

Population Density of Yellow Crazy Ants (*Anoplolepis gracilipes*) and Terrestrial Arthropod Diversity and Abundance in the Singapore American School Rainforest

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Abstract

This study investigated the relationship between Yellow Crazy Ant (*Anoplolepis gracilipes*) density and terrestrial arthropod species richness and abundance in the Singapore American School (SAS) Rainforest to explore the ecological impact of the potentially invasive *A. gracilipes* in Singapore. Four different 5x5 meter sampling plots were established in randomized

locations throughout the SAS Rainforest, and 1m² quadrats were used to sample *A. gracilipes* population density and terrestrial arthropod species richness and abundance in five specified, predetermined areas of each plot. Linear regression analysis found a significant negative correlation between higher *A. gracilipes* density and lower terrestrial arthropod species richness and abundance, proving the existence of a relationship between the variables. Additionally, there was strong evidence that many arthropods in the SAS Rainforest are displaced from areas with higher *A. gracilipes* density and an indication that *A. gracilipes* could be having a significant, negative impact on native ant species richness in the SAS Rainforest. Future studies should further investigate the ecological impact of *A. gracilipes* across Singapore, attempt to identify established supercolonies and assess the possibility of *A. gracilipes* being an invasive species in Singapore.

Keywords

Yellow Crazy Ant (*Anoplolepis gracilipes*), Species Richness, Invasive Species, Population Density, Ecological Impact

Introduction

In the Southeast Asian city-state of Singapore, a country with little land and a very dense human population, identifying invasive species and studying their ecological impact to formulate effective population management plans is paramount to safeguarding local biodiversity (National Parks Board, 2000). This study examined the ecological impact of the potentially invasive Yellow Crazy Ant (*Anoplolepis gracilipes*) in Singapore by exploring the relationship between

the population density of *Anoplolepis gracilipes* and the species richness and abundance of terrestrial arthropods in the Singapore American School rainforest.

The Yellow Crazy Ant or Long-legged Ant (*Anoplolepis gracilipes*) is among the 100 worst invasive species on Earth (invasives.org). A yellowish-orange ant around 5mm in length, *A. gracilipes* can form extensive supercolonies with exceptionally dense populations of workers and queens; up to 20 million per hectare (Stork, 2014). Supercolonies are often located aboveground, occasionally carpeting hundreds of hectares of forest floor as on Christmas Island, Australia, where a 750-hectare supercolony was identified in 2001 (Abbott, 2004).

Not only is *A. gracilipes* able to form extensive, densely populated supercolonies, but it can also quickly establish itself in new locations, where it is often transported and introduced accidentally through human cargo (Abbott, 2004). Due to *A. gracilipes*' ability to rapidly colonize new areas, it is now found in many tropical and subtropical regions worldwide (Harris & Berry, 2010). Where it is considered invasive, *A. gracilipes*' presence is detrimental to the populations and abundance of many vertebrates and invertebrates, such as red land crabs on Christmas Island, small skinks in Queensland, and nesting seabirds on Johnston Atoll and the Seychelles (Vanderwoude, 2006; Lach, 2022; Hoffman, 2009; Stork, 2014). On Christmas Island alone, *A. gracilipes* was directly responsible for the deaths of up to 20 million endemic land crabs (Stork, 2014).

Though *A. gracilipes* workers subsist off of honeydew and other sweet liquids, brood and queens require protein, provided by workers that hunt and scavenge for the colony (Harris & Berry, 2010). *A. gracilipes* has a versatile diet and will consume anything from crickets, earthworms, and slugs to small lizards and nestling birds, with the bulk of their diet consisting of arthropods and other invertebrates (Csurhes, 2012). As they are unable to bite or sting, *A.*

gracilipes employs formic acid, a highly corrosive liquid, as a means of self-defence and for hunting (Csurhes, 2012). Workers spray overwhelming quantities of formic acid at prey and potential predators; blinding, crippling, and eventually killing their quarries (invasives.org). *A. gracilipes*' utilization of formic acid has been one of the cornerstones of their success as invasives, enabling the ants to effectively topple ecosystems in several locations (Csurhes, 2012). The ecological impact of *A. gracilipes* extends beyond its predatory behavior, however.

To obtain necessary carbohydrates for survival, *A. gracilipes* workers farm sap-eating scale insects (Infraorder *Coccoidea*) in vast quantities, protecting the bugs from parasites and predators in exchange for sweet honeydew (Boland, 2011). Unnaturally high populations of scale insects harm and kill trees, altering and damaging forests (Stork, 2014). On Christmas Island, scale insect predation creates gaps in the rainforest canopy where sunlight can penetrate down to the forest floor, allowing invasive weeds to colonize (O'Dowd, 2003) quickly. Excess honeydew produced by the insects drips down into the forest below, causing sooty mold to form and carpet foliage (O'Dowd, 2003).

In Singapore, more is needed to know about *A. gracilipes* and its role in the ecosystem. The native geographic range of the ants is the subject of debate and uncertainty in the scientific community (Csurhes, 2012). Wang (2022) did not mention *A. gracilipes* being an invasive species in Singapore in her comprehensive list of the ants of Singapore. She explained that they are 'cosmopolitan tramps presumed native to Indomalaya' but whose exact native range 'remains uncertain' (Wang, 2022). A type specimen—a specimen of an unclassified organism used to describe a new species or genus—of *A. gracilipes* was collected in Singapore in 1854, suggesting that the ants are either an old introduction to Singapore or are native (Smith, 1857). On the contrary, Hoffman (2009) explains the likelihood of the entire *Anoplolepis* genus, including

Anoplolepis gracilipes, being of African rather than Southeast Asian origin, based on the ‘devastating impacts of Yellow Crazy Ants on native ant species in India’ and *A. gracilipes* being the only member of its genus found outside Africa. What is known is that *A. gracilipes* is a quickly-established tramp species that is widespread around Singapore in a variety of different habitats (Wang, 2022).

Altogether, *A. gracilipes* is prevalent across Singapore and possesses highly destructive invasive capabilities. Additionally, there is a lack of conclusive information on the status of *A. gracilipes* as a native or invasive species locally. Thus, this study was important for addressing gaps in the scientific literature regarding *A. gracilipes* in Singapore and for exploring the potential ecological impact of *A. gracilipes*.

Method

The location chosen for conducting a study investigating the impact of *A. gracilipes* population density on terrestrial arthropod diversity and abundance was the Singapore American School (SAS) rainforest, a 1.2-hectare plot of regenerating secondary rainforest on the SAS campus, home to a population of *A. gracilipes*.

The SAS student researcher who conducted the study needed a convenient location for data collection where *A. gracilipes* was present. The SAS Rainforest was chosen as it is ideally located on the SAS campus, accessible to students, and requires no permits or permissions for collecting data within the forest.

Figure 1

The SAS Rainforest



Data acquisition occurred through randomized plot sampling with the end goal of gathering quantifiable, numerical data on the diversity and abundance of terrestrial arthropods and the density of *A. gracilipes* in the SAS rainforest.

Inclusion Criteria of Sample(s) and Sampling Method

This study examined the relationship between *A. gracilipes* density and the species richness and abundance of terrestrial arthropods, a major food source and source of competition for *A. gracilipes*. These metrics were employed in a similar study by (Vanderwoude, 2006), which examined invasive *A. gracilipes* populations on Nuulua Island, Samoa.

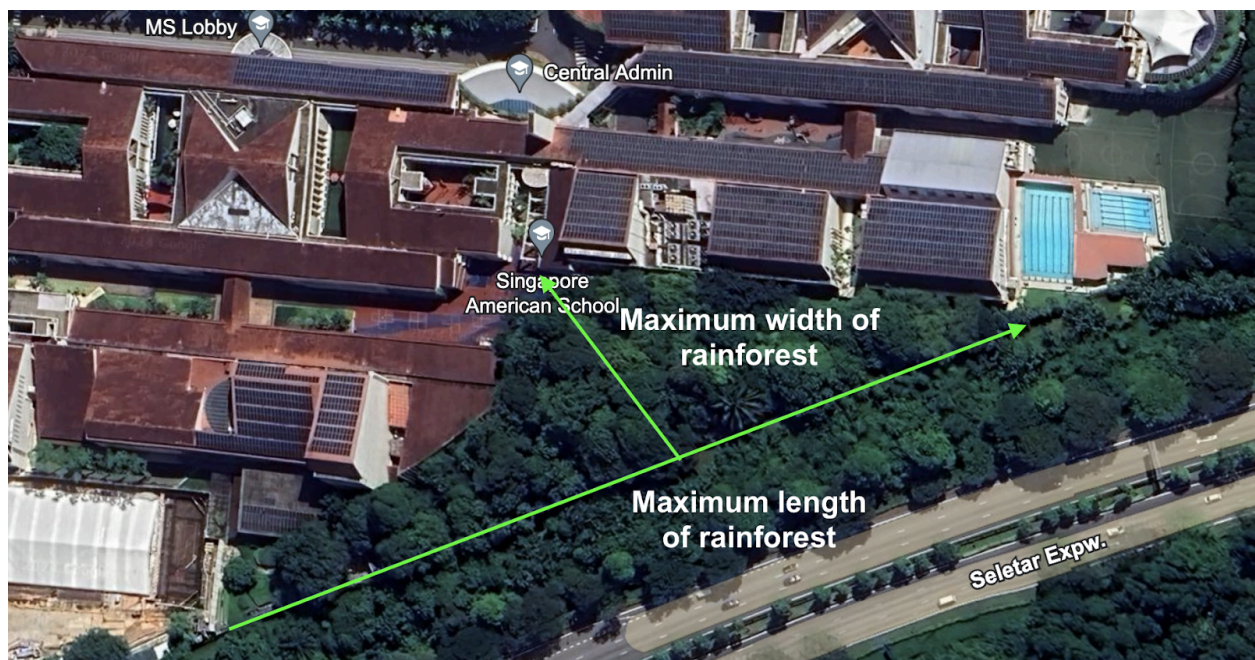
Four different 5x5 meter sampling plots were established in randomized locations throughout the SAS Rainforest, where all three variables were recorded (*A. gracilipes* density per 1m² and terrestrial arthropod abundance and richness). The rationale for the establishment of specifically four 5x5 meter plots was on several grounds. Valid quantifiable data was needed to

explore the correlation between ant density and arthropod diversity and abundance, and this meant obtaining a large sample size of all three variables. More sampling plots meant more data; however, the small size of the forest in addition to time constraints on the study limited the size of the plots and the number of plots that could be established. Four 5x5 meter plots, and a total of 20 samples of each of the three variables, provided simplicity in demarcating plots, ease in compiling data, and the ability to sample a large portion of the rainforest using plots of modest dimensions. The size of the sample provided a sufficient amount of data to perform a quantifiable analysis.

Determining the exact locations for sampling plot establishment was through the use of length (L) and width (W) coordinates. These coordinates ranged from (0,0) to the maximum length and width (in meters) of the rainforest (238L x 57W) as shown below in Figure 2.

Figure 2

Dimensions of the SAS Rainforest (length & width)



Since the rainforest's maximum length is 238 meters and maximum width is 57 meters as

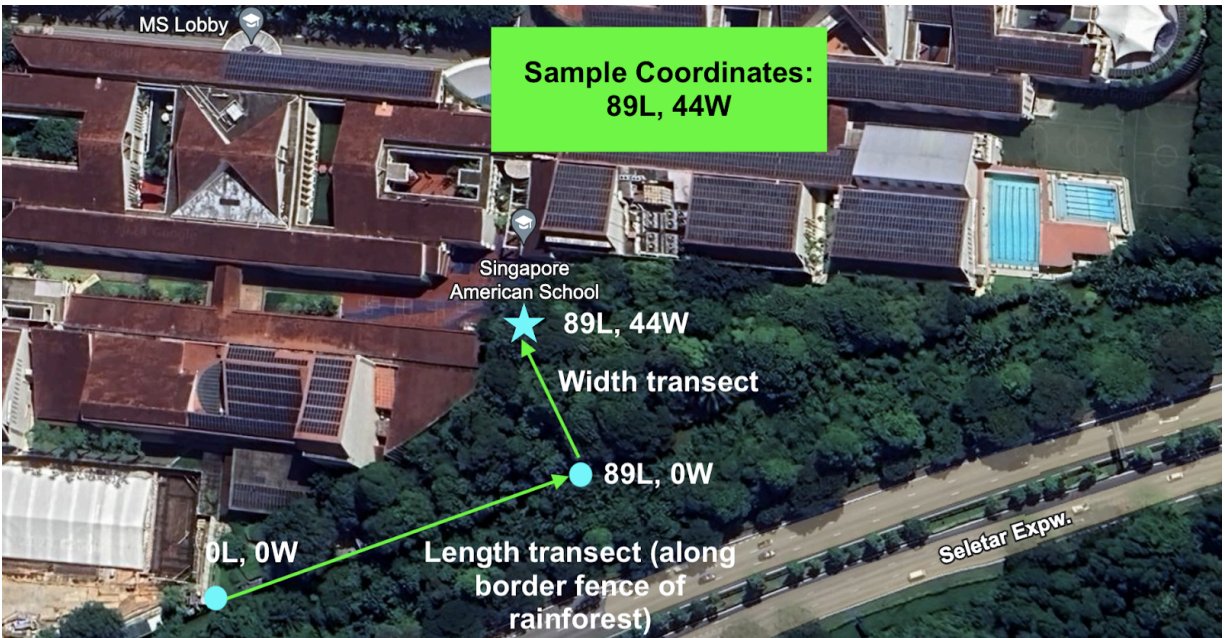
determined by Google Earth, length coordinates initially ranged anywhere from 0-238, and width coordinates from 0-57. A random number generator was used to generate length and width coordinates for the center of each plot to permit randomized sampling of the rainforest.

To locate the coordinates of the plot establishment sites, length and width transects were established. Length transects were situated along the border fence of the rainforest so width (the distance from the border fence inwards) could be accurately assessed. The start of the length transect was from the most southwesterly accessible part of the rainforest onwards. The justification for this is that the other possible starting point, the northeastern part of the forest, is topographically inaccessible.

As Figure 3 displays below, to locate the sample coordinates of 89L, 44W, one would walk from the start point of the length transect at 0L, 0W, to the 89th meter along the border fence. This is the relevant length coordinate (89L, 0W). From here, one would follow a second, perpendicular transect (width transect) 44 meters away from the border fence to find the location of the center of the plot at 89L, 44W.

Figure 3

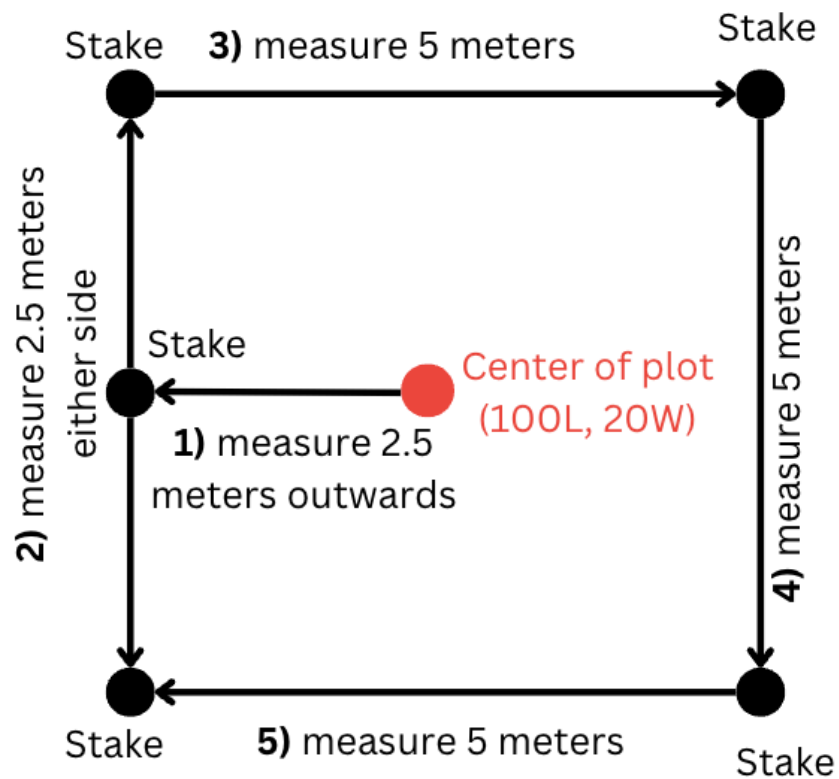
Locating a sample pair of coordinates in the SAS Rainforest



After locating the coordinates, the dimensions of the square-shaped plot (5x5 meters) were measured out and demarcated with twine strung between wooden stakes. One researcher would place a wooden stake in the center of the plot and stand in the center, holding a measuring tape while the other researcher measured 2.5 meters from the center outwards and placed a stake at this new location. Then both researchers would shift positions, where one would stand on the stake 2.5 meters from the center, holding the measuring tape, and the other would measure 2.5 meters in front of and behind the stationary researcher, placing stakes in each new location. Now that two out of four corners of the plot had been established, the stake in the middle would be removed, and 5 meters of twine would be strung between the top and bottom stakes. One researcher would tie one end, and the other researcher the other end. The positions of the researchers would shift again, and the other three, 5-meter sides of the plot would be measured out, the corners staked, and then demarcated with twine. Figure 4 below shows detailed instructions for how plot boundaries were demarcated in the SAS Rainforest.

Figure 4

Step-by-step instructions for demarcating plot boundaries



The plot establishment process was repeated four more times until four 25m² plots were established in random locations throughout the forest. The plots were labelled 1-4 in order of their establishment, and the final locations of the plots are shown below in Figure 5.

Figure 5

The final locations of all four plots in the SAS rainforest



Figure 6

Demarcating one side of Plot 2 with twine

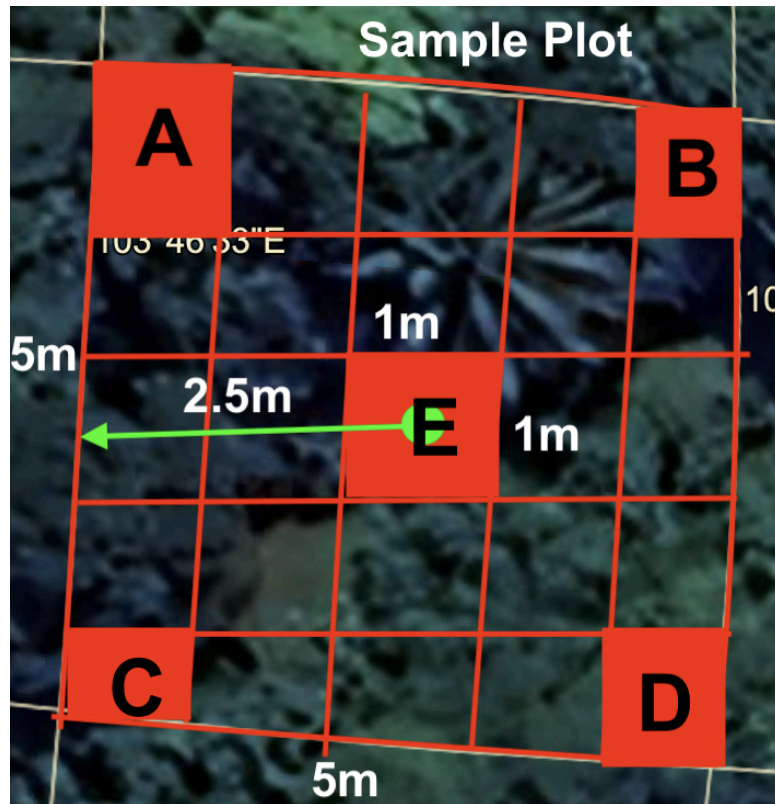


Data Collection Procedure

Once Plots 1-4 were demarcated, 1m² quadrats were used to sample population density and terrestrial arthropod diversity and abundance in five separate areas of the plot. The reasoning for specifically five sampled areas per plot was the ability to sample all four corners of each plot, plus the center, to allow data acquisition from all major sections of each plot without necessitating a larger number of quadrat samples. In all four plots, the five different 1m² locations were labeled A, B, C, D, and E for ease of reference, as shown below in Figure 7.

Figure 7

The 5 (A, B, C, D, and E) 1m² areas that were sampled in each 25m² plot



The exact locations of areas A, B, C, D, and E of certain plots during data collection were sometimes obstructed by dense vegetation, tree trunk, or dead branches, preventing a quadrat from being laid. In these circumstances, the nearest location to the original alphabetized sampling area where a quadrat sample could be taken was used instead.

A. gracilipes population was counted in all five areas of each plot, to calculate the average density of the ants per 1m². In addition to *A. gracilipes* density, the number of terrestrial arthropod species and the population of each species per 1m² was also recorded for areas A, B, C, D, and E in each plot. All 1m² quadrats laid down were photographed to more easily identify arthropod species and count individual ants. Figure 8 below shows area A of Plot 1 being sampled.

Figure 8

Area A of Plot 1 being sampled



Data from the five, alphabetized areas in all five plots (25 samples total) was compiled onto the same spreadsheet.

Data Analysis

Quantifiable data analysis was conducted through a linear regression analysis of *A. gracilipes* density on the diversity of terrestrial arthropod species and the abundance of each species to determine whether arthropod diversity or abundance was significantly impacted by varying population densities of *A. gracilipes*. This data analysis methodology was adapted by Vanderwoude (2006). Four regression models were constructed using the 20 samples of each variable, one for arthropod abundance and ant density, and another for arthropod diversity and ant density, with an additional two regression lines comparing native ant abundance and species richness with *A. gracilipes* density.

Ethical Considerations and Safety

As the study was focused on measuring populations of arthropods and had no human implications, there were no ethical considerations encountered. Safety was of cardinal importance throughout the study. Two researchers at a time took samples and demarcated plots to reduce the risk of performing these actions alone in the rather isolated and rarely visited SAS rainforest. In addition, long pants and bug spray were donned by researchers to reduce the risk of mosquito-borne illnesses transmitted by the prevalent mosquito population of the forest.

Results

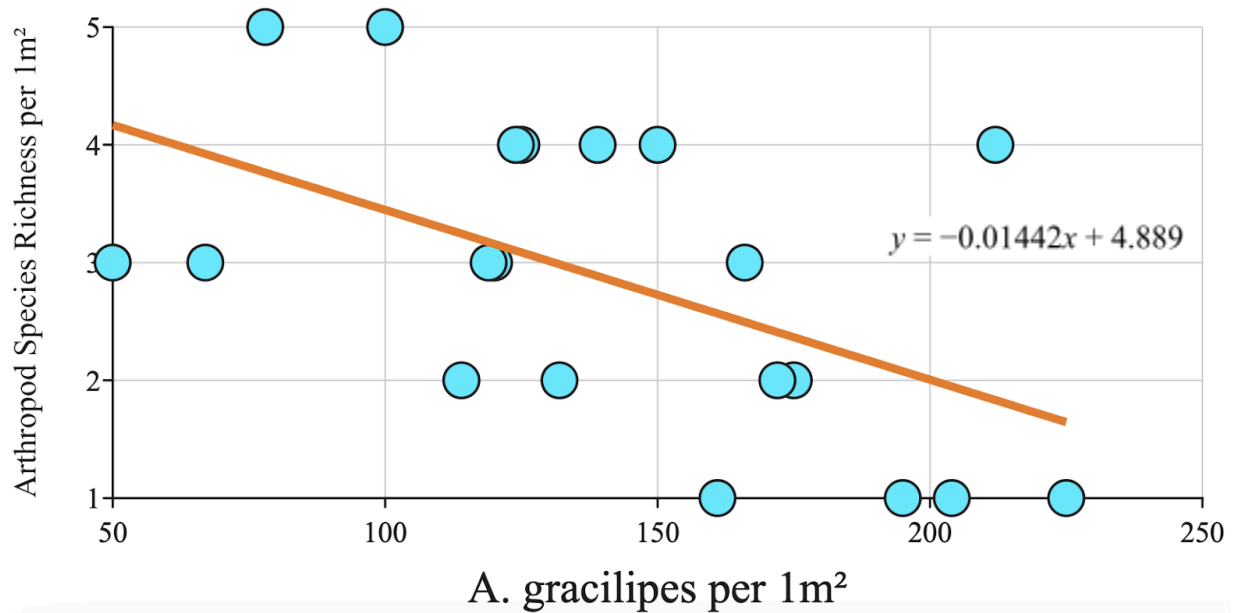
To investigate a relationship between the population density of *Anoplolepis gracilipes* and the species richness and abundance of terrestrial arthropods in the Singapore American School rainforest, linear regression analysis was conducted. For each of the regression analyses conducted, the researcher stated a null hypothesis that there was no significant relationship between *A. gracilipes* density and the biodiversity variable being compared.

***A. gracilipes* Density and Arthropod Species Richness**

The first regression analysis explored *A. gracilipes* density and arthropod species richness per 1m². The findings are represented in Figure 9 below.

Figure 9

First regression analysis graph and linear equation



As the explanatory variable or the density of *A. gracilipes* increased, the response variable or arthropod species richness decreased. Table 1 below displays the regression analysis table for this comparison.

Table 1

First regression analysis table

IV	DV	r	r ²	Slope	P-value
A. gracilipes per 1m ²	Arthropod Species Richness	-0.529	0.28	-0.0144	0.02

Note: IV is defined as independent variable, DV as dependent variable, r as the strength of the correlation, P-value as the significance of the relationship between between the variables, r² as variation in arthropod species richness impacted by *A. gracilipes* density, and r as the strength of the correlation between the variables.

The r² value is 0.28, meaning 28% of the variation in arthropod species richness was impacted by *A. gracilipes* density. This indicates that a low percentage of the variation in arthropod species

richness can be explained by *A. gracilipes* density. On the other hand, the r-value is -0.529, meaning there was a strong, negative correlation between *A. gracilipes* density and arthropod species richness. Since the P-value is 0.02, which is less than 0.05, there was a significant relationship between *A. gracilipes* density and arthropod species richness. Because of this, the null hypothesis can be rejected.

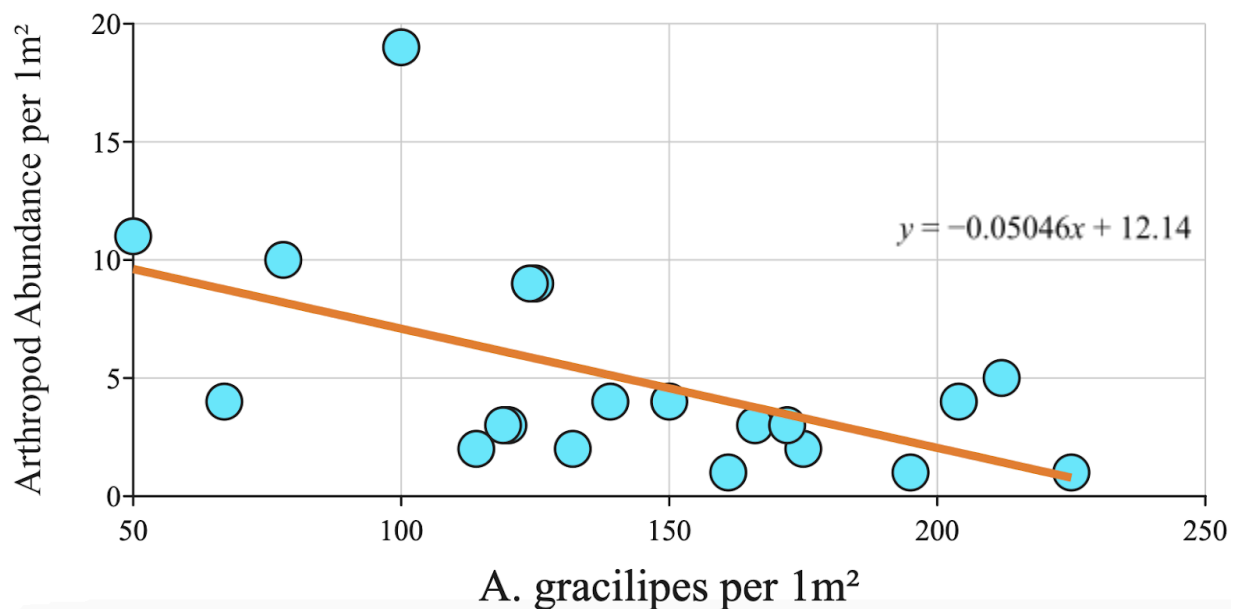
***A. gracilipes* Density and Arthropod Abundance**

The second regression analysis explored *A. gracilipes* density and arthropod abundance per 1m².

The findings are represented in Figure 10 below.

Figure 10

Second regression analysis graph and linear equation



As the explanatory variable or the density of *A. gracilipes* increased, the response variable or arthropod abundance decreased. Table 2 below displays the regression analysis table for this comparison.

Table 2

Second regression analysis table

IV	DV	r	r ²	Slope	P-value
A. gracilipes per 1m ²	Arthropod Abundance	-0.539	0.291	-0.0505	0.01

Note: IV is defined as independent variable, DV as dependent variable, r as the strength of the correlation, P-value as the significance of the relationship between the variables, r² as variation in arthropod abundance impacted by A. gracilipes density, and r as the strength of the correlation between the variables.

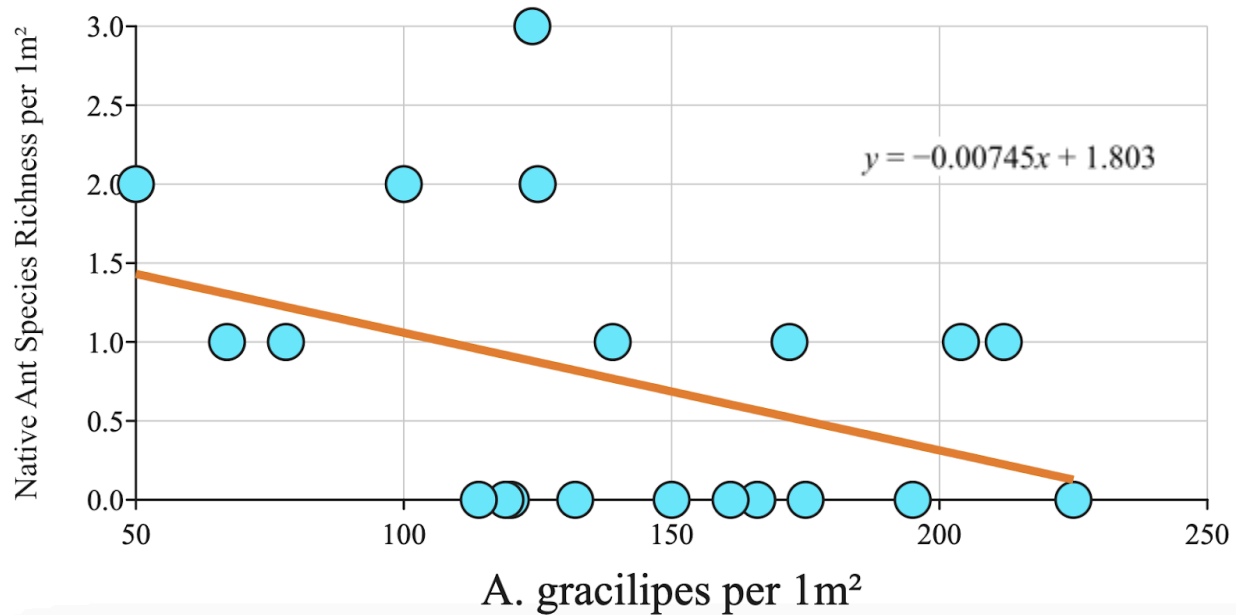
The r² value is 0.291, meaning 29.1% of the variation in arthropod abundance was impacted by *A. gracilipes* density. This indicates that a low percentage of the variation in arthropod abundance can be explained by *A. gracilipes* density. The r-value is -0.539, meaning there was a strong, negative correlation between *A. gracilipes* density and arthropod abundance. Since the P-value is 0.01, which is less than 0.05, there is a significant relationship between *A. gracilipes* density and arthropod abundance. Because of this, the null hypothesis can be rejected.

***A. gracilipes* Density and Native Ant Species Richness**

The third regression analysis explored *A. gracilipes* density and native ant species richness per 1m². The findings are represented in Figure 11 below.

Figure 11

Third regression analysis graph and linear equation



As the explanatory variable or the density of *A. gracilipes* increased, the response variable or native ant species richness decreased. Table 3 below displays the regression analysis table for this comparison.

Table 3

Third regression analysis table

IV	DV	r	r ²	Slope	P-value
A. gracilipes per 1m ²	Native Ant Species Richness	-0.393	0.155	-0.00745	0.09

Note: IV is defined as independent variable, DV as dependent variable, r as the strength of the correlation, P-value as the significance of the relationship between the variables, r² as variation in native ant species richness impacted by *A. gracilipes* density, and r as the strength of the correlation between the variables.

The r² value is 0.155, meaning 15.5% of the variation in native ant species richness was impacted by *A. gracilipes* density. This indicates that a very low percentage of the variation in native ant species richness can be explained by *A. gracilipes* density. The r-value is -0.393, meaning there

was a moderate, negative correlation between *A. gracilipes* density and native ant species richness. Since the P-value is 0.09, which is more than 0.05, there is inconclusive evidence of a significant relationship between *A. gracilipes* density and native ant species richness. Because of this, the null hypothesis fails to be rejected.

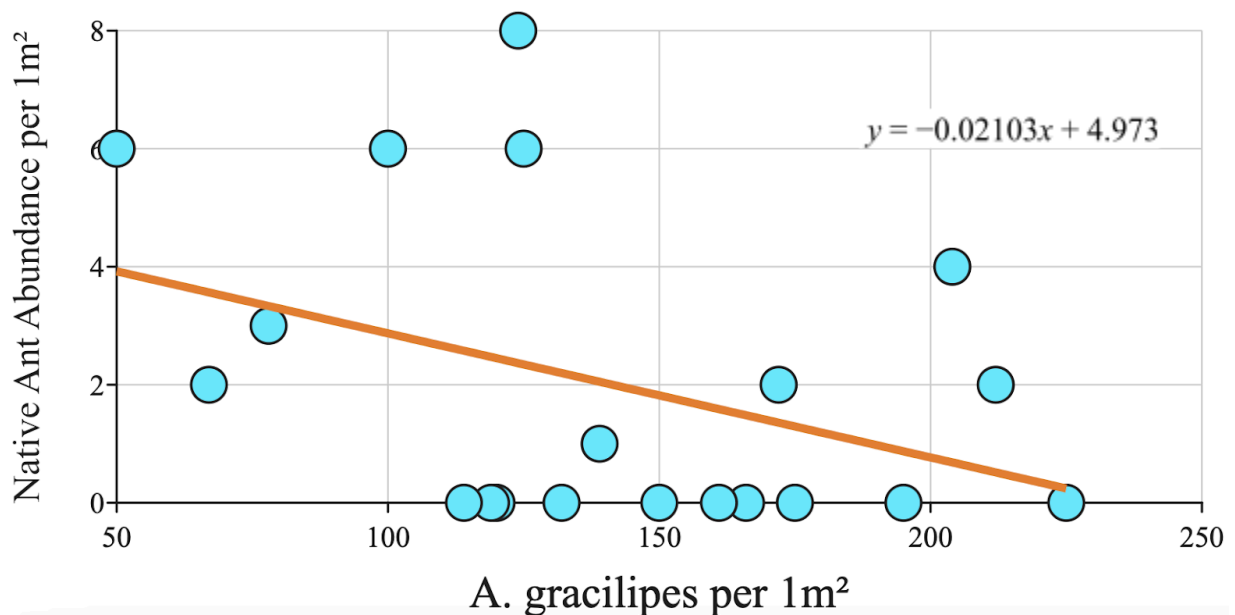
***A. gracilipes* Density and Native Ant Abundance**

The fourth regression analysis explored *A. gracilipes* density and native ant abundance per 1m².

The findings are represented in Figure 12 below.

Figure 12

Fourth regression analysis graph and linear equation



As the explanatory variable or the density of *A. gracilipes* increased, the response variable or native ant abundance decreased. Table 4 below displays the regression analysis table for this comparison.

Table 4

Fourth regression analysis table

IV	DV	r	r ²	Slope	P-value
A. gracilipes per 1m ²	Native Ant Abundance	-0.386	0.149	-0.021	0.09

Note: IV is defined as independent variable, DV as dependent variable, r as the strength of the correlation, P-value as the significance of the relationship between the variables, r² as variation in native ant abundance impacted by A. gracilipes density, r as the strength of the correlation between the variables.

The r² value is 0.149, meaning 14.9% of the variation in native ant abundance was impacted by *A. gracilipes* density. This indicates that a very low percentage of the variation in native ant abundance can be explained by *A. gracilipes* density. The r-value is -0.386, meaning there was a moderate, negative correlation between *A. gracilipes* density and native ant abundance. Since the P-value is 0.09, which is more than 0.05, there is inconclusive evidence of a significant relationship between *A. gracilipes* density and native ant abundance. Because of this, the null hypothesis fails to be rejected.

Discussion

Data was collected and analyzed to investigate the relationship between the population density of *Anoploepis gracilipes* and the species richness and abundance of terrestrial arthropods in the SAS Rainforest. Regression analysis found that higher 1m² densities of *A. gracilipes* in the SAS Rainforest were significantly correlated with lower species richness and abundance of terrestrial arthropods. There is evidence from these findings that higher 1m² densities of *A. gracilipes* displace many terrestrial arthropods from the forest floor of the SAS Rainforest.

The impact of *A. gracilipes* density on native ant species richness and abundance was also examined through regression analysis. Though the results were too insignificant to extrapolate from, there is an indication based on the data collected that *A. gracilipes* has diminished the original native ant species richness of the SAS Rainforest.

The findings of this study, though limited in scope, provide evidence that *A. gracilipes* has a broad and heavy ecological impact on terrestrial arthropods in the SAS Rainforest, which is explored further in the subsections below.

Impact of *A. gracilipes* Population Density on Terrestrial Arthropods in the SAS Rainforest

The regression analyses examining the relationship between the population density of *Anoploepis gracilipes* and the species richness and abundance of terrestrial arthropods in the SAS Rainforest show that higher densities of *A. gracilipes* correlate to significantly lower terrestrial arthropod species richness and abundance. The findings indicate that many arthropods in the SAS Rainforest are displaced from areas with higher *A. gracilipes* density, which is comparable to Christmas Island, where typically abundant red land crabs are excluded from areas with dense *A. gracilipes* populations (Vanderwoude, 2006)

Plots with fifty or fewer *A. gracilipes* workers had on average four or more terrestrial arthropod species present and ten or more individuals of all non-*A. gracilipes* terrestrial arthropods combined. Both arthropod variables dropped starkly when more *A. gracilipes* workers were present. In plots with two hundred or more *A. gracilipes* individuals, there was an average of two or fewer individuals of 1-2 species of terrestrial arthropods; less than half the species richness and a fifth of the abundance of terrestrial arthropods in areas with fewer *A. gracilipes* workers. Additionally, many terrestrial arthropod species were excluded from samples with

higher 1m² densities of *A. gracilipes*. The four samples with 100 or fewer *A. gracilipes* individuals per 1m² had a combined total of 11 terrestrial arthropod species; contrastingly, only 5 species of terrestrial arthropod were recorded across the four samples with more than 190 *A. gracilipes* individuals per 1m². These findings suggest that higher densities of *A. gracilipes* displace many terrestrial arthropods on the forest floor of the SAS Rainforest, possibly due to over-competition for resources, predation, or a combination of both. A study by (Lach, 2022) supports this theory. The study compared varying densities of an invasive population of *A. gracilipes* with native skink abundance in Queensland, Australia. Lach reported that *A. gracilipes* displaces a range of invertebrates anywhere it is present in high densities. The findings of this study showed that higher densities of *A. gracilipes* were correlated with a significantly lower abundance of small native skinks. Small skinks are preyed upon by *A. gracilipes* and must compete with *A. gracilipes* for food— both the skinks and ants have a similar diet (Lach, 2022). Though vertebrates in the SAS Rainforest weren't the focus of this study, the significant reductions in skink abundance in areas with dense *A. gracilipes* populations in Queensland found by (Lach, 2022) provide a framework for analyzing findings from the SAS Rainforest.

In summary, similar to how predation and competition for resources displace small skinks and red land crabs from areas with dense *A. gracilipes* populations in Queensland and Christmas Island (Lach, 2022), (Vanderwoude, 2006), findings from this study indicate that higher densities of *A. gracilipes* displace many species of terrestrial arthropods on the forest floor of the SAS Rainforest.

Impact of *A. gracilipes* on Native Ant Species Richness in the SAS Rainforest

In addition to the regression analyses comparing *A. gracilipes* density and terrestrial arthropod species richness and abundance, more specific data on *A. gracilipes* density and native

ant species richness and abundance within the 20 samples was also analyzed. Though the findings were not statistically significant enough to conclude from, there is still an indication that *A. gracilipes* could be having a significant, negative impact on native ant species richness in the SAS Rainforest. A study by (Evans, 2018), which examined ant and termite species composition in different habitats around Singapore, found there was an average of 6 ant species per 1m² across 25 forest floor quadrat samples from a secondary rainforest with a similar vegetation composition as the SAS Rainforest. *A. gracilipes* was absent from the forest. Conversely, in the SAS Rainforest, where *A. gracilipes* is abundant, a combined total of three native ant species were recorded across all 20 1m² quadrat samples. There was an average of 0.6 native ant species per 1m² quadrat sample, ten times fewer species on average than the secondary rainforest in Singapore without an established *A. gracilipes* population. This may indicate that *A. gracilipes* is outcompeting native ant species on the forest floor of the SAS Rainforest, possibly diminishing the original species richness of native ants by a significant degree. A similar finding from India was cited by (Hoffman, 2009), where the presence of *A. gracilipes* was correlated with far fewer native ants.

A further indication of the negative impacts of *A. gracilipes* on native ant species richness in the SAS Rainforest was the complete absence of the locally ubiquitous Asian weaver ant, (*Oecophylla smaragdina*), across all four plots. *O. smaragdina*, a highly aggressive, native, primarily arboreal ant, is typically an abundant and ecologically dominant species in forests throughout Singapore (Wang, 2022). (Exélis, 2023) remarked that *O. smaragdina*, though being a primarily arboreal ant, frequently forages on the forest floor for sustenance throughout its range. (Wang, 2022) explained that *O. smaragdina* is a dominant and common ant species in a broad range of habitats across Singapore, and actively forages within and atop leaf litter. The

complete absence of *O. smaragdina* on sampled areas of the forest floor of the SAS Rainforest, where *A. gracilipes* is by far the most conspicuous and abundant ant species, means *A. gracilipes* may have outcompeted and driven *O. smaragdina* populations away from suitable habitat within the SAS Rainforest.

Altogether, though there was no significant correlation between *A. gracilipes* density and native ant species richness and abundance, the complete absence of common native ant species like *O. smaragdina* in sampled areas of the SAS Rainforest together with a low native ant fauna when compared to a similar habitat in Singapore indicates *A. gracilipes* could be having a significant, negative impact on native ant species richness within the SAS rainforest.

Limitations

Throughout this study, several major limitations faced by the student researcher hindered gathering the most accurate data possible on *A. gracilipes* density and terrestrial arthropod species richness and abundance in the SAS Rainforest. The largest limitation present was the relatively small number of samples that were collected due to time constraints on the study. Ideally, more plots would have been established and more than 20 samples of data would have been compiled to provide the broadest and most accurate range of data possible across the entirety of the rainforest's area. The data collected may not have been accurately representative of the terrestrial arthropod fauna of the SAS Rainforest, nor conclusive about the relationship between *A. gracilipes* density and terrestrial arthropod abundance and species richness as the number of samples and plots only partially encompassed the SAS Rainforest's land area, and excluded large chunks of habitat from data collection. Another major limitation of this study was the methodology used to collect data on *A. gracilipes* density and arthropod species richness and abundance. Pitfall traps and Berlese funnels, two standardized materials employed by

entomologists in similar studies (Evans, 2018), (Vanderwoude, 2006), and (Bos et al. 2008), were unable to be utilized in this study due to time constraints and a lack of materials necessary to conduct data collection this way. Pitfall traps and Berlese funnels are among the most effective and efficient methods for identifying and counting terrestrial arthropods above and below leaf litter (Vanderwoude, 2006). Likewise, they provide much more accurate data on terrestrial arthropod species richness and abundance than simply counting the terrestrial arthropods on the surface of the forest floor; the methodology employed by this study. In all, though there were several major limitations to accurate data collection on *A. gracilipes* density and terrestrial arthropod species richness and abundance in the SAS Rainforest, the collected and analyzed data still shows a few significant relationships between the variables.

Conclusion

This study investigated the relationship between *A. gracilipes* density and terrestrial arthropod species richness and abundance in the Singapore American School Rainforest. Regression analysis found a significant negative correlation between *A. gracilipes* density and terrestrial arthropod species richness and abundance, proving the existence of a relationship between the variables. Additionally, there was strong evidence that many arthropods in the SAS Rainforest are displaced from areas with higher *A. gracilipes* density.

The relationship between *A. gracilipes* density and native ant species richness and abundance in the SAS Rainforest was also investigated through regression analysis. Though the findings were not statistically significant enough to conclude, there is still an indication that *A. gracilipes* could be harming native ant species richness in the SAS Rainforest.

The findings of this study contributed markedly to the minimal preexisting cache of information on *A. gracilipes* in Singapore (Wang, 2022), (Smith, 1857). The findings, though

limited in scope, provide implications for possible future studies that may conduct comprehensive scientific assessment of *A. gracilipes* status and ecological impact across Singapore. Additionally, parts of the methodology used for collecting and analyzing data in this study could be replicated in future studies.

Implications

This study was the first to investigate the ecological impact of *A. gracilipes* in Singapore, providing implications for future research on this topic. In Singapore, the local status of *A. gracilipes* as either a native or invasive species remains unclear. This study provided strong evidence that *A. gracilipes* harms the arthropod fauna of a Singaporean ecosystem, which is a possible indication that *A. gracilipes* is an invasive species in Singapore and is destructive to a breadth of Singaporean biodiversity; however, the findings were limited, and conclusions this broad cannot be made. A recommendation related specifically to this study's research question and findings is for more experienced researchers to investigate *A. gracilipes'* impact on terrestrial arthropods and native ants locally to better conclude the ecological impact and status of *A. gracilipes* in Singapore.

Researchers conducting future studies that comprehensively investigate this relationship can determine locations across Singapore with established *A. gracilipes* populations, attempt to identify supercolonies where present, and conduct data collection using scientific methods defined in (Evans, 2018), (Vanderwoude, 2006), and (Bos et al. 2008). Additionally, sampling plot establishment using randomized length and width coordinates, the methodology outlined in this study, can be employed by future studies investigating *A. gracilipes* in Singapore.

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Appendix

Table 5

Sample table showcasing A. gracilipes density, arthropod diversity, and arthropod abundance for Plot 1.

Sample	Arthropod 1 Abundance (# of individuals per 1m ²)	Arthropod 2 Abundance (# of individuals per 1m ²)	Arthropod 3 Abundance (# of individuals per 1m ²)	Total arthropod diversity (# of species per 1m ²)	<i>A. gracilipes</i> per 1m ²
A	2	0	0	1	50
B	4	6	2	3	10
C	0	0	1	1	30
D	0	1	0	1	70
E	0	0	2	1	60
Total	6	7	5	3	220 (avg. 44 per 1m ²)

Figure 13

Real-life demonstration of locating Width coordinate (62L, 23W) from the Length coordinate of (62L, 0W) in the SAS Rainforest

