5 Risk characterisation

5.1 Damage – pest density relationships in pest management

The assumption that underlies all pest management is that damage is positively related to pest density. Management therefore aims to reduce damage by reducing pest density. However, “damage” is often due to other factors besides pest density. For example, the destructive potential of the pest may vary with its genotype, environmental condition, duration of exposure, resistance of the species under attack, social behaviour of predator (pest) and prey (species to protect), prey numbers and so on. Under these conditions simple damage-pest density relationships will rarely manifest. In a review of quantitative pest damage studies reported in the literature, however, Hone (1994) found that 43% showed no correlation between the level of damage (Y-variable) and pest density (X-variable), whilst 57% did. In addition, most damage-pest density relationships may be nonlinear and exhibit threshold effects because of the underlying assumptions of predator-prey dynamics (Bayliss & Choquenot 1999).

The mortality of nesting seabird chicks on Ashmore Reef islands, therefore, may be partly related to tropical fire ant abundance and partly related to a host of other mortality agents, most of which should be natural. The difficulty, however, is to isolate confounding mortality effects under non-experimental field conditions (ie ‘control’ islands where tropical fire ants are lacking, or unplanned at least). However, if sample units (either whole colonies on islands, or artificial grid cells over colonies) have spatial variation in damage level and their multiple causes, then it may be possible to tease out the effects of pest density using partial multiple regression analysis. Additionally, if there are sufficient sample replicates across a range of damage and pest density levels, then more complex multivariate models can be used to examine other sources of variation. For example, covariance analysis can be used to stabilise pest density effects in order to examine other factors, such as differences between developmental stages of chicks, species, islands and, in future, management treatments (via the adaptive management approach).

5.2 Number of dead seabird chicks and the abundance of tropical fire ants

5.2.1 Working hypotheses and model assumptions

The main question that the field component of our risk assessment attempts to answer is whether or not tropical fire ants, at current densities, have a negative impact on the nesting success of seabirds on Ashmore Reef. tropical fire ant populations have the potential to erupt (eg introduced ants on Christmas Island) and, hence, detectable impacts at low density could transform to far more serious impacts on seabird recruitment in future given the right environmental triggers.

Tropical fire ants could lower recruitment rate indirectly by interfering with nesting behaviour, and/or directly by killing birds, particularly the more susceptible young nestlings. The literature and expert opinion suggests that tropical fire ants are aggressive hunters and are capable of killing young nestlings for food. However, other factors also influence nesting success, such as adult food supply out at sea, disease, predation of young and adult birds by other species (eg raptors), the availability of suitable breeding habitat (eg the density of nesting sites, or the overall cover of vegetation), or all factors in combination and interaction. As discussed above, we have assumed at the outset that many other ecological factors will influence seabird chick mortality besides tropical fire ant abundance and, hence, adopt a multifactorial approach as the most efficient means of analysis with our data set. The most powerful approach in ecology is to test 'multiple working hypotheses' defined *a priori*. It reflects the multivariate nature of reality and, just as important, avoids statistical Type I errors when "fishing" for significance among a large number of poetntial correlates. A multifactorial model that encompasses a group of working hypotheses incorporating the above effects, for eample, may take the form (where F denotes some function of):

> Mortality rate seabirds = F1 (size class of seabirds) + F2 (Habitat) + F3 (abundance tropical fire ants) + F4 (other mortality factors) + F5 (interaction of all effects)

The short time allowed for island visits during the breeding season, and the need to minimise human disturbance as an additional mortality factor, precluded the use of standard methods to directly estimate seabird mortality rates from eggs to fledglings (eg the life table cohort approach), and to partition mortality caused by tropical fire ants from other mortality agents (eg comparison of mortality rates in similar areas with and without tropical fire ants, or at low and high levels of tropical fire ants). Nevertheless, we used key seabird–ant attribute data obtained during our 'snapshot' baseline surveys of the islands in order to characterise risk to nesting success of colonies exposed to 'low-level' tropical fire ant populations (ie there are no current records of eruptions to very high numbers). Key data were: the number of dead chicks by species in a 10 m radius circle (0.03 ha) at the centre of the grid cell, and in each of four developmental or age/size classes (see Section 3.5.1), where size may index the degree of susceptibility to tropical fire ant predation; the percentage cover of vegetation in the same 0.03 ha circular plots, indexing the availability of suitable nesting habitat, and the abundance of tropical fire ants estimated by pitfall traps, visual abundance ranks at bait stations and the number of ant nests (Section 4.3.1). The total number of dead young seabirds was hence used as an index of mortality rate, although this could not be adjusted for initial numbers of hatchlings. In all statistical analyses we used data collected in each grid cell on each island as sample replicates, although such data are not spatially independent. A reduced model encompassing testable working hypotheses amenable to our data set is:

> Density of dead seabird chicks (per grid cell) = F1 (size class chick) + F2 (% vegetation cover) + F3 (density of tropical fire ants) + F4 ((interaction of all effects)

Only dead chicks of the Common Noddy and Brown Booby were found at the time of survey, and only the Common Noddy on East and Middle Islands had sufficient numbers for statistical analyses (Table 4). Not all grid cell data were used in analyses because of the following two a *priori* exclusion criteria: (1) the grid cell could not be sampled because of excessive disturbance to birds; and (2) the grid cell was outside the nesting colony because the habitat was totally unsuitable for nesting. However, grid cells with zero ants and dead seabirds, and zero dead seabirds and ants, were included in analysis. Overall there were 40 grid cells for analysis, 23 for Middle Island and 17 for East Island. Grid cells varied in size between islands (50 m x 50 m for Middle and 40 m x 40 m for East) and, hence, sample fraction per grid cell varied by 12% and 8%, respectively.

Table 4 Summary of the number of dead common noddy chicks found in the sample grids of East and Middle islands

	East Island				**Middle Island**			
Seabird species	**Developmental stage**				**Developmental stage**			
	1	2	3	4	1	2	3	4
Common noddy	5	7	8	9	13	11	9	10
Brown booby	0	11	0	0	0	2	0	0

5.2.2 Methods of analysis

Comparison and calibration of different ant survey methods

Ant nests were only surveyed in 21% of grid cells (6 of 23) on Middle Island, and were not surveyed on East Island. Hence, estimates of the number of tropical fire ant nests in each sample grid cell could not be used in subsequent analysis although it may be a more stable index of ant population abundance (see section 2.4.6), especially if adjusted to an optimal sample cell size.

For comparison of ant survey methodologies, visual abundance ranks (n=5, see Section 4.3.1) at bait stations were converted to counts using the highly significant nonlinear regression of mid-point abundance range of each abundance rank (Y) on rank (X) (Figure 42). Pitfall trap data were collected for night and day activity periods (see Section 4.3.1), and were significantly correlated (R^2=30.4%, n=23, P< 0.004). Twice as many tropical fire ants were caught at nights compared to days (6.6 cf 3.1; $t_{1/43\ (0.05}$ =1.88, P<0.05), suggesting more ant activity at nights rather than abundance *per se*. Night-time pitfall data were used in all subsequent analyses in preference to day-time pitfall data or a combination of day and night-time data because they were precise and potentially more relevant to assessing future management strategies (ie bait exposure at nights when tropical fire ants are most active & possibly with a lower risk of incidental take by birds).Additionally, in contrast to instantaneous counts at bait stations, night-time pitfall traps integrated ant activity over a longer period of time and so would be more stable. Nevertheless, mean indices of tropical fire ant abundance derived by pitfall traps at night were, overall, significantly correlated to the corresponding visual ranks at bait stations (Figure 43).

Statistics

Statistical analyses were undertaken with Statistica™ software (2001). Normality tests (Lilliefors & Shapiro Wilks test; examination of normal probability plots) on all variables indicated that data transformations were necessary (arcsine for % vegetation cover converted to proportions, $\log_e$+1 for counts of dead chicks and ant abundance data; Zar 1974). However, the means (observed unweighted) of untransformed data are presented in figures.

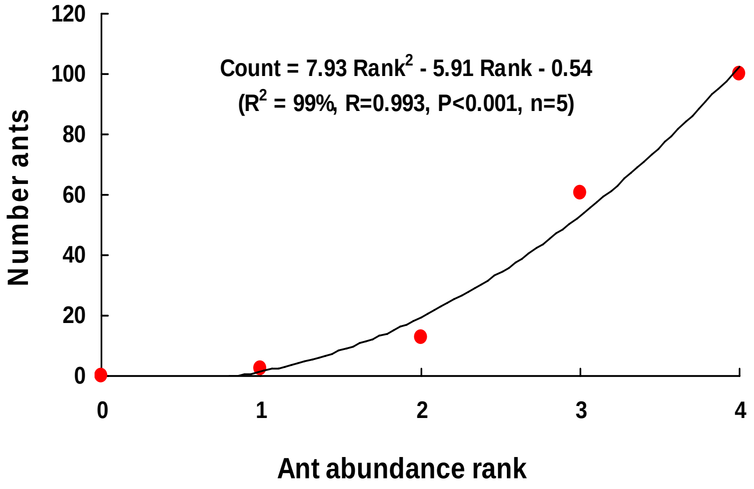


Figure 42 Nonlinear regression between mid-point range of tropical fire ant counts and abundance rank at bait stations

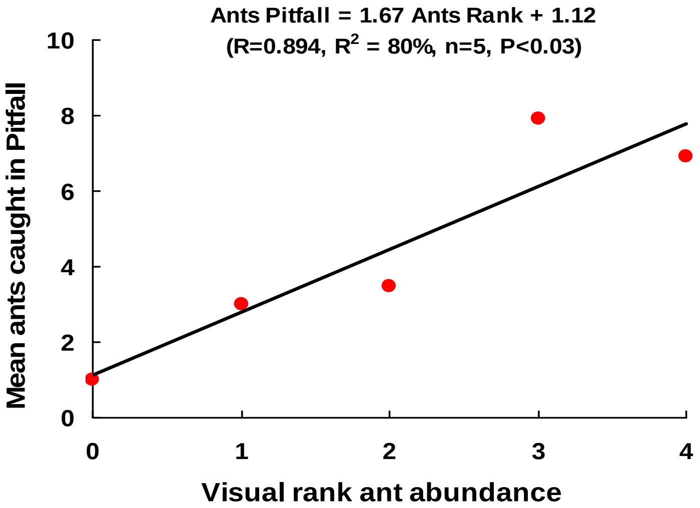


Figure 43 Regression between the mean number of ants caught in pitfall traps at night (Y-Ants Pitfall) and the visual abundance rank (X-Ants Rank) at bait stations

A 1-ANOVA was used to compare survey attributes (density of all dead Common Noddy chicks, % vegetation cover, density index of tropical fire ants derived from pitfall traps at night & bait stations) between Middle and East islands, and a 2-ANOVA was used to compare differences in the density of dead chicks by age/size class and islands.

General Linear Models (GLM) were used to test for differences in response variables between factors and key variables of interest. The response variable was either single (total dead chicks) or multiple (the four dead size classes of chicks, comprising a repeated measures design). The only factor was ‘Island’ (Middle cf East), and the two covariates of interest were vegetation cover (%) and tropical fire ant density. The mixed GLM is referred to as a MANCOVA (multivariate analysis of covariance).

As mentioned, univariate analyses of correlations between pest damage (Y) and pest density (X) is questionable because the effect of one variable may be influenced by the levels of other intercorrelated variables; that is, there would be no single level of importance. For example, the death of a Common Noddy chick may depend on other key variables besides the abundance of tropical fire ants and, hence, the correlation between dead chicks (our impact or damage) and ant abundance (pest density) may not be at first apparent. Multiple *partial* regression analysis was therefore used to tease out and enhance the correlation between dead chicks and ants by statistically holding the effects of vegetation cover constant. All hypotheses were *a priori*, hence no adjustment (eg Bonferoni) needs to be made for Type I error in this multiple and simultaneous contrast approach.

5.2.3 Results: common noddy on East and Middle islands

Island differences

The density of all dead common noddy chicks, the density of tropical fire ants and the percentage cover of vegetation were similar between East and Middle Islands (Figure 44).

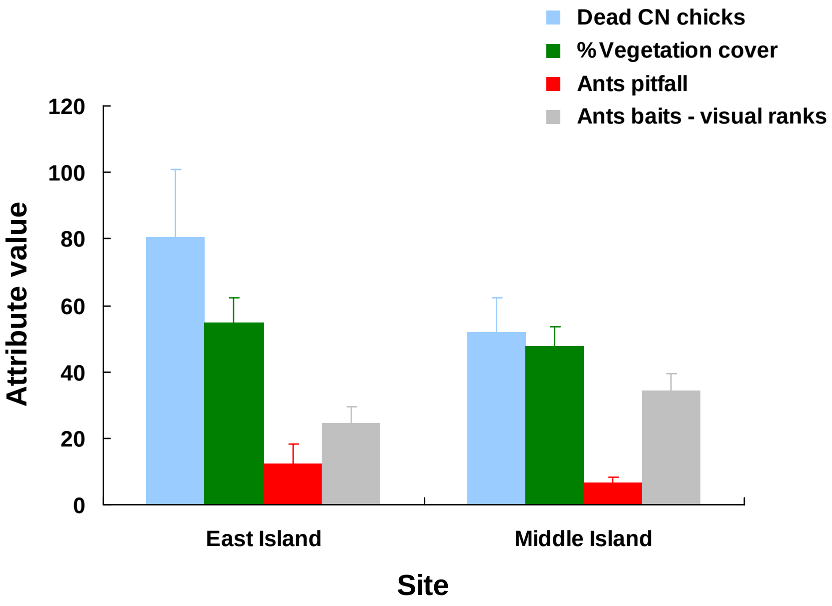


Figure 44 Comparison of the mean density (per 0.25 ha) of total dead common noddy chicks (scaled-up by a factor of 10), vegetation cover (%) and tropical fire ant density (via night-time pitfall traps & visual abundance ranks at bait stations converted to counts, respectively) between East and Middle islands. Vertical bars are standard errors.

However, a 2-ANOVA between age/size class of dead chicks (n=4) and island (n=2) shows that the density of dead chicks in the smallest, most vulnerable size class 1 was 3.9 times higher on Middle Island than on East Island, but similar between all other size classes (interaction term using untransformed data: $F_{3/152}$= 3.97, P<0.009; that for Log_e transformed data P<0.05; Figure 45a & b).

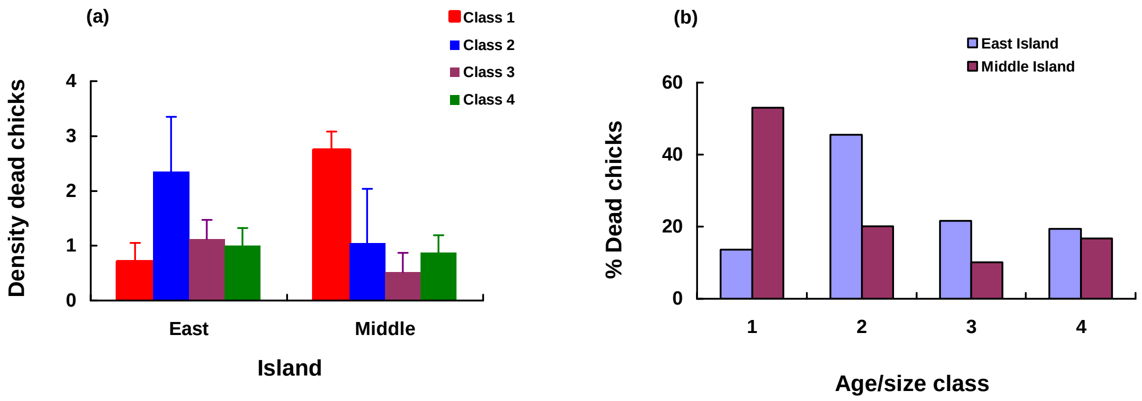


Figure 45 a & b Comparison of the (a) mean density (per 0.03 ha) of total dead common noddy chicks in each of the four age/size classes between East and Middle islands (vertical bars are standard errors), and (b) the percentage of dead chicks in each age/size class by island.

Damage-density relationships

Multiple regression analysis showed that across both islands the density (per 0.25 ha cell) of dead common noddy chicks increased with an increase in the density of tropical fire ants (Figure 46a, Table 5), and decreased with increasing amounts of percentage vegetation cover (Figure 46b, Table 5). The combined regression relationship was highly significant and explained 26% of the variability in the data. The partial regression plots for both ants and vegetation show a uniform spread of data along the lines and the absence of very extreme outliers (Figure 46a & b).

Table 5 Summary of the multiple regression relationship between the density (per 0.03 ha) of all dead common noddy chicks, with the density index of tropical fire ants and percentage vegetation cover, across East and Middle islands. Regression statistics are above the Table (B is the slope coefficient with standard error SE B, and P the significance of variables in the equation). Shaded Table cells highlight sigificant results.

R= 0.542, R^2= 25.6%, F(2,37)= 7.71, P<.001, SE =0.36

variable	B	SE B	P
Intercept	0.81	0.16	<0.001
% veg cover	-0.48	0.17	0.011
Ants	0.30	0.11	0.01

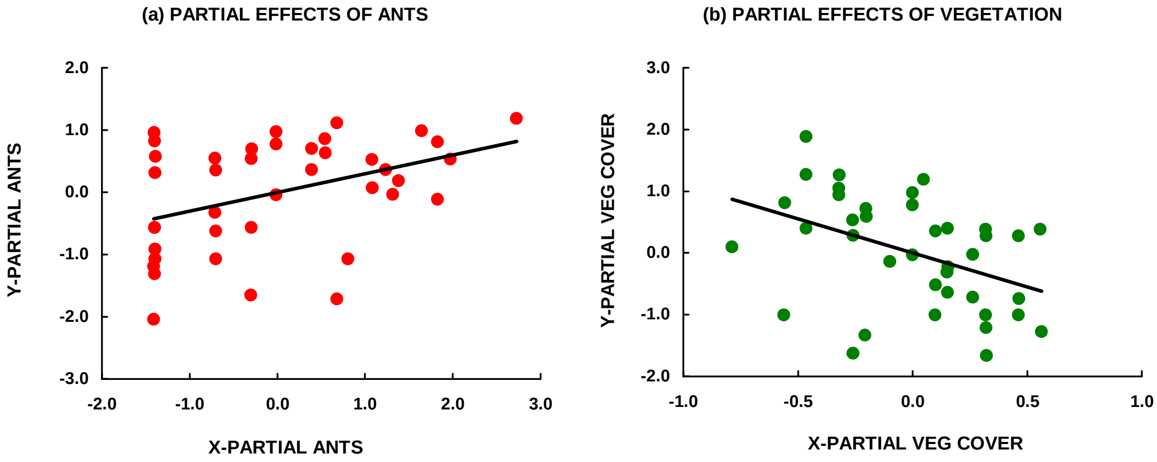


Figure 46a & 46b Partial regression plots between the density (per 0.03 ha) of all dead common noddy chicks (Y-PARTIALs) with: (a) the density index of tropical fire ants (X-PARTIAL ANTS) and (b) percentage vegetation cover (X-PARTIAL VEG COVER). Data across both islands are combined.

Further multivariate analysis (a mixed MANCOVA model) shows, however, that the relationship between the density of dead common noddy chicks and the abundance of both tropical fire ants and vegetation cover, differed in a complex manner between age/size class and island (Table 6; the significant highest order interaction).

Partial multiple regression analysis was therefore performed separately for each class of dead common noddy for each island, in order to identify the main sources of variation (or explanation) of the general relationships described above. Results (Table 7) show that, across the two islands, the negative effects of vegetation cover and the positive effects of tropical fire ants on the mortality of common noddies were greatest for the first two size classes. However, this depended on which island; vegetation cover only influenced common noddy mortality on East Island, and that for tropical fire ant abundance, only on Middle Island. The reason for the complex interaction between tropical fire ants, vegetation and islands is unknown and requires further study. The relationships could be independent and coincidental, or as a result of real ecological interaction. For example, noddies could simply prefer nesting sites that have less vegetation and, similarly, tropical fire ants could prefer less vegetation to forage in. Alternatively, or additionally, ants may affect vegetation by farming hemipterans.

Table 6 MANCOVA of the density (per 0.03 ha) of dead common noddy chicks in each age/size class (n=4 response variables) with vegetation cover (%) and tropical fire ant density index (n=2 regression covariates, respectively), and island (n=1 factor, Middle vs. East). Shaded table cells highlight significant effects and interactions.

Source	F	df	P
Intercept	8.92	4/29	0.0001
Island	4.48	4/29	0.0061
Veg cover regression	4.42	4/29	0.0066
Ant regression	2.89	4/29	0.0394
Island*Veg	2.78	4/29	0.0454
Island*Ants	4.46	4/29	0.0062
Veg*Ants	2.05	4/29	0.1140
Island*Veg*Ants	2.55	4/29	0.0606

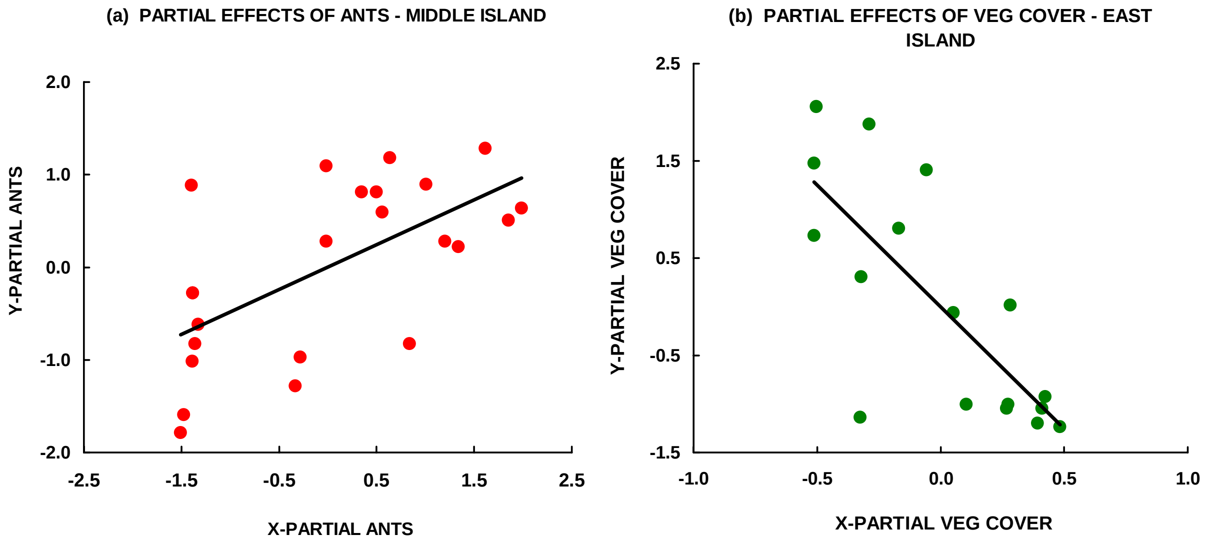
Table 7 Summary of multiple regression analyses between the density of dead common noddy chicks (per 0.03 ha) with vegetation cover (%) and tropical fire ant density index, for each size class on each island. Highlighted table cells indicate significant regression variables and overall regression equations.

ISLAND	DEAD CLASS	REGRESSION SUMMARY					
		Variable	Coefficient	P	Reg DF	Reg %R^2	Reg P
EAST	**1**	**Intercept**	**1.45**	**<0.001**	**2/14**	**50.0**	**0.003**
		Veg cover	**-1.05**	**0.002**			
		Tropical fire ant		**NS**			
	2	**Intercept**	**1.63**	**0.01**	**2/14**	**29.8**	**0.033**
		Veg cover	**-1.50**	**0.02**			
		Tropical fire ant		**NS**			
	3	**Intercept**	**1.28**	**0.008**	**2/14**	**8.9**	**NS**
		Veg cover		**NS**			
		Tropical fire ant		**NS**			
	4	**Intercept**		**NS**	**2/14**	**<1.0**	**NS**
		Veg cover		**NS**			
		Tropical fire ant					
MIDDLE	**1**	**Intercept**	**0.94**	**0.07**	**2/20**	**21.3**	**0.035**
		Veg cover		**NS**			
		Tropical fire ant	**0.35**	**0.027**			
	2	**Intercept**	**0.41**	**NS**	**2/20**	**24.3**	**0.024**
		Veg cover		**NS**			
		Tropical fire ant	**0.26**	**0.011**			
	3	**Intercept**		**NS**	**2/20**	**2.1**	**NS**
		Veg cover		**NS**			
		Tropical fire ant		**NS**			
	4	**Intercept**		**NS**	**2/20**	**1.6**	**NS**
		Veg cover		**NS**			
		Tropical fire ant		**NS**			

To further isolate sources of unexplained variability, all partial multiple regression analyses above were repeated using the combined counts of dead chicks from Classes 1 and 2 only. Results (Table 8) show that the simultaneous relationship between common noddy mortality of the first two vulnerable size classes with tropical fire ant density on Middle Island (Figure 47a), and vegetation cover (%) on East Island (Figure 47b), are considerably tightened.

Table 8 Summary of multiple regression analyses between the density (per 0.03 ha) of dead Common Noddy chicks in vulnerable age/size Classes 1 and 2 combined, with vegetation cover and tropical fire ant density index, separately for both islands and combined, respectively. Highlighted table cells indicate significant regression variables and overall regression equations.

ISLAND	REGRESSION SUMMARY CLASS 1 & 2 COMBINED					
	Variable	Coefficient	P	Reg df	Reg %R^2	Reg P
BOTH ISLANDS	Intercept	1.71	<0.001	2/37	38.0	<0.001
	Veg cover	-1.45	<0.001			
	Tropical fire ant	0.33	0.003			
EAST	Intercept	2.99	<0.001	2/14	58.0	0.002
	Veg cover	-2.50	<0.001			
	Tropical fire ant	0.11	NS			
MIDDLE	Intercept	1.10	0.031	2/20	38.0	0.003
	Veg cover	-0.80	NS			
	Tropical fire ant	0.48	0.002			



(a) PARTIAL EFFECTS OF ANTS - MIDDLE ISLAND

(b) PARTIAL EFFECTS OF VEG COVER - EAST ISLAND

Figure 47a & 47b Partial regression relationships between the total density (per 0.03 ha) of dead common noddy chicks in Classes 1 and 2 combined (Y-PARTIALs) with: (a) the density index of tropical fire ants on Middle Island (X-PARTIAL ANTS); and (b) vegetation cover (%) on East Island (X-PARTIAL VEG COVER). Regression equations for each island retained both variables to preserve complex interactions detected in previous multivariate analyses.

Mortality rates

The above analyses showed that the abundance of tropical fire ants and vegetation cover were highly correlated to the mortality of younger, more vulnerable common noddy chicks: mortality increased with increasing densities of tropical fire ants and decreased with increasing amounts of vegetation cover. However, the effect of ants was only statistically evident on Middle Island and, in contrast, the effect of vegetation cover was only statistically evident on East Island. Additionally, the density of dead chicks of the most vulnerable age/size (Class 1) was greater, statistically, on Middle Island than on East island (Figure 45). This provides a fortuitous study 'control' to examine the differential effects of tropical fire ants on the mortality of common noddy chicks in the more vulnerable age/size class (Classes 1 & 2 combined).

A 2-ANOVA showed that the mean proportion of total dead common noddy chicks varied significantly between islands and the two new size/age classes (ie Classes 1+2 vs. 3+4; interaction: $F_{1/76} = 3.88$, $P<0.05$). Hence, the mortality of each new combined age/size class on each island can be estimated directly as that proportion of the total dead chicks found in selected sample cells (Table 6, adjusted for different grid cell size on East island). The frequency distribution of counts of dead chicks in each new age/size class on each island was non-uniform ($\chi^2 = 5.52$, df=1, $P<0.019$). On Middle Island, where the impacts of ants was statistically detected and assumed greater, mortality of the more vulnerable younger chicks was 73%, and that for East Island 59% (Figure 48). However, these proportions are not particularly useful when it comes to partitioning mortality rates between agents. Nevertheless, if we assume that on Middle Island total mortality (M_{total}) is a combination of natural mortality ($M_{natural}$) and mortality caused by tropical fire ants (M_{ants}), and that on East island mortality is mostly natural ($M_{natural}$), then the isolated mortality rate of each age/size class attributable to tropical fire ants can be estimated by (after Caughley 1980):

$$M_{total} = M_{natural} + M_{ants} - (M_{natural}.M_{ants})$$

This model accounts for the fact that the proportion of each age/size class killed by natural agents and tropical fire ants are not independent of each other. In the absence of tropical fire ants, many chicks that would otherwise have been killed by ants are now at risk of death from natural agents. The proportions killed in each age class by each mortality agent can, therefore, be recast as 'isolated' mortality rates by using the interactive term of the above model.

Hence, for the younger age/size class, if $M_{total} = 0.73$ and $M_{natural}$ 0.59 then $M_{ants} = 0.34$. That is, the isolated rate of mortality of the more vulnerable chicks attributed to tropical fire ants is at least 34%, which is a significant amount. The isolated rate of mortality attributed to tropical fire ants for the less vulnerable age/size class, however, is not independent also of the deaths in the previous age/size class. The proportion of dead older chicks on Middle Island was 27% compared to 41% for East Island. Hence, less chicks would have died in the older age class on Middle Island because there were less of them that reached this class to die in the first place. That is, they simply died in the previous younger age class due to ant predation, not because survival was better in the older age class. Therefore the total mortality of older chicks was adjusted upwards by the amount equal to the interaction term in the previous age/size class, or the proportion of young chicks killed by ants that otherwise would have survived to be at risk from all mortality agents (= 0.20). This logic returned an estimate of isolated mortality rate attributed to tropical fire ants for the less vulnerable age/size class of 11% (Table 9).

Table 9 The frequency distribution of dead common noddy chicks by two vulnerability classes (age/size Classes 1 & 2 combined vs. 3 & 4 combined) and by island, and associated estimates of mortality rate. Estimates of mortality rates attributed only to tropical fire ants are also provided (see text).

Island	Age/size class	Frequency	Proportion dead	Moratlity from ants
East (natural)	1	52	0.59	
	2	36	0.41	
	Total	88		
Middle (ants + natural)	1	87	0.73	0.34
	2	32	0.27	0.11
	Total	119		

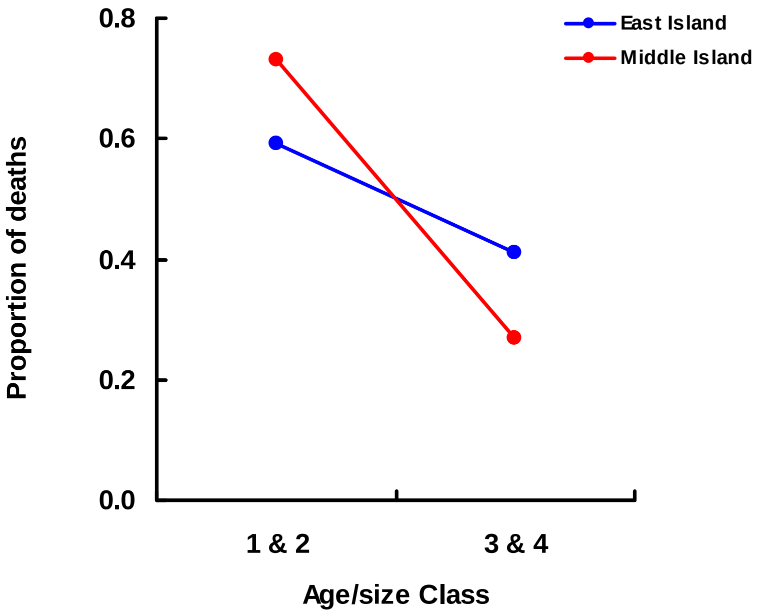


Figure 48 Proportion of deaths in each age/size class (1+2 & 3+4) of common noddy chicks on East and Middle islands