Monitoring of the 2009 aerial baiting of yellow crazy ants (*Anoplolepis gracilipes*) on non-target invertebrate fauna on Christmas Island

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May 11

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Version control:

Date	Version	Description	Author	Reviewed By
31/3/2011	1.0	Draft final report	AW, SM	MS, PU
18/5/2011	1.1	Final Report	AW, SM	

Abbreviations:

Abbreviations	Description
NMDS	Nonmetric multidimentional scaling
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
NDVI	Normalised difference vegetation index
YST	Yellow sticky traps
ANOVA	Analysis of variance
MRPP	Multi-response permutation procedures

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EXECUTIVE SUMMARY

The highly invasive yellow crazy ant, *Anoplolepis gracilipes*, has caused widespread destruction and ecosystem changes on Christmas Island in the Indian Ocean over the last 20 years. This small ant, which forms highly aggressive supercolonies, has caused major changes in the islands rainforest, as they have displaced and killed many of the islands unique fauna such as the islands keystone species, the endemic red crabs, *Gecaroidea natalis*. Fipronil baits have been used extensively on the island to control the yellow crazy ant since 2000 with over 4500 hectares baited in the last ten years. An aerial baiting program was undertaken in 2002, with over 2500 hectares and 11000 kg of bait distributed on Christmas Island. This effectively depressed the yellow crazy ant colony for many years, but in 2009 over 800 hectares of the island was again covered by super colonies. A new fipronil aerial baiting program was again undertaken in September/October 2009. Relatively little information is known about fipronil, and especially the potential impacts that the bait formulation may have on non-target organisms. The potential for bioaccumulation of the pesticide in the environment of Christmas Island is also unknown. CESAR Consultants were commissioned by National Parks Australia to undertake an assessment of the effects of the 2009 fipronil aerial baiting on non-target fauna and potential bioaccumulation of fipronil in the environment on Christmas Island. Invertebrate surveys were undertaken on three separate occasions to assess the affects of the aerial fipronil baiting program. The first survey was immediately prior to the aerial baiting in August 2009, the second immediately after the aerial baiting was completed in October 2009 and the final survey was approximately 6 months later in May 2010. Invertebrate communities were surveyed in three different environments: pitfall traps were used intensively to assess fipronil baiting impacts on ground-dwelling invertebrates; yellow sticky traps were used to assess the potential effects on canopy invertebrates; and freshwater/sediments were surveyed for macroinvertebrates to determine whether the fipronil had entered the freshwater springs on the island. In addition, to determine whether fipronil is bioaccumulating in the environment on Christmas Island, soil, water and sediment samples were analysed using liquid chromatography tandem mass spectrometry (LC-MS/MS) for the presence of fipronil and

three key toxic degradates (fipronil sulfide, fipronil sulfone and fipronil desulfinyl). The key findings of the surveys were:

1. Strong seasonal (collection) effects on invertebrates communities were found for ground-dwelling, canopy-dwelling and freshwater arthropods.

2. The fipronil aerial baiting undertaken in September/October 2009 had significantly negative impacts on *A. gracipiles*, with over a 98% reduction at sites that were baited.

3. The LC-MS/MS analyses provided no evidence that fipronil or three toxic degradation by-products, fipronil sulfide, fipronil sulfone and fipronil desulfinyl, are accumulating in the environment on Christmas Island.

4. No evidence was found that the fipronil aerial baiting undertaken in September/October 2009 caused significantly negative impacts on arthropod communities.

NATURE OF WORK

Arthropod communities are sensitive to alterations in their environment such as land use changes, habitat fragmentation and degradation, nutrient enrichment and environmental stress (Perner and malt 2003, Hoonbok and Moldenke 2005, Schowalter and Zhang 2005, Nash et al. 2008). Changes in land use, predominantly driven by agriculture, are among the most immediate drivers of species diversity (Perner and Malt 2003). Within agricultural environments, arthropod communities can be greatly affected by a range of factors, including crop, cultivars, tillage practices, weeds and cover crops, surrounding vegetation, and applications of pesticides to control arthropod pests, diseases and weeds (Olson and Wackers 2007, Thomson and Hoffmann 2007, Sharley et al. 2008). The prophylactic use of pesticides within agroecosystems, however, is often considered to have the largest impact on the distribution of arthropods in an environment. Detrimental effects of chemicals on specific nontarget arthropods in agroecosystems have been widely documented (Croft and Brown 1975, Theiling and Croft 1988, Bunemann et al. 2006). These include decline in species diversity (Everts et al. 1989), resurgence and outbreak of secondary pests (Theiling and Croft 1988), and reduction in natural enemies (Thomson and Hoffmann 2006).

The awareness of such harmful effects of pesticides and knowledge of the role beneficial invertebrates play in agroecosystems, from biological control of pests through to ecosystem services such as pollination, has led to many agricultural industries adopting different strategies to limit the use of these detrimental pesticides (Thomson and Hoffmann 2006). The environmental impact of these pesticides can reach far beyond the area of intended use, affecting invertebrate communities in nearby remnant vegetation (Thomson and Hoffmann 2009), as well as entering streams, rivers, and wetlands through farm run-off events and potentially affecting fauna living in these areas (Sharley et al. 2008, Thomson et al. 2010). Accumulation of pesticide residues can also occur in sediments, with slow degradation of pesticides in these anoxic and dark environments, potentially exposing fauna for long periods of time (Konwick et al. 2006, Siriwong et al. 2009). The use of pesticides, therefore, can have vast impacts on arthropod communities in both terrestrial and aquatic environments, and can change the composition of those communities.

Our knowledge on the impacts of pesticides on arthropod communities and vertebrate fauna is largely derived from agroecosystems, where they are applied routinely. The effects of pesticides on fauna in natural systems such as rainforests or remnant vegetation are limited to areas where they are adjacent to agricultural land (Nash et al. 2008). Therefore the impact on arthropod assemblages, and the bioaccumulation of pesticides in these natural environments is largely unknown.

Christmas Island, a territory of Australia, is a 134 km^2 tropical island located in the northeastern Indian Ocean. Due to its unique geographical history and minimal human disturbance, this island has a high level of endemism amongst its flora and fauna. Since human settlement in the 1900's, this unique ecosystem has seen the introduction of the exotic yellow crazy ant, *Anoplolepis gracilipes*, a species that can have large impacts on native flora and fauna and therefore pose a major threat to the islands ecosystem (O'Dowd et al. 2003). This highly aggressive species has caused widespread environmental damage on numerous tropical islands and continents through its impact on native flora and fauna, often causing ecosystem changes (Green et al. 1999, O'Dowd et al. 2003).

The yellow crazy ant remained in relatively low densities on Christmas Island until 1989, when the first supercolony containing multiple queen ants was discovered (O'Dowd et al. 2003). These supercolonies have spread rapidly and in 2001 they occupied approximately 20% of the islands rainforests. Major changes in the islands rainforest have resulted from these ants, as they have displaced (and killed) many of the islands fauna such as the islands endemic red crabs, *Gecaroidea natalis* (O'Dowd et al 2003, Davis et al. 2010). This displacement and death of red crabs has resulted in a dramatic change to the forest ecosystem, by promoting seedling recruitment that would otherwise be supressed by the red crabs. Their mutualistic association with invasive honey-dew producing scale insects is also likely to have caused canopy dieback and tree death, as well as the growth of sooty moulds (O'Dowd et al 2003, Abbott and Green 2007).

Fipronil solid baits (Presto®01, active ingredient fipronil 0.1g/kg Bayer Environmental Science Pty Ltd) have been used extensively on Christmas Island to control the yellow crazy ant (Stork et al. 2003, Marr et al. 2003; Figure 1). Baiting programs have been conducted since 2000, with a large scale aerial baiting program conducted in 2002 resulting in 11,000 kg of fipronil baits distributed to all yellow crazy ant supercolonies on the island (covering ~ 2500 ha). This baiting program resulted in a dramatic decrease in yellow crazy ant abundance on the island within weeks. Unfortunately, however, by 2009 ant numbers had increased substantially and were estimated to cover approximately 833 ha of rainforest on the island (DNP, unpubl. data). An aerial baiting program was therefore undertaken again in September/October 2009, to distribute Presto®001 baits (active ingredient 0.01g/kg fipronil).

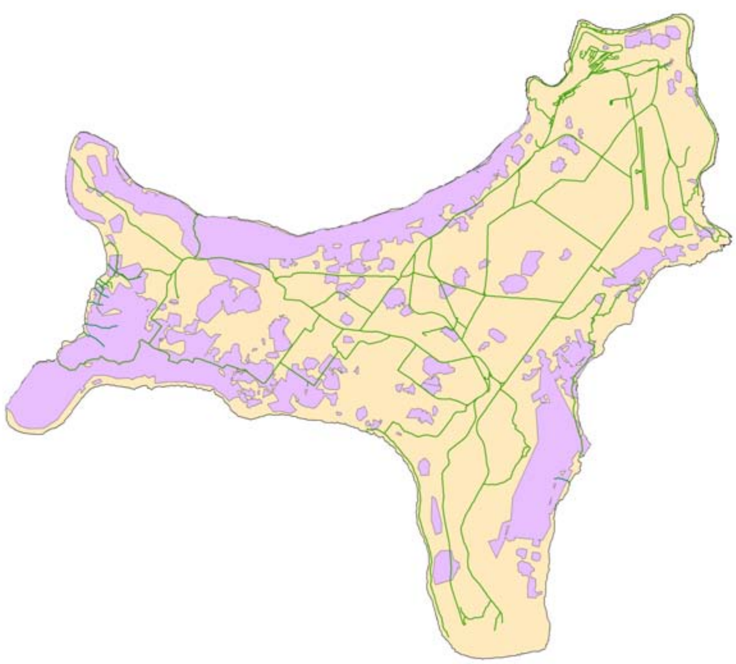

Figure 1. Christmas Island showing areas where fipronil baits (Presto®01 and Presto®001) have been distributed between 2000-2009 (including aerial baiting undertaken in Sept-Oct 2009).

Fipronil is a phenylpyrazole insecticide used to control a broad spectrum of insects including cockroaches, mosquitoes, locusts, ticks, fleas, termites and ants (Gunasekara et al. 2007). Fipronil is effective at low field application rates against insects that are often resistant to other insecticides such as pyrethroids, organophosphates and carbamates. Its mode of action is interference with the normal function of γ-aminobutyric acid (GABA)-gated channels (a neurotoxin). Fipronil degrades by means of oxidation, reduction, hydrolysis and photolysis to form four major products; fipronil-sulfone (oxidation at soil surface), fipronil-sulfide (reduction in soils), fipronil-amide (hydrolysis in water and soils), and fipronil-desulfinyl, (photolysis) (Gunasekara et al. 2007). Fipronil, and its degradation by-products, can be highly toxic to many non-target species (Konwick et al. 2006) and is also known to bioaccumulate in some organisms (Konwick et al. 2006, Beggel et al. 2010, Mullin et al. 2010). Fipronil is more toxic to invertebrates than mammals (Hainzl et al 1998) and can impact aquatic environments at low concentrations. In addition, fipronil's degradation products, which are suggested to have similar toxic potential (Hainzl et al. 1998) and are more environmentally stable (Hainzl and Casida 1996), increase the threat of fipronil to the environment. It is known to persist in soils, water and sediments (Gunasekara et al. 2007) and therefore poses a significant risk when used in high doses to control a pest species such as the yellow crazy ant.

Previous research conducted around the 2002 aerial baiting program (Marr et al. 2003, Stork et al. 2003) indicated that the fipronil baiting program was not a significant risk to fauna found on the island. There were no detectable effects of the aerial baiting program on litter invertebrates (Marr et al. 2003), despite the apparent toxicity of fipronil in laboratory assays on four common invertebrate groups found in litter. Similarly, there were no detectable effects found on canopy arthropods and several vertebrate species (Stork et al. 2003; although they suggest that there may be an effect on one bird species, the Christmas Island imperial pigeon). It was recommended that fipronil baits only be used in areas where supercolonies are found, and that further research should be conducted to determine the impacts of the fipronil baiting program on the highly endemic and unique fauna of Christmas Island (Marr et al. 2003).

The aerial baiting program undertaken in September/October 2009 provided an opportunity to monitor the effects of fipronil on Christmas Islands fauna. Invertebrate surveys combined with new methods for pesticide detection and the detection of residual by-products, can provide a more sensitive picture of the effects and persistence of fipronil (and degradates) on Christmas Island. CESAR Consultants were commissioned by National Parks Australia to undertake an assessment of the effects of the proposed 2009 fipronil aerial baiting on non-target fauna on Christmas Island. The objectives of the study were to:

1. Assess the immediate effects of the fipronil aerial baiting on non-target invertebrate fauna.
2. Determine longer-term impacts of the fipronil aerial baiting on non-target invertebrate fauna.
3. Assess the possible bioaccumulation of fipronil and its degradates (sulfide, sulfone and desulfinyl) in soil, water and sediment from Christmas Island using LC-MS/MS (liquid chromatography tandem mass spectrometry).

METHODS

Several invertebrate survey methods were used to determine the impacts of the fipronil aerial baiting program on non-target organisms. The impacts of the fipronil aerial baiting are likely to be greatest on ground-dwelling terrestrial invertebrates, which will be exposed directly to the fipronil baits. We therefore used pitfall traps to sample the ground-dwelling invertebrate community. It is also possible that the fipronil baits can affect invertebrates in the canopy, especially given that the fipronil baits will be delivered from a helicopter flying immediately over the canopy. We trapped invertebrates using sticky traps at a height of ~10-12 m above ground. Finally, fipronil is known to persist for longer periods in areas where breakdown is less likely (i.e. conditions with a lack of light and oxygen; Gunasekara et al. 2007). We therefore also sampled macroinvertebrates that live in sediment found in permanent freshwater pools on Christmas Island. If fipronil or its degradates enter the freshwater systems on Christmas Island, then sediment is the most likely place where it will persist and accumulate through time.

Terrestrial Invertebrate Collections

To collect ground-dwelling terrestrial invertebrates, six transects were established within Christmas Island National Park. Each transect consisted of thirteen sampling points separated by a distance of 200 to 300 metres. Transects were numbered from T1 to T6, and sites within each transect were numbered from S1 to S13. Transects were positioned to encompass the full range of habitat types and baiting histories on the island (*A. gracilipes* present or absent; historical baiting, baited during the 2009 aerial baiting program or never baited) (Figure 2; Appendix 1, Table A1), and with accessibility taken into account. Given the difficult terrain across much of the island, transects were either set up parallel to a road/track (T1-T4), or in areas where access on foot was possible (T5 and T6).

Invertebrate collections took place during three trips to Christmas Island by CESAR Consultants staff. The initial trip (22nd – 29th August, 2009) took place prior to the fipronil aerial baiting program (September/October 2009). The second trip (21st – 28th October, 2009) occurred ~ 3 weeks after the baiting program had been completed, and the final trip (29th April – 8th May, 2010) was timed to take place after the wet season (approximately 6 months after the fipronil aerial baiting). Four of the six transects were setup and sampled during the initial trip (T1-T4), and the remaining two (T5 and T6) were established during the October 2009 trip (Appendix 1, Table A1).

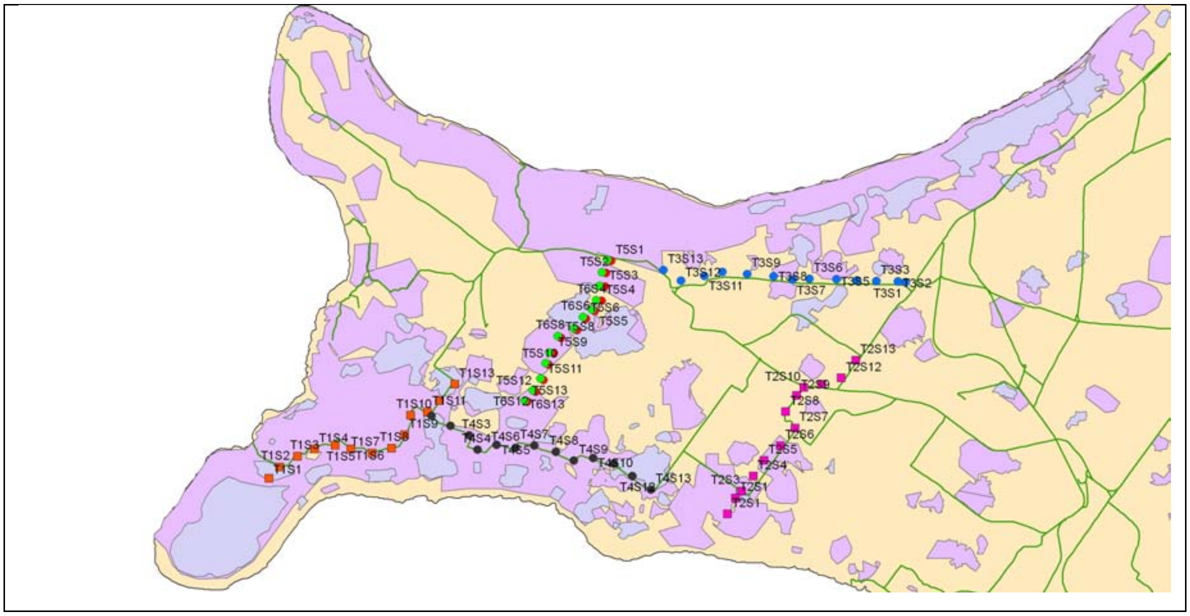

Figure 2. Location of transects on Christmas Island where terrestrial invertebrates were sampled during August 2009, October 2009 and May 2010. Purple indicates areas that have been baited since 2000. Blue indicates areas that were baited during the aerial baiting in Sept/Oct 2009.

At each site along each transect, pitfall traps were set to sample ground dwelling invertebrates occupying the forest floor. Pitfall traps enable rapid and efficient collection of data amenable to statistical analysis (Topping, Sunderland, 1992). Pitfall traps consisted of a 120 ml polypropylene vial inserted into a polyvinyl chloride (PVC) sleeve (45 mm), buried flush with the surface. Traps contained 40 ml of 70% ethanol solution. Initially, traps contained a mixture of 70% ethanol and 100% ethylene glycol in a 1:1 ratio. However, this attracted both robber crabs (*Birgus latro*) and red crabs, which subsequently attempted to remove the traps. We therefore removed the ethylene glycol component and applied baits (prawn paste) to trees ~ 50-100 m away from traps. This seemed to largely stop the crabs from attempting to remove traps. Traps were left open for 4 nights (Table A1), after which the vials were removed, capped, and transported back to the laboratory. Any bias caused by 'digging in' effects (Greenslade, 1973) of pitfalls traps were negated by removing the pitfall sleeve and then reinstalling on subsequent collection trips.

Yellow sticky traps (YSTs) were used to sample invertebrates present near the forest canopy. Traps consist of a rectangular plastic card (21 cm x 10 cm) with a sticky surface covering the surface of both sides. An initial pilot study with YSTs was conducted during the August 2009 trip at seven sites along Transect 4 (Appendix 1, Table A1). This was used to assess whether vertebrates (reptiles) may be caught be the sticky traps after consultation with the Christmas Island National Parks Team (Chris Boland and Michael Smith). No reptiles were found on these YSTs, and therefore they were deployed on subsequent trips. During the October 2009 and May 2010 trips, YSTs were set up at half of the total sites (39 sites) spread across 4 of the 6 transects (Appendix 1, Table A1). At each site, two yellow sticky traps were suspended with a short piece of wire (approx. 30cm long) on a small branch within the canopy. A 10-metre telescopic fibreglass pole was used to set and retrieve traps, and each trap was placed between 10 and 12 metres above ground level, depending on the availability of suitable branches.

Freshwater Invertebrate Collections

Collections were also made of macroinvertebrates occupying freshwater habitats on Christmas Island. Freshwater areas were generally low in water and sediment, particularly during the August 2009 and October 2009 collection trips. Normal macroinvertebrate collection methods (rapid bioassessment, kick and sweep sampling techniques; O'Brien et al. 2010) in edge and riffle habitats could not be undertaken due to the low amounts of water and sediment. Macroinvertebrates were therefore sampled with a 250 µm net, by either 'sweeping' through pooled water, or by placing the net on the substrate in running water and using a hand trowel to dislodge invertebrates, which subsequently flowed into the net. This process was performed over a 10 m interval at each site. Samples were then briefly washed in the laboratory and stored in 70% ethanol until identification.

Soil, Water & Sediment Collection

Soil was collected from various sites on Christmas Island (Appendix 1, Table A2). Leaf litter on the soil surface was first removed and soil from the top 20 mm was collected and sieved through a 4 mm test sieve, then placed into a 250 ml glass sample jar. Soil samples were stored at 4˚C or frozen at -20˚C, before transportation, under quarantine procedures to the analytical laboratory.

Sediment and water samples were also collected from several permanent freshwater sites on Christmas Island (Appendix 1, Table A2). Hosnies Spring was the first site on Christmas Island to be recognised under the Ramsar Convention on Wetlands. This site is located on the western end of the island and contains a unique stand of mangroves some 120 m inland and > 20 m above sea level. Jones Spring is another spring located at the eastern end of Christmas Island, close to the site of the Christmas Island casino and resort. The Dales are a series of watercourses running down to the coastal cliffs at the western end of Christmas Island and contain most of the surface water on the island. The Dales are also listed as an internationally significant wetland under the Ramsar convention. The sites we sampled at The Dales were Hugh's Dale, above and below the waterfall, and Anderson's Dale. The Ross Hill Gardens site was developed in the late 1920's when the springs were harvested to constitute a back up water supply for the island. At this site we sampled near both the southern and northern tanks/springs.

Depositional sediment was collected with a hand trowel and filtered through a 63 µm Nybolt mesh net into a 10 L bucket on site. Filtering prevents any macroinvertebrates passing through and also means that the most biologically available particle size for macroinvertebrate species (and hence the most toxic component) is analysed (O'Brien et al. 2010). After settling, water was decanted, and sediments were transferred to a 1 L glass collection jar. Sediments were stored at 4˚C or frozen at -20˚C, before transportation to the analytical laboratory under quarantine restrictions. Water samples were collected directly from pools or running streams/waterfalls into a 1L glass bottle, stored at 4˚C or frozen at -20˚C, and then transported to the analytical laboratory under quarantine restrictions.

Australian quarantine restrictions prevented the immediate analysis of the soil, water and sediment samples at the analytical laboratory. Ideally, these samples would have been analysed immediately after collection. Samples were therefore stored in lightproof glass containers (to prevent breakdown by light) and frozen to limit degradation via microbial activity. Samples were analysed between four and eight months after collection at the analytical laboratory. Due to the extended timeframe before analysis, some sample degradation is likely to have occurred. Therefore, soil, water and sediment samples were analysed for the presence of fipronil, as well as three fipronil degradates (fipronil sulphide, fipronil sulfone and fipronil desulfinyl) via liquid chromatography-tandem mass spectrometry (LC-MS/MS). Detection limits for analyses were 2 µg/kg for soil and sediment, and 0.01 µg/L (fipronil and fipronil desulfinyl) or 0.005 µg/L (fipronil sulfide/sulfone) for water samples. Fipronil can also be broken down into fipronil-amide, however a suitable standard is not available in Australia and therefore it was not included in analyses.

All analyses were undertaken in Dr Gavin Rose's laboratory at the Department of Primary Industries (DPI), Future Farming Systems Research Division (Werribee, Victoria). Appropriate Australian quarantine permits were obtained for transport and processing at the DPI laboratories (import permit number IP10008943). The DPI laboratory is accredited by the National Association of Testing Authorities (http://www.nata.asn.au) for all chemical analyses (ISO 17025: 2005).

Invertebrate Processing and Sorting

Following collection, pitfall traps were rinsed and filled with 70% ethanol, and transported to the CESAR Consultants laboratory in Melbourne. The contents of each trap was placed in a 250 µm sieve and washed thoroughly with running water. Samples were initially sorted to Order level or guild level under a dissecting microscope at 40X magnification, following the key of Harvey and Yen (1989). Sorting to lower taxonomic levels took place for some groups: all ants (Hymenoptera: Formicidae) were sorted to species level using the key of Framenau and Thomas (2008), whereas beetles (Coleoptera), spiders (Araneae) and mites (Acari) were sorted to Family level.

Yellow sticky traps were placed into an individual clear zip-lock plastic bag upon collection, and then stored in a refrigerator. Samples were sorted on Christmas Island using a dissecting microscope at 40X magnification. Invertebrates collected on yellow sticky traps were sorted to the same taxonomic levels as those collected in pitfall traps.

Macroinvertebrate samples were transported in 70% ethanol to the CESAR Consultant laboratories in Melbourne. The contents of each trap was transferred to a 250 μm sieve and washed thoroughly with running water. Samples were then sorted under a dissecting microscope at 40X magnification. Various taxonomic keys were used to identify macroinvertebrates from freshwater sediments to Family and morphospecies levels (e.g. Gooderham and Tsyrlin 2002, Dean et al. 2004). Chironomid larvae were also checked for deformities. Deformities are a common indicator of environmental stress and can be used as a sub-lethal effect with comparisons being made between sampling periods (before and after aerial baiting).

Christmas Island NDVI

Vegetation is known to greatly affect arthropod communities (Perner and Malt 2003) and could potentially cause experimental error in analyses of the arthropod community data. We therefore calculated an objective measure of greenness - the normalised difference vegetation index (NDVI) for each sample location. This measure is derived from satellite images that show the amount of photosynthesising vegetation present (Jenson, 2000). Data from QUICKBIRD satellite imagery was captured on the 4th March 2006 commissioned by the Department of Environment and Heritage. The Quickbird satellite recorded reflectance data of the island in the red, green, blue, near infra-red (2.39 m resolution) and panchromatic bands (0.6 m resolution). The red channel (band 3: 630 to 690 nm) and the near infrared channel (band 4: 760 to 900 nm) of the registered image were processed to create the QB NDVI imagery (NDVI = Band 4 - Band 3 / Band 4 + Band 3) for the study area.

We used an NDVI approach as the index has been shown to have a high correlation with green leaf biomass. The photosynthetic pigments (primarily chlorophyll) in green plants absorb light from the blue and red portions of the spectrum, whilst a large proportion of the infrared is reflected or scattered. Therefore healthy vegetation with vigorous growth has high NR reflectance and low red spectrum values resulting in high NDVI values. Impervious surfaces (roads, buildings) and cleared land (mine sites, bare soil) have simialar reflectance values in red and NIR components of the spectrum, so we would expect values closer to zero.

NDVI values were derived for individual sample localities (n = 78) from 6 transects. A 10 m, 20 m and 50 m buffer was created around each site location to obtain a mean NDVI value. These buffer distances were chosen as measures of vegetation cover and used as covariates in all analyses of invertebrate community structure. Eight sites were omitted from the analysis due to cloud cover / shading distorting NDVI values for these locations. We used a buffer NDVI approach to reduce heterogeneous spectral-radiometric characteristics in vegetation cover and to normalise potential atmospheric effects within the captured imagery for site localities.

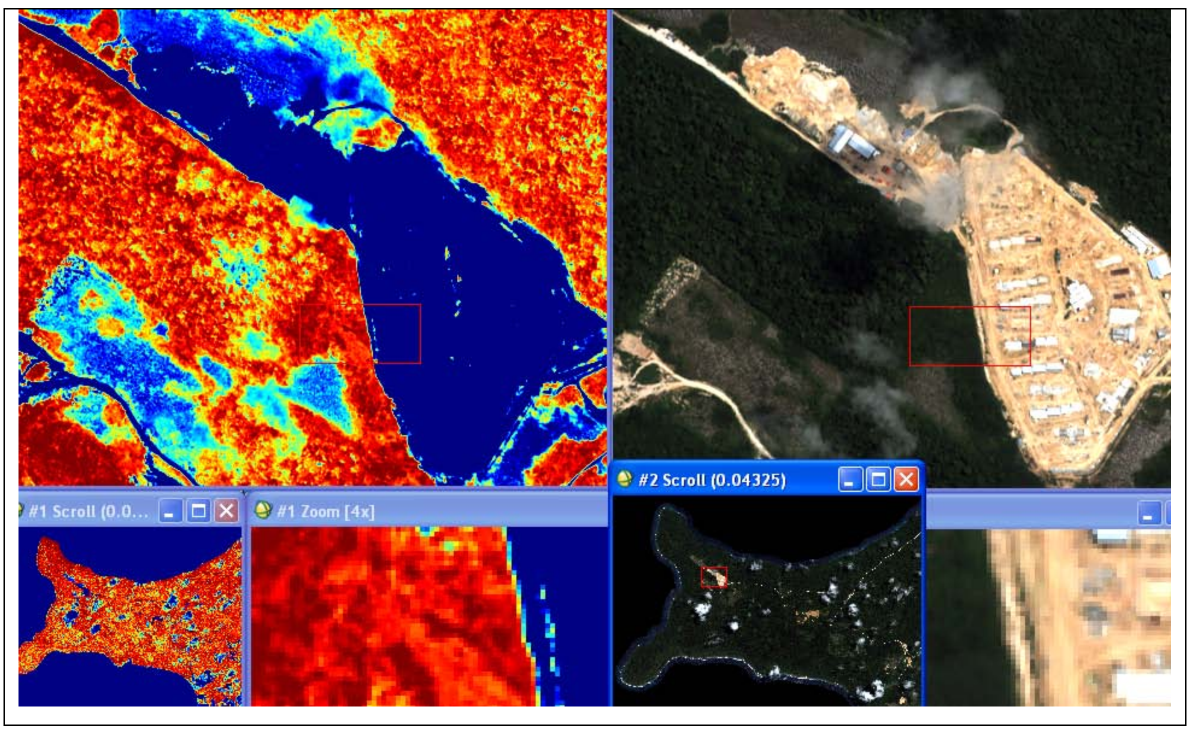

Figure 3. QUICKBIRD satellite imagery used to derive NDVI buffer zones of 10 m, 20 m and 50 m around each invertebrate sampling point.

Data Analysis

Ideally, to determine the impact of fipronil baiting on non-target taxa, experiments would be designed with adequate controls. However, due to the previous baiting undertaken on the island and the nature of the fipronil baiting program (i.e. baits are only set in areas that have high densities of *A. gracilipes*), ideal controls for our experiments could not be achieved. We therefore focused on arthropod community analyses to determine impacts of the aerial fipronil baiting as arthropod communities are very sensitive to changes in their environment (e.g. Hoonbok and Moldenke 2005, Schowalter and Zhang 2005).

The mean abundance of each arthropod group (taxon or guild) collected per site for each collection was used in the statistical analyses. Taxa and guilds present in low numbers were excluded from analyses (McCune and Grace 2002). Nonmetric multidimensional scaling (NMDS) was undertaken to describe the structure in arthropod communities at sites and transects on Christmas Island. This ordination procedure was completed with PC-ORD version 5.0 (MjM Software, Glenden Beach, Oregon, USA). Nonmetric multidimensional scaling is an effective method for analysing ecological data sets because it does not assume linear relationships and can be performed with data that are non-normally distributed, arbitrary, or discontinuous or that contain numerous samples with a value of zero (McCune and Grace 2002). Sorensen's (Bray-Curtis) distance measure was used in the autopilot "slow and thorough" mode of PC-ORD to avoid the issue of local minima. This calculated the best solution via a random starting configuration and 250 real data runs, involving up to six dimensions and stepping down in dimensionality. A Monte Carlo significance test based on 250 runs established final dimensionality. Axis scores from the final run provided information on stress, instability, and scores for subsequent analyses. Analyses were also rerun with the Relative Sorensen's distance measure, as this corrects for large differences in abundance.

To determine effects of collection, transect, baiting history and the 2009 aerial baiting on arthropod community structure, a number of different analyses were undertaken. NMDS axis scores were graphed for each treatment group to visualize effects. Significance of treatment was then determined using either Multi-Response Permutation Procedures (MRPP) in PC-ORD or by Generalised Linear Models (GLM) in PASW-SPSS version 18 for the Mac. MRPP analyses were used to verify effects of different groups (collection, transect, fipronil baiting etc). MRPP is a nonparametric procedure for testing the hypothesis that two or more groups are not significantly different. MRPP has the advantage of not being based on assumptions of distribution (such as normality and homogeneity of variances) that are seldom met in ecological community data (Mielke and Berry 2001). Where the overall MRPP indicated significant differences among groups, we tested pairs of groups to see which differed from each other. For MRPP, analyses also included non-parametric MANOVA (McCune and Grace 2002) to look at two factors (collection and transect), so that we could determine the interaction between collection and transect in analyses. GLM (multivariate analyses of variance) were undertaken so that NDVIs could be included in analyses as a covariate. In these analyses, NMDS axis scores were used to represent arthropod communities. Spearman's rank (r_s) correlations were computed to describe associations between mean abundance per trap of each ground or canopy arthropod taxon or guild and NMDS axis scores.

There are 54 ant species recognised on Christmas Island (Framenau and Thomas 2008). None of these ant species are considered endemic to Christmas Island, with the ant fauna composed of species that are regarded worldwide as tramps. Given that the yellow crazy ant is the target of the fipronil baiting program, other ant species may represent the most vulnerable invertebrate group. Ants were therefore identified to species using the key of Framenau and Thomas (2008) and analysed at the species level using the above procedures to determine the effects of the 2009 aerial baiting program.

FINDINGS

Soil, water and sediment analyses

Soil samples from 18 sites were analysed using LC-MS/MS for the presence of fipronil and three fipronil degradates, fipronil sulfide, fipronil sulfone and fipronil desulfinyl. Seven samples were analysed from the first collection trip (August 2009), seven from the second collection trip (October 2009) and four from the last collection trip (May 2010). Samples spanned areas that had been baited between 2000-2008, areas that were aerial baited in 2009, and areas that had no history of baiting (see Figure 4 and Appendix 1, Table A2).

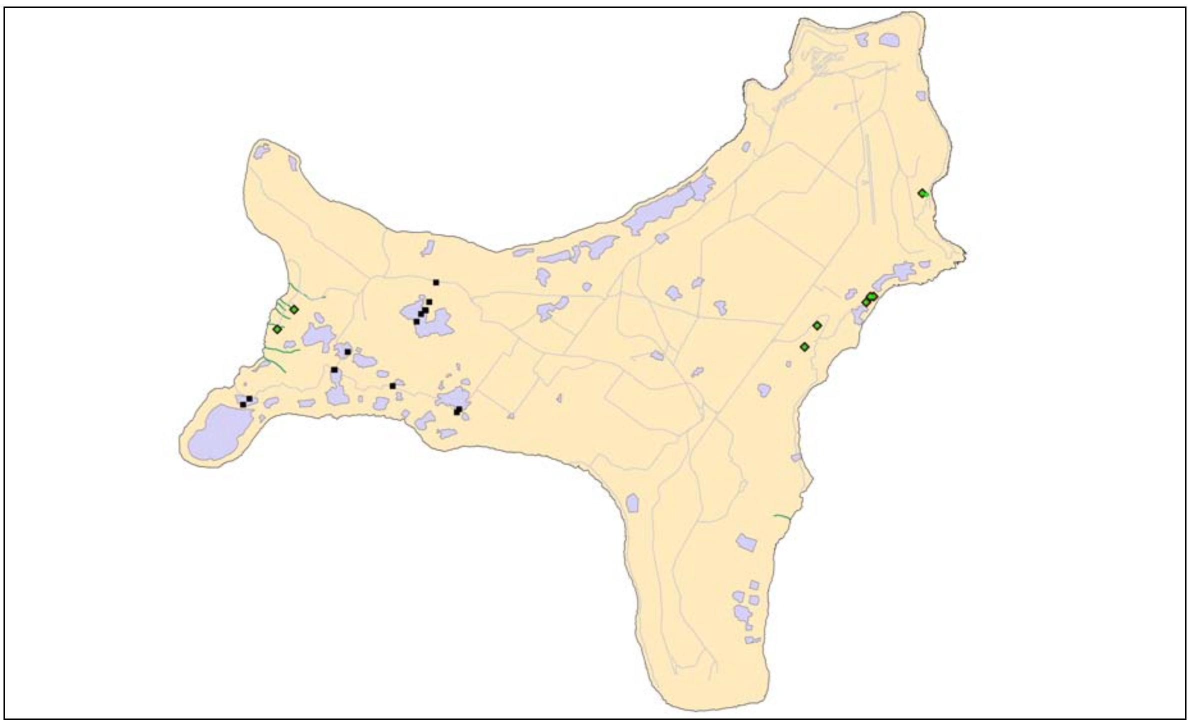

Figure 4. Locations from where soil (closed black squares) and water/sediment samples (green diamonds) were collected for LC-MS/MS analysis.

In all soil samples, there were no detectable levels of fipronil, nor fipronil breakdown products fipronil sulphide, fipronil sulfone or fipronil desulfinyl (Table 1, Appendix 2). Five samples were collected within three weeks of aerial baiting (samples SS8-10, SS12, SS13), indicating that the fipronil broke down quickly and is undetectable after a short period of time, at least at the sites where samples were taken.

Table 1. LC-MS/MS analysis results for soil samples collected from Christmas Island during August 2009, October 2009 and May 2010 (see Appendix 2). No samples exceeded detection limits for fipronil or its' degradates.

		LC-MS/MS analysis (µg/kg)			
Date	Site	Fipronil	Fipronil sulfide	Fipronil sulfone	Fipronil desulfinyl
August 2009	SS1	<2	<2	<2	<2
	SS2	<2	<2	<2	<2
	SS3	<2	<2	<2	<2
	SS4	<2	<2	<2	<2
	SS5	<2	<2	<2	<2
	SS6	<2	<2	<2	<2
	SS7	<2	<2	<2	<2
October 2009	SS10	<2	<2	<2	<2
	SS11	<2	<2	<2	<2
	SS12	<2	<2	<2	<2
	SS13	<2	<2	<2	<2
	SS14	<2	<2	<2	<2
	SS8	<2	<2	<2	<2
	SS9	<2	<2	<2	<2
May 2010	SS15	<2	<2	<2	<2
	SS16	<2	<2	<2	<2
	SS17	<2	<2	<2	<2
	SS18	<2	<2	<2	<2

Sediment and water samples were taken from eleven freshwater sites (Figure 4 and Appendix 1, Table A2), with 16 sediment and 23 water samples analysed over the three collection periods. For sediment (water) samples, four (five) samples were analysed from the August 2009 collections, six (eleven) from the October 2009 collections and six (seven) from the May 2010 collections. All sites, except Jones Spring (upper and lower), have not been directly baited with fipronil, although areas immediately adjacent to each site have been baited over the 10 year period in which baiting has been conducted. Similar to the soil samples, LC-MS/MS analyses did not detect any fipronil or it's degradates in the sediment or water samples from the eleven freshwater sites (Table 2, Appendix 2).

Table 2. LC-MS/MS analysis results for water samples collected from Christmas Island during August 2009, October 2009 and May 2010 (see Appendix 2). No samples exceeded detection limits for fipronil or its' degradates.

		LC-MS/MS analysis (µg/L)			
Date	Site	Fipronil	Fipronil sulfide	Fipronil sulfone	Fipronil desulfinyl
August 2009	Anderson's Dale	<0.01	<0.005	<0.005	<0.01
	Hosnies Spring C	<0.01	<0.005	<0.005	<0.01
	Hugh's Dale Waterfall (below)	<0.01	<0.005	<0.005	<0.01
	Ross Hill Gardens_1	<0.01	<0.005	<0.005	<0.01
	Ross Hill Gardens_2	<0.01	<0.005	<0.005	<0.01
October 2009	Anderson's Dale	<0.01	<0.005	<0.005	<0.01
	Hosnies Spring A	<0.01	<0.005	<0.005	<0.01
	Hosnies Spring B	<0.01	<0.005	<0.005	<0.01
	Hosnies Spring C	<0.01	<0.005	<0.005	<0.01
	Hosnies Spring D	<0.01	<0.005	<0.005	<0.01
	Hugh's Dale Waterfall (above)	<0.01	<0.005	<0.005	<0.01
	Hugh's Dale Waterfall (below)	<0.01	<0.005	<0.005	<0.01
	Jones Spring Lower	<0.01	<0.005	<0.005	<0.01
	Jones Spring Upper	<0.01	<0.005	<0.005	<0.01
	Ross Hill Gardens_1	<0.01	<0.005	<0.005	<0.01
	Ross Hill Gardens_2	<0.01	<0.005	<0.005	<0.01
May 2010	Anderson's Dale	<0.01	<0.005	<0.005	<0.01
	Hugh's Dale Waterfall (above)	<0.01	<0.005	<0.005	<0.01
	Hugh's Dale Waterfall (below)	<0.01	<0.005	<0.005	<0.01
	Jones Spring (lower)	<0.01	<0.005	<0.005	<0.01
	Jones Spring (upper)	<0.01	<0.005	<0.005	<0.01
	Ross Hill Gardens_1	<0.01	<0.005	<0.005	<0.01
	Ross Hill Gardens_2	<0.01	<0.005	<0.005	<0.01

The results of the LC-MS/MS analyses of soil, water and sediment samples suggest that fipronil is breaking down quickly within the Christmas Island environment and not accumulating in the environment over a 10 year baiting history. The results also suggest that fipronil is fully degraded and not merely being broken down and persisting as the volatile and lethal degradates fipronil sulfide, fipronil sulfone or fipronil desulfinyl. The environment of Christmas Island is such that breakdown is probably facilitated by a number of factors including photoperiod (photolysis), rain (hydrolysis) and oxygenation (Gunasekara et al. 2007). The organic composition of soil, as well as temperature and moisture will ultimately dictate the rate at which fipronil is broken down on the soil surface and whether it enters the soil profile. Microbial activity in soil, sediment and water will also contribute substantially to the breakdown of fipronil and it's degradates (Gunasekara et al. 2007). It is possible the fipronil baits are taken immediately by *A. gracilipes*, leaving little opportunity for breakdown on the soil surface. The half-life of fipronil has been shown to vary, from a few days (Bobe et al. 1998, Gunasekara et al. 2007) to several weeks (Belayneh 1998, Gunasekara et al. 2007), although it has never been estimated in the current bait formulation. Some breakdown products (e.g. fipronil sulfide) are known to persist for years in soil (Gunasekara et al. 2007). If fipronil enters the freshwater system and deposits in sediment, then the anoxic, dark environment could also substantially delay the breakdown of fipronil. However, no sediment samples had detectable amounts of fipronil or it's degradates, suggesting that fipronil is not entering the freshwater system of the island, or that breakdown still occurs rapidly in these areas. A caveat to the results is the time between sampling and analysis of samples, which took between 3 and 8 months. Samples were frozen at -20 °C until analysis, which should largely prevent degradation of fipronil.

Pitfall Trap Data: Ground-dwelling arthropods

In the pitfall traps, 92 363 organisms were collected across the three collections. Of these, 48 718 organisms were collected in the August 2009 samples, 17 271 in the October 2009 samples and 24 374 in the May 2010 samples. The most dominant group in all collections were the ant family Formicidae (Hymenoptera), with 31 813, 9 734 and 8 714 collected in the August 2009, October 2009 and May 2010 collections, respectively.

Arthropod groups that were also present in the pitfall data in relatively high frequency were the Acari (Mesostigmata, Oribatida and Prostigmata), Coleoptera (Curculionidae, Nitidulidae, Ptiliidae and Staphylinidae), Collembola, Diptera, Gastropoda, Isopoda and Blattodea. Other groups in relatively low frequency were Aranae, Chilopoda, Diplopoda, Hemiptera, Isoptera, Lepidoptera, Oligochaeta, Orthoptera, Pscoptera, Symphyla and Thysanoptera (Table 3).

Table 3. Ground-dwelling arthropods sampled from three collections at 78 sites on Christmas Island.

Order and family	Sites present	Collections present	Total abundance	r_s (NMDS axis score) Axis 1	Axis 2	Axis 3
Acari						
Mesostigmata	78	3	6 379	0.260**	-0.464***	-0.771***
Oribatida	76	3	2 580	0.291**	-0.257**	-0.627***
Prostigmata	34	3	119	…	…	…
Araneae						
Combined	71	3	312	0.360***	-0.240**	…

Blattodea						
Combined	78	3	984	0.458***	0.525***	0.352***
Chilopoda						
Combined	31	3	…	…	…	…
Coleoptera						
Curculionidae	78	3	1 791	0.228**	-0.290**	…
Nitidulidae	77	3	1 358	-0.217**	-0.438***	-0.230**
Ptiliidae	38	3	168	0.276**	-0.253**	…
Staphylinidae	73	3	431	…	-0.271**	…
Collembola						
Combined	78	3	8 936	0.382***	-0.782***	-0.350***
Diplopoda						
Combined	66	3	271	0.522***	0.201*	…
Diptera						
Combined	78	3	6 674	0.457***	-0.737***	-0.332***
Gastropoda						
Combined	78	3	5 307	…	…	-0.638***
Hemiptera						
Combined	60	3	369	0.321***	…	-0.329***
Hymenoptera						
Formicidae	78	3	50 270	0.225**	…	…
Others (Scelionidae, parasitoids)	58	3	172	…	-0.340***	…
Isopoda						
Combined	77	3	3 122	0.580***	…	-0.356***
Isoptera						
Combined	10	2	39	…	…	…
Lepidoptera						
Combined	68	3	305	0.267**	…	…
Oligochaeta						
Combined	68	3	428	…	-0.426***	-0.514***
Orthoptera						
Combined	17	3	24	…	…	…
Pscoptera						
Combined	30	3	46	…	…	…
Symphyla						
Combined	17	3	54	…	…	…
Thysanoptera						
Combined	1	1	10	…	…	…

Notes: Spearmans correlations (r_s) are used to test order/taxon correlations with axis scores derived from nonmetric multidimensional scaling (NMDS) ordination of the arthropod community data.
***P < 0.05; **P < 0.01; ***P < 0.001**

Ordination analysis of the pitfall data for the collections (only transects T1-4 were included in the ordination due to T5-6 not being sampled in the first collection) indicated a three-dimensional solution (P = 0.004) for which the lowest stress was 16.09, requiring 76 iterations to reach the default instability of 10^{-4}. These three axes accounted for 86% of the variance.

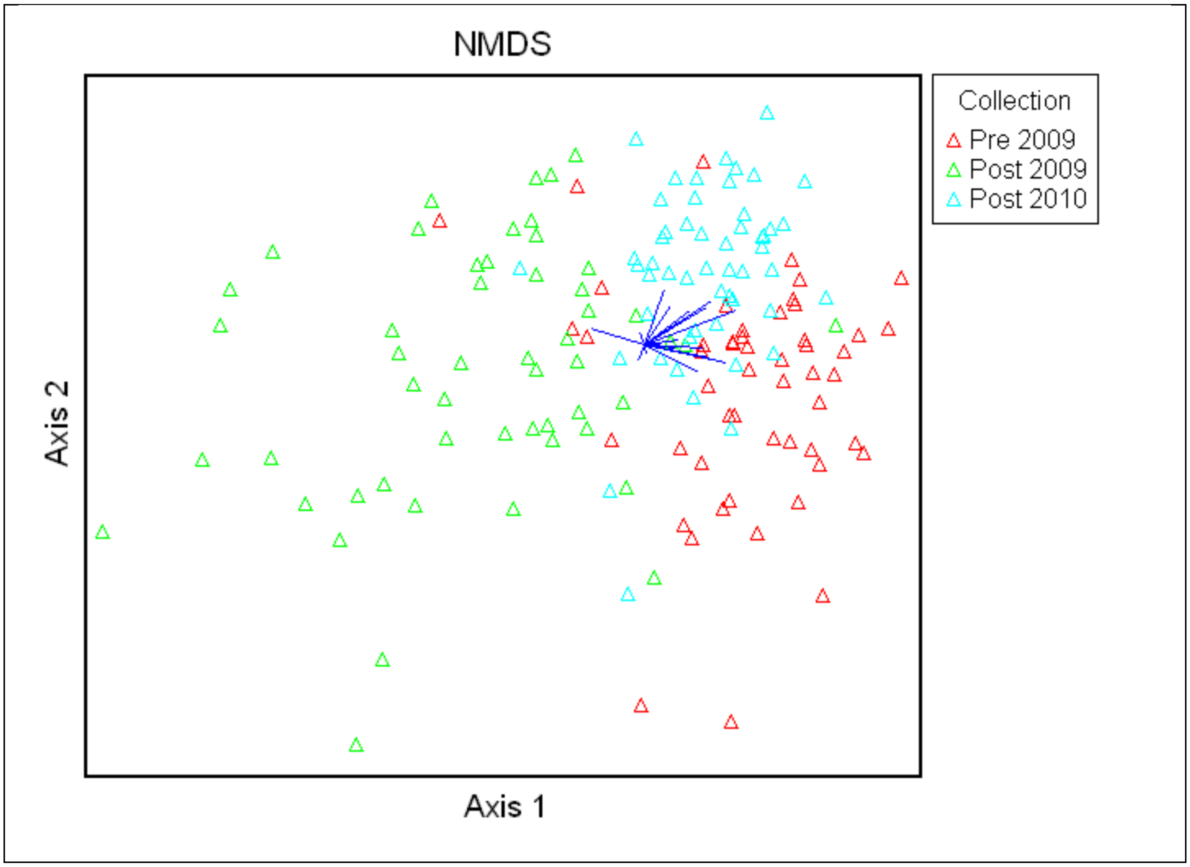

Figure 5. Nonmetric multidimensional scaling (NMDS) of arthropod communities in three collection periods on Christmas Island. Only 4 transects are represented in this ordination.

NMDS ordination depicted a clear separation between arthropod communities for the different collections (Figure 5). This separation was confirmed by the MRPP and GLM analyses. MRPP analysis indicated that collections were highly significantly different for arthropod community structure (T = -50.118, A = 0.12159, P < 0.001), with all collections different from each other (P < 0.001 for all comparisons). The nonparametric permutation multivariate ANOVA showed a significant effect of collection ($F_{2,155}$ = 25.359, P < 0.001), and transect ($F_{3,155}$ = 4.396, P < 0.001), but no collection by transect interaction ($F_{6,155}$ = 0.9789, P > 0.05). The GLM multivariate ANOVA analysis on the axes scores from the NMDS with NDVI scores used as a covariate also showed a significant effect of collection (on all three axes scores) and transect (on two axes scores), but no interaction between collection and transect on any axes scores (Appendix 3, Table A3). This indicates that the collection period affects the arthropod communities and that transects are behaving similarly within collection periods (i.e. no difference between transects for arthropod community structure within collections). The significant effect of collection time on arthropod community structure could be due to fipronil baiting or simply differences in community structure through time.

To determine if arthropod communities changed due to the aerial fipronil baiting conducted in September/October 2009, or due to seasonal changes in invertebrate assemblages, we reran the above analyses on the four transects, but removed the sites where baiting occurred. The NMDS ordination again indicated a three-dimensional solution (P = 0.004), for which the lowest stress was 15.68, requiring 117 iterations to reach the default instability of 10^{-4}. The three axes accounted for 87% of the variance. The NMDS ordination again depicted a clear separation of collections on arthropod community structure (Figure 6). The MRPP analysis showed a clear effect of collection (T = -44.614, A = 0.128, P < 0.001) and also transect (T = -8.271, A = 0.029, P < 0.001), with both significant in all comparisons. A permutation MANOVA could not be performed because of the unbalanced design. The GLM multivariate ANOVA of axes scores, collection and transect (with NDVI as a covariate) again showed a significant effect of collection on all three axes, an effect of transect on one axis, but no significant interaction between collection and transect for any axes (Appendix 3, Table A4). These results show that the variation in arthropod community structure is due to changes through time (collection period), and not the fipronil aerial baiting program conducted in September/October of 2009.

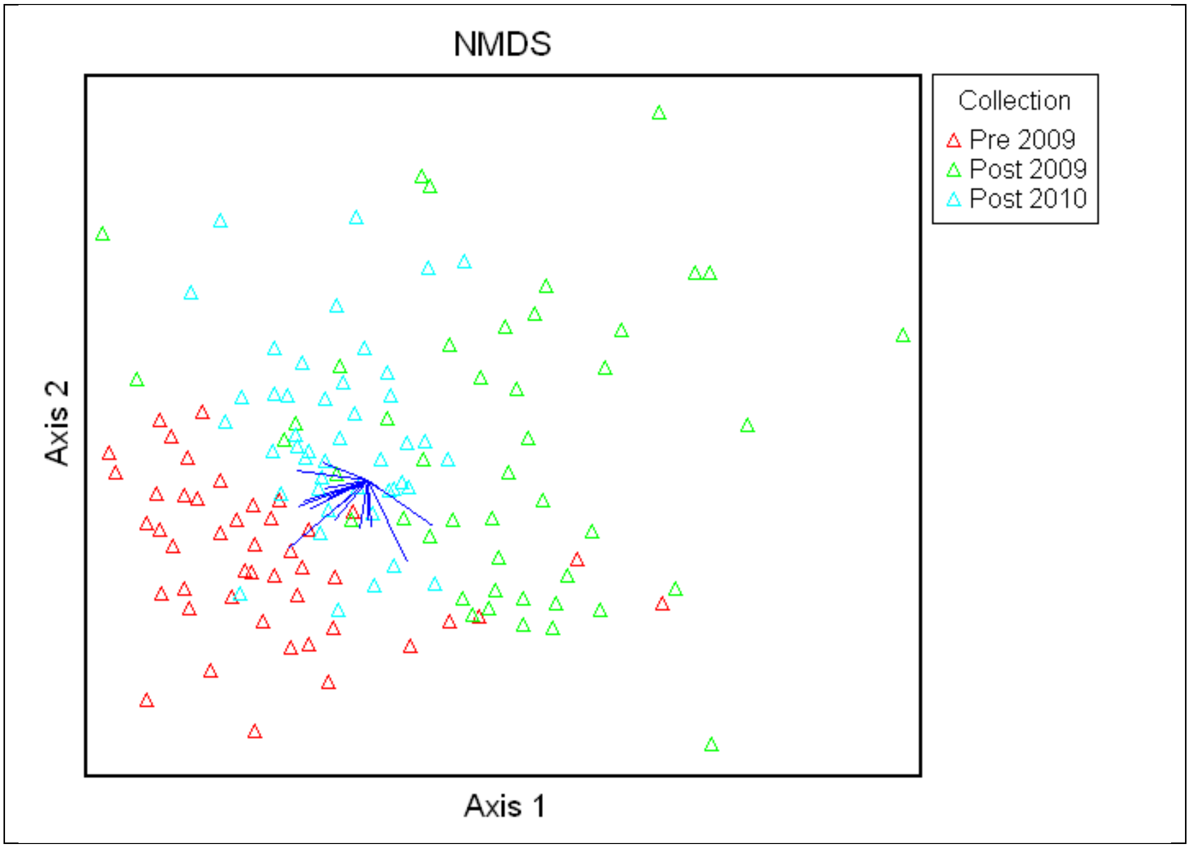

Figure 6. Nonmetric multidimensional scaling (NMDS) of arthropod communities in three collection periods on Christmas Island with sites where fipronil baiting occurred in Sept/Oct 2009 removed. Only 4 transects are represented in this ordination.

To determine if the fipronil aerial baiting program has affected the ground-dwelling arthropod community, we performed an NMDS ordination on both the October 2009 and May 2010 collections separately and compared sites baited with fipronil in the aerial baiting conducted in September/October 2009 with sites that were not baited in an MRPP analysis. Data from all six transects were included in these analyses. Ordinations for the October 2009 (May 2010) indicated a three-dimensional solution (P = 0.004) for which the lowest stress was 17.67 (18.17), requiring 86 (102) iterations to reach the default instability of 10^{-4}. These three axes accounted for 80% (78%) of the variance. The NMDS ordination for the October 2009 and May 2010 ground-dwelling arthropod collections showed no clear pattern associated with the aerial fipronil baiting (Figure 7). Similarly, the MRPP analysis indicated no difference between fipronil baited and unbaited sites for the October 2009 collection (T = -0.805, A = 0.003, P = 0.192) or for the May 2010 collection (T = -1.505, A = 0.005, P = 0.084). The GLM multivariate ANOVA also found no effect of fipronil baiting on the three axes scores derived from the NMDS ordination for both the October 2009 and May 2010 collections (Appendix 3, Tables A5 & A6).

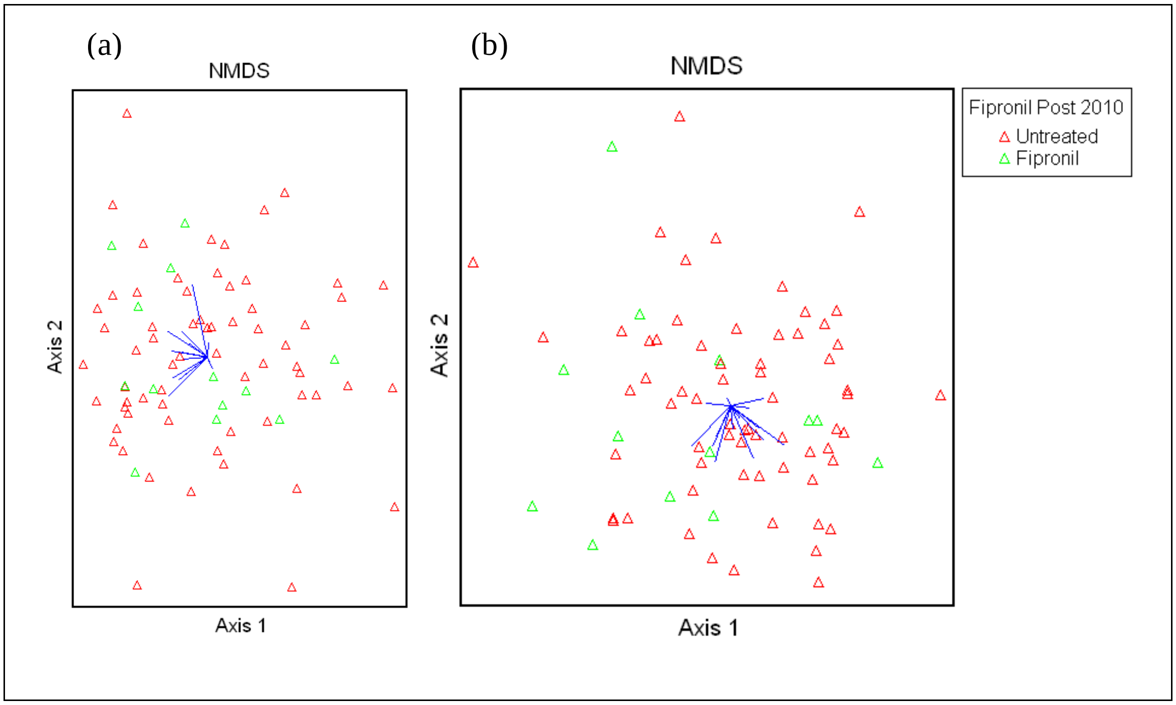

Figure 7. Nonmetric multidimensional scaling (NMDS) of arthropod communities collected in October 2009 (a) and May 2010 (b) with fipronil treated and untreated sites indicated.

The results show clearly that there is no effect of the fipronil aerial baiting conducted in September/October 2009 on the total ground-dwelling arthropod community.

Effects on Formicidae

The Formicidae were the dominant arthropod group found in the pitfall trap data. They were generally dominated in all collections by the yellow crazy ant, *A. gracilipes*, which was found at 64% of sites in August 2009 (28 821 collected), 56% of sites in October 2009 (3 164 collected) and 48% of sites in May 2010 (2 564). Overall, there was a 91% reduction in yellow crazy ants over all sites surveyed between August 2009 and May 2010. At sites where fipronil baits were distributed, there was an overall 98% reduction in yellow crazy ants between August 2009 and May 2010, indicating a very high success of the aerial fipronil baiting program. Other ant species were relatively abundant in samples (Table 4), constituting ~ 48% of organisms collected after the removal of yellow crazy ants.

Table 4. Formicidae species sampled from three collections at 78 sites on Christmas Island.

Species	Sites present	Collections present	Numbers	r_s (NMDS axis score) Axis 1	Axis 2	Axis 3
Anoplolepis gracilipes	40	3	33706	-.427**	-.470**	.424**
Camponotus sp. (*melichloros* group)	4	1	48	…	…	…
Camponotus sp. (*novaehollandiae* group)	26	3	432	…	.266**	.476**
Camponotus sp. (*reticulatus* group)	1	1	3	…	…	…
Paratrechina sp. (*bourbonica* group)	18	2	40	.217**	-.299**	…
Paratrechina sp. (*minutula* group)	39	3	182	…	…	.472**
Paratrechina sp. (*vaga* group)	42	3	722	.373**	…	.597**
Paratrechina vividula	11	2	20	…	…	.180*
Tapinoma melanocephalum	14	3	26	…	…	.248**
Tapinoma sp. (*minutum* group)	3	2	3	…	…	…
Technomyrmex vitiensis	8	3	9	…	…	…
Amblyopone zwaluwenburgi	1	1	2	…	…	…
Cerapachys biroi	1	1	2	…	…	…
Anochetus sp. (*graeffei* group)	17	3	34	.290**	…	.195*
Hypoponera confinis	3	2	3	…	…	…
Hypoponera punctatissima	1	1	1	…	…	…
Leptogenys harmsi	25	3	79	…	…	…
Odontomachus simillimus	16	3	180	…	.224**	-.461**
Pachycondyla christmasi	42	3	3579	-.772**	.780**	-.243**
Ponera swezeyi	35	3	98	.239**	-.216**	.257**
Cardiocondyla wroughtonii	7	2	7	…	…	…
Monomorium cf. *subcoecum*	23	3	98	…	-.219**	…
Pheidole megacephala	5	3	8	…	…	…
Pheidole sp. (*variabilis* group)	44	3	3650	.837**	-.652**	.310**
Pyramica membranifera	4	3	44	…	.164*	…
Strumigenys emmae	28	3	59	…	-.199*	.229**
Strumigenys godeffroyi	3	2	5	…	…	…
Tetramorium bicarinatum	1	1	1	…	…	…
Tetramorium insolens	38	3	350	…	-.208**	.473**
Tetramorium pacificum	2	1	2	…	…	…
Tetramorium simillimum	29	3	529	.510**	-.410**	…
Tetramorium smithi	1	1	1	…	…	…
Tetramorium walshi	14	3	43	.308**	-.258**	.199*
Leptanilla sp.	3	1	4	…	…	…

A total of 34 different ant species were collected in pitfall traps across the three collections, with eight species generally dominating collections (*Pheidole* sp. *variabilis* group, *Pachycondyla christmasi*, *Paratrechina* sp. *vaga* group, *Tetramorium simillimum*, *Camponotus* sp. *novaaehollandiae* group, *Tetramorium insolens*, *Paratrechina* sp. *minutula* group and *Odontomachus simillimus*). The high diversity and abundance of ant species found allowed data analysis to be conducted at the species level on this group to test for effect of the aerial fipronil baiting program.

NMDS ordination for transects T1-T4 over three collections (with yellow crazy ants removed from the analysis) indicated a three-dimensional solution (P = 0.004) for which the lowest stress was 13.4, requiring 200 iterations to reach the default instability of 10^{-4}. These three axes accounted for 87% of the variance. The NMDS ordination (Figure 8) showed no clear pattern of collection (unlike for total ground-dwelling invertebrates). However, the nonparametric multivariate permutation ANOVA showed an affect of collection ($F_{2,155}$ = 3.834, P = 0.001) and transect ($F_{3,155}$ = 13.072, P < 0.001) but no interaction between collection and transect ($F_{6,155}$ = 0.844, P = 0.678) on ant species structure. Similarly, the GLM multivariate ANOVA showed an effect of transect on all three ordination axes scores, an effect of collection on two axes scores, but no interaction between collection and transect (transects did not differ for ant communities within collections). Spearmans correlations indicated a strong correlation between *A. gracilipes* and all three axes from the NMDS analyses (Table 4), suggesting that *A. gracilipes* numbers are affecting ant structure at some sites.

We repeated the NMDS ordination on the October 2009 and May 2010 collections to determine effects of the aerial fipronil baiting. No clear patterns were apparent in the ordination (data not shown). The MRPP analysis showed a non-significant, but borderline effect of fipronil baiting (T = -2.008, A = 0.013, P = 0.051) in the October 2009 collection on ant species structure, but no effect in the May 2010 collection (T = -0.982, A = 0.005, P = 0.14). A similar result was found for the GLM multivariate ANOVA, where there was a borderline effect of fipronil on one axes (P = 0.023) in the October 2009 collection, but no effect in the May 2010 collection. It is likely that the small (non significant) effect is due to *A. gracilipes* affecting ant numbers at some sites (see above).

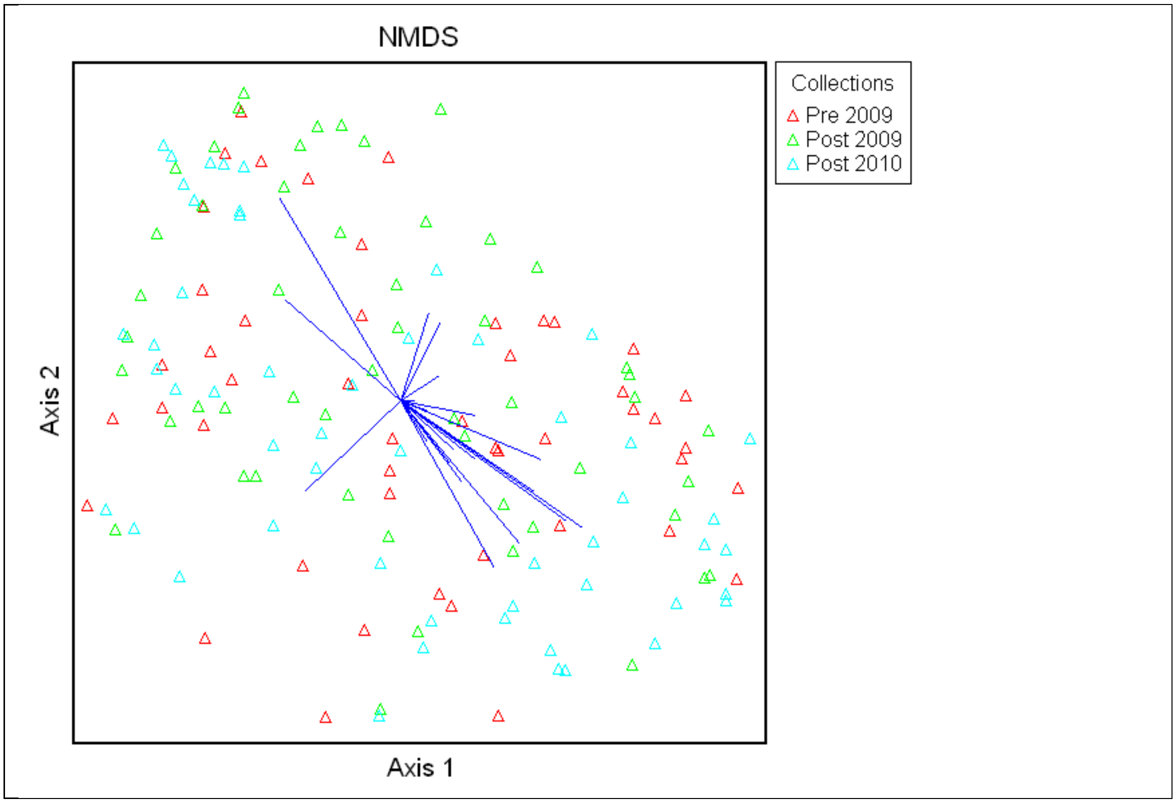

Figure 8. Nonmetric multidimensional scaling (NMDS) of Ant species in three collection periods on Christmas Island. Only 4 transects are represented in this ordination.

Canopy-dwelling arthropods

The diversity of arthropods collected from sticky traps in October 2009 and May 2010 was low compared with pitfall traps. A total of 20 978 organisms were trapped by the sticky traps (9 528 in October 2009 and 11 450 in May 2010). Generally, sticky traps were dominated by dipterans (13 952; 67%), leaf hopper hemipterans (2 401; 11%) and small parasitoids (1 703; 8 %). The diversity is likely to have been affected by the height at which the sticky traps were set. Most sticky traps were set between 10-12 m above ground, whereas the canopy height in these areas was generally around 20 m in height. While the sticky traps allowed multiple replicates to be easily set (as opposed to other methods of canopy surveys e.g. Stork et al. 2003), ideally the sticky traps would need to be located within the canopy.

Thus, due to the low diversity, we did not perform NMDS ordination on these samples. MRPP analyses, using the Sorensen (Bray Curtis) distance measure was undertaken to determine if there was an affect of sites that were baited in the aerial fipronil baiting program in 2009 compared with areas that were not baited. There was no effect of baiting on either the October 2009 collections (T = -1.659, A = 0.006, P = 0.063) or the May 2010 collections (T = 0.215, A = -0.001, P = 0.522).

Freshwater/sediment dwelling macroinvertebrates

Freshwater and sediments were sampled for macroinvertebrates at 11 permanent freshwater sites on Christmas Island (Appendix 1, Table A2) on all three collection trips. A broad variety of macroinvertebrates were found, despite the low amounts of water and sediments at each site. A total of 34 229 macroinvertebrates were identified, representing 58 morphospecies, 13 families and 6 orders. Several species dominated at most sites, with a mayfly species (Order: Ephemeroptera) making up 41% (14134) of organisms identified. The non biting midge family Chironominae (Order: Diptera) were the next most abundant group, with five species constituting 39% (13 201) of organisms identified. Table 5 depicts the most common macroinvertebrates identified. Taxon abundances are correlated to NMDS axes scores (see below), with axis 3 generally negatively correlated to most taxon abundance scores.

Numerous macroinvertebrate species are thought to be novel uncharacterised taxa that may be endemic to Christmas Island (V. Pettigrove, pers. comm.). Interestingly, many cosmopolitan taxa (e.g. *Polypedilum* sp.), are extremely small as adults on Christmas Island compared with other parts of the world. This may be related to the seasonal occurrence of some freshwater sites on the island and limited access to nutrients in sediments. It may also be related to the unique fauna present on the island (e.g. various crab species). Deformities (indicators of stress) were also assessed in the Chironominae group of species. However, very few deformities were found, indicating that development of larvae occurred with sufficient nutrients and relatively low levels of stress.

Table 5. Freshwater sediment-dwelling macroinvertebrates sampled from three collections at 11 sites on Christmas Island.

						r_s (NMDS axis score)		
Class/Order	SubFamily/Family	Taxa	Sites Present	Collections Present	Total Abundance	Axis 1	Axis 2	Axis 3
Oligochaeta		*Oligochaeta* spp.	11	3	717	…	…	-0.508**
Mites	Oribatida	*Oribatida* spp.	5	2	145	…	…	-0.385*
Mites	Limnesiidae	*Limnesiidae* sp.	3	2	218	-0.483**	…	…
Ephemeroptera	Caenidae	*Caenidae* imm.	9	3	10956	-0.711**	…	…
Ephemeroptera	Caenidae	*Tasmanocoenis* sp.	9	3	3178	-0.851**	…	…
Hemiptera	Mesoveliidae	*Mesoveliidae* sp. 1	8	2	159	…	…	-0.701**
Hemiptera	Mesoveliidae	*Mesovelia* sp. 2	8	3	313	…	…	-0.631**
Diptera	Tipulidae	*Tipulidae* x sp. 2	7	3	51	…	…	…
Diptera	Ceratopogonidae	*Ceratopogonidae* imm/dam	9	3	280	…	…	-0.641**
Diptera	Ceratopogonidae	*Ceratopogonidae* x sp. 1	11	2	150	…	0.426*	-0.494**
Diptera	Ceratopogonidae	*Ceratopogonidae* x sp. 2	6	3	107	…	…	-0.492**
Diptera	Simuliidae	*Simuliidae* sp. 1	8	3	642	…	…	-0.664**
Diptera	Simuliidae	*Simuliium* sp. 2	10	3	1093	…	…	-0.766**
Diptera	Orthocladiinae	nr Parametriocnemis	6	3	436	…	…	…
Diptera	Chironominae	Chironominae	11	3	1426	…	…	-0.741**
Diptera	Chironominae	*Cladotanytarsus* sp.	11	3	1393	…	0.410*	-0.562**
Diptera	Chironominae	*Neozavrelia* sp.	9	3	1735	-0.762**	…	…
Diptera	Chironominae	*Chironomus* sp.	6	3	1645	0.356*	0.447*	…
Diptera	Chironominae	*Polypedilum* sp.	11	3	7002	…	…	-0.807**
Diptera	Tanypodinae	*Tanypodinae* imm	10	3	1113	-0.547**	…	-0.588**
Diptera	Tanypodinae	*Paramerina* sp.	11	3	220	…	…	-0.642**
Diptera	Tanypodinae	*Ablabesymia* sp.	7	3	1026	-0.689**	…	…
Trichoptera	Leptoceridae	*Oecetis* sp.	5	3	88	-0.580**	…	…
Trichoptera	Hydroptilidae	Hydroptilidae	5	2	136	…	…	-0.390*

Ordination analysis of the freshwater macroinvertebrate data indicated a three dimensional solution (*P* = 0.004) for which the lowest stress was 11.49, requiring 62 iterations to reach the default instability of 10^{-4}. These three axes accounted for 88% of the variance (Figure 9).

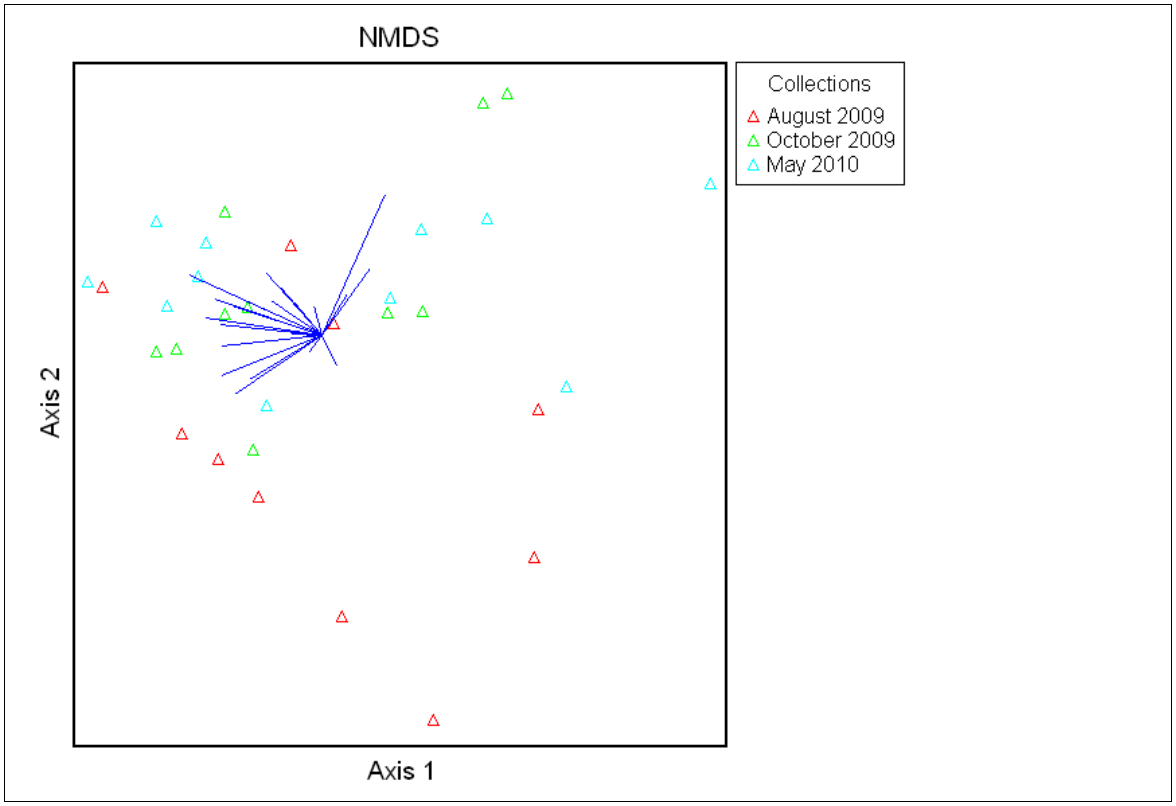

Figure 9. Nonmetric multidimensional scaling (NMDS) of macroinvertebrates from three collections at 11 sites on Christmas Island. Colours/symbols represent different collection periods.

MRPP analysis indicated that there were significant differences between collections in the macroinvertebrate communities (T = -6.09, A = 0.092, P < 0.001). Comparisons between collections showed that this was due to the first collection being significantly different from the other collections (P < 0.001), but there was no difference between the October 2009 and May 2010 collections. Analyses of NMDS axes scores using GLM multivariate ANOVA also found significant differences in axes scores for collections, with Tukey's *b* posthoc tests identifying that the August 2009 collection (prior to aerial baiting) was significantly different from the October 2009 and May 2010 collections (which were not significantly different). However, looking at Shannon's diversity index (PC-ORD, MjM Software Design) for the different collections indicates that the August 2009 collection had a much lower diversity (H = 0.867) than the October 2009 (H =

1.664) and May 2010 collections (H = 1.311). The lower diversity in the initial collection (August 2009) probably reflects the drier conditions at this time on the island, leading to low freshwater and sediment levels. This was certainly apparent when undertaking the collections at this time. The data therefore indicates that there was no apparent effect of the fipronil aerial baiting conducted in September/October 2009 on freshwater macroinvertebrates.

CONCLUSIONS

The results indicate that the fipronil aerial baiting program undertaken during September/October 2009 did not adversely influence arthropod community structure on Christmas Island. The extensive pitfall trapping, in which over 90 000 invertebrates were identified from 78 sites spread throughout the main baited areas of the rainforest, showed no effects of the aerial fipronil baiting on invertebrate structure. Seasonal (collection) differences were identified, which is not surprising given the varied lifecycles of ground-dwelling invertebrates. For instance, some Oribatid mite species are known to live for years (Behan-Pelletier 1999), while others undergo rapid generation times of less than two weeks, especially in tropical areas (Behan-Pelletier 1999). Other species of ground-dwelling invertebrates will only reproduce once a year (Harvey and Yen 1989), or seasonally (Shintani et al. 2010), highlighting the potential for differences in community composition to change with time. Marr et al. (2003) also found differences in arthropod numbers through time in their surveys on Christmas Island. Interestingly, there was no difference between arthropod communities along transects within our collections. This indicates that the sites within transects likely reflect the majority of habitats found on the island (at least in survey areas). Sites along transects T1 and T4 were in the most heavily baited areas of the island over the last 10 years, but these transects did not differ significantly in arthropod composition from other transects sampled.

The results confirm previous findings by Stork et al. (2003) and Marr et al. (2003) who investigated effects of the aerial baiting conducted in 2002 on arthropods in the canopy and litter, respectively. Using a randomised block design, Marr et al. (2003) were unable to detect an effect of fipronil baiting, although there were differences in abundance, largely due to the removal of yellow crazy ants. Our experimental design differed, largely because of the history of baiting on the island since the Marr et al. (2003) study. Ideally a randomised sampling design would have been used over the island. However, a lack of access made this difficult and the results from pitfall invertebrate collections (no differences between transects within collections) justified our approach.

Fipronil is known to be broken down relatively quickly under some conditions (Gunasekara et al. 2007), although there is no information known about breakdown rates in the bait formulation used on Christmas Island. Fipronil breakdown products (fipronil sulfide, fipronil sulfone, fipronil desulfinyl and fipronil amide) are known to be just as toxic to many organisms as fipronil itself (Gunasekara et al 2007, Miguel et al. 2008). We tested samples from a range of habitats on the island, including sites that had fipronil baits applied three weeks previously, for the presence of fipronil and three of its degradates (fipronil sulfide, fipronil sulfone, fipronil desulfinyl) using liquid chromatography tandem mass spectrometry. This method had low detection rates, especially in water samples (0.01 ug/L and 0.005 ug/L). Fipronil and its metabolites were not detected in any samples, indicating that fipronil is broken down to undetectable levels very quickly in the environment on Christmas Island. There was also no evidence that fipronil and its degradates were accumulating in areas that may increase the half-life of these components. Sediments, which are widely known as sinks for a large range of pollutants (O'Brien et al. 2010) did not have any detectable levels of fipronil or its degradates. Similarly, there was no evidence that fipronil was affecting the macroinvertebrate communities found in sediments, or causing deformities in larvae/nymphs.

The highly invasive yellow crazy ant has caused widespread changes to the forest ecosystem on Christmas Island. Their lethal effects on the islands keystone species, the red crab, have resulted in the promotion of seedling recruitment in the understory of the forest, changing the forest ecosystem and the dynamics of species living within that ecosystem. Without intervention, the yellow crazy ant will continue to cause large destruction to the forest ecosystem and potential cause the loss of many of the islands endemic fauna. Currently, given the technical hurdles present on Christmas Island, the only realistic method of controlling the yellow crazy ant is through fipronil baits, which have been used effectively since 2000. Eradication of yellow crazy ants from Christmas Island will be difficult and therefore it is likely that fipronil baiting is the only foreseeable option for limiting the impact of these ants on the unique fauna.

The aerial fipronil baiting program in September/October 2009 used baits that contain less active ingredient (0.01g/kg fipronil) than all baiting exercises previously, yet our results indicate that there was a 98% reduction in yellow crazy ants at sites where baiting took place. This supports the results of Christmas Island National Parks, where an overall 99% reduction was found (Chris Boland, pers. comm.) in yellow crazy ants two months after baiting. Future baiting is recommended using this bait formulation to limit any effects on non-target fauna found on Christmas Island. While we found no direct evidence for the bioaccumulation of fipronil or its degradates on Christmas Island, we recommend that monitoring is undertaken in the future to continue to evaluate the potential for this insecticide to accumulate and affect endemic fauna on the island.

ACKNOWLEDGEMENTS

CESAR Consultants would like to thank staff from Christmas Island National Park, and in particular Chris Boland, Michael Smith and Dion Maple for help, support and all the logistical issues that arose throughout this project. We also thank Glenn Johnstone (Dept. of Sustainability, Environment, Water, Population and Communities) for providing satellite imagery of Christmas Island, Daniel Ierodiaconou (Deakin University) for the NDVI analysis and Chee Seng Chong (University of Melbourne) for ant species identification.

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APPENDIX 1. Sampling site locations

Table A1. Location of transects and sites where pitfall and sticky traps sampling took place in August 2009, October 2009 and May 2010.

Transect	Site	Coordinates		August 2009				October 2009				May 2010			
		Latitude	Longitude	Set up	Pick up	Days	YST	Set up	Pick up	Days	YST	Set up	Pick up	Days	YST
1	1	8839085.781	559964.451	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
	2	8839244.544	560117.253	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
	3	8839393.794	560367.448	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
	4	8839494.262	560608.094	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
	5	8839548.359	560897.732	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
	6	8839495.050	561116.773	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
	7	8839440.108	561414.224	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
	8	8839508.457	561685.195	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
	9	8839694.292	561870.187	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
	10	8839969.384	561953.884	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
	11	8840015.202	562192.835	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
	12	8840173.590	562358.548	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
	13	8840402.978	562575.913	23/08/09	27/08/09	4	N	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
2	1	8838584.468	566401.889	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
	2	8838808.810	566513.571	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
	3	8838905.954	566602.262	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
	4	8839114.414	566764.227	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
	5	8839334.208	566911.952	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
	6	8839542.719	567147.079	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
	7	8839789.006	567350.342	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
	8	8840017.848	567219.525	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
	9	8840245.104	567370.912	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
	10	8840358.238	567476.335	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
	11	8840406.790	567728.375	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
	12	8840492.579	567999.715	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
	13	8840740.656	568203.336	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	Y	1/05/10	5/05/10	4	Y
3	1	8841834.490	568903.825	23/08/09	27/08/09	4	N	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N
	2	8841842.793	568797.646	23/08/09	27/08/09	4	N	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N
	3	8841850.036	568492.840	23/08/09	27/08/09	4	N	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N
	4	8841861.976	568220.140	23/08/09	27/08/09	4	N	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N
	5	8841877.698	567932.440	23/08/09	27/08/09	4	N	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N
	6	8841878.014	567549.485	23/08/09	27/08/09	4	N	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N
	7	8841870.640	567315.228	23/08/09	27/08/09	4	N	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N
	8	8841916.943	567049.437	23/08/09	27/08/09	4	N	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N
	9	8841947.138	566676.951	23/08/09	27/08/09	4	N	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N
	10	8841980.577	566330.694	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N
	11	8841923.370	566074.941	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N
	12	8841852.701	565745.155	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N

	13	8842004.134	565499.498	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	2/05/10	6/05/10	4	N
4	1	8839960.813	562246.066	24/08/09	28/08/09	4	Y	22/10/09	26/10/09	4	Y	30/04/10	4/05/10	4	Y
	2	8839818.852	562512.992	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
	3	8839688.531	562779.333	24/08/09	28/08/09	4	Y	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
	4	8839484.850	562894.147	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
	5	8839550.584	563164.733	24/08/09	28/08/09	4	Y	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
	6	8839513.517	563428.892	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
	7	8839543.547	563694.093	24/08/09	28/08/09	4	Y	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
	8	8839455.796	563994.388	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
	9	8839332.733	564248.250	24/08/09	28/08/09	4	Y	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
	10	8839368.380	564513.482	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
	11	8839290.186	564800.601	24/08/09	28/08/09	4	Y	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
	12	8839117.298	565066.078	24/08/09	28/08/09	4	N	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
	13	8838927.073	565328.364	24/08/09	28/08/09	4	Y	22/10/09	26/10/09	4	N	30/04/10	4/05/10	4	N
5	1	8842134.411	564767.741	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
	2	8841961.266	564695.712	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
	3	8841772.883	564677.705	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
	4	8841570.648	564631.994	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
	5	8841429.188	564535.206	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
	6	8841321.145	564412.619	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
	7	8841161.158	564291.070	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
	8	8841053.115	564065.634	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
	9	8840835.990	563962.786	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
	10	8840677.042	563892.142	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
	11	8840460.956	563818.382	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
	12	8840299.931	563711.378	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
	13	8840156.566	563596.062	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	Y	1/05/10	5/05/10	4	Y
6	1	8842145.493	564716.490	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N
	2	8841973.732	564638.920	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N
	3	8841782.579	564602.906	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N
	4	8841584.500	564555.810	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N
	5	8841451.005	564498.845	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N
	6	8841345.039	564373.141	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N
	7	8841185.052	564251.593	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N
	8	8841081.165	564020.963	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N
	9	8840846.379	563904.608	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N
	10	8840696.781	563850.587	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N
	11	8840480.695	563777.866	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N
	12	8840318.630	563670.862	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N
	13	8840175.266	563557.624	N/A	N/A	N/A	N/A	23/10/09	27/10/09	4	N	1/05/10	5/05/10	4	N

Table A2. Site names and coordinates where water, sediment and/or soil collections were taken.

	Site	Latitude	Longitude
Water and/or sediment	Anderson's Dale	8840968.823	560809.651
	Hugh's Dale Waterfall (above)	8841459.548	561231.483
	Hugh's Dale Waterfall (below)	8841514.564	561201.478
	Hosnies Spring A	8841790.506	575695.182
	Hosnies Spring B	8841781.569	575663.903
	Hosnies Spring C	8841788.272	575618.102
	Hosnies Spring D	8841648.635	575509.743
	Jones Spring (lower)	8844328.582	577015.553
	Jones Spring (upper)	8844373.604	576907.498
	Ross Hill Gardens_1	8840532.698	573977.602
	Ross Hill Gardens_2	8841065.529	574288.420
Soil	SS1	8839962.557	562234.861
	SS2	8839239.521	560117.307
	SS3	8841442.766	564524.530
	SS4	8841652.902	564608.892
	SS5	8839551.093	563697.931
	SS6	8838896.539	565296.515
	SS7	8838978.276	565353.941
	SS8	8839960.813	562246.066
	SS9	8839085.781	559964.451
	SS10	8840402.978	562575.913
	SS11	8841656.229	564606.412
	SS12	8841348.415	564402.022
	SS13	8841451.840	564500.523
	SS14	8842136.421	564778.788
	SS15	8841451.840	564500.523
	SS16	8840402.978	562575.913
	SS17	8839960.813	562246.066
	SS18	8841161.158	564291.070

APPENDIX 2. Results of soil, sediment, and water analyses

Department of Primary Industries

ANALYTICAL REPORT

Report No: 6594
Report Date: 18-Nov-2010

Final Report

Public Submission

Andrew Weeks
University Of Melbourne
University of Melbourne, CESAR, Bio21 Institute
30 Flemington Road
Parkville
VIC 3010

Sample(s) received on 6 August 2010 from Andrew Weeks.

Number of samples for testing: 38

Submission No: P10-08-0002

Supervising Analyst: Colin Cook
Senior Scientist

Colin Cook

Test results apply only to the sample(s) submitted for analysis.

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Samples received

Laboratory ID (Sample No.)	Date Sampled/Client ID/Description	General Sample Type, Specific Sample Type
P10-08-0002-001P (82,195)	27/08/2009 SS#4 Sample No.1, SS_4	Soil, Soil
P10-08-0002-002P (82,196)	27/08/2009 SS5 Sample No.2, SS_5	Soil, Soil
P10-08-0002-003P (82,197)	26/08/2009 SS1 Sample No.3, SS_1	Soil, Soil
P10-08-0002-004P (82,198)	27/08/2009 SS6 Sample No.4, SS_6	Soil, Soil
P10-08-0002-005P (82,199)	26/08/2009 SS#2 Sample No.5, SS_2	Soil, Soil
P10-08-0002-006P (82,200)	28/08/2009 SS#9 Sample No.6, SS_9	Soil, Soil
P10-08-0002-007P (82,201)	27/08/2009 SS7 Sample No.7, SS_7	Soil, Soil
P10-08-0002-008P (82,202)	28/08/2009 SS#10 Sample No.8, SS_10	Soil, Soil
P10-08-0002-009P (82,203)	27/08/2009 SS#3 Sample No.9, SS_3	Soil, Soil
P10-08-0002-010P (82,204)	28/08/2009 SS8 Sample No.10, SS_8	Soil, Soil
P10-08-0002-011P (82,205)	26/10/2009 SS-A Sample No.11, SS_11	Soil, Soil
P10-08-0002-012P (82,206)	26/10/2009 SSB Sample No.12, SS_12	Soil, Soil
P10-08-0002-013P (82,207)	26/10/2009 SSC Sample No.13, SS_13	Soil, Soil
P10-08-0002-014P (82,208)	27/10/2009 SS-D Sample No.14, SS_14	Soil, Soil
P10-08-0002-015P (82,209)	27/10/2009 SS-E Sample No.15, SS_15	Soil, Soil
P10-08-0002-016P (82,210)	27/10/2009 SS-F Sample No.16, SS_16	Soil, Soil
P10-08-0002-017P (82,211)	27/10/2009 SS-G Sample No.17, SS_17	Soil, Soil
P10-08-0002-018P (82,212)	27/10/2009 SS-H Sample No.18, SS_18	Soil, Soil
P10-08-0002-019P (82,213)	27/10/2009 SS-I Sample No.19, SS_19	Soil, Soil
P10-08-0002-020P (82,214)	27/10/2009 SSJ Sample No.20, SS_20	Soil, Soil
P10-08-0002-021P (82,215)	27/10/2009 SS-K Sample No.21, SS_21	Soil, Soil
P10-08-0002-022P (82,216)	27/10/2009 SS-L Sample No.22, SS_22	Soil, Soil
P10-08-0002-023P (82,217)	27/10/2009 SS-M Sample No.23, SS_23	Soil, Soil
P10-08-0002-024P (82,218)	28/10/2009 SS-N Sample No.24, SS_24	Soil, Soil
P10-08-0002-025P (82,219)	28/10/2009 SSO Sample No.25, SS_25	Soil, Soil
P10-08-0002-026P (82,220)	28/10/2009 SSP Sample No.26, SS_26	Soil, Soil
P10-08-0002-027P (82,221)	28/10/2009 SS-Q Sample No.27, SS_27	Soil, Soil
P10-08-0002-028P (82,222)	28/10/2009 SSR Sample No.28, SS_28	Soil, Soil
P10-08-0002-029P (82,223)	28/10/2009 SSS Sample No.29, SS_29	Soil, Soil
P10-08-0002-030P (82,224)	05/05/2010 T5 S5 Sample No.30, SS_30	Soil, Soil
P10-08-0002-031P (82,225)	05/05/2010 T5/6 S13 Sample No.31, SS_31	Soil, Soil
P10-08-0002-032P (82,226)	04/05/2010 T1 S13 Sample No.32, SS_32	Soil, Soil
P10-08-0002-033P (82,227)	04/05/2010 T1 S1 Sample No.33, SS_33	Soil, Soil
P10-08-0002-034P (82,228)	04/05/2010 T4 S8 Sample No.34, SS_34	Soil, Soil
P10-08-0002-035P (82,229)	04/05/2010 T4 S5 Sample No.35, SS_35	Soil, Soil
P10-08-0002-036P (82,230)	04/05/2010 T4 S1 Sample No.36, SS_36	Soil, Soil
P10-08-0002-037P (82,231)	05/05/2010 T5 S7 Sample No.37, SS_37	Soil, Soil
P10-08-0002-038P (82,232)	04/05/2010 T1 S6 Sample No.38, SS_38	Soil, Soil

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Fipronil in soil

Analyte	Units	P10-08-0002-002	P10-08-0002-004	P10-08-0002-005	P10-08-0002-006
Fipronil	ug/kg	<2	<2	<2	<2
Fipronil sulfide	ug/kg	<2	<2	<2	<2
Fipronil sulfone	ug/kg	<2	<2	<2	<2
Fipronil desulfinyl	ug/kg	<2	<2	<2	<2

A blank space indicates no test performed.

Fipronil in soil

Analyte	Units	P10-08-0002-008	P10-08-0002-009	P10-08-0002-010	P10-08-0002-013
Fipronil	ug/kg	<2	<2	<2	<2
Fipronil sulfide	ug/kg	<2	<2	<2	<2
Fipronil sulfone	ug/kg	<2	<2	<2	<2
Fipronil desulfinyl	ug/kg	<2	<2	<2	<2

A blank space indicates no test performed.

Fipronil in soil

Analyte	Units	P10-08-0002-014	P10-08-0002-016	P10-08-0002-019	P10-08-0002-020
Fipronil	ug/kg	<2	<2	<2	<2
Fipronil sulfide	ug/kg	<2	<2	<2	<2
Fipronil sulfone	ug/kg	<2	<2	<2	<2
Fipronil desulfinyl	ug/kg	<2	<2	<2	<2

A blank space indicates no test performed.

Fipronil in soil

Analyte	Units	P10-08-0002-021	P10-08-0002-023	P10-08-0002-030	P10-08-0002-032
Fipronil	ug/kg	<2	<2	<2	<2
Fipronil sulfide	ug/kg	<2	<2	<2	<2
Fipronil sulfone	ug/kg	<2	<2	<2	<2
Fipronil desulfinyl	ug/kg	<2	<2	<2	<2

A blank space indicates no test performed.

Fipronil in soil

Analyte	Units	P10-08-0002-036	P10-08-0002-037
Fipronil	ug/kg	<2	<2
Fipronil sulfide	ug/kg	<2	<2
Fipronil sulfone	ug/kg	<2	<2
Fipronil desulfinyl	ug/kg	<2	<2

A blank space indicates no test performed.

Sample Logged

Analyte	Units	P10-08-0002-001	P10-08-0002-003	P10-08-0002-007	P10-08-0002-011
Sample Logged	-	Logged	Logged	Logged	Logged

A blank space indicates no test performed.

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Sample Logged

Analyte	Units	P10-08-0002-012	P10-08-0002-015	P10-08-0002-017	P10-08-0002-018
Sample Logged	-	Logged	Logged	Logged	Logged

A blank space indicates no test performed.

Sample Logged

Analyte	Units	P10-08-0002-022	P10-08-0002-024	P10-08-0002-025	P10-08-0002-026
Sample Logged	-	Logged	Logged	Logged	Logged

A blank space indicates no test performed.

Sample Logged

Analyte	Units	P10-08-0002-027	P10-08-0002-028	P10-08-0002-029	P10-08-0002-031
Sample Logged	-	Logged	Logged	Logged	Logged

A blank space indicates no test performed.

Sample Logged

Analyte	Units	P10-08-0002-033	P10-08-0002-034	P10-08-0002-035	P10-08-0002-038
Sample Logged	-	Logged	Logged	Logged	Logged

A blank space indicates no test performed.

Method references

The sample(s) referred to in this report were analysed by the following method(s):

Analyte(s)	Method	Laboratory
Fipronil in soil		Organic Chemistry
Sample Logged		Organic Chemistry

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ANALYTICAL REPORT

Report No: 6595
Report Date: 18-Nov-2010

Final Report

Public Submission

Andrew Weeks
University Of Melbourne
University of Melbourne, CESAR, Bio21 Institute
30 Flemington Road
Parkville
VIC 3010

Sample(s) received on 6 August 2010 from Andrew Weeks.

Number of samples for testing: 25

Submission No: P10-08-0003

Submission Comments

Samples 022 and 023 had test removed as per client request on 17/09/10.

Supervising Analyst: Colin Cook
Senior Scientist

Test results apply only to the sample(s) submitted for analysis.

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Samples received

Laboratory ID (Sample No.)	Date Sampled/Client ID/Description	General Sample Type, Specific Sample Type
P10-08-0003-001P (82,233)	25/08/2009 1st Water Tank Sample No.39, F_1	Water, Other
P10-08-0003-002P (82,234)	25/08/2009 2nd Water Tank Sample No.40, F_2	Water, Other
P10-08-0003-003P (82,235)	24/08/2009 Hugh Dale Sample No.41, F_3	Water, Other
P10-08-0003-004P (82,236)	24/08/2009 2nd Dale Sample No.42, F_4	Water, Other
P10-08-0003-005P (82,237)	24/08/2009 HS-2 Sample No.43, F_5	Water, Other
P10-08-0003-006P (82,238)	24/10/2009 Hosnie springs D Fallen Tree Sample No.44, F_6	Water, Other
P10-08-0003-007P (82,239)	24/10/2009 Hosnie springs A Pool Sample No.45, F_7	Water, Other
P10-08-0003-008P (82,240)	25/10/2009 Hosnie springs B Ridge Sample No.46, F_8	Water, Other
P10-08-0003-009P (82,241)	25/10/2009 Hosnie springs C Pool above Sample No.47, F_9	Water, Other
P10-08-0003-010P (82,242)	25/10/2009 Jones spring Lower Sample No.48, F_10	Water, Other
P10-08-0003-011P (82,243)	25/10/2009 Jones spring Upper Sample No.49, F_11	Water, Other
P10-08-0003-012P (82,244)	26/10/2009 Ross Hill gardens Tank 2 Sample No.50, F_12	Water, Other
P10-08-0003-013P (82,245)	26/10/2009 Ross Hill gardens H20 #1 Sample No.51, F_13	Water, Other
P10-08-0003-014P (82,246)	26/10/2009 The Dales (6 or 7) Sample No.52, F_14	Water, Other
P10-08-0003-015P (82,247)	26/10/2009 Hughes Dales (above Falls) Sample No.53, F_15	Water, Other
P10-08-0003-016P (82,248)	26/10/2009 Hughes Dales (below Falls) Sample No.54, F_16	Water, Other
P10-08-0003-017P (82,249)	04/05/2010 ANDERSONS Sample No.55, F_17	Water, Other
P10-08-0003-018P (82,250)	04/05/2010 DALES ABOVE FALLS Sample No.56, F_18	Water, Other
P10-08-0003-019P (82,251)	04/05/2010 DALES BELOW FALLS Sample No.57, F_19	Water, Other
P10-08-0003-020P (82,252)	02/05/2010 JONES SPRING LOWER Sample No.58, F_20	Water, Other
P10-08-0003-021P (82,253)	02/05/2010 JONES SPRING UPPER Sample No.59, F_21	Water, Other
P10-08-0003-022P (82,254)	03/05/2010 Hosnies Springs 2 (fallen tree) Sample No.60, F_22	Water, Other
P10-08-0003-023P (82,255)	03/05/2010 Hosnie Springs - upper Sample No.61, F_23	Water, Other
P10-08-0003-024P (82,256)	03/05/2010 Water Supply #1 (South) Sample No.62, F_24	Water, Other
P10-08-0003-025P (82,257)	03/05/2010 Water Supply #2 (North) Sample No.63, F_25	Water, Other

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Fipronil in water

Analyte	Units	P10-08-0003-001	P10-08-0003-002	P10-08-0003-003	P10-08-0003-004
Fipronil	ug/L	<0.01	<0.01	<0.01	<0.01
Fipronil sulfide	ug/L	<0.005	<0.005	<0.005	<0.005
Fipronil sulfone	ug/L	<0.005	<0.005	<0.005	<0.005
Fipronil desulfinyl	ug/L	<0.01	<0.01	<0.01	<0.01

A blank space indicates no test performed.

Fipronil in water

Analyte	Units	P10-08-0003-005	P10-08-0003-006	P10-08-0003-007	P10-08-0003-008
Fipronil	ug/L	<0.01	<0.01	<0.01	<0.01
Fipronil sulfide	ug/L	<0.005	<0.005	<0.005	<0.005
Fipronil sulfone	ug/L	<0.005	<0.005	<0.005	<0.005
Fipronil desulfinyl	ug/L	<0.01	<0.01	<0.01	<0.01

A blank space indicates no test performed.

Fipronil in water

Analyte	Units	P10-08-0003-009	P10-08-0003-010	P10-08-0003-011	P10-08-0003-012
Fipronil	ug/L	<0.01	<0.01	<0.01	<0.01
Fipronil sulfide	ug/L	<0.005	<0.005	<0.005	<0.005
Fipronil sulfone	ug/L	<0.005	<0.005	<0.005	<0.005
Fipronil desulfinyl	ug/L	<0.01	<0.01	<0.01	<0.01

A blank space indicates no test performed.

Fipronil in water

Analyte	Units	P10-08-0003-013	P10-08-0003-014	P10-08-0003-015	P10-08-0003-016
Fipronil	ug/L	<0.01	<0.01	<0.01	<0.01
Fipronil sulfide	ug/L	<0.005	<0.005	<0.005	<0.005
Fipronil sulfone	ug/L	<0.005	<0.005	<0.005	<0.005
Fipronil desulfinyl	ug/L	<0.01	<0.01	<0.01	<0.01

A blank space indicates no test performed.

Fipronil in water

Analyte	Units	P10-08-0003-017	P10-08-0003-018	P10-08-0003-019	P10-08-0003-020
Fipronil	ug/L	<0.01	<0.01	<0.01	<0.01
Fipronil sulfide	ug/L	<0.005	<0.005	<0.005	<0.005
Fipronil sulfone	ug/L	<0.005	<0.005	<0.005	<0.005
Fipronil desulfinyl	ug/L	<0.01	<0.01	<0.01	<0.01

A blank space indicates no test performed.

Fipronil in water

Analyte	Units	P10-08-0003-021	P10-08-0003-024	P10-08-0003-025
Fipronil	ug/L	<0.01	<0.01	<0.01
Fipronil sulfide	ug/L	<0.005	<0.005	<0.005
Fipronil sulfone	ug/L	<0.005	<0.005	<0.005
Fipronil desulfinyl	ug/L	<0.01	<0.01	<0.01

A blank space indicates no test performed.

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Sample Logged

Analyte	Units	P10-08-0003-022	P10-08-0003-023
Sample Logged	-	Logged	Logged

A blank space indicates no test performed.

Method references

The sample(s) referred to in this report were analysed by the following method(s):

Analyte(s)	Method	Laboratory
Fipronil in water		Organic Chemistry
Sample Logged		Organic Chemistry

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Department of Primary Industries

ANALYTICAL REPORT

Report No: 6596
Report Date: 18-Nov-2010

Final Report

Public Submission

Andrew Weeks
University Of Melbourne
University of Melbourne, CESAR, Bio21 Institute
30 Flemington Road
Parkville
VIC 3010

Sample(s) received on 6 August 2010 from Andrew Weeks.

Number of samples for testing: 27

Submission No: P10-08-0004

<u>Submission Comments</u>

Samples P-10-08-0004-004P and -021P could not be found when samples were processed.

Supervising Analyst: Colin Cook
Senior Scientist

Test results apply only to the sample(s) submitted for analysis.

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Samples received

Laboratory ID (Sample No.)	Date Sampled/Client ID/Description	General Sample Type, Specific Sample Type
P10-08-0004-001P (82,258)	26/08/2009 CASINO CRK (Jones Spring) Sample No.64, S_1	Soil, Sediment
P10-08-0004-002P (82,259)	23/08/2009 HS-2 Sample No.65, S_2	Soil, Sediment
P10-08-0004-003P (82,260)	24/08/2009 Hugh Dale Sample No.66, S_3	Soil, Sediment
P10-08-0004-004P (82,261)	24/08/2009 2nd Dale Sample No.67, S_4	Soil, Sediment
P10-08-0004-005P (82,262)	25/08/2009 Tank#2 Sample No.68, S_5	Soil, Sediment
P10-08-0004-006P (82,263)	24/10/2009 Hosnie Springs - pool above cliff C Sample No.69, S_6	Soil, Sediment
P10-08-0004-007P (82,264)	24/10/2009 Hosnie Springs - A Sample No.70, S_7	Soil, Sediment
P10-08-0004-008P (82,265)	24/10/2009 Hosnie Springs - B Sample No.71, S_8	Soil, Sediment
P10-08-0004-009P (82,266)	24/10/2009 Hosnie Springs - D Sample No.72, S_9	Soil, Sediment
P10-08-0004-010P (82,267)	25/10/2009 Jones Spring - upper Sample No.73, S_10	Soil, Sediment
P10-08-0004-011P (82,268)	25/10/2009 Jones Spring - lower Sample No.74, S_11	Soil, Sediment
P10-08-0004-012P (82,269)	26/10/2009 The Dales (6 or 7) Sample No.75, S_12	Soil, Sediment
P10-08-0004-013P (82,270)	26/10/2009 Hughes Dale (B) - above falls Sample No.76, S_13	Soil, Sediment
P10-08-0004-014P (82,271)	26/10/2009 Hughes Dale (C) - below falls Sample No.77, S_14	Soil, Sediment
P10-08-0004-015P (82,272)	26/10/2009 Rose Hill Gardens Tank 2 Sample No.78, S_15	Soil, Sediment
P10-08-0004-016P (82,273)	03/05/2010 Hosnie spring #2 fallen tree Sample No.79, S_16	Soil, Sediment
P10-08-0004-017P (82,274)	03/05/2010 Hosnie spring - upper Sample No.80, S_17	Soil, Sediment
P10-08-0004-018P (82,275)	03/05/2010 Water Supply #1 South Sample No.81, S_18	Soil, Sediment
P10-08-0004-019P (82,276)	03/05/2010 Water Supply #2 North-1 Sample No.82, S_19	Soil, Sediment
P10-08-0004-020P (82,277)	03/05/2010 Water Supply #2 North-2 Sample No.83, S_19	Soil, Sediment
P10-08-0004-021P (82,278)	02/05/2010 Jones Spring upper Sample No.84, S_20	Soil, Sediment
P10-08-0004-022P (82,279)	04/05/2010 Andersons Dale-1 Sample No.85, S_21	Soil, Sediment
P10-08-0004-023P (82,280)	04/05/2010 Andersons Dale-2 Sample No.86, S_21	Soil, Sediment
P10-08-0004-024P (82,281)	04/05/2010 Dales Above Falls-1 Sample No.87, S_22	Soil, Sediment
P10-08-0004-025P (82,282)	04/05/2010 Dales Above Falls-2 Sample No.88, S_22	Soil, Sediment
P10-08-0004-026P (82,283)	04/05/2010 Dales Below Falls-1 Sample No.89, S_23	Soil, Sediment

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Samples received

Laboratory ID (Sample No.)	Date Sampled/Client ID/Description	General Sample Type, Specific Sample Type
P10-08-0004-027P (82,284)	04/05/2010 Dales Below Falls-2 Sample No.90, S_23	Soil, Sediment

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Fipronil in sediment

Analyte	Units	P10-08-0004-001	P10-08-0004-002	P10-08-0004-003	P10-08-0004-005
Fipronil	ug/kg	<2	<2	<2	<2
Fipronil sulfide	ug/kg	<2	<2	<2	<2
Fipronil sulfone	ug/kg	<2	<2	<2	<2
Fipronil desulfinyl	ug/kg	<2	<2	<2	<2

A blank space indicates no test performed.

Fipronil in sediment

Analyte	Units	P10-08-0004-006	P10-08-0004-007	P10-08-0004-010	P10-08-0004-012
Fipronil	ug/kg	<2	<2	<2	<2
Fipronil sulfide	ug/kg	<2	<2	<2	<2
Fipronil sulfone	ug/kg	<2	<2	<2	<2
Fipronil desulfinyl	ug/kg	<2	<2	<2	<2

A blank space indicates no test performed.

Fipronil in sediment

Analyte	Units	P10-08-0004-013	P10-08-0004-015	P10-08-0004-016	P10-08-0004-017
Fipronil	ug/kg	<2	<2	<2	<2
Fipronil sulfide	ug/kg	<2	<2	<2	<2
Fipronil sulfone	ug/kg	<2	<2	<2	<2
Fipronil desulfinyl	ug/kg	<2	<2	<2	<2

A blank space indicates no test performed.

Fipronil in sediment

Analyte	Units	P10-08-0004-018	P10-08-0004-019	P10-08-0004-022	P10-08-0004-024
Fipronil	ug/kg	<2	<2	<2	<2
Fipronil sulfide	ug/kg	<2	<2	<2	<2
Fipronil sulfone	ug/kg	<2	<2	<2	<2
Fipronil desulfinyl	ug/kg	<2	<2	<2	<2

A blank space indicates no test performed.

Sample Logged

Analyte	Units	P10-08-0004-004	P10-08-0004-008	P10-08-0004-009	P10-08-0004-011
Sample Logged	-	Logged	Logged	Logged	Logged

A blank space indicates no test performed.

Sample Logged

Analyte	Units	P10-08-0004-014	P10-08-0004-020	P10-08-0004-021	P10-08-0004-023
Sample Logged	-	Logged	Logged	Logged	Logged

A blank space indicates no test performed.

Sample Logged

Analyte	Units	P10-08-0004-025	P10-08-0004-026	P10-08-0004-027
Sample Logged	-	Logged	Logged	Logged

A blank space indicates no test performed.

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Method references

The sample(s) referred to in this report were analysed by the following method(s):

Analyte(s)	Method	Laboratory
Fipronil in sediment		Organic Chemistry
Sample Logged		Organic Chemistry

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APPENDIX 3. Results of multivariate analyses

Table A3. Multivariate ANOVA investigating effects of collection and transect on the axes scores from the NMDS with NDVI scores used as a covariate. All sites from four transects were included in the analyses.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	X1	12.432[a]	12	1.036	4.574	.000
	X2	48.417[b]	12	4.035	22.231	.000
	X3	10.153[c]	12	.846	4.539	.000
Intercept	X1	.112	1	.112	.495	.483
	X2	.003	1	.003	.019	.892
	X3	.525	1	.525	2.815	.096
NDVI_50	X1	.114	1	.114	.502	.480
	X2	.003	1	.003	.019	.891
	X3	.531	1	.531	2.851	.093
Collection	X1	8.601	2	4.301	18.986	.000
	X2	42.999	2	21.499	118.458	.000
	X3	7.920	2	3.960	21.247	.000
Transect	X1	.972	3	.324	1.431	.236
	X2	2.341	3	.780	4.299	.006
	X3	1.466	3	.489	2.622	.053
Collection * Transect	X1	2.185	6	.364	1.608	.149
	X2	2.893	6	.482	2.657	.018
	X3	.445	6	.074	.397	.880
Error	X1	32.392	143	.227		
	X2	25.953	143	.181		
	X3	26.653	143	.186		
Total	X1	44.824	156			
	X2	74.370	156			
	X3	36.806	156			
Corrected Total	X1	44.824	155			
	X2	74.370	155			
	X3	36.806	155			

a. R Squared = .277 (Adjusted R Squared = .217)

b. R Squared = .651 (Adjusted R Squared = .622)

c. R Squared = .276 (Adjusted R Squared = .215)

Table A4. Multivariate ANOVA investigating effects of collection and transect on the axes scores from the NMDS with NDVI scores used as a covariate. Sites aerial baited with fipronil during September/October 2009 have been removed from the analyses.

GLM Multivariate ANOVA

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	X1	9.629[a]	12	.802	4.276	.000
	X2	28.560[b]	12	2.380	12.937	.000
	X3	26.544[c]	12	2.212	10.825	.000
Intercept	X1	.586	1	.586	3.124	.080
	X2	.015	1	.015	.082	.775
	X3	.029	1	.029	.142	.707
ndvi_50	X1	.593	1	.593	3.161	.078
	X2	.009	1	.009	.048	.827
	X3	.027	1	.027	.130	.719
Collection	X1	6.694	2	3.347	17.837	.000
	X2	19.992	2	9.996	54.335	.000
	X3	22.110	2	11.055	54.100	.000
Transect	X1	.893	3	.298	1.587	.196
	X2	3.213	3	1.071	5.822	.001
	X3	.956	3	.319	1.560	.203
Collection * Transect	X1	1.028	6	.171	.913	.488
	X2	2.735	6	.456	2.478	.027
	X3	1.613	6	.269	1.315	.255
Error	X1	22.892	122	.188		
	X2	22.445	122	.184		
	X3	24.930	122	.204		
Total	X1	32.521	135			
	X2	51.005	135			
	X3	51.474	135			
Corrected Total	X1	32.521	134			
	X2	51.005	134			
	X3	51.474	134			

a. R Squared = .296 (Adjusted R Squared = .227)

b. R Squared = .560 (Adjusted R Squared = .517)

c. R Squared = .516 (Adjusted R Squared = .468)

Table A5. Multivariate ANOVA investigating effects of fipronil and transect on the axes scores from the NMDS on the October 2009 collections with NDVI scores used as a covariate.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	X1	3.845[a]	10	.384	1.528	.149
	X2	3.902[b]	10	.390	1.267	.267
	X3	12.685[c]	10	1.268	4.236	.000
Intercept	X1	.005	1	.005	.021	.884
	X2	.202	1	.202	.655	.421
	X3	.227	1	.227	.759	.387
ndvi_50	X1	.002	1	.002	.008	.927
	X2	.217	1	.217	.703	.405
	X3	.268	1	.268	.893	.348
Fip_2009	X1	.098	1	.098	.389	.535
	X2	.105	1	.105	.340	.562
	X3	.015	1	.015	.049	.825
Transect	X1	2.644	5	.529	2.101	.076
	X2	2.787	5	.557	1.809	.123
	X3	8.338	5	1.668	5.568	.000
Fip_2009 * Transect	X1	.357	3	.119	.473	.702
	X2	.320	3	.107	.346	.792
	X3	1.008	3	.336	1.122	.347
Error	X1	16.862	67	.252		
	X2	20.641	67	.308		
	X3	20.065	67	.299		
Total	X1	20.707	78			
	X2	24.543	78			
	X3	32.750	78			
Corrected Total	X1	20.707	77			
	X2	24.543	77			
	X3	32.750	77			

a. R Squared = .186 (Adjusted R Squared = .064)

b. R Squared = .159 (Adjusted R Squared = .033)

c. R Squared = .387 (Adjusted R Squared = .296)

Table A6. Multivariate ANOVA investigating effects of fipronil and transect on the axes scores from the NMDS on the May 2010 collections with NDVI scores used as a covariate.

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	X1	10.753[a]	10	1.075	5.260	.000
	X2	2.862[b]	10	.286	.782	.646
	X3	11.066[c]	10	1.107	4.910	.000
Intercept	X1	.111	1	.111	.544	.463
	X2	.493	1	.493	1.346	.250
	X3	2.914	1	2.914	12.929	.001
ndvi_50	X1	.177	1	.177	.865	.356
	X2	.527	1	.527	1.441	.234
	X3	2.708	1	2.708	12.015	.001
Fip_2009	X1	.574	1	.574	2.810	.098
	X2	.130	1	.130	.354	.554
	X3	.807	1	.807	3.578	.063
Transect	X1	5.345	5	1.069	5.229	.000
	X2	.514	5	.103	.281	.922
	X3	7.691	5	1.538	6.825	.000
Fip_2009 * Transect	X1	.469	3	.156	.765	.518
	X2	1.679	3	.560	1.529	.215
	X3	.746	3	.249	1.103	.354
Error	X1	13.697	67	.204		
	X2	24.521	67	.366		
	X3	15.102	67	.225		
Total	X1	24.450	78			
	X2	27.382	78			
	X3	26.168	78			
Corrected Total	X1	24.450	77			
	X2	27.382	77			
	X3	26.168	77			

a. R Squared = .440 (Adjusted R Squared = .356)

b. R Squared = .105 (Adjusted R Squared = -.029)

c. R Squared = .423 (Adjusted R Squared = .337)