

Habitat augmentation drives secondary invasion: an experimental approach to determine the mechanism of invasion success

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Abstract. The entry of secondary invaders into, or their expansion within, native communities is contingent on the changes wrought by other (primary) invaders. When primary invaders have altered more than one property of the recipient community, standard descriptive and modeling approaches only provide a best guess of the mechanism permitting the secondary invasion. In rainforest on Christmas Island, we conducted a manipulative field experiment to determine the mechanism of invasion success for a community of land snails dominated by non-native species. The invasion of rainforest by the yellow crazy ant (*Anoplolepis gracilipes*) has facilitated these land snails, either by creating enemy-free space and/or increased habitat and resources (in the form of leaf litter) through the removal of the native omnivorous-detritivorous red land crab (*Gecarcoidea natalis*). We manipulated predator densities (high and low) and leaf litter (high and low) in replicated blocks of four treatment combinations at two sites. Over the course of one wet season (five months), we found that plots with high leaf litter biomass contained significantly more snails than those with low biomass, regardless of whether those plots had high or low predation pressure, at both the site where land crabs have always been abundant, and at the site where they have been absent for many years prior to the experiment. Each site was dominated by small snail species (<2 mm length), and through handling size and predation experiments we demonstrated that red crabs tend not to handle and eat snails of that size. These results suggest that secondary invasion by this community of non-native land snails is facilitated most strongly by habitat and resource augmentation, an indirect consequence of red land crab removal, and that the creation of enemy-free space is not important. By using a full-factorial experimental approach, we have confidently determined—rather than inferred—the mechanism by which primary invaders indirectly facilitate a community of secondary invaders.

Key words: *Anoplolepis gracilipes*; *Christmas Island*; *Gecarcoidea natalis*; *invasional meltdown*; *land snails*; *leaf litter*; *predation pressure*; *top-down and bottom-up*.

INTRODUCTION

The invasion success of non-native plants and animals is determined in part by the properties of the recipient community they are attempting to invade (Catford et al. 2009, Lockwood et al. 2009, Blackburn et al. 2011, Moles et al. 2012). Abiotic drivers, such as disturbance, are thought to be a strong determinant of invasion success by simultaneously altering niche availability, decreasing competition, and releasing pulses of resources (Davis et al. 2000). However, disturbance per se is not a static property of a community, and information on how a particular disturbance regime has changed is expected to be most informative (Moles et al. 2012). Similarly, properties of the recipient community can be altered by biotic components, particularly the presence and influence of resident invasive species. Successful invaders are known to alter niche availability through occupation (Richardson and Pyšek 2006, O'Loughlin et al. 2015) and local species

extinctions (Green et al. 2011), to simplify ecosystems, which reduces competition (Grosholz 2005, Flory and Bauer 2014), and to increase resources through altering belowground processes (Vitousek and Walker 1989, Flory and Bauer 2014). The invasion success of subsequent non-native species will be the outcome of these altered properties of the recipient community that are a direct result of the impacts of previous invaders. Although a key component of the “invasional meltdown hypothesis” (Simberloff and Von Holle 1999), the idea of invasion success being contingent on the presence and influence of previously successful invaders (secondary invasion) remains only loosely defined (D'Antonio and Dudley 1993) and under-researched (see Green et al. 2011, Jeschke et al. 2012).

Identifying and predicting secondary invasion requires an understanding of how species interact directly and indirectly (Traveset and Richardson 2006) following biotic and abiotic changes associated with the impacts of resident invasive species (Simberloff et al. 2013). In some cases, successful invaders will only alter a single key property of the recipient community and so the mechanism of secondary

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invasion can easily be identified; for example, as reduced competition (Grosholz 2005) or increased resources (Flory and Bauer 2014). However, rarely are ecosystem dynamics that simple and successful invaders can have myriad impacts that alter multiple properties of invaded communities (Simberloff and Von Holle 1999, O'Dowd et al. 2003). A potential secondary invader is likely to respond to many altered properties afforded by the impacted community and observational studies comparing invaded to non-invaded areas will not identify which of these mechanisms has the greatest importance in determining invasion success. In such cases, the mechanism of invasion success will only be confidently determined through full-factorial experiments that create all possible combinations of the various properties of the altered community.

The giant African land snail (*Achatina* [*Lissachatina*] *fulica*) is a known secondary invader of rainforest on Christmas Island (Green et al. 2011). Using a combination of modeling and experimentation, Green et al. (2011) determined the mechanism of invasion success was the creation of enemy-free space afforded by an invasive ant (yellow crazy ant *Anoplolepis gracilipes*), in mutualism with non-native honeydew-producing scale insects, extirpating a native omnivorous-detritivorous terrestrial crab (red land crab *Gecarcoidea natalis*). However, the deletion of the red crab also indirectly increases resource and habitat availability in the form of leaf litter (O'Dowd et al. 2003). There is a whole community of non-native land snails on Christmas Island that are able to enter intact rainforest, but are found in significantly higher densities in areas impacted by the invasive ant (O'Loughlin and Green 2015). The majority of these species are much smaller than *A. fulica* (Kessner 2006) and might therefore escape predation by the relatively large native crabs. On Christmas Island, the naturally abundant red crab is a recognized ecosystem engineer; responsible for species absences through top-down actions (direct predation of invertebrates; O'Dowd et al. 2003, Green et al. 2011) and ecosystem structure through bottom-up forces (consumption of leaf litter and seedling germinants; Green et al. 1997, O'Dowd et al. 2003). For the smaller species of non-native land snail on the island, the mechanism of their invasion success may be the removal of the bottom-up influence of the crab, which augments habitat and resource availability, rather than the removal of any top-down influence.

The aim of our study was to determine experimentally the relative importance of the direct and indirect changes to the recipient community by the actions of primary invaders on the invasion success of secondary invaders. This study specifically asked; (1) what is the relevant strength of the creation of enemy-free space vs. habitat augmentation (increased leaf litter) as the mechanisms of invasion success for land snails on Christmas Island, and (2) is the mechanism of invasion success dependant on whether snails are directly handled and preyed on by the native crab?

METHODS

Study system

Christmas Island (105°40' E, 10°30' S) is an isolated oceanic island (135 km², maximum elevation 360 m), 360 km south of Java in the northeastern Indian Ocean. Located in the humid tropics, the island experiences a monsoonal climate with most of the 2,000 mm mean annual rainfall occurring between December and May (Falkland 1986). Approximately 74% of the island supports broad-leaved, structurally simple tropical rainforest (Du Puy 1993). Sites for this study were located on the central plateau (>200 m a.s.l.) of the island in tall (~34 m height) closed forest on deep soils (forest type 1; Mitchell 1975).

On Christmas Island, the endemic red land crab (*Gecarcoidea natalis*) plays a key functional role in shaping the forest understory structure by largely regulating seedling recruitment and litter decomposition (Green et al. 1997, 1999, 2008). The omnivorous red land crab also opportunistically preys on some non-native species, providing the community with biotic resistance against invaders (Lake and O'Dowd 1991, Green et al. 2011). The yellow crazy ant (*Anoplolepis gracilipes*) is a pantropical invader that has spread rapidly across the Indo-Pacific region (Wetterer 2005) and, in association with honeydew-secreting insects (Neumann et al. 2016), has formed expansive high-density supercolonies on Christmas Island (O'Dowd et al. 2003, Abbott 2006). Where supercolonies have formed, red land crabs are extirpated through predation by yellow crazy ants, which has led to considerable changes in forest understory structure (O'Dowd et al. 2003). These changes offer a recipient community with increased habitat and resources in the form of leaf litter and enemy-free space. Non-native land snails establish abundant populations in impacted rainforest because they are not preyed on by yellow crazy ants and are likely facilitated by one or both of these altered properties (Green et al. 2011, O'Loughlin and Green 2015).

Mechanism experiment

In order to assess the mechanism of invasion success for the land snail community in rainforest on Christmas Island we conducted two manipulative experiments with two opposing yet complimentary aims. In one experiment, we decreased predation pressure and increased litter resources in an area where red crabs were present in naturally high densities; we hypothesized that our manipulations would increase the low-density snail community (facilitation experiment). In the other experiment, we increased predation pressure and decreased litter resources in an area where red crabs occurred in very low densities; we hypothesized that our manipulations would decrease the high-density snail community (inhibition experiment). We approached our hypothesis from both

directions to increase the likelihood of observing a response to our experimental treatments and increase confidence in our conclusions.

The facilitation experiment was undertaken at an intact site where yellow crazy ants had never invaded, and red land crabs had always been abundant (105°39'32.4" E, 10°27'2.7" S). Red land crabs were excluded from plots in order to mimic the effects of the yellow crazy ant invasion and facilitate the low-density snail population. The inhibition experiment was undertaken at a "ghosted" site where yellow crazy ants had indirectly caused the local extinction of red land crabs (105°40'5.9" E, 10°29'11.0" S). Ghosted rainforest on Christmas Island is where yellow crazy ant supercolonies have never formed, but in which red land crabs are absent or rare (<0.015 crabs/m²) because the local population had been killed while en route to the ocean during their annual breeding migration (Davis et al. 2008, Green et al. 2011, O'Loughlin and Green 2015). Red land crabs were enclosed in plots in order to mimic their density in intact rainforest and inhibit the presumably high-density snail population. Site history was determined through exploration of survey data collected by Parks Australia as part of an ongoing monitoring program (Maple 2012). Red land crabs enclosed for the inhibition experiment were all large adults (>90 mm carapace width) while abundant crabs at the facilitation experiment site were more varied in their size (60–105 mm carapace width).

Experimental design

For both the facilitation (crab exclusion) and inhibition (crab enclosure) experiments we manipulated predation pressure (crab density; high—low) and resources (leaf litter biomass; high—low) in 10 replicated blocks of four treatment plots (Fig. 1). The four treatments were the pairwise combinations of these properties; (1) predation high—resources low (P+ R-), (2) predation high—resources high (P+ R+), (3) predation low—resources high (P- R+), and (4) predation low—resources low (P- R-). Due to the current crab densities of each site, P+ R- was the control (unmanipulated) treatment for the facilitation experiment (Fig. 1A), and P- R+ was the control treatment for the inhibition experiment (Fig. 1B). Each treatment plot was 2 × 2 m. Plots within a replicate block were haphazardly arranged within an area, approximately 5–10 m apart. Blocks were randomly placed within a site, a minimum of 30 m apart.

Red crabs were enclosed or excluded using fences, so that each block consisted of two fenced plots and two unfenced plots. Semi-permeable fences (see Green et al. (1997)) were constructed from sheet metal and wire mesh that either denied access or confined red crabs while allowing litter invertebrates and small reptiles unimpeded access to the plots. Sheet metal (60 cm wide) was attached to wooden corner posts 10 cm above the ground. Wire mesh (10 × 10 mm aperture) was attached to the sheet metal extended to the ground and bent outwards to form

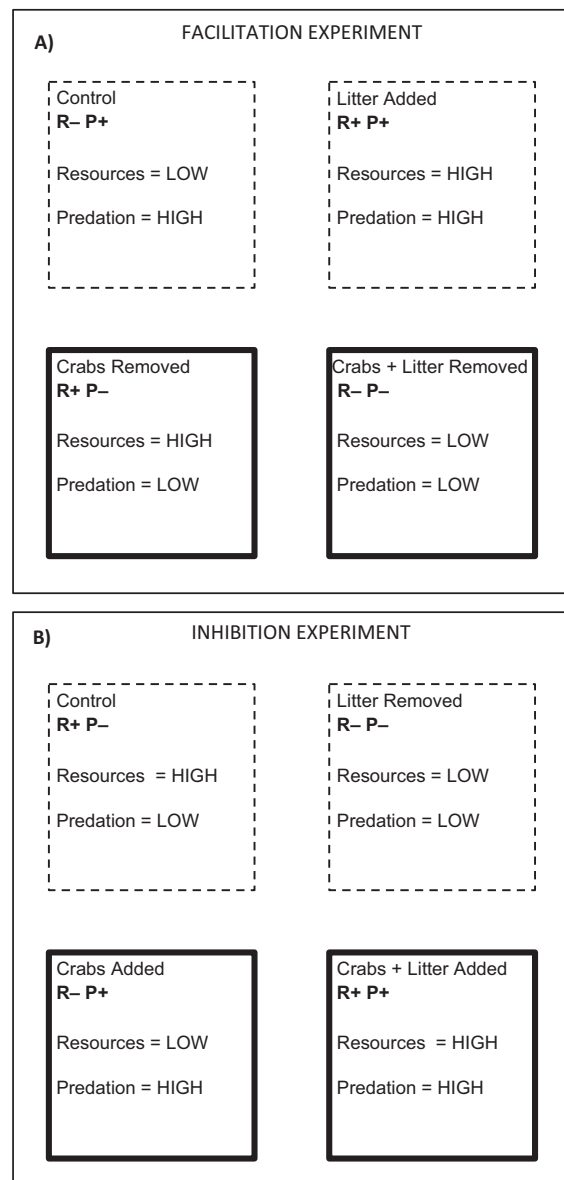


FIG. 1. Experimental treatments for (A) the facilitation experiment, and (B) the inhibition experiment. In both cases, two plots were open (dashed lines) and two plots were fenced (solid lines) in each block ($n = 10$). These fenced plots either excluded abundant crabs present at the site (facilitation experiment) or enclosed crabs reintroduced to a site of low crab abundance (inhibition experiment). Litter was manipulated (added or removed) from plots in order to achieve a full-factorial experiment of predation and resources—high and low. Photo examples of each of these treatments in the field are presented in Appendix S1.

an apron 50 cm wide around the fence. Unfenced plots were marked with wooden corner posts. In the case of the inhibition experiment, adult red crabs were taken from a nearby location and enclosed within fenced plots at a density of 0.75 crabs/m² (three crabs per plot), a density within the range observed in intact forest (Green 1997).

An indirect effect of removing red land crabs is the build up of leaf litter (Green et al. 1999), so we established the P- R- treatment in each experiment by excluding litter input. Litter excluders were built using a wooden frame and wire mesh (10 × 10 mm aperture) and suspended over each P- R- plot with the aid of fishing line. Wire mesh was bent into the shape of a gable roof, supported by a 2 m high wooden frame. The four corners were held out over the plot by fishing line attached to nearby trees. This shape meant fallen litter rolled down the excluder and did not shade the plot. The low edges of the suspended wire mesh were bent up slightly in order to capture the fallen litter, which became part of the litter input for the P+ R+ plots.

Both the facilitation and inhibition experiments were conducted over the course of a single wet season for a period of five months. The inhibition experiment was established first and began on January 15, 2013. The facilitation experiment followed, beginning on February 7, 2013.

Sampling methods

Sites were visited three times per week to record crab density and maintain treatments. Crab density was recorded in the morning as the number of crabs active on each plot at the time the observer arrived at the plot. At the inhibition experiment, if crabs had escaped from fenced plots, new ones were added and repairs made to the fence. Crabs would occasionally escape by burrowing under the wire mesh apron or creating holes in the mesh. For the facilitation experiment, if crabs were found within fenced plots they were simply removed. Red crab densities (and therefore predation pressure) were significantly altered relative to the natural state (Appendices S2 and S3).

To maintain high litter biomass in plots with high crab density (P+ R+), litter was constantly added to the plots over the course of the experiment. Litter was haphazardly added as was required in order to maintain the plots at approximately 100% litter cover. This involved adding a few generous handfuls of litter on most visits to the plots. The primary source of litter for these plots was leaves caught in the trap above the P- R- plots, which was added directly as snails were never found on this freshly fallen, suspended litter. Litter was collected from around the site as a secondary source because crabs removed more litter than fell into the plots. This litter was taken to the laboratory, shock dried (80°C for 20 min) and coarsely sieved (10 mm) which dislodged snails from the litter. Snail-free leaves remaining in the sieve were then added to the plots the following day. No litter was added or removed from plots of the other treatments. Percentage ground cover of leaf litter and bare ground was also recorded each month in each plot (Appendices S2 and S3), using a 1 × 1 m frame with a grid of fishing line placed in the approximate centre of each plot. Each of 100 intersecting grid points was recorded as being either leaf litter, bare ground or other (rock, log/stick or plant).

The land snail community of each plot was quantified monthly using a destructive quadrat approach. Each month, leaf litter and loose surface soil of one randomly placed 0.25 × 0.25 m quadrat per plot was collected and processed in the laboratory. Quadrats were collected away from the edges of plots and not from areas sampled in previous months. This method was chosen as visual searches were determined to be inaccurate in estimating abundance of this land snail community dominated by small individuals (<2 mm). Only 9.4% of the plot was disturbed over the six sampling occasions. Samples were oven dried (60°C) for ~20 min in order to dislodge snails from the wet leaves, prior to being separated into three fractions using a set of graded soil sieves (10, 2 and 0.5 mm). Leaf litter was retained and dried (55°C for 48 hr) from the 10 mm fraction for a coarse-litter-biomass estimate (Appendices S2 and S3). Land snail shells were visually picked from the 2 mm fraction. The 0.5 mm fraction was immersed in water with floating material being collected, oven dried, and then searched for land snails under a dissecting microscope. The grades of the sieves used meant separate samples of “large” snails (>2 mm in size) and “small” snails (<2 mm in size) were collected for each quadrat. All snails determined as alive at the point of sampling (visible foot and an intact and non-discolored shell) were collected and preserved in ethanol for later counts and identification.

Handling size and predation experiments

To assess the minimum handling size and test for direct predation of land snails by red crabs, two field-based experiments were undertaken. Minimum handling size was determined by presenting beads of various sizes to red crabs and recording their rate of removal. Beads were used because red crabs will collect, handle and investigate most objects encountered, irrespective of whether or not there is a food reward (O’Dowd and Lake 1991). Direct predation was determined by presenting tethered snails of various species to red crabs and recording their rate of removal. Both experiments were undertaken in February 2014, immediately adjacent to the site that used for the facilitation (crab exclusion) experiment the previous wet season.

The handling size experiment was conducted at four plots (8 × 8 m), approximately 50 m apart. In each plot, 10 perspex plates (0.5 × 0.5 m) were placed on the ground, each containing eight round, white, plastic beads—one each of 14, 12, 10, 8, 6, 4, 3 and 1.8 mm diameter. Slight depressions were drilled into the plates for the beads to rest in so that they would not roll off, and differently sized beads were randomly allocated to these depressions. The plates were randomly placed in each plot 2 d before the experiment so resident red crabs became accustomed to them. The experiment began at 07:20 h when beads were placed on each plate and ran for 9 h. One observer rotated around the four plots every hour; observing each for 15 min. Each hour, beads that were

TABLE 1. Parameter estimates for predictors of land snail abundance from the facilitation experiment. Results obtained by generalized linear mixed models (GLMMs) with Poisson distribution using block, litter biomass, litter cover, and crab density (Total and Small individuals models) as random effects. A "random observation" was also included as a random effect in the Total and Small individuals models in order to correct for over dispersion. Control treatment = P+ R-. Only significant Month:Treatment interactions are included.

Response variable	Parameter	Estimate	SE	Z
Land snail abundance				
<i>Total</i>	Intercept	2.67	0.23	11.66***
	Month (linear)	0.45	1.90	0.24
	Month (quadratic)	-8.08	1.82	-4.45***
	P+ R+	0.39	0.14	2.77**
	P- R+	0.43	0.14	3.09**
	P- R-	0.22	0.14	1.57
	Month (linear) : P+ R+	7.53	2.67	2.82**
	Month (linear) : P- R+	6.21	2.65	2.34*
<i>Large individuals</i> (≥2 mm)	Intercept	-7.23	3.38	-2.14*
	Month (linear)	-20.20	39.34	-0.51
	Month (quadratic)	-26.33	51.89	-0.51
	P+ R+	1.81	3.48	0.52
	P- R+	2.47	3.41	0.73
	P- R-	2.47	3.42	0.72
<i>Small individuals</i> (<2 mm)	Intercept	2.67	0.23	11.45***
	Month (linear)	0.48	1.88	0.26
	Month (quadratic)	-8.06	1.79	-4.49***
	P+ R+	0.38	0.14	2.75**
	P- R+	0.41	0.14	2.97**
	P- R-	0.20	0.14	1.44
	Month (linear) : P+ R+	7.39	2.63	2.80**
	Month (quadratic) : P+ R+	5.05	2.47	2.04*
Month (linear) : P- R+	5.95	2.62	2.27*	

* $P < 0.05$; ** $P < 0.01$ *** $P < 0.001$.

removed since the previous hour were recorded and direct observations of red crab activity made. Where possible, observations were made away from the plot using binoculars in order to minimize disturbance. Surveying ceased after 9 h as red crab activity was minimal.

The snail predation experiment was conducted on the same plots (three of the four) as the handling size experiment using the same set-up and sampling methodology. Each of the 10 plates in a plot contained one of each of seven species of land snail, tethered to the plate via a short length of cotton thread and secured to both the snail and the plate with a small amount of glue. Snails were attached 1 d before the experiment commenced. Land snail species used were juvenile *Achatina fulica* (Length (L) = 30.35 ± 0.79 SE mm, Width (W) = 18.19 ± 0.77 mm), *Bradybaena similaris* (L = 10.69 ± 0.41 mm, W = 6.66 ± 0.20 mm), *Japonia wallacei* (L = 9.52 ± 0.20 mm, W = 7.97 ± 0.23 mm), *Succinea solidula* (L = 10.03 ± 0.43 mm, W = 6.52 ± 0.31 mm), *Subulina octona* (L = 9.45 ± 0.38 mm, W = 3.24 ± 0.08 mm), *Liardetia scandens* (L = 2.26 ± 0.13 mm, W = 1.79 ± 0.12 mm) and *Georissa williamsi* (L = 1.53 ± 0.04 mm, W = 1.33 ± 0.05 mm). All species are invasive on Christmas Island except for *J. wallacei* and *S. solidula*, which are considered native (Kessner 2006). Plots were observed for 20 min every hour for 7 h (beginning at 0720), and then once at 24 h since

the start. Each hour, snails that were removed since the previous hour were recorded and direct observations of red crab activity made.

Data analysis

Linear mixed models with analysis of variance were used to test whether our treatments, and the interaction of time, were good predictors of crab density, litter biomass and litter cover (Appendix S3). This analysis was undertaken to assess the appropriateness of our treatments. Land snail abundance was modeled using generalized linear mixed models with Poisson distributions and logit link functions. Total land snail abundance, as well as abundance of large (>2 mm) and small (<2 mm) individuals, were modeled as a function of time and treatment (and the interaction), with replicate block, litter biomass, litter cover and crab density included as random effects. Time (Month) was modeled as a polynomial factor (linear and quadratic) to improve explanatory power. Plots of residuals against fitted values, residual frequency histograms, quantile-quantile plots and residual variation box plots were examined to verify homogeneity and expected properties of residuals (Zuur et al. 2009). Tests for overdispersion were

undertaken to assess whether there was additional variance in the data than assumed by the Poisson distribution (Crawley 2013). If models were overdispersed, a random observation was included as a random effect in order to correct for the unexplained variance (Zuur et al. 2009). One-way ANOVA's with Bonferroni post-hoc tests were used to test for differences in removal of beads and snails in the handling size and predation experiments. All models were performed using the lme4 package (Bates et al. 2014) in R version 3.1.1 (R Core Team 2014).

RESULTS

A total of 47,625 snails representing 17 species and 9 families were sampled (Appendix S4). Of these, 13 species were invasive, three were native, and one species was of uncertain biogeographic status. Land snails were almost 6-fold more abundant at the inhibition experiment site, accounting for 84% (40,138) of individuals sampled. Despite this difference in land snail abundance, both the facilitation and inhibition experiment recorded 16 species. The cryptogenic *Georissa* sp. (Hydrocenidae)

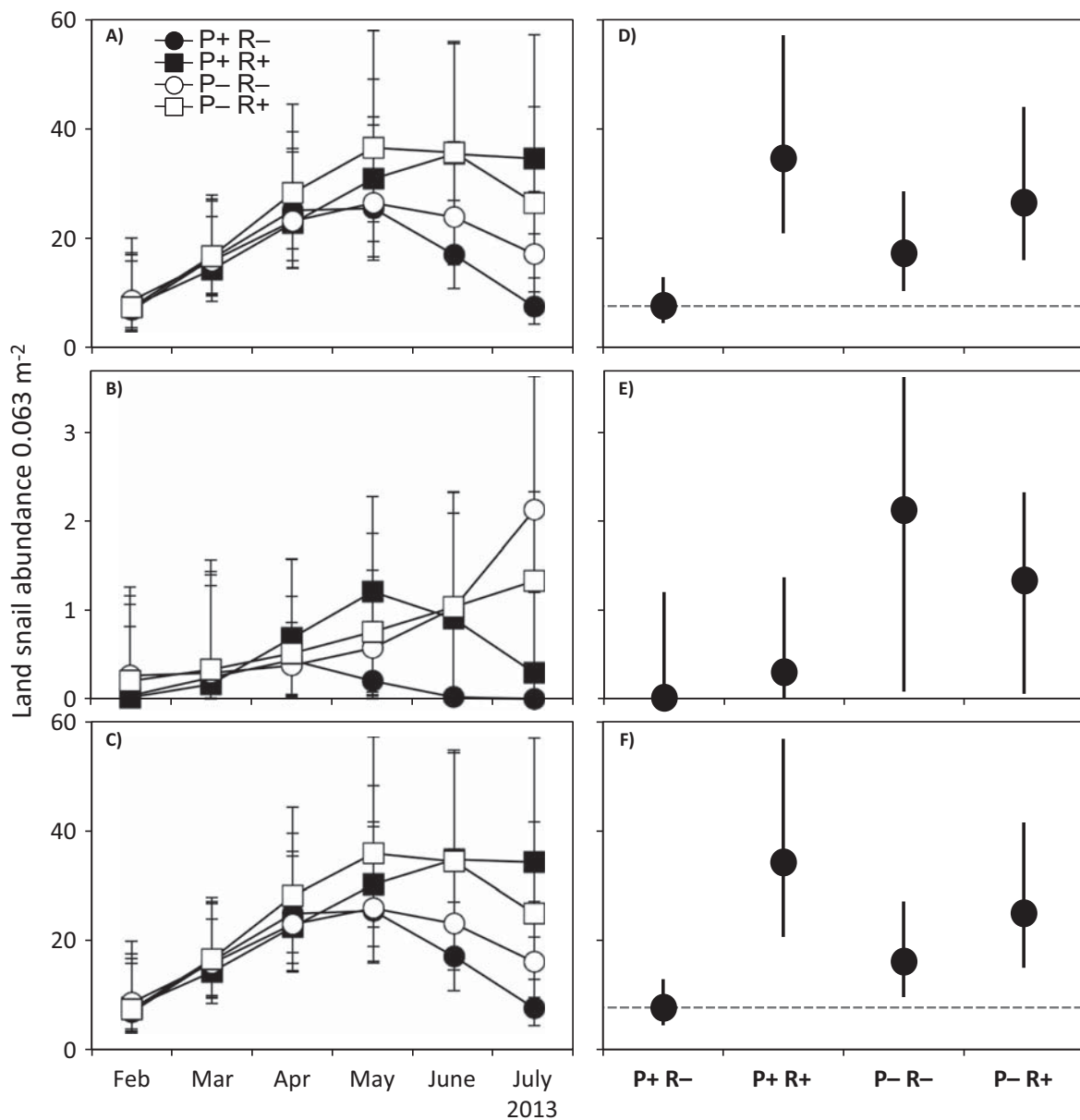


FIG. 2. Facilitation experiment. Mean ($\pm 95\%$ CI) land snail abundance in experimental treatments for the entire experiment (left hand column) and for just the final month (right hand column) as calculated by generalized linear mixed models. (A, D) the total population, (B, E) large individuals (≥ 2 mm size), and (C, F) small individuals (< 2 mm size). Dashed lines (D-F) indicate mean abundance of control treatment (P+ R-).

TABLE 2. Parameter estimates for predictors of land snail abundance from the inhibition experiment. Results obtained by generalized linear mixed models (GLMMs) with Poisson distribution using block, litter biomass, litter cover, and crab density as random effects in all models. A random observation was also included as a random effect in the Total and Small individuals models in order to correct for over dispersion. Control treatment = P- R+. Only significant Month:Treatment interactions are included.

Response variable	Parameter	Estimate	SE	Z
Land snail abundance				
<i>Total</i>	Intercept	5.13	0.17	30.26***
	Month (linear)	2.38	1.62	1.46
	Month (quadratic)	-1.02	1.53	-0.67
	P+ R+	0.22	0.12	1.85
	P+ R-	-0.50	0.12	-4.20***
	P- R-	-0.72	0.12	-5.99***
	Month (linear) : P+ R-	-5.57	2.30	-2.30*
	Month (linear) : P- R-	-6.85	2.31	-2.97**
<i>Large individuals</i> (≥2 mm)	Intercept	2.23	0.18	12.52***
	Month (linear)	4.72	2.07	2.29*
	Month (quadratic)	-2.72	2.13	-1.28
	P+ R+	0.07	0.14	0.51
	P+ R-	-0.46	0.15	-3.15**
	P- R-	-0.74	0.15	-4.93***
	Month (quadratic):P- R-	-7.07	3.32	-2.13*
	Intercept	5.07	0.17	29.27***
<i>Small individuals</i> (<2 mm)	Month (linear)	2.37	1.70	1.39
	Month (quadratic)	-1.24	1.60	-0.77
	P+ R+	0.23	0.12	1.85
	P+ R-	-0.51	0.13	-4.04***
	P- R-	-0.72	0.13	-5.70***
	Month (linear) : P+ R-	-5.68	2.41	-2.35*
	Month (linear) : P- R-	-6.94	2.42	-2.87**

* $P < 0.05$; ** $P < 0.01$ *** $P < 0.001$

dominated plots at the inhibition site, accounting for 73% (29,488) of all individuals observed. The next most common species, the invaders *Georissa williamsi*, *Subulina octona* (Subulinidae) and *Paropeas achatinaceum* (Subulinidae), made up a further 22% (8,734 individuals). Similarly, *G. williamsi* was the most common species at the facilitation site (55%; 3,616 individuals), with *Georissa* sp. and the invaders *Liardetia scandens* and *L. doliolum* (Helicarionidae) also abundant (35%; 2,261 individuals). In both experiments, the remaining 12 species contributed only 5–10% of total land snail abundance at a site. Small individuals (<2 mm) dominated at each site, accounting for 98.5% and 95% of the total sample for the facilitation and inhibition experiments respectively.

Facilitation experiment

Total land snail abundance and abundance of the smallest individuals (<2 mm) were both significantly influenced by time and treatment (Table 1, Fig. 2). Total abundance was significantly higher in the two resource high treatments compared to the control treatment (P+ R-) (Fig. 2A), and by the fifth month, there was around a 4-fold increase in snail abundance in those treatments (Fig. 2D). Abundance of the larger individuals (≥2 mm length) was not influenced by time or treatment (Table 1,

Fig. 2B). By the fifth month, abundance of larger individuals appeared higher in those treatments where predation pressure was low, although confidence intervals were overlapping because there were few large snails at the site (Fig. 2E). As the community was dominated by the smaller individuals, the predicted response of the abundance of those snails <2 mm in size was the same as for the total sample (Table 1, Fig. 2C, F).

Inhibition experiment

Total land snail abundance, abundance of the larger individuals (≥2 mm) and abundance of the smallest individuals (<2 mm) were all significantly influenced by both treatment and the interaction of time and treatment for low resource treatments (Table 2, Fig. 3). Only the abundance of the larger individuals was significantly influenced by time alone (Table 2). Total land snail abundance was significantly lower in the two resource low treatments than in the control treatment (P- R+) (Fig. 3A), and by the fifth month there was around a 4-fold decrease in snail abundance in those treatments (Fig. 3D). The abundances of both the larger and smaller individuals were also significantly decreased in those treatments where resources were lower than the control (Fig. 3B, E, C, F respectively).

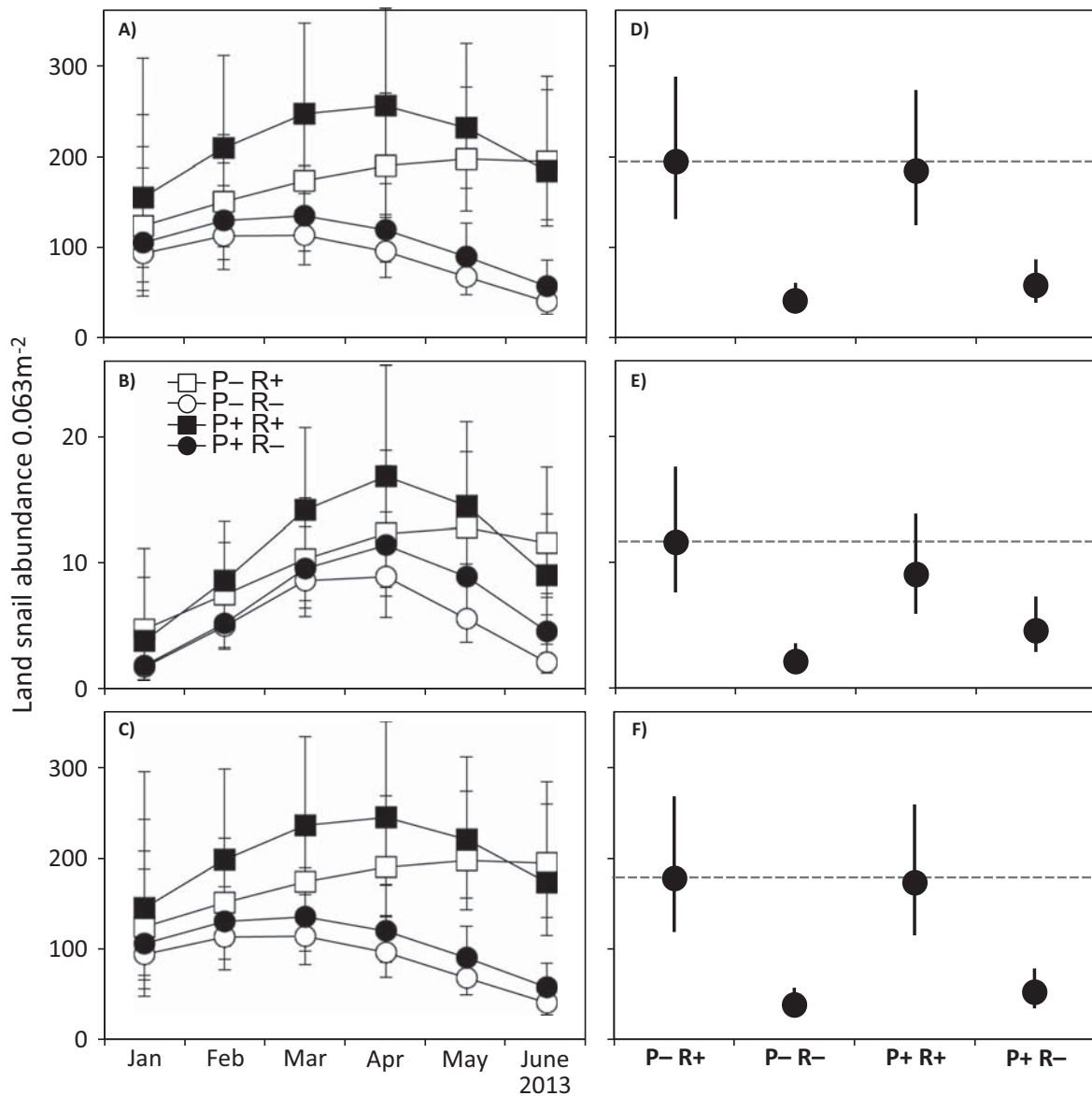


FIG. 3. Inhibition experiment. Mean ($\pm 95\%$ CI) land snail abundance in experimental treatments for the entire experiment (left hand column) and for just the final month (right hand column) as calculated by generalized linear mixed models. (A, D) the total population, (B, E) large individuals (≥ 2 mm size), and (C, F) small individuals (< 2 mm size). Dashed lines (D–F) indicate mean abundance of control treatment (P– R+).

Handling size and predation experiments

There were significant differences in the percent removal of both beads ($F_{7, 24} = 12.18, P < 0.001$) and snails ($F_{6, 14} = 17.4, P < 0.001$) of different sizes by the end of each experiment. In both cases, a significantly higher percentage of the large objects were removed compared to the small (Fig. 4). Red crabs removed over 50% of those beads > 10 mm diameter while only intercepting fewer than 20% of beads ≤ 6 mm in diameter (Fig. 4A). Similarly, over 50% of the largest four snail species (≥ 9.5 mm mean length) were removed compared to the

smallest two species (≤ 2 mm mean length), which were not taken at all (Fig. 4B).

DISCUSSION

Through manipulation of both predation pressure and resource availability in two experiments, we found that increased leaf litter was the more important mechanism driving the abundance of land snail species following the impacts of the invasive yellow crazy ant. These findings are significant for two reasons. First, we determined—rather

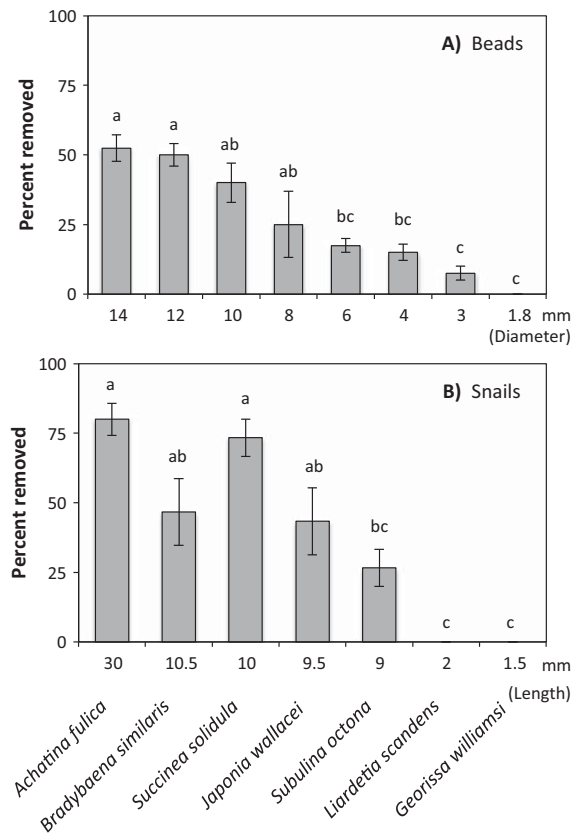


FIG. 4. Mean (\pm SE) percent removed by red crabs at the end of the experiment for (A) the bead handling size experiment ($n = 4$, end time = 9 h), and (B) the snail predation experiment ($n = 3$, end time = 24 h). Beads/snail species ordered from largest to smallest based on diameter and mean length (mm), respectively. Letters above denote significant differences as determined by Bonferroni post-hoc tests.

than inferred—the mechanism of invasion success in a case in which the recipient community offered multiple altered properties to which non-native land snails could respond. Second, our results coupled with those of Green et al. (2011) who found strong predatory effects of red crabs on *A. fulica*, demonstrate that red crabs inhibit the land snail community on Christmas Island through a combination of direct top-down and indirect bottom-up processes.

Increased habitat and resources

The experiment that aimed to facilitate the land snail community found snail abundance to increased significantly in plots where litter biomass was manipulated to remain high, regardless of the level of predation pressure. The finding that snails are promoted by increased leaf litter was not surprising as higher snail abundance is commonly found in higher amounts of litter (Aubry et al. 2005, Liew et al. 2010, de Chavez and de Lara 2011), including on Christmas Island (O'Loughlin and Green 2015). Leaf litter is a key habitat and food resource for

these ground-dwelling detritivores, which play a key functional role in nutrient cycling through litter consumption and promotion of microbial growth (Meyer et al. 2013). Bultman and Uetz (1982) demonstrated through leaf litter manipulation experiments that litter depth increased structural complexity that was important for supporting high abundances of litter-dwelling spiders. A more heterogeneous environment supports a greater amount and diversity of niche space that in turn will support a greater number of individuals (Bultman and Uetz 1982, Liew et al. 2010). Of the two key community changes we investigated, higher biomass of leaf litter in rainforest impacted by the yellow crazy ant was clearly the more important mechanism, facilitating the increased abundance of land snails on Christmas Island.

Similarly, the experiment that aimed to inhibit an already highly abundant land snail community found snail abundance decreased significantly where litter biomass was manipulated to be low, regardless of the level of predation pressure. Our ability to demonstrate the mechanism in two directions adds significant strength to the conclusion that habitat augmentation drives invasion success for land snails in this impacted community. Litter removal studies have demonstrated leaf litter presence, and decomposition to be vital parts of abiotic ecosystem function (Sayer 2006). However, few studies experimentally decrease leaf litter and search for a biological response. Most commonly, leaf litter is experimentally added and a corresponding increase in biological activity observed (Bultman and Uetz 1982, Melody and Richardson 2004, Batzer and Palik 2007, Tieggs et al. 2008). The results of our inhibition experiment demonstrated that the return of the red crab to rainforest previously impacted by the yellow crazy ant would decrease the abundant land snail community, as leaf litter once again becomes a limited resource.

On plots where leaf litter biomass was high the abundance of land snails was remarkably variable. In particular, snail abundance on resources-high (R+) plots in the inhibition experiment ranged from $c. 150$ – 300 individuals $0.063/\text{m}^2$. This variability could be due to two factors. First, land snail abundance can differ considerably across relatively small patches of rainforest without a clear mechanism (de Winter and Gittenberger 1998, O'Loughlin and Green 2015) suggesting that due to either chance or much finer-scale processes, some plots would contain significantly more individuals than others. Second, litter biomass sometimes varied considerably among plots of the same treatment (Appendix S2) because of the inherent difficulties establishing experimental treatments within a natural setting. Within-treatment variability can be high in litter manipulation experiments (Melody and Richardson 2004, Sayer 2006) and sometimes treatments may not work at all (Tieggs et al. 2008). These difficulties highlight the importance of treatment replication when conducting manipulative ecosystem experiments.

The land snail community in rainforest on Christmas Island is dominated by small ground-dwelling species

(<2 mm size) and as such, results were similar when considering the abundance patterns of the whole community or only the smallest individuals. Our results suggest that these snails are able to avoid direct predation by red crabs and are only limited by the availability of litter resources in areas where red crabs dominate. This is further supported by our handling size and predation experiments in which no beads or snails ≤ 2 mm in size were handled by red crabs. Relatively small crabs (<50 mm carapace width) will actively prey on small snails in intertidal ecosystems (Bertness et al. 1981, Trussell 1996). Red crabs observed within our experimental site were all larger than 50 mm and the largest male crabs in plateau rainforest can grow to >120 mm carapace width and >500 g mass (Green 2004). The tips of the chelae of larger crabs are relatively blunt compared to those of smaller crabs, making them unlikely to have the dexterity to handle very small objects. Incidental predation of these smallest snails could possibly occur through red crabs consuming leaves with land snails attached. Although possible, snail abundance was lower, or remained low, where both predation pressure and resources were low (P– R–) leading us to conclude that the removal of red crab predation alone is not a strong enough mechanism to facilitate abundance increases for the majority of land snail species on Christmas Island.

Creation of enemy-free space

Although creation of enemy-free space as a direct consequence of the invasive yellow crazy ant was not a mechanism explaining the invasion success of the land snail community broadly, other studies have found it does explain invasion success of the *A. fulica*, the largest species on Christmas Island (Lake and O'Dowd 1991, Green et al. 2011). Feeding experiments and modeling *A. fulica* spread have shown that the probability of invasion was facilitated 253-fold in ant supercolonies but impeded in intact forest where predaceous red crabs remained abundant (Green et al. 2011). Neither of our experimental sites contained this species, and only a small percentage of the total community were individuals >2 mm in size. Although low leaf litter biomass was the significant inhibiting property of intact rainforest for these snails, the low density of larger individuals, relative to smaller individuals, at both sites may indicate these species are more frequently encountered and consumed by red crabs.

Our handling size and predation experiments demonstrated that red crabs would more readily handle larger objects. For both beads and the live snails, the highest removal was seen for the largest item offered and percent removal decreased with size until the smallest items were handled rarely or not at all. Red crabs are generalist and opportunistic feeders (O'Dowd and Lake 1989, 1991, Lake and O'Dowd 1991) and all direct observations of either bead or snail handling involved the red crab moving its chelae along the substrate in a sweeping

motion until it encountered something it was interested in (L. O'Loughlin pers. obs.). In an earlier experiment, O'Dowd and Lake (1991) found removal rates of plastic beads was similar to the removal rates of several kinds of fruits, which suggests that red crabs are likely to collect and investigate most objects encountered, irrespective of whether there is a food reward. Therefore, our pattern of decreased handling with size was probably the result of decreased opportunity for a crab to encounter the object; a larger item is probably encountered more readily than a smaller one.

CONCLUSIONS

Increased resource levels in recipient communities can significantly influence invasion success (Davis et al. 2000, Rowles and Silverman 2009). In this study, we found that habitat augmentation and increased resources in the form of leaf litter was the most important factor in influencing the land snail community as a whole. Our finding is in contrast to those manipulative studies that find increasing resources and removing predation will both increase a population independently, yet the most significant response is when both are altered (Dyer and Letourneau 1999, Byrom et al. 2000, Melody and Richardson 2004). We experimentally determined that the increased abundance of the majority land snail species in rainforest on Christmas Island is facilitated exclusively by indirect habitat augmentation as a result of the invasive ant-scale mutualism.

Furthermore, the combination of this study with that of Green et al. (2011)—who found strong predatory effects of red crabs on *A. fulica*—documents a community influenced by both top-down and bottom-up forces that are driven by the actions of a single organism. The omnivorous red crab provides top-down control directly through predation (for the largest species; Green et al. 2011) and bottom-up control indirectly by consuming leaf litter, thereby limiting resources (all other species; this study). Omnivores use many different energy sources within the nested hierarchy of food webs, and the simplified view of a trophic chain may seriously underestimate their role in stabilizing and determining ecosystem properties (Terradas and Penuelas 2011). By removing the red crab, yellow crazy ant supercolonies on Christmas Island have altered both top-down and bottom-up forces (O'Dowd et al. 2003) that inhibit the land snail community. As the land snail community of Christmas Island is dominated by non-native species, their invasion success in terms of significant population growth as a result of the impacts of the invasive ant-scale mutualism constitutes a community-scale secondary invasion.

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