of the walking trail along the ridge and some accessible steeper terrain between *A. gracilipes* detections. An assessment comprised an approximately four second visual survey of surrounds following agitation of the ground. All assessments were recorded in GPS.

Assessments of population boundaries were able to be conducted for only three of the five *A. gracilipes* populations found, and those surveys were restricted to accessible areas (approximately <30° slope and within penetrable vegetation). Delimiting surveys comprised assessments spaced <10 m apart, haphazardly made along survey paths spaced approximately 10 m apart. Wherever possible, surveys were conducted up to 100 m away in all directions from the peripheral *A. gracilipes* detections (the perceived boundary).

The area covered by the *A. gracilipes* population at Nu'utele beach was found to be greatly reduced from when it had last been assessed (Abbott 2006; Vanderwoude et al. 2008), so it could not be used to determine rate of spread. Instead the 2010 and 2011 determinations of the Vini beach population's western boundary were compared. Only this location is used for assessment because it is the only relevant boundary that was intensively and appropriately surveyed at both time periods.

2.3 Reproductive phenology

The reproductive strategy of this species is particularly problematic and unresolved (Drescher et al. 2007; Gruber et al. in press), and there is also enough variation in reproductive timing in different locations to necessitate the local determination of its phenology. The reproductive phenology of *A. gracilipes* was assessed by quantifying the annual patterns of male and queen pupae production. During both the October

2010 and May 2011 field trips, all pupae found within ten nests were collected, then determined in the laboratory as being either a worker, male or queen. For the October sample, five nests each were sampled from Nu'utele beach and Vini beach, but all 10 nests in the May sample were from Vini beach. Additional monthly collections were to be conducted by MNRE staff, but unfortunately this did not occur.

2.4 Annual abundance cycle

The *A. gracilipes* annual abundance cycle was measured indirectly from pupae counts (Section 2.2), and directly from worker counts on cards and at fish lures at the Vini beach infestation. Card and lure counts were conducted at the same sample points along transects, with the card assessments being conducted prior to fish lure assessments. Eleven sample points were spaced 5m apart along four 50m transects.

Cards were 20 cm x 20 cm laminated paper divided into four 10 cm x 10 cm squares. At each assessment point a card was placed on the ground with the edges in contact with substrate as far as possible to allow easy access for the ants to walk on card. For 20 seconds the card was observed, and the first square accessed by an *A. gracilipes* worker was the only square used for the assessment. The number of *A. gracilipes* workers walking over that square were counted over the following 30 seconds. If no ant walked over the grid in the first 20 second assessment period, then the square to be used was determined by the first ant that walked over the grid in the 30 second quantifying period. The abundance counts were pooled for each transect, then averaged among transects.

Fish lures were a teaspoon amount of canned fish. *A. gracilipes* abundance at each lure was scored after 20 minutes according to the following scale: 0 = no ants; 1 = 1 ant; 2 = 2-5 ants; 3 = 6-10 ants; 4 = 11-20 ants; 5 = 21 – 50 ants; 6 = 50 -100 ants; and 7 = >100 ants. The scaled abundance measures were averaged for each transect, then averaged among transects. Additional monthly collections of both card and lure counts were to be conducted by MNRE staff, but unfortunately this did not occur.

2.5 Annual nest density cycle

Nest density was quantified in four 5 x 5m plots within the Vini beach infestation, with plot location differing in the two sample times. Within each plot, nests were

located by disturbing all leaf litter and surface materials. Nests were defined as locations from where ants were recruiting (i.e. a hole in the ground), or where pupae were aggregated. Nests < 50cm apart were considered to be the same nest. Additional monthly collections were to be conducted by MNRE staff, but unfortunately this did not occur. Nest density data on Nu'utele were compared with data from identical assessments from Arnhem Land and Christmas Island.

2.6 Impacts

All impact studies were conducted within the Vini beach infestation (Figure 1) and the nearby uninfested area to the southwest of the fales. These areas were paired as far as practicable by: (1) elevation, being near the base of the steep incline; (2) vegetation structure having an interlocking canopy and a dense understorey; and (3) the vegetation of all strata being comprised of numerous species (i.e. not a near monoculture of Coconut *Cocos nucifera* or *Pisonia grandis*). The vegetation structure and composition of these sites appears (by eye) comparable to all vegetation covering the island, other than the peripheral vegetation near the shoreline.

There are two important considerations for the impact studies. First, the absence of pre-invasion data means that impacts can only be inferred from analyses of data from areas invaded vs areas uninvaded, and differences between these areas are not necessarily caused by the invader. Second, univariate analyses in studies of invasions such as this suffer from inherent pseudoreplication because the invasion is not replicated (Hurlbert 1984). However, within comparative mensurative experiments such as this the issue of pseudoreplication is minimized when samples are conducted throughout the entire area, not just within one part of an infestation (Hurlbert 1984). Accordingly, for the main component of the impact analysis (pitfall trap data of epigeic fauna) I have used 20 small plots comprised of only three pitfall traps in both the infested and uninfested sites, instead of the more typical ant community sampling regime utilising a few large plots typically comprised of 12 or 15 traps. To further reduce pseudoreplication issues, I lowered the probability level of statistical significance to $P = 0.025$.

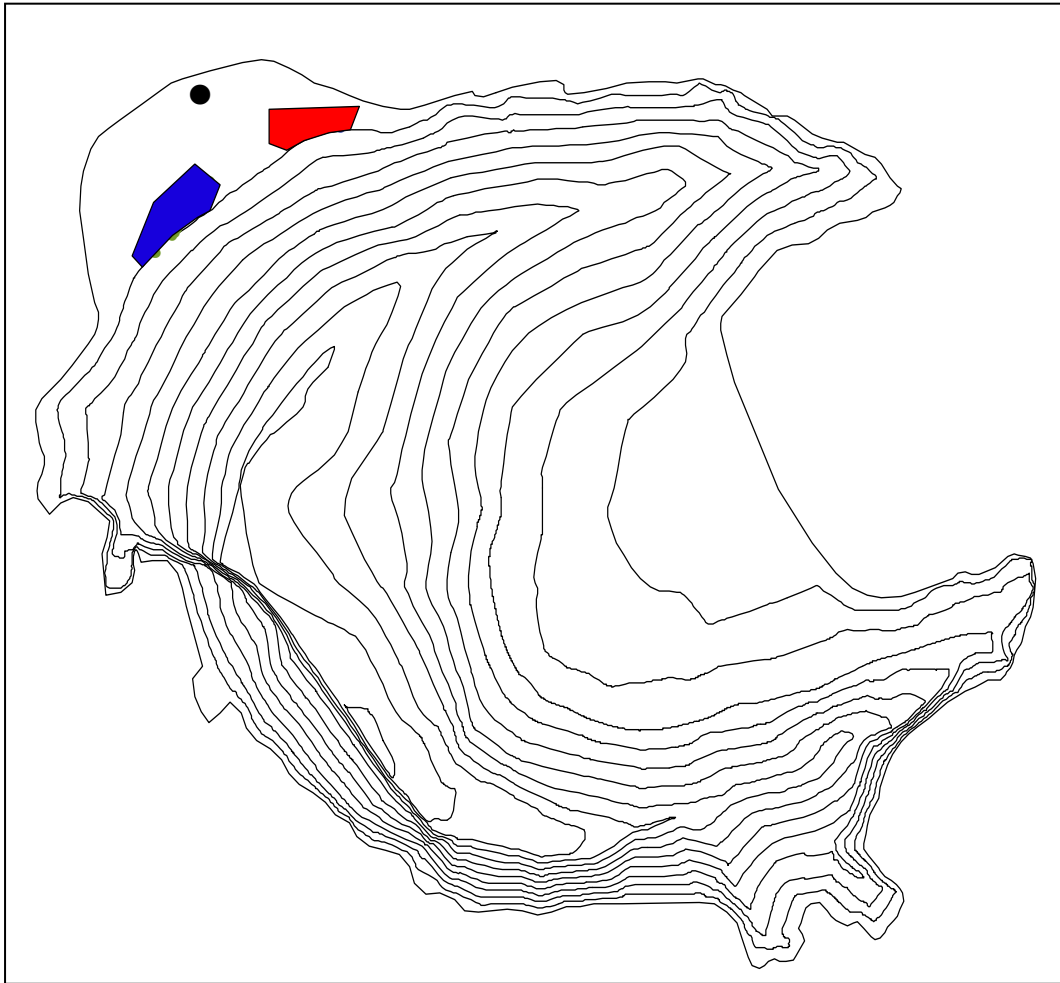


Figure 1. Location of sites at Vini beach infested with *A. gracilipes* (red polygon) and uninfested (blue polygon) used to measure *A. gracilipes* environmental impacts. The point indicates the location of the fales.

The epigeic invertebrate fauna was sampled using pitfall traps, which were plastic containers with an internal diameter of 65 mm, one third filled with ethylene glycol as a preservative. The three traps per plot were placed in triangle formation, spaced approximately 2 m apart. Plots were spaced no less than 10 m apart. All macroinvertebrates (taxa > 1 mm) were identified to ordinal level, except ants, which were identified to species level. Pitfall trap data were pooled for each plot. Foliage beats were conducted to sample the arboreal invertebrate fauna. Samples were collected along a single transect within each of the infested and uninfested sites. Where possible, assessments were made every 4 m along the transect using the

closest tree (>2 m high), or low lying branch of an established tree. The transect was extended as far as needed to collect 12 samples in each site. The selected foliage was beaten four times over a 1 x 1 m white canvas, and all invertebrates that fell onto the canvas were collected.

The potential impact of *A. gracilipes* on hermit crabs was assessed by counting the number of crabs found within one minute in 20 5 x 1 m transects during the early evening between 7 and 9 pm. Crabs were divided into two arbitrary size classes: small (<5 mm across the carapace) and large (>5 mm across the carapace).

The non-parametric Mann-Whitney U-Test was used when comparing data from infested and uninfested plots, and the Wilcoxon matched pairs T-test was used when analysing data from infested samples only.

2.7 Phytophagous insects and extrafloral nectar

All phytophagous insects, as well as plants observed with extra floral nectaries (EFNs) or with nectar sources were collected and identified. Any associations of these with *A. gracilipes* were noted. Additionally a brief survey was conducted attempting to quantify vegetation composition between the infested and uninfested sites on Vini beach. In both locations vegetation was sampled every two metres along the same transects used for foliage beat measures of *A. gracilipes* impacts (Section 2.5). At each sample location the closest tree (> 2m high) was identified, and observations were made of the presence/absence of phytophagous insects and EFNs, as well as any interaction with *A. gracilipes*.

3. Results and Discussion

3.1 Distribution and rate of spread

A total of 1546 point assessments were conducted, with *A. gracilipes* detected in 190 (Figure 2). The *A. gracilipes* detections were primarily within three populations. The largest was at Vini beach covering 2.64 ha of accessible terrain which could be assessed, and continuing for an unknown distance into terrain that could not be assessed. The next largest infestation was on the western ridge top, covering 1.36 ha, and the third at Nu'utele beach covered 0.37 ha. An additional two isolated detections were made along the trail on the northern slope of the central ridge. It remains unclear if these detections are part of the Vini beach population or not. Interestingly, these two detections were made in the October 2010 sampling period, but were not detected again in the April 2011 sampling period despite multiple attempts to find them.

The *A. gracilipes* distribution found here contrasts greatly to the findings of the survey conducted in 2003 (Abbott 2006). In 2003, only a single population was found on Nu'utele beach, compared to at least three populations found in 2010/2011. Also, the population on Nu'utele beach covered approximately 8 ha in 2003 but now covers < 0.4 ha. The reason for the decline of this population remains unclear. It is possible that this dramatic reduction of infested area is partly a result of the 2009 tsunami, but it cannot be the whole reason because *A. gracilipes* was not detected within previously infested areas well above the tsunami-affected zone.

The maximum rate of spread determined from the only reliable measure, being the comparison of the western boundary of the Vini beach population between the October 2010 and May 2011 samples, was 20 m. It is assumed here that population expansion is negligible between April and October in Samoa, and thus consistent with this ant's population dynamics globally, thus the figure of 20 m is the current maximum annual figure. This distance is well below the expansion rates of well established populations (> 10 years old) which can disperse more than 100 m per year (Haines & Haines 1978), but is consistent with the expected expansion rate of a population approximately six years old (Hoffmann unpublished data).

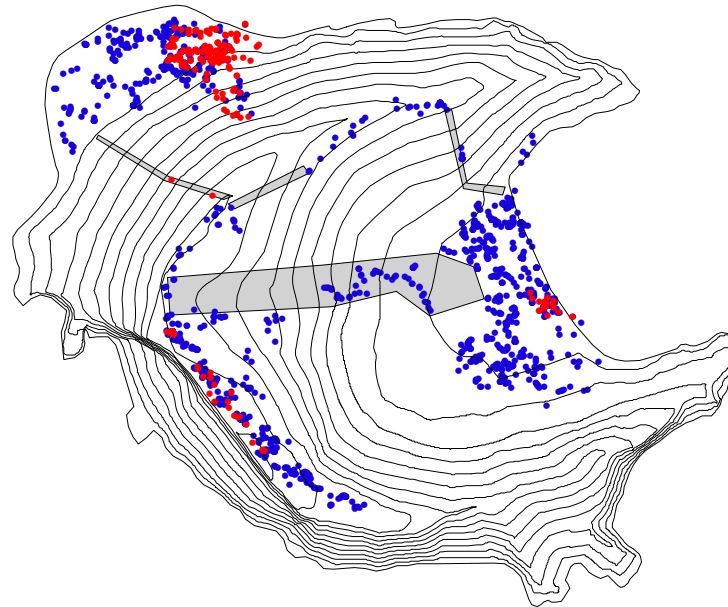


Figure 2. Locations where *A. gracilipes* was detected (red points) or not detected (blue points) using visual inspections. Shading indicates areas that were assessed, but where GPS locations could not be obtained. Note: because GPS signal was often difficult to maintain under the vegetation canopy, many GPS points overlay each other and thus don't accurately display their continual dispersion throughout assessed areas.

Overall, the significant decline in the Nu'utele beach population, the clear rise of other greatly dispersed populations, as well as the detection and subsequent absence of isolated nests suggests that *A. gracilipes* populations on Nu'utele are undergoing substantial flux.

3.2 Reproductive phenology

The unfortunate lack of monthly sampling means that little can be confirmed about *A. gracilipes* reproductive phenology in Samoa, but there are two noteworthy points. First, male reproduction patterns in Samoa appear to be consistent with places elsewhere globally, with the relative abundance of male pupae in October and May being extremely similar to that recorded in Arnhem Land, Australia in the same period (Figure 3), and October being within the period of male reproduction recorded from many places throughout the world. Only a single male pupa was found in the May sample from 4224 pupae collected.

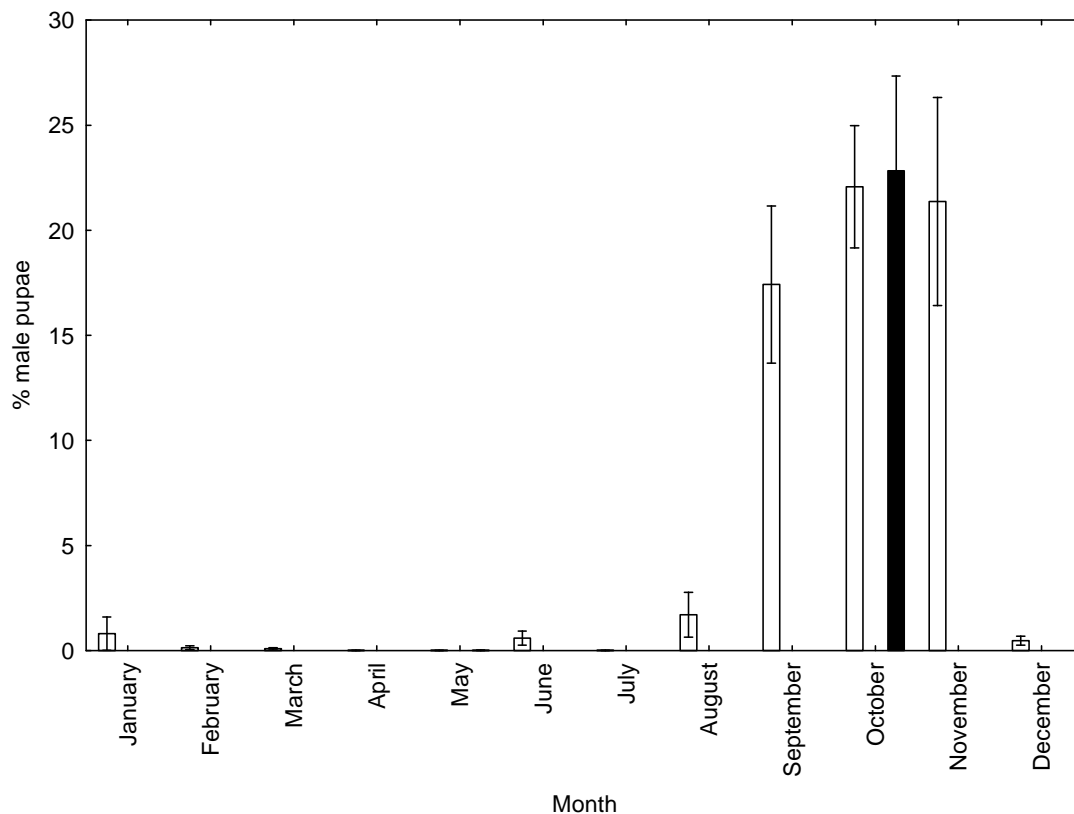


Figure 3. Proportion of male pupae collected from monthly nest samples of pupae in Arnhem Land, Australia (grey bars) and Nu'utuele (black bar). Note: December data for Arnhem Land are incomplete, and only a single male pupa was found in the May sample from Samoa.

Second, such patterns for male reproduction are not consistent for queen reproduction. The only queen pupae that was collected was from a nest excavated in May, which is outside of the known reproductive period for this species, and none were excavated in October, which was when queen reproduction was anticipated to occur. The determination of the timing of queen reproduction remains as an important requirement for any management decision because treatment should be timed around the queen reproductive phase.

2.3 Annual abundance cycle

The unfortunate lack of monthly sampling means that the complete *A. gracilipes* annual abundance cycle in Samoa cannot be shown, but clear and expected trends are apparent. Worker counts on cards and tuna lures confirmed the expectation that worker abundance would be greatest in the May sample. Abundance from card counts

was on average 30 ants in October, compared to 83 in May. The average abundance score from tuna lures was 4 (being between 11-20 ants) in October and over 7 (> 100 ants) in May. As a comparison, high-density crazy ant populations on Christmas island are defined as where ant abundance exceeds 38 in card counts. Thus *A. gracilipes* population levels on Nu'utele during their times of high abundance are as great as those seen on Christmas Island, but fall below this critical level during the time of low abundance. As a further comparison, within Nhulunbuy, Australia, card counts rarely exceed 38, and are on average only 17.

From pupal samples it is clear that the abundance levels from Samoa are much greater than those from Arnhem Land, Australia during comparable time periods, and it appears likely that there is also a difference in the period of greatest ant abundance with pupal abundance increasing earlier in Samoa than in Arnhem Land (Figure 4).

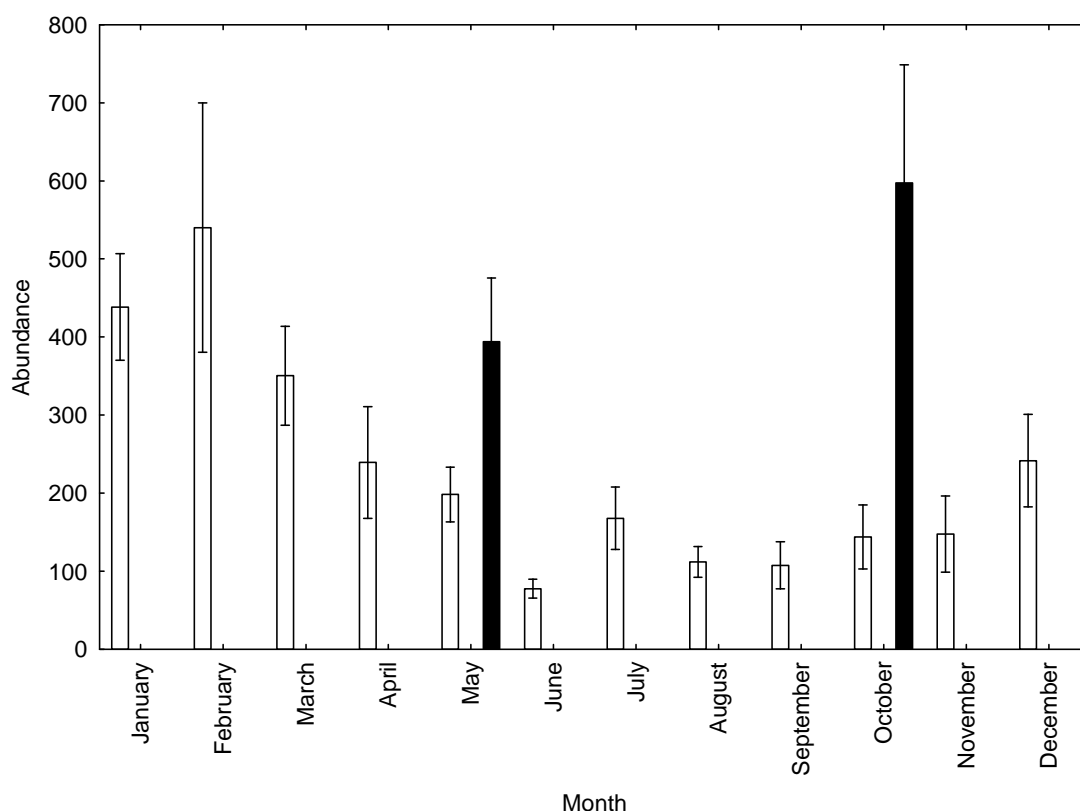


Figure 4 . Average monthly abundance of *A. gracilipes* pupae per nest sample in Arnhem Land, Australia (open bars) and Nu'utele (black bar). Note: December data for Arnhem Land are incomplete.

3.4 Annual nest density cycle

Because monthly sampling was not conducted, the exact annual cycle of nest density cannot be demonstrated. However, nest densities quantified in the two sample times conformed to expectations, with the nest density being greater in May when population levels were also greater.

The four plots from the October sampling contained 6, 2, 5 and 6 nests respectively. The plot containing only two nests is considered to be atypical as it was within a stand of *Pisonia grandis*, which is known to be unfavourable for invasive ants (Gerlach 2004; Hoffmann & Kay 2009). Therefore, excluding this plot, the average nest density was one per 4.4 m². The nest density within the four differently located plots in the May sample was approximately double of that in October, containing 17, 12, 10 and 7 nests respectively, equating to an average nest density of one per 2.2 m².

This nest density on Nu'utele is among the highest recorded throughout the world. In the Seychelles, maximum nest density was one per 14.9 m², none being underground (Haines and Haines 1978a). In comparable rainforest habitat in Arnhem Land *A. gracilipes* nest densities were one per 6.3 m² (Hoffmann unpublished data). In New Guinea coconut palm plantations, Young (1996) found *A. gracilipes* ephemeral nests in leaf litter could occur up to one per 2 m². Finally, on Christmas island, Abbott (2005) found nest entrance densities reached 10.5 per m², however at this density these entrances would not constitute discrete nests. Indeed what constitutes a discrete nest within the high density populations on Christmas Island is not clear (personal observation).

3.5 Impacts

3.5.1 Ants in pitfall traps

A total of 24 ant species from 15 genera were collected within pitfall traps within both sampling times, 18 species from 13 genera within the 2010 sample and 20 species from 13 genera within the 2011 sample (Appendix 1). The most abundant species (excluding *A. gracilipes*) were *Pheidole umbonata* (46.2% of total abundance of all species excluding *A. gracilipes* within both sample times), the exotic tramp

Paratrechina longicornis (18.6%), *Odontomachus simillimus* (17.4%) and another exotic tramp, *Tetramorium bicarinatum* (7.2%). The relative contribution of these four species was very similar between the two sample times.

Anoplolepis gracilipes abundance within the infested plots was always much greater than the abundance of all other ants combined, being 7.6 and 5.9 times greater than native ant abundance within infested and uninfested plots respectively in the 2010 sample, and 2.7 and 3.5 times greater respectively in the 2011 sample (Figure 5), with these differences being statistically significant in all cases (Tables 1, 2).

Interestingly, *A. gracilipes* abundance within pitfall traps was lower within the May sample, not greater as found by card counts and tuna lures, but this is solely due to an exceptionally large number of *A. gracilipes* (815) falling into a single trap within the 2010 sample, presumably because the trap was placed directly beside a nest.

Other ant abundance was not statistically different between infested and uninfested plots in both sample times (Figure 5, Table 2). However, other ant abundance was dominated by a single species, *Pheidole umbonata* (51% and 44% in the 2010 and 2011 samples respectively), and with this species excluded from analysis, other ant abundance was significantly lower within the infested plots (average 5 ants per plot) compared to the uninfested plots (11 ants) within the 2010 sample, and lower albeit not significantly (15 vs 19 ants) in the 2011 sample (Figure 6, Table 2). This lack of significance in the 2011 sample is predominantly attributable to a very high number of *Tetramorium bicarinatum* (48 ants) caught within a single trap, presumably placed beside a nest, but even with this trap removed, the difference between the two sites remained statistically insignificant (Mann-Whitney U-test, $P = 0.08$).

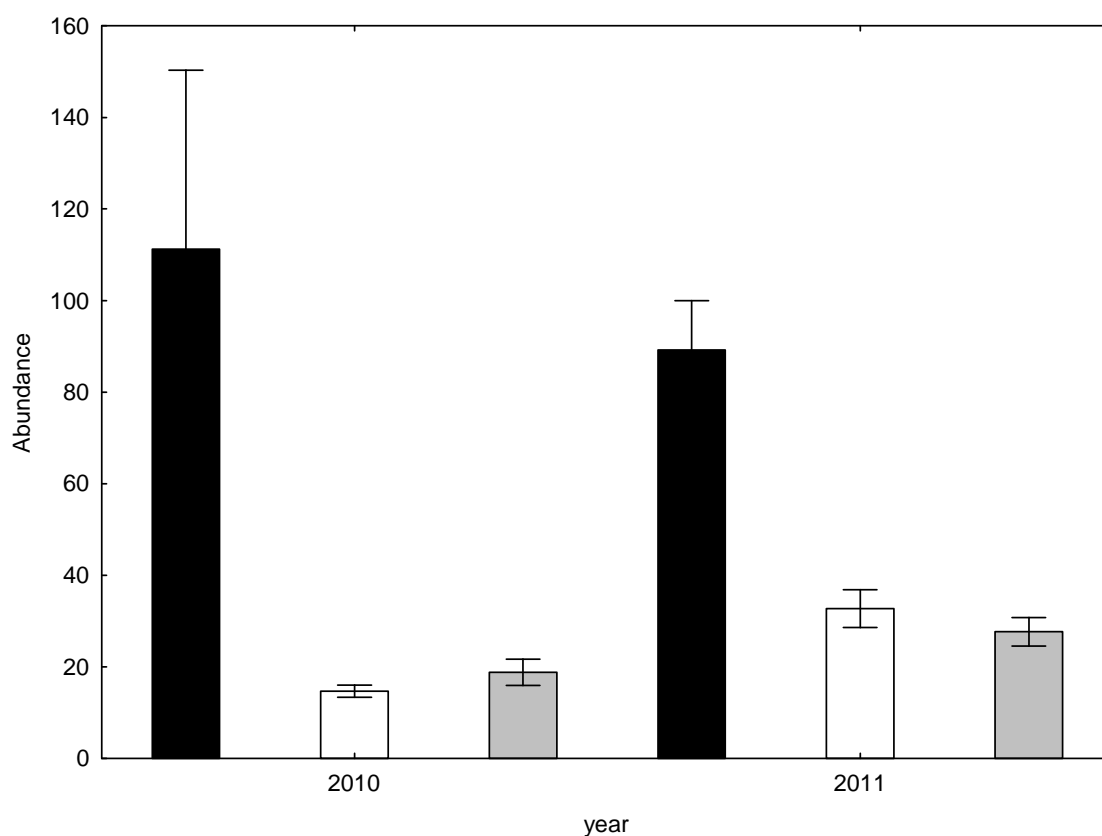


Figure 5. Mean (\pm SE) *Anoplolepis gracilipes* abundance (black bar) and the abundance of all other ants within plots in the infested site (white bar) and uninfested site (grey bar) within pitfall traps during the 2010 and 2011 sampling periods.

Table 1. Results of Wilcoxon matched pairs T-tests for comparisons of *A. gracilipes* abundance vs native ant abundance within infested plots, for the two sample times.

Bold indicates significance of $P < 0.025$.

Sample time	T	z	P
2010 sample	0	3.92	< 0.0001
2011 sample	0	3.72	< 0.0002

Table 2. Results of Mann-Whitney U-tests of ant pitfall trap data between infested and uninfested plots for the two sample times. Bold indicates significance of $P < 0.025$.

	U	z	P
<i>2010 sample</i>			
<i>A. gracilipes</i> abundance vs other ant abundance	27	4.667	< 0.0001
Non- <i>A. gracilipes</i> ant abundance	175.5	-0.649	0.516
Ant species richness excluding <i>A. gracilipes</i>	166	0.906	0.365
Non- <i>A. gracilipes</i> ant abundance excluding <i>Pheidole umbonata</i>	92	-2.91	0.0035
<i>2011 sample</i>			
<i>A. gracilipes</i> abundance vs other ant abundance	35.5	4.21	< 0.0001
Non- <i>A. gracilipes</i> ant abundance	157	0.658	0.511
Ant species richness excluding <i>A. gracilipes</i>	27.5	4.444	< 0.0001
Non- <i>A. gracilipes</i> ant abundance excluding <i>Pheidole umbonata</i>	124	-1.623	0.105

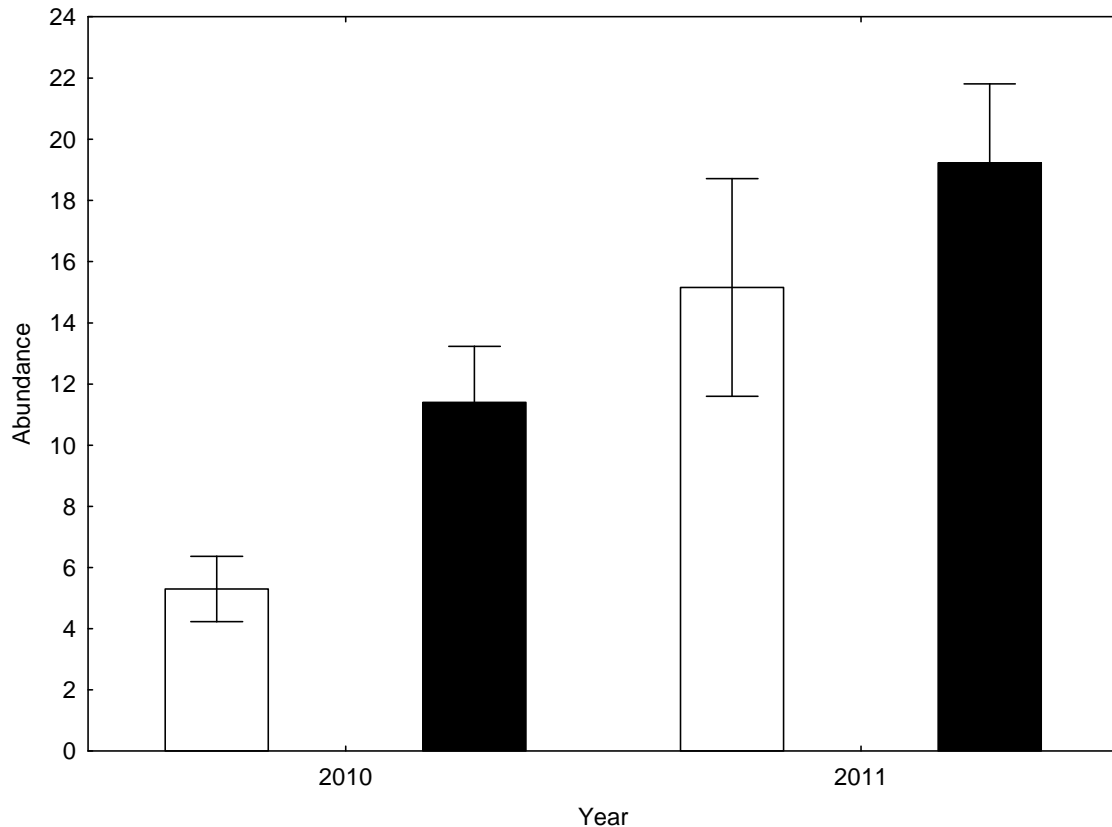


Figure 6. Mean (\pm SE) non-*Anoplolepis gracilipes* ant abundance, excluding *Pheidole umbonata*, within plots in the infested (white bar) and unfested site (black bar) within pitfall traps during the 2010 and 2011 sampling periods.

Ant species richness per plot, excluding *A. gracilipes*, was always greater within the infested site, statistically significantly so in the 2011 sample (Table 2), having an average of six species per plot in the infested site vs three in the unfested site. The greater species richness in the infested plot in the 2011 sample was predominantly due to other native ant species rather than other exotic species (8 species vs 4 respectively). A total of 14 species were found within the infested site and 11 in the unfested site in the 2010 sample, and 18 vs 7 in the 2011 sample.

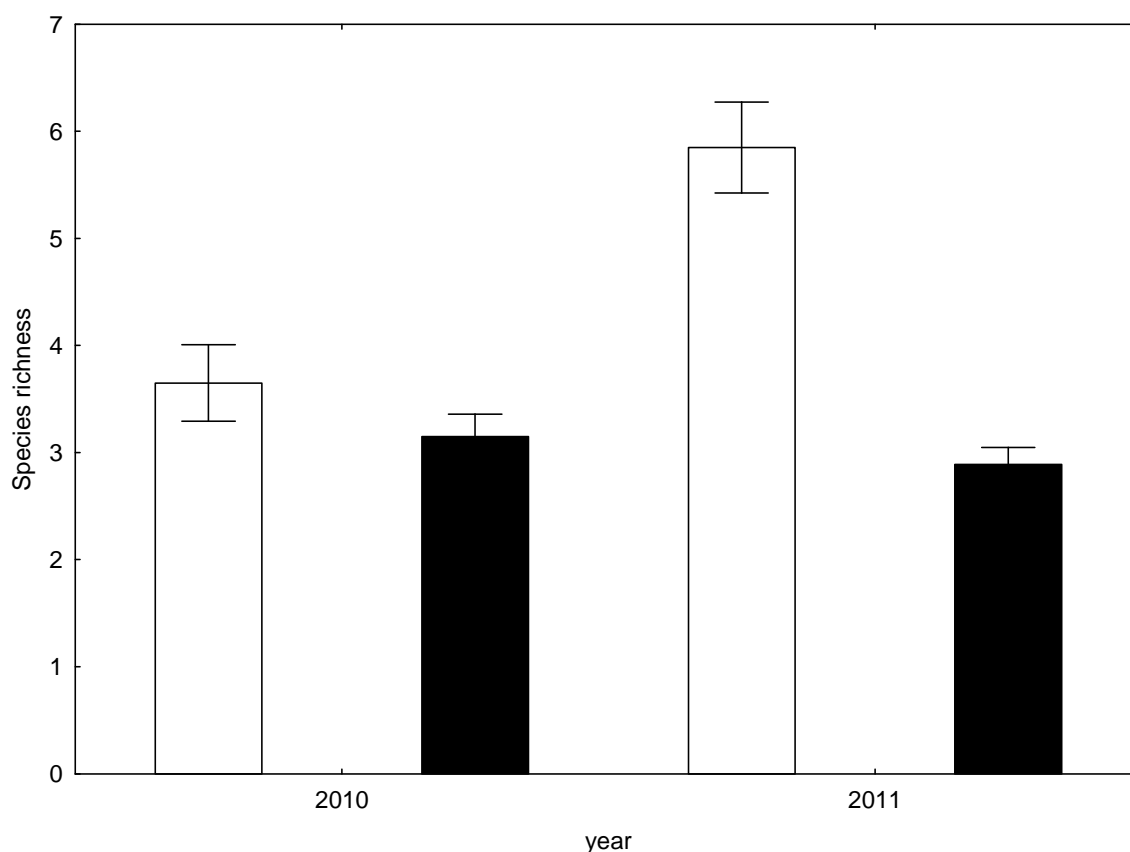


Figure 7. Mean (\pm SE) ant species richness, excluding *Anoplolepis gracilipes*, within plots in the infested (white bar) and uninfested site (black bar) within pitfall traps during the 2010 and 2011 sampling periods.

3.5.2 *Ants in foliage beats*

Nine ant species from seven genera were collected within foliage beats of both sample times combined, with the 2010 and 2011 samples each having only seven species (Appendix 1). Excluding *A. gracilipes*, four exotic tramps comprised 86% of total abundance within both samples combined, being *Tapinoma melanocephalum* (39%), *Paratrechina longicornis* (23%), *Monomorium floricola* (19%) and *Tetramorium bicarinatum* (5%). The contribution of these species within the two sample times varied greatly, with that of *Paratrechina longicornis* being 34% and 9% in the 2010 and 2011 samples respectively, 31% and 6% respectively for *Monomorium floricola*, 28% and 52% for *Tapinoma melanocephalum*, and 0% and 12% for *Tetramorium bicarinatum*.

Within the infested site the abundance of other ants was 2.6 and 1.8 times greater than that of *A. gracilipes* in the 2010 and 2011 samples respectively, (Figure 8), but these differences were not statistically significant (Wilcoxon matched pairs T-test, $T = 14.5$, $z = 1.325$, $P = 0.185$ for 2010 and $T = 17$, $z = 1.423$, $P = 0.155$ for 2011) due to great variation among the samples. Similarly, other ant abundance within the uninfested site was not statistically different from *A. gracilipes* abundance in the infested site in the 2010 sample, but was statistically greater in the 2011 sample (Figure 8, Table 3). There was no significant difference between the abundance or species richness of other ants between the infested and uninfested sites in both sample times (Figures 8, 9; Table 3).

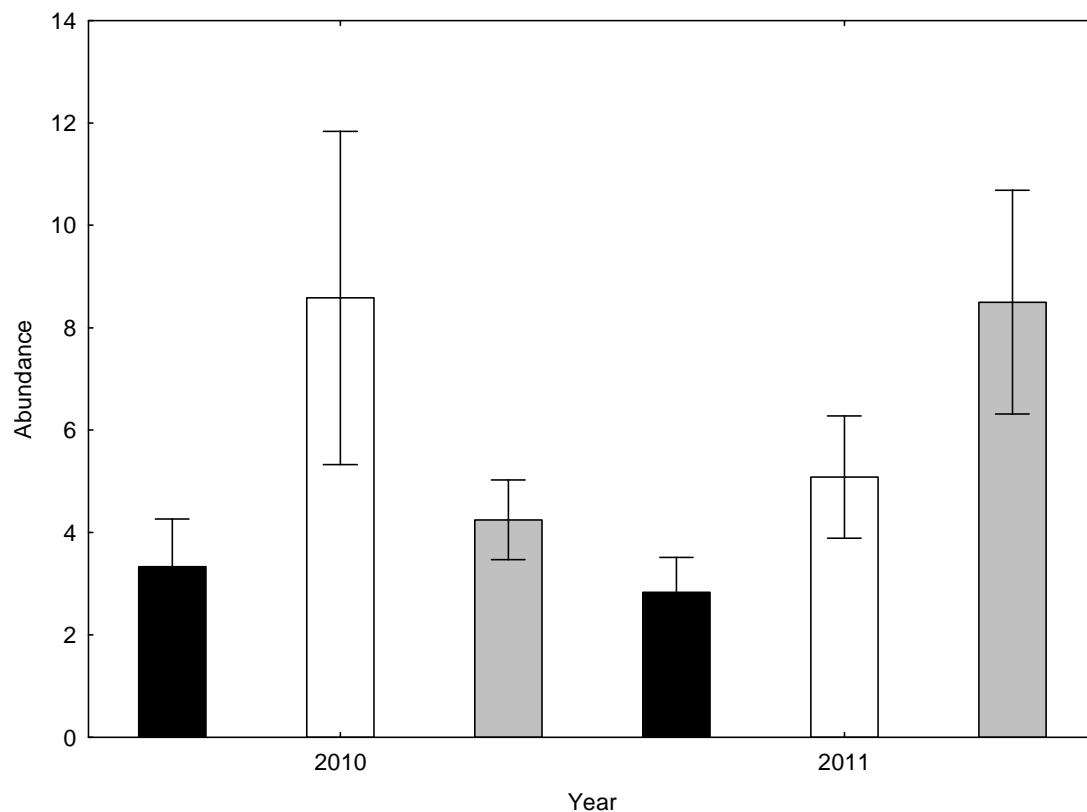


Figure 8. Mean (\pm SE) *Anoplolepis gracilipes* abundance (black bar) and the abundance of all other ants (white bar) in the infested site (white bar) and uninfested site (grey bar) within foliage beats during the 2010 and 2011 sampling periods.

Table 3. Results of Mann-Whitney U-tests of ant foliage beat data between infested and uninfested plots. Bold indicates significance of $P < 0.025$.

	U	z	P
<i>2010 sample</i>			
<i>A. gracilipes</i> abundance vs other ant abundance	59	-0.722	0.466
Non- <i>A. gracilipes</i> ant abundance	64	0.433	0.665
Ant species richness excluding <i>A. gracilipes</i>	51	1.184	0.237
<i>2011 sample</i>			
<i>A. gracilipes</i> abundance vs other ant abundance	31	-2.338	0.019
Non- <i>A. gracilipes</i> ant abundance	54.5	-0.981	0.326
Ant species richness excluding <i>A. gracilipes</i>	54.5	-0.981	0.326

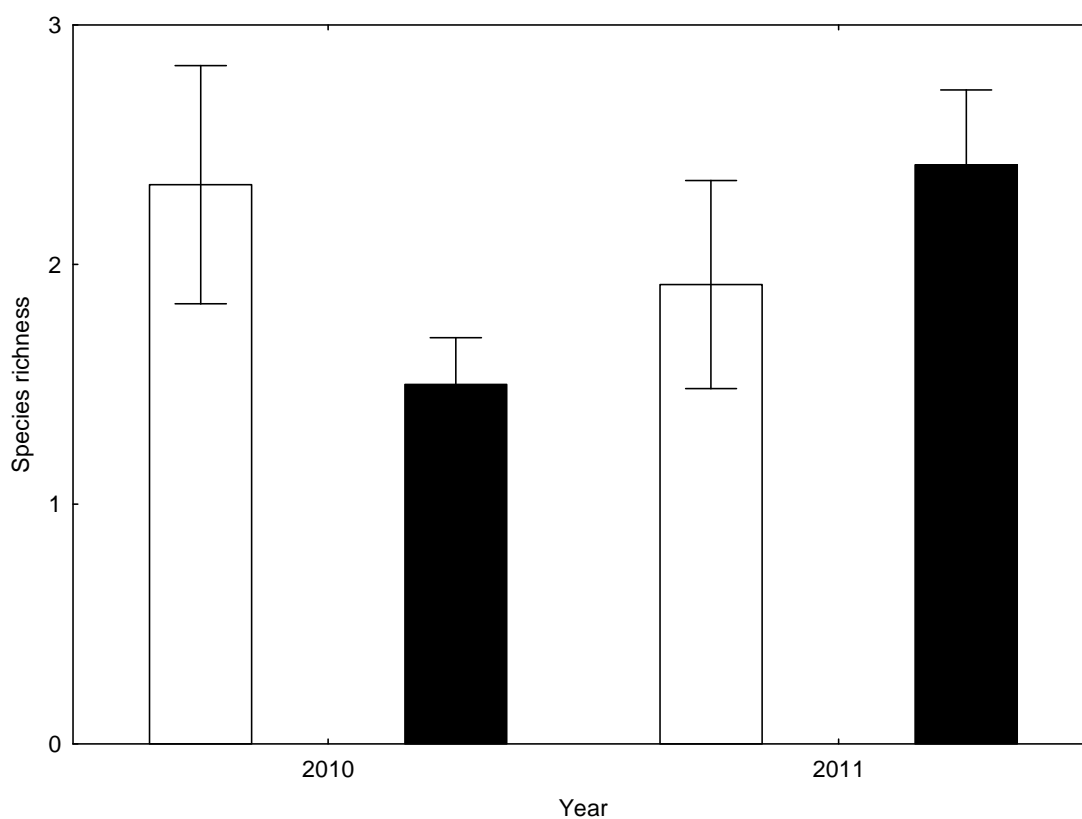


Figure 9. Mean (\pm SE) ant species richness, excluding *Anoplolepis gracilipes*, within foliage beats in the infested (black bar) and uninfested site (white bar) during the 2010 and 2011 sampling periods.

2.5.3 Other macro-invertebrates in pitfall traps

Other macro-invertebrates from 11 orders were collected in pitfall traps. Flies were the predominate group collected (46% of all samples combined), followed by isopods (14%), moths and butterflies (8%) and crickets (7%) (Figure 10). There was no difference in the overall abundance or ordinal richness of other macro-invertebrates between the infested and uninfested sites within any of the two sample times (Figures 11, 12, Table 4). Variation in the abundance of individual orders both between sites and between sample times is present (Figure 10), but most specimens were capable of flight and thus are highly mobile, so such variation (at least within such a small infested area) should be interpreted with caution. The exception are spiders (Arachnida) and isopods (Isopoda) which are relatively sedentary, and are well known to be sensitive to exotic ant invasions.

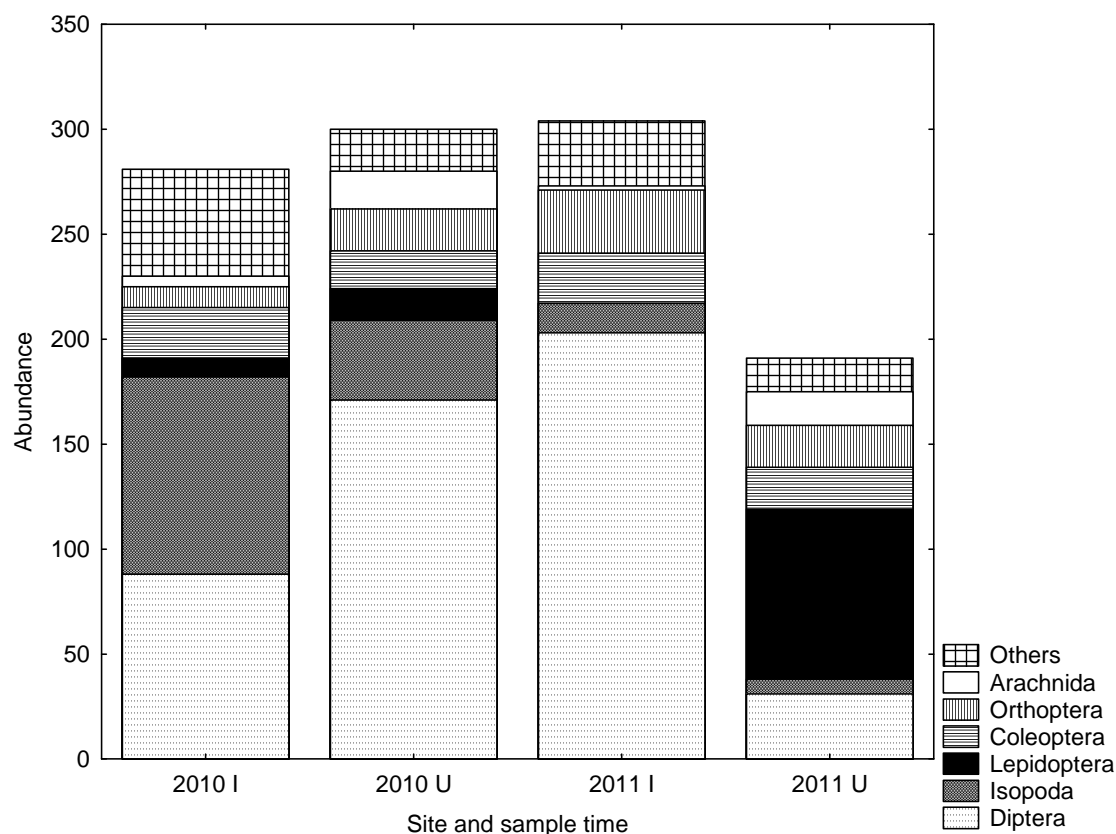


Figure 10. Abundance of the other macro-invertebrate orders collected in pitfall traps within sites infested (I) or uninfested (U) with *Anoplolepis gracilipes* during the 2010 and 2011 sampling periods.

There were consistently fewer spiders within the infested site (5 vs 18 individuals in 2010 and 2 vs 16 in 2011), however, because of the imposed lower level of probability for statistical significance ($P = 0.025$) these differences were only statistically significant in the 2011 sample (Table 4). Consistent with research globally, there were more isopods in the infested site in both sample times, but the differences were not statistically significant (Table 4).

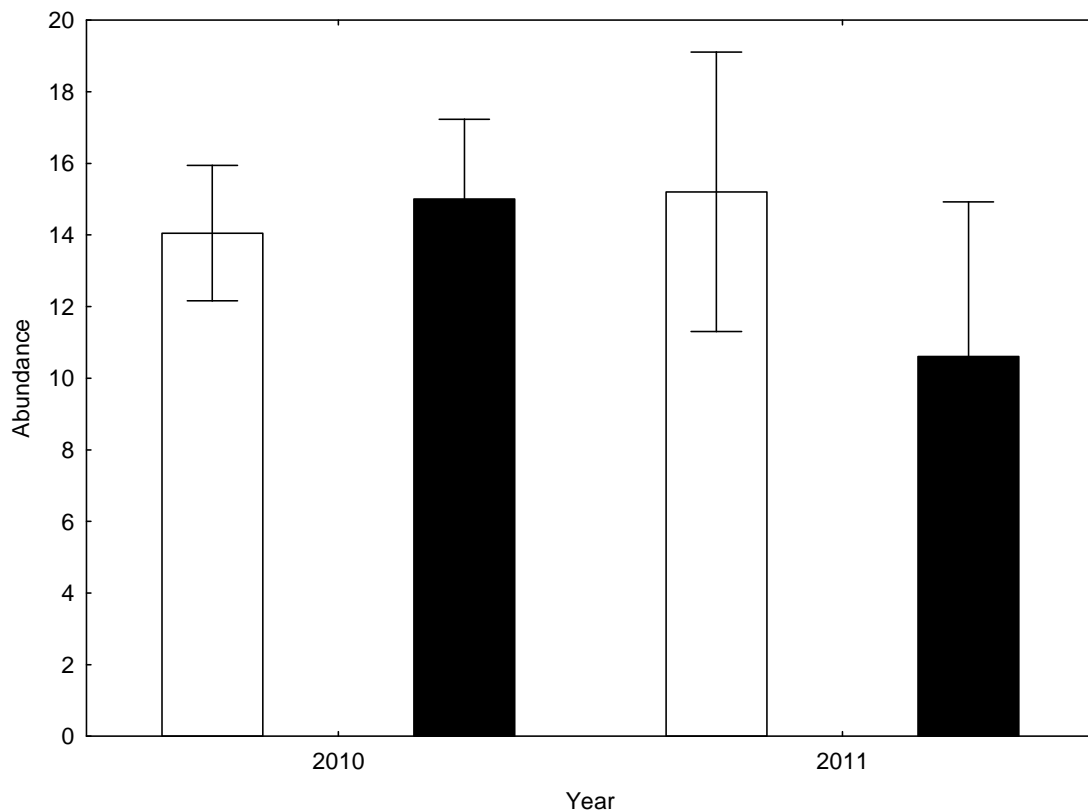


Figure 11. Mean (\pm SE) other macro-invertebrate abundance within plots in the infested (white bar) and uninfested site (black bar) within pitfall traps during the 2010 and 2011 sampling periods.

Table 4. Results of Mann-Whitney U-tests of other macro-invertebrate data from pitfall traps between infested and uninfested plots in the 2010 and 2011 samples. Bold indicates significance of $P < 0.025$.

	U	z	P
<i>2010 sample</i>			
total abundance	196	-0.09	0.924
ordinal richness	164	0.96	0.337
Spider abundance	124.5	-2.03	0.042
Isopod abundance	139.5	1.62	0.105
<i>2011 sample</i>			
total abundance	109	2.06	0.039
ordinal richness	178	-0.03	0.977
Spider abundance	74	-3.08	0.002
Isopod abundance	178.5	0.029	0.977

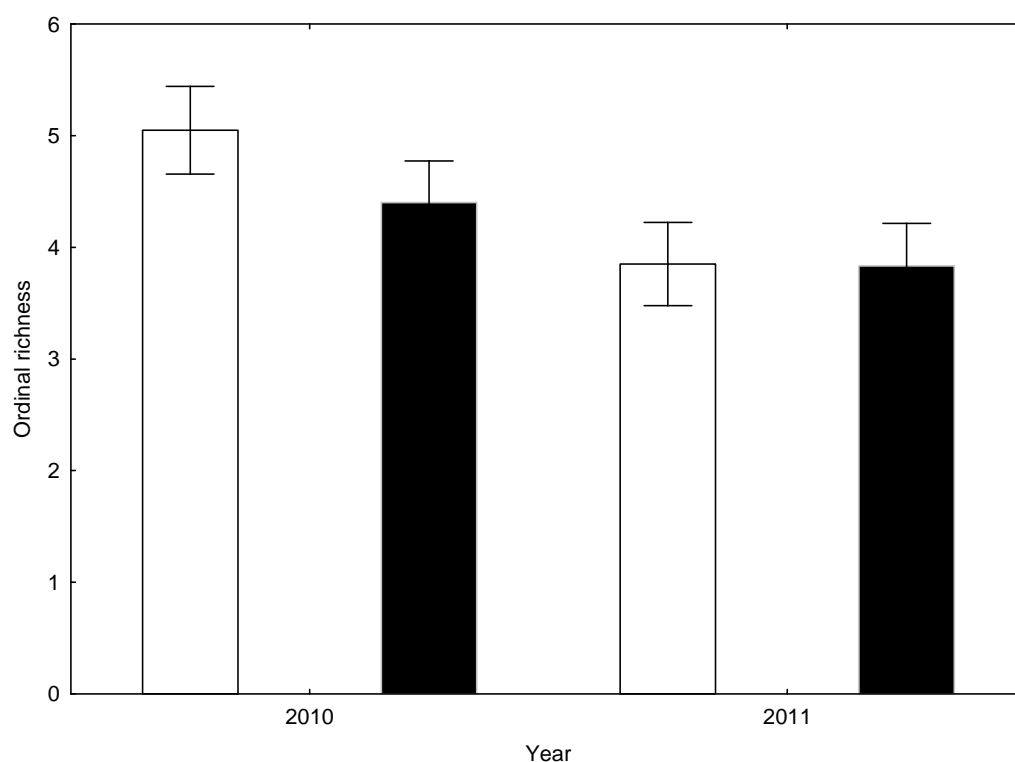


Figure 12. Mean (\pm SE) other macro-invertebrate ordinal richness within plots in the infested (white bar) and uninfested site (black bar) within pitfall traps during the 2010 and 2011 sampling periods.

3.5.3 Other macro-invertebrates in foliage beats

Other macro-invertebrates from eight orders were collected in foliage beats. Spiders were the predominate group collected (37% of all samples combined), followed by crickets (21%), and beetles (14%) (Figure 13). The variation in the abundance of most individual orders is not addressed here because of the mobility of the fauna. However, for spiders (Arachnida) which are both relatively sedentary and well known to be sensitive to exotic ant invasions, there is a clearly fewer individuals within the infested site in both sample times, and this abundance difference was statistically significant in the 2011 sample (Table 4). This difference in spider abundance had no effect on combined macro-invertebrate data, and just as for pitfall trap data there was no difference in overall abundance or ordinal richness between the infested and uninfested sites within either of the two sample times (Figures 14, 15, Table 5).

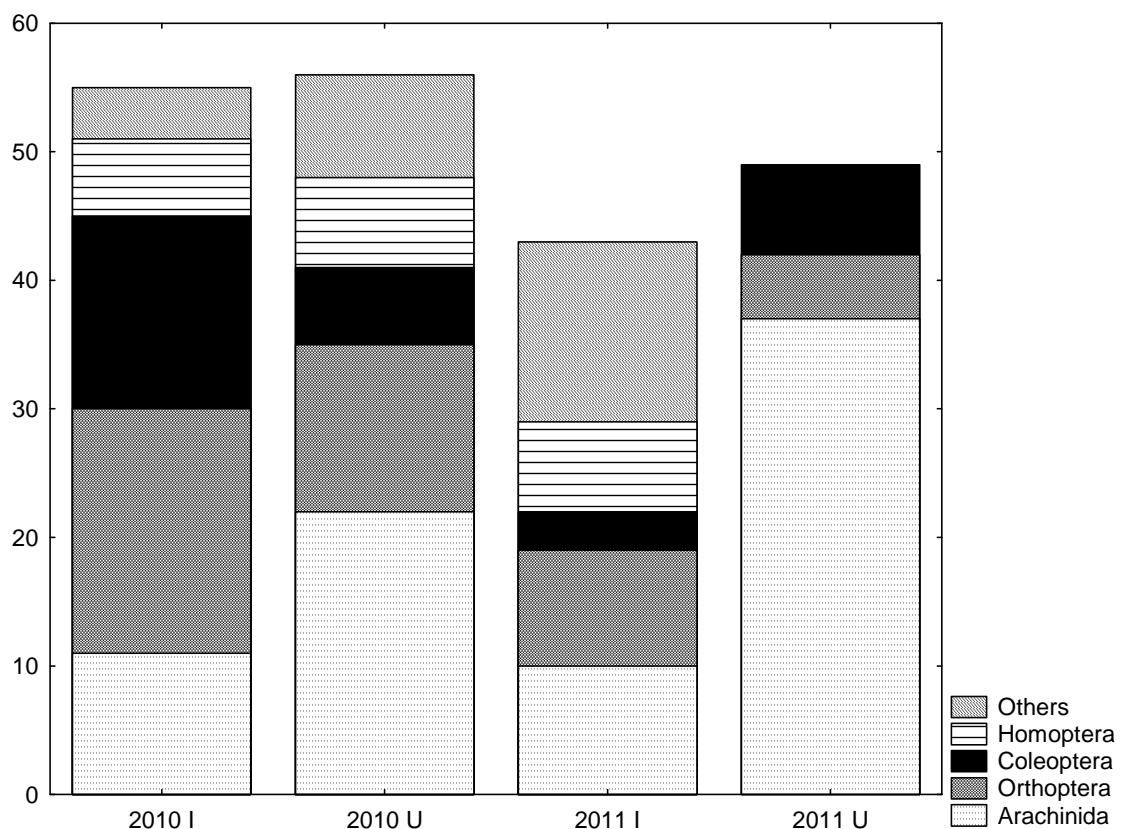


Figure 13. Abundance of the other macro-invertebrate orders collected in foliage beats within sites infested (I) or uninfested (U) with *Anoplolepis gracilipes* during the 2010 and 2011 sampling periods.

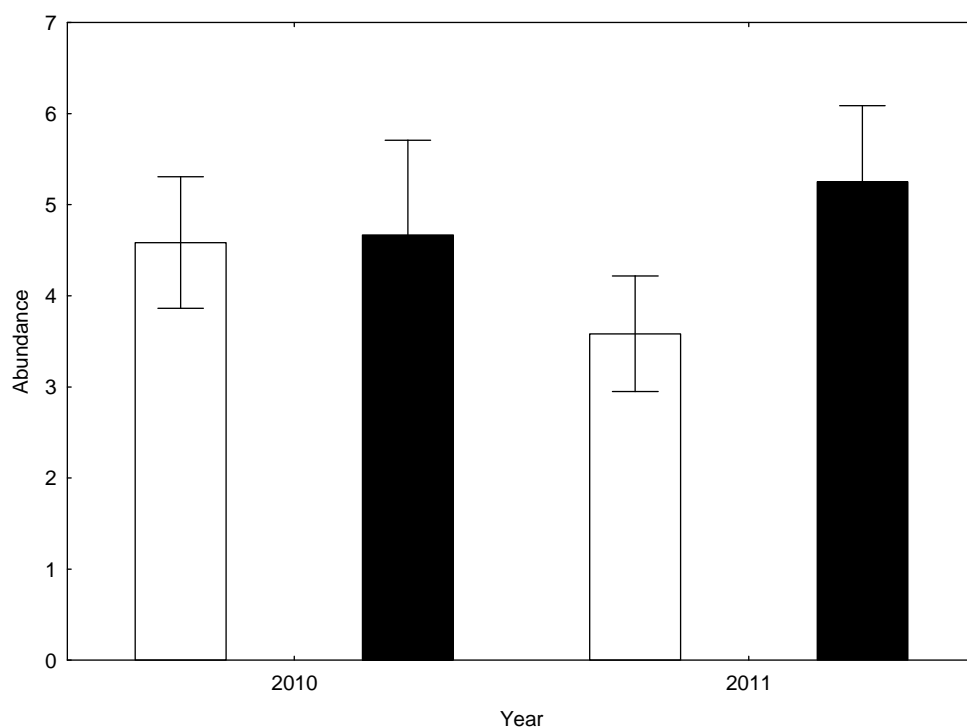


Figure 14. Mean (\pm SE) other macro-invertebrate abundance within foliage beats in the infested (white bar) and uninfested site (black bar) during the 2010 and 2011 sampling periods.

Table 4. Results of Mann-Whitney U-tests of other macro-invertebrate data from foliage beats between infested and uninfested plots in the 2010 and 2011 samples. Bold indicates significance of $P < 0.025$.

	U	z	P
<i>2010 sample</i>			
total abundance	63.5	0.462	0.644
ordinal richness	63.5	-0.462	0.644
Spider abundance	55.5	-0.924	0.356
<i>2011 sample</i>			
total abundance	48	-1.357	0.175
ordinal richness	64.5	-0.404	0.686
Spider abundance	30	-2.397	0.017

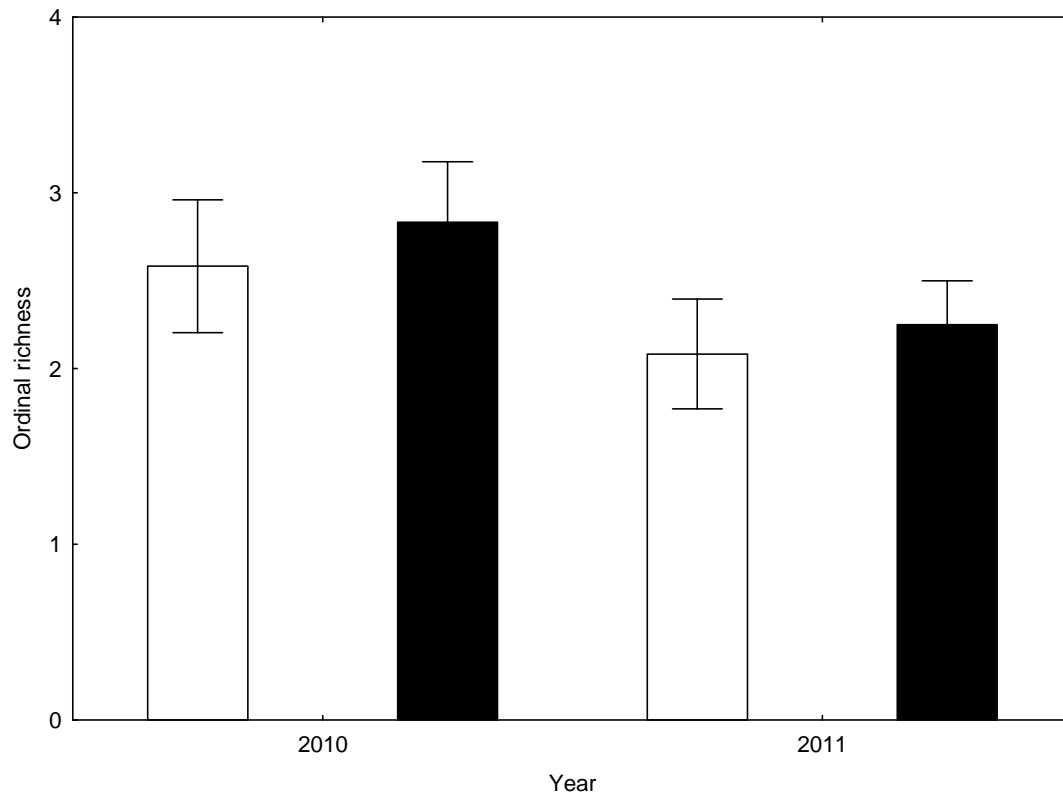


Figure 15. Mean (\pm SE) other macro-invertebrate ordinal richness within foliage beats in the infested (white bar) and unfested site (black bar) during the 2010 and 2011 sampling periods.

3.5.4 Hermit crab counts

There was a clear difference in total hermit crab abundance between infested and unfested sites in both sample times. In the 2010 sample, when *A. gracilipes* abundance was lowest, the infested site had approximately one quarter of the crabs per plot (average = 1.3 ± 0.43) of the unfested site (average = 5.3 ± 1.62), being greatly statistically different; Mann-Whitney U Test: $U = 104$, $Z = -2.5$, $P = 0.0098$. This statistical result was driven by large crabs (Mann-Whitney U test: $U = 98.5$, $Z = -2.73$, $P = 0.0063$) as there were too few small crabs collected to produce a statistical difference in this size class (Mann-Whitney U test: $U = 179.5$, $Z = -0.54$, $P = 0.5885$). Only seven small crabs were found in the infested site compared to 28 in the unfested site, but the proportion of small crabs to the total count was consistent between the two sites (27% and 26% respectively), indicating that any factor affecting hermit crab abundance applied equally to both size classes.

The difference in crab abundance between the infested and uninfested sites were even more pronounced in the 2011 sample when *A. gracilipes* abundance was greater, with only four large crabs being found in the infested site, compared to an average of 2.7 crabs per sample in the uninfested site. Naturally this difference was highly statistically significant (Mann-Whitney U Test: $U = 34$, $Z = -4.477$, $P < 0.0001$).

3.6 Phytophagous insects and extrafloral nectar

Multiple unidentified species of scale and at least one mealy bug species were found on six tree species (Table 3). The only interaction noticed between *A. gracilipes* and these insects was with scales on Indian Mulberry (Nonu) *Morinda citrifolia*, but all of the insect species were found within the infested areas.

Six plant species were found to have extra floral nectaries or carbohydrate sources accessible to ants (Table 3), but *A. gracilipes* was found attending these sources only on the Indian Mulberry (Nonu) *Morinda citrifolia*.

There was a marked difference in the abundance of extrafloral nectar sources between the infested and uninfested sites. Within the infested site, 50% and 32% (average of 41%) of the trees sampled along the two transects had EFNs, being approximately double than that within the uninfested site (17% and 34% respectively, average of 26%). This difference was not attributable to a single species, with four of the six species being encountered more within the infested site. Similarly, the occurrence of phytophagous insects differed greatly between the two sites, with 24% and 33% respectively (average of 29%) of assessable trees within the infested site harbouring phytophagous insects compared to only 7% and 0% (average of 4%) within the uninfested site. The abundance of phytophagous insects were also clearly different between the two sites, with those in the infested site predominantly occurring as clusters of many individuals, whereas only two individual scales were found within the uninfested site on two trees.

It is not possible to state whether the current distribution of *A. gracilipes* solely at the north-eastern end of Vini beach is a consequence of the vegetation composition (and hence EFN availability), or if this distribution is merely by chance and in time the ant will infest the entire beach. Similarly, it is unclear whether the phytophagous insect

density is a cause or consequence of the *A. gracilipes* distribution. However, carbohydrate sources from both plants and phytophagous insects are well-known drivers of invasive ant abundance, and interestingly the greatest ant diversity was also found within the infested site where carbohydrate sources were greatest. Such a deterministic relationship has never been demonstrated before between invasive ants and vegetation composition, and thus this is an exciting observation worthy of further investigation.

Table 3. Plants with extra floral nectar sources and phytophagous insects observed on Nu’utele, as well as observed interactions with *A. gracilipes*.

Scientific name	Samoan name	English name	Description	<i>A. gracilipes</i> interaction observed
Plants				
<i>Morinda citrifolia</i>	Nonu	Indian Mulberry	Nectar supply at floral inserts on fruit	Yes
<i>Passiflora foetida</i>	Pāsio vao	Passionfruit	EFN location unclear, but <i>Passiflora</i> known to have EFN	No
<i>Passiflora</i> sp.	Pāsio	Passionfruit	EFN location unclear, but <i>Passiflora</i> known to have EFN	No
<i>Terminalia catappa</i>	Talie	Tropical almond	EFN pair at base of leaf	No
<i>Macaranga harveyana</i>	Lau pata		EFN at base of leaf	No
<i>Hibiscus tiliaceus</i>	Fau	Beach hibiscus	EFNs at base of leaf	No
Insects				
		Mealy bug	Found on <i>Barringtonia asiatica</i> (Futu), <i>Cocos nucifera</i> (coconut, Niu), <i>Mikanika micrantha</i> (Fue Saina), <i>Omalanthus nutans</i> (Mamala),	No Only mealy bugs on <i>Cocos nucifera</i> were within the infested areas
		scale	Found on <i>Barringtonia asiatica</i> (Futu), <i>Macaranga harveyana</i> (Lau pata), <i>Morinda citrifolia</i> (Nonu), unidentified tree	Yes on <i>Morinda citrifolia</i> , no for all others. Scales on <i>Macaranga harveyana</i> were within an uninfested area

4. Management implications

The presence of *A. gracilipes* on the Aleipata islands is potentially of great concern, given the conservation significance of the islands, as well as the global reputation of this ant for its negative and often severe ecological impacts. Indeed the abundance levels and nest densities of this ant found in the surveys are among the highest recorded in the world. However, this did not translate directly into clear impacts for anything other than hermit crabs, and large ants such as *Odontomachus simillimus*. Importantly, this invasion is within an establishment phase when impacts are very localised and restricted to relatively sedentary or slow fauna, thus as the invasion expands and matures effects could be expected to increase, especially for more mobile fauna such as other invertebrates, birds and reptiles. However, the *A. gracilipes* populations on Nu'utele do not appear to be establishing well, and it remains unclear if any population on Nu'utele will be self-sustaining for more than a few years. Rather than expanding, the original population at Nu'utele beach appears to have been almost completely extirpated by natural causes, and two other populations along the walking trail disappeared altogether during the timeframe of this study. Also, between the two sampling periods the rate of expansion of the Vini beach population was quite negligible (20 m at maximum).

Should the impacts of an invader be determined to be great enough to consider management of the species, the decision to apply management actions or not should be dependent upon five criteria: 1) technical possibility; 2) practical feasibility; 3) environmental acceptability of treatments; 4) economic sensibility; and 5) political and social acceptability. Economic, political and social factors are not discussed here, as these are issues outside of the scope of this study.

Controlling and even eradicating *A. gracilipes* is definitely technically possible, as it has now been confirmed eradicated from 30 locations around the world (Hoffmann et al. in press; Hoffmann unpublished data), and ongoing efforts on Christmas island are well documented to be highly successful for short-term control (Green et al. 2004, 2009). Management actions on Nu'utele could also be argued to be feasible, depending upon the goal and area. Not all terrain on Nu'utele, including some infested areas, are accessible, thus any ground-based actions are only feasible for short-term

management. However, aerial operations are feasible for broad scale treatments anywhere over the island, potentially for an eradication attempt.

The environmental acceptability of treatments is probably the greatest issue for *A. gracilipes* management on Nu'utele, and any other island ecosystem. Currently, all ant baits that are efficacious against *A. gracilipes*, also negatively affect land crabs (Wegmann 2008), marine invertebrates and many other ants. Whilst the impacts on marine invertebrates are presumably negligible (if at all) due to the dilution effect of the sea, treatment effects on land crabs and native ants can be greater than the impact of *A. gracilipes*, and this would be especially so if an entire island was to be treated multiple times for an eradication attempt. All but one *A. gracilipes* eradications to date have been achieved on mainland systems with no non-target issues, and there are no published details about the sole eradication that was achieved on a part of an island within the Seychelles (Haines & Haines 1978). There is no doubt that broad-scale treatments using toxic bait over Nu'utele would have a significant impact on the island's hermit crabs (Wegmann 2008), and may well cause the local extinction of the coconut crab (*Birgus latro*). Unfortunately, the product with the least non-target issues (Distance), which utilises an Insect Growth Regulator rather than a toxicant, has thus far only achieved high levels of control rather than eradication, even after five treatments over two years within trials in Arnhem Land. Thus there is currently no product that can safely remove *A. gracilipes* from Nu'utele without causing significant environmental impact.

5. Management recommendations

Considering holistically the great flux of *A. gracilipes* populations, the restricted impacts, the great likelihood of severe non-target impacts from broad-scale baiting and the impossibility of conducting hand-treatments over all infested terrain, I do not recommend eradication from the island as a management goal. However, I do consider it feasible to at least suppress the spread and establishment of *A. gracilipes* on Nu'utele by conducting treatments aimed at locally eradicating the small populations on Nu'utele beach and on the western ridge, and to contain the population on Vini beach so that it does not further infest the lowland area. Such management would re-contain the ant to a single location, thereby suppressing its ability to completely establish over the island, and restrict its impacts to a single and small area.

Containment also provides better scope for complete eradication in the event that a treatment product is developed that can achieve eradication without inducing severe non-target impacts.

6. Research recommendations

Regardless of whether control measures are implemented or not, I highly recommend the continuation of some research conducted here, as well as additional research into other aspects of *A. gracilipes* biology, to address its invasiveness and potential management.

First, monthly sampling of crazy ant nest contents (e.g. pupae counts, queen counts) and nest density should be continued to fill the knowledge gaps of the biology of the ant, especially to determine the timing of queens reproduction. Such information is critical for effective management, and should be known prior to any broad-scale management operation, because treatments should be timed around the queen reproductive phase.

Second, the distribution of the ant should be monitored annually to bi-annually to either ensure that management actions are achieving their goals or to re-assess its status and risk on the island.

Third, additional research should be instigated to address the apparent relationship found between *A. gracilipes* distribution and the supply of carbohydrate resources from both plants and phytophagous insects. Such a deterministic relationship (if it really does exist) has never been demonstrated before between invasive ants and vegetation composition, and thus this is an exciting observation worthy of further investigation. For example, if a strong correlation was found to exist, then the distribution *A. gracilipes* within any area could be predicted based on vegetation composition. This research would require comparative work to be conducted on Nu'ulua, where *A. gracilipes* seems to be well-established island-wide (Vanderwoude et al. 2006). Any such work would also be best designed with an impact component to elucidate any relationship between vegetation composition, *A. gracilipes* abundance and ecological impacts.

7. Acknowledgements

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Appendix 1. Ant species found within the infested (I) and uninfested (U) sites in the 2010 and 2011 sampling periods within pitfall traps and foliage beats.

Species	Classification	Pitfall traps				Foliage beats			
		2010 I	2010 U	2011 I	2011 U	2010 I	2010 U	2011 I	2011 U
<i>Anochetus graeffei</i>	Native	X	X						
<i>Anoplolepis gracilipes</i>	Exotic	X	X	X		X		X	
<i>Brachymyrmex obscurior</i>	Native	X							
<i>Camponatus</i> sp. <i>maculatus</i> group	Native			X					
<i>Hypoponera punctatissima</i>	Native	X	X	X	X				
<i>Monomorium destructor</i>	Exotic		X						
<i>Monomorium floricola</i>	Exotic	X		X		X	X	X	X
<i>Monomorium pharaonis</i>	Exotic		X						
<i>Odontomachus simillimus</i>	Native	X	X	X	X				
<i>Oligomyrmex atomus</i>	Native				X				
<i>Paratrechina longicornis</i>	Exotic	X	X	X	X	X	X	X	X
<i>Pheidole fervens</i>	Native			X					
<i>Pheidole oceania</i>	Native	X		X			X		
<i>Pheidole sexspinosa</i>	Native	X		X					
<i>Pheidole umbonata</i>	Native	X	X	X	X	X		X	X
<i>Rogeria stigmatica</i>	Native	X	X	X					
<i>Rogeria sublevinodis</i>	Native			X					
<i>Strumigenys rogeri</i>	Native	X	X	X	X				
<i>Tapinoma melanocephalum</i>	Exotic	X	X	X	X	X	X	X	X
<i>Technomyrmex vitiensis</i>	Native			X					X
<i>Tetramorium bicarinatum</i>	Exotic	X	X	X				X	
<i>Tetramorium lanuginosum</i>	Exotic	X		X					
<i>Tetramorium pacificum</i>	Native			X			X		
<i>Tetramorium simillimum</i>	Exotic			X					

**Contact Us**

Phone: 1300 363 400

+61 3 9545 2176

Email: enquiries@csiro.auWeb: www.csiro.au**Your CSIRO**

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