

Predator-induced collapse of niche structure and species coexistence

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Biological invasions are both a pressing environmental challenge and an opportunity to investigate fundamental ecological processes, such as the role of top predators in regulating biodiversity and food-web structure. In whole-ecosystem manipulations of small Caribbean islands on which brown anole lizards (*Anolis sagrei*) were the native top predator, we experimentally staged invasions by competitors (green anoles, *Anolis smaragdinus*) and/or new top predators (curly-tailed lizards, *Leiocephalus carinatus*). We show that curly-tailed lizards destabilized the coexistence of competing prey species, contrary to the classic idea of keystone predation. Fear-driven avoidance of predators collapsed the spatial and dietary niche structure that otherwise stabilized coexistence, which intensified interspecific competition within predator-free refuges and contributed to the extinction of green-anole populations on two islands. Moreover, whereas adding either green anoles or curly-tailed lizards lengthened food chains on the islands, adding both species reversed this effect—in part because the apex predators were trophic omnivores. Our results underscore the importance of top-down control in ecological communities, but show that its outcomes depend on prey behaviour, spatial structure, and omnivory. Diversity-enhancing effects of top predators cannot be assumed, and non-consumptive effects of predation risk may be a widespread constraint on species coexistence.

Humans have accelerated the rate at which predators colonize formerly isolated ecosystems such as islands and lakes¹, but the biological consequences of these invasions are variable and difficult to predict². The effects are often substantial: introduced predators are a leading cause of extinction³, which in turn alters food-web structure, ecosystem functions, and evolutionary trajectories^{2,4}. In other cases, however, colonizing populations naturalize with little effect on resident communities⁵. Theory suggests how such variability might arise. For example, predators can either facilitate or impede the coexistence of prey species—and thus augment or diminish biodiversity—under a range of plausible assumptions^{6–8}. Similarly, the addition of a predator species can have opposing effects on community properties such as food-chain length, depending on the ecological context and the dietary breadth of the predator^{9–11}. There is a need for empirical tests of the effects of introduced predators and the mechanisms through which these effects emerge, both to advance our understanding of basic ecological and evolutionary questions¹² and to guide environmental management⁵. However, such tests are challenging because invasions can rarely be studied from the outset as they occur, much less with experimental control and replication.

Predators can influence the coexistence of competing prey species through multiple mechanisms. Classic studies suggested that predators promote prey diversity by depleting abundant or dominant competitors, thereby preventing competitive exclusion^{13–15}. Although more-recent work has shown that this outcome is neither theoretically general^{6,16,17} nor empirically commonplace¹⁸, the idea that predators generally facilitate coexistence remains widespread in the literature^{19,20}.

The discovery that non-consumptive effects of predators on prey behaviour often outweigh the effects of direct consumption^{21,22} further complicates efforts to predict the consequences of predator introductions⁸. Species coexistence is stabilized by differentiation in resource use (niche partitioning), such that intraspecific competition outweighs interspecific competition¹⁶. However, fear-driven behavioural avoidance of predators might disrupt such niche structure by forcing prey species to converge on a narrower suite of resources that is associated with low predation risk^{23,24}, thereby intensifying interspecific competition and destabilizing coexistence.

Any effects of introduced predators on prey abundance and behaviour are likely to influence community properties—but, again, theory offers contrasting predictions. Intuition suggests that adding a top predator should lengthen food chains. But the opposite outcome can arise if the predator eliminates intermediate-level consumers, feeds at multiple trophic levels (trophic omnivory), or both²⁵. Which of these mechanisms prevails may depend on environmental attributes (notably ecosystem size) and how food webs reorganize in response to the introduced predator^{9–11,26–30}.

Here we present results from a six-year-long whole-ecosystem manipulation designed to investigate the dynamics of predator introductions and their effects on island communities.

Experimental design and initial conditions

Small Bahamian islands on which lizards are top predators³⁰ are ideal model systems for studying species introductions under natural conditions^{31–34}. On 16 islands that were initially occupied by a widespread,

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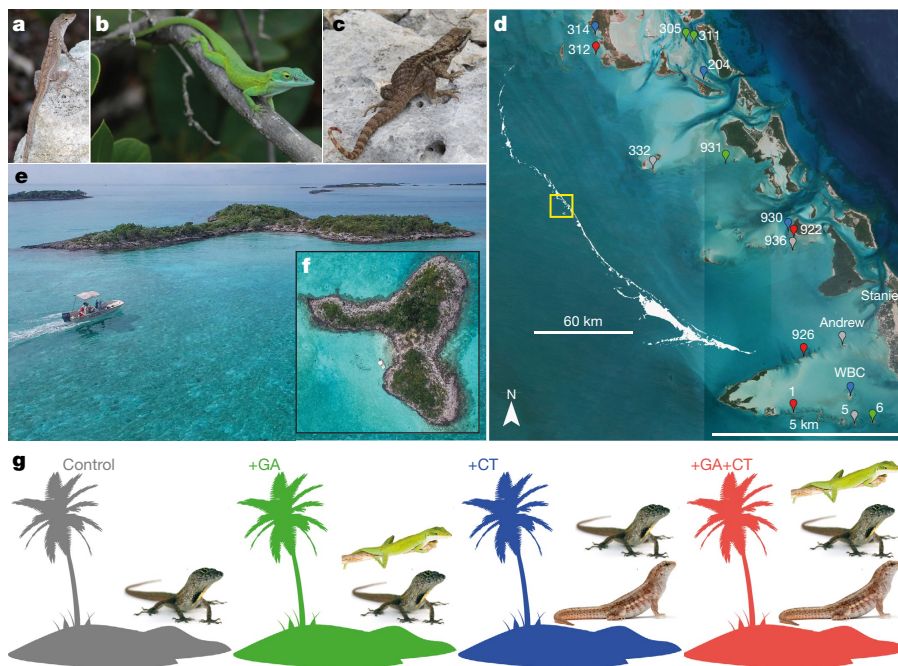


Fig. 1 | Study system and experimental design. **a–c**, Study species: brown anole (**a**); green anole (**b**); and curly-tailed lizard (**c**). **d**, Location of study area (yellow box) within the Exuma island chain, Bahamas (white silhouette), and distribution of the 16 experimental islands near Staniel Cay (satellite image). Marker colours indicate the experimental treatments schematized in **g**, and the name of each island is shown. WBC, White Bay Cay. **e–f**, Oblique (**e**) and top-down (**f**) photographs of island 922, a representative +GA+CT island with a vegetated area of 1,648 m² (experiment-wide mean, 1,635 m²). The boat, 5.5-m long, provides an indication of scale. **g**, Schematic of the factorial experimental treatments ($n = 4$ islands each). Imagery in **d**, Google Earth imagery copyright 2019 DigitalGlobe, map silhouette created using open-access data from the Global Administrative Areas Database (<http://gadm.org>); **e, f**, Day's Edge Productions; **g**, Island image designed by www.Vexels.com; lizard images, Dreamstime, Shutterstock, and Alamy.

semi-terrestrial lizard species (brown anole, *A. sagrei*), we introduced combinations of a ground-dwelling predator (curly-tailed lizard, *L. carinatus*)³³ and/or an arboreal competitor (green anole, *A. smaragdinus*)³² in a 2×2 factorial design (Fig. 1, Extended Data Fig. 1). Caribbean *Anolis* lizards are an adaptive radiation, and brown anoles and green anoles exhibit traits associated with specialization in more-terrestrial and more-arboreal niches, respectively³⁵. All three lizard species eat arthropods that span multiple trophic levels^{30,36}. Adult curly-tailed lizards can also eat anoles³⁶, but the reverse is not true; this makes curly-tailed lizards both the top predator²⁵ and an intraguild predator (one that potentially both eats and competes with its prey³⁷) in the context of this experiment. Because curly-tailed lizards are ground-dwelling, we expected their consumptive effects to fall disproportionately on brown anoles. Thus, for resident brown anoles, the experiment tests the independent and interactive effects of introducing intraguild top predators and congeneric competitors ($n = 4$ islands for each of the 4 factorial treatment combinations: addition of curly-tailed lizards (+CT), addition of green anoles (+GA), addition of both species (+GA+CT), and unmanipulated control islands) (Fig. 1). For introduced green anoles, the experiment tests whether top predators influence establishment success on islands that are already occupied by congeneric competitors—a frequent real-world colonization scenario³⁵. Species coexistence is operationally defined by the ability of invading populations to increase from low initial abundances and persist¹⁶.

We conducted baseline censuses of brown-anole populations in May 2011 (range, 42–457 individuals per island; mean \pm s.e.m., 210 ± 29 individuals). We then randomly assigned treatments to islands and introduced 5–7 curly-tailed lizards and/or 10–11 green anoles (all collected from nearby larger islands on which these species occur naturally) to simulate colonization by small founding populations. All of the islands had similar vegetation structure and were stratified by area (range, 487–3,320 m²; mean \pm s.e.m., $1,635 \pm 198$ m²) during treatment assignment (see Methods). Neither island area nor initial brown-anole population size differed by treatment (analysis of variance (ANOVA) $F_{3,12} = 0.19$, $P = 0.90$ for island area; $F_{3,12} = 1.46$, $P = 0.27$ for initial population size). In subsequent years, we quantified population sizes, habitat use, diet composition, and trophic position of all three lizard species.

Population trajectories and species coexistence

We hypothesized that on +GA islands, the two anole species would compete³²—reducing brown-anole abundance relative to control

islands—but that green-anole populations would establish and grow because spatial niche separation prevents competitive exclusion (Extended Data Fig. 2a, b). On +CT islands, we expected curly-tailed lizards to reduce the abundance of brown anoles and force individuals to become more arboreal^{33,34} (Extended Data Fig. 2c). For +GA+CT islands, we considered two alternative hypotheses (Extended Data Fig. 2d, e). Under a 'keystone-predation' model¹³, predicated on strong consumptive effects, predators rapidly reduce the abundance of brown anoles and facilitate the establishment of green anoles by relaxing interspecific competition. Alternatively, if consumptive effects are weak and/or slow to emerge, then the non-consumptive effects of predators on the behaviour of brown anoles (increased arboreality) might collapse spatial niche structure and intensify interspecific competition within arboreal refuges²⁴. Under this 'refuge-competition' model, curly-tailed lizards suppress the populations of both anole species and impede coexistence, resulting in the failure of green-anole populations to establish and increase.

Our results refute the keystone-predation model and support the refuge-competition model. From 2011–2016, curly-tailed lizard populations increased by more than fivefold, to an average of 30 individuals, irrespective of treatment (Fig. 2a). All green-anole populations increased on +GA islands, by an average of eightfold (range, 55–161 individuals per island in 2016), which indicates stable coexistence (Fig. 2b). On +GA+CT islands, however, green anoles were heavily suppressed: one population grew moderately, one remained static, and two went extinct (Fig. 2b, c). Thus, curly-tailed lizards destabilized the coexistence of brown and green anoles, falsifying the central prediction of the keystone-predation model. All brown-anole populations on control islands increased, by an average of 177% (range, 136–230%). Brown-anole populations in other treatments showed significant deficits relative to this standard: an increase of only 40% on +GA islands (range, 11–71% increase), no net growth on +CT islands, and a 42% decrease on +GA+CT islands (range, 21–63% decrease) (Fig. 2d). Thus, competitors and predators independently suppressed brown-anole populations, and suppression was strongest where both competitors and predators were present.

Predator-induced collapse of niche structure

Data on the habitat use and diet composition of each lizard species indicate that non-consumptive effects of the top predator increased interspecific competition for space and food, consistent with the refuge-competition model. Habitat use by brown anoles was similar on all

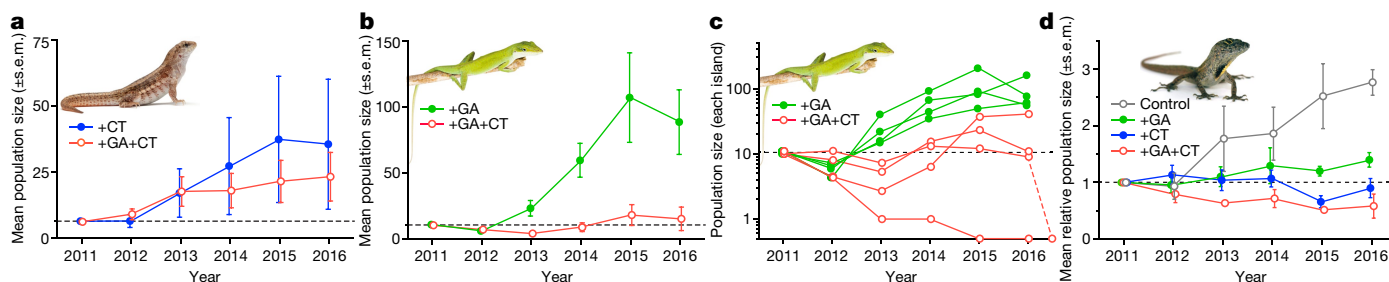


Fig. 2 | Population trajectories of each lizard species by experimental treatment. **a**, Introduced populations of curly-tailed lizards increased, with no significant difference between islands with (+GA+CT) and without (+CT) green anoles ($t = 0.40$, $P = 0.70$, degrees of freedom (d.f.) = 5, $n = 7$ islands). **b**, On average, introduced populations of green anoles increased more than eightfold on +GA islands, but did not increase on +GA+CT islands ($t = -2.81$, $P = 0.031$, d.f. = 6, $n = 8$ islands). **c**, Trajectories of green-anole populations on each island, showing increases of 5–15-fold on all islands without curly-tailed lizards; on islands with curly-tailed lizards, 1 population increased, another exhibited no net growth, and 2 went extinct within 6 years (the population on island 312 disappeared by January 2017, after the final census, dashed red line).

islands before the manipulation and subsequently changed little on islands without curly-tailed lizards, aside from a tendency for brown anoles to occur closer to the ground on +GA islands (Fig. 3). However, on +CT and +GA+CT islands, brown anoles rapidly became arboreal: mean perch height doubled, and the proportion of individuals observed on the ground decreased from 37% to 9% (Fig. 3). Thus, there was limited spatial overlap between the anole species on +GA islands, but frequent overlap on +GA+CT islands. On +GA+CT islands, green anoles occupied the highest and narrowest perches, whereas brown anoles were confined to the middle third of vegetation height (Fig. 3g–i) and exhibited sharply reduced variability in habitat use (Extended Data Fig. 3). We observed multiple agonistic interspecific interactions on +GA+CT islands, including dewlap displays, chasing, and displacement (Extended Data Fig. 1a–d).

We quantified diet composition using DNA metabarcoding³⁸ of arthropod prey in faecal samples from 315 individual lizards, taken from all species–treatment combinations (Supplementary Data 1, 2). In addition, we conducted a quantitative PCR assay to test for the presence of anole DNA in faecal samples from 51 curly-tailed lizards, taken from 7 of the 8 islands on which curly-tailed lizards were present ($n = 28$ individuals from +GA+CT islands; $n = 23$ individuals from +CT islands).

The three lizard species consumed overlapping suites of arthropod prey, but in different relative abundances (Fig. 4, Supplementary Table 1). Curly-tailed lizards and green anoles typified ‘terrestrial’ and ‘arboreal’ diets, respectively, and these diets were extremely dissimilar (Fig. 4a, b, Extended Data Table 1). Indicator-species analysis identified 33 prey taxa that were uniquely associated with curly-tailed lizards, 31 taxa that were uniquely associated with green anoles, and no taxa that were associated with both of these species (Supplementary Table 1). The diets of brown anoles were intermediate between these extremes, comprising both terrestrial and arboreal components: four prey taxa were uniquely associated with brown anoles, two with brown anoles and curly-tailed lizards, and three with brown and green anoles (Supplementary Table 1). The top three prey taxa overall were detected on every island and collectively accounted for 30% of estimated diet across all lizard species. These taxa illustrate how habitat use shaped diet composition in lizards. The ground-dwelling cockroach *Hemiblabera pabulator* (Blaberidae) was the most abundant prey of curly-tailed lizards and brown anoles; ground-nesting ants (*Brachymyrmex* spp., Formicidae) were the second- and fifth-most abundant prey of curly-tailed lizards and brown anoles, respectively; and the arboreal leaf-notcher beetle *Artipus floridanus* (Curculionidae) was the first- and second-most abundant prey of green and brown anoles, respectively (Fig. 4, Supplementary Table 1).

Note the log scale on y axis. **d**, Populations of brown anoles (plotted relative to the 2011 pre-manipulation baseline) increased nearly threefold from 2011 to 2016 on control islands, and were suppressed by competitors and predators in the other treatments (effect of curly-tailed lizards $t = -7.49$, $P < 0.001$; effect of green anoles $t = -5.49$, $P < 0.001$; interaction $t = 2.67$, $P = 0.024$; all d.f. = 10, $n = 14$ islands). In **a**, **b**, and **d**, data are mean \pm 1 s.e.m. of $n = 7$ or 8 islands (curly-tailed lizards), $n = 8$ islands (green anoles), and $n = 14$, 15, or 16 islands (brown anoles); underlying data are in Supplementary Data 3. All statistical effect tests are two-sided t -tests from generalized least-squares linear models of data from the terminal census in 2016 (see Methods); full model results are in Extended Data Table 3. Images are from Dreamstime, Shutterstock, and Alamy.

The diets of curly-tailed lizards and green anoles did not differ significantly across treatments (Fig. 4d, e). However, the diets of brown anoles were independently and interactively affected by the presence of curly-tailed lizards and green anoles (Fig. 4c, Extended Data Fig. 4). On control islands, brown anoles consumed substantial quantities of both terrestrial and arboreal prey. On +GA islands, the diets of brown anoles shifted in a terrestrial direction, becoming more similar to those of curly-tailed lizards (Fig. 4c, e)—notably through reduced consumption of arboreal beetles (which accounted for 25% of diet in green anoles but <1% of diet in curly-tailed lizards) (Fig. 4i–k) and elevated consumption of ground-nesting ants (which accounted for 20% of diet in curly-tailed lizards but <1% of diet in green anoles) (Fig. 4l–n). On +CT islands, the diets of brown anoles shifted in an arboreal direction, becoming more similar to those of green anoles (Fig. 4c, d): consumption of cockroaches and ants decreased relative to control and +GA islands (Fig. 4f, l), whereas consumption of beetles peaked (Fig. 4i). On +GA+CT islands, brown anoles were squeezed into a hybrid dietary niche—resembling a narrower version of that on control islands (Fig. 4c)—with intermediate consumption of the dominant terrestrial and arboreal prey taxa (Fig. 4f–l). We also detected 11 indicator-prey taxa that were uniquely associated with the +GA+CT treatment (for example, the planthopper *Acanalonia bivittata*) (Supplementary Table 1). The results in Fig. 4 are based on analyses of relative sequence-read abundances (see Methods); analyses of presence–absence data yielded equivalent inferences (Extended Data Fig. 5).

These treatment effects on diet composition paralleled those on habitat use (Fig. 3), which indicates that lizards are generalist consumers wherever they occur, and that dietary niche partitioning emerges through spatial segregation. Green anoles and curly-tailed lizards occupied the top and bottom habitat strata, respectively, and neither significantly affected the diet of the other. By contrast, the diets of brown anoles were constrained from above and below by competition with and avoidance of green anoles and curly-tailed lizards (Fig. 4, Extended Data Figs. 4, 5).

Quantitative PCR revealed the DNA of brown anoles in faecal samples from 2 of the 51 curly-tailed lizards that we tested, and the DNA of green anoles in 1 of the 28 individuals from +GA+CT islands. We are unable to calculate a rate of lethal predation because we do not know the rate at which anoles are digested (previous work suggests that transit time is probably more than 150 h for a 27-g lizard³⁹, but it may take even longer for the DNA of large vertebrate prey to disappear from gut contents), and because non-lethal consumption can occur (for example, of autotomized tails or carcasses). We interpret these results as indicating that consumption was infrequent—at least in 2013–2014, when

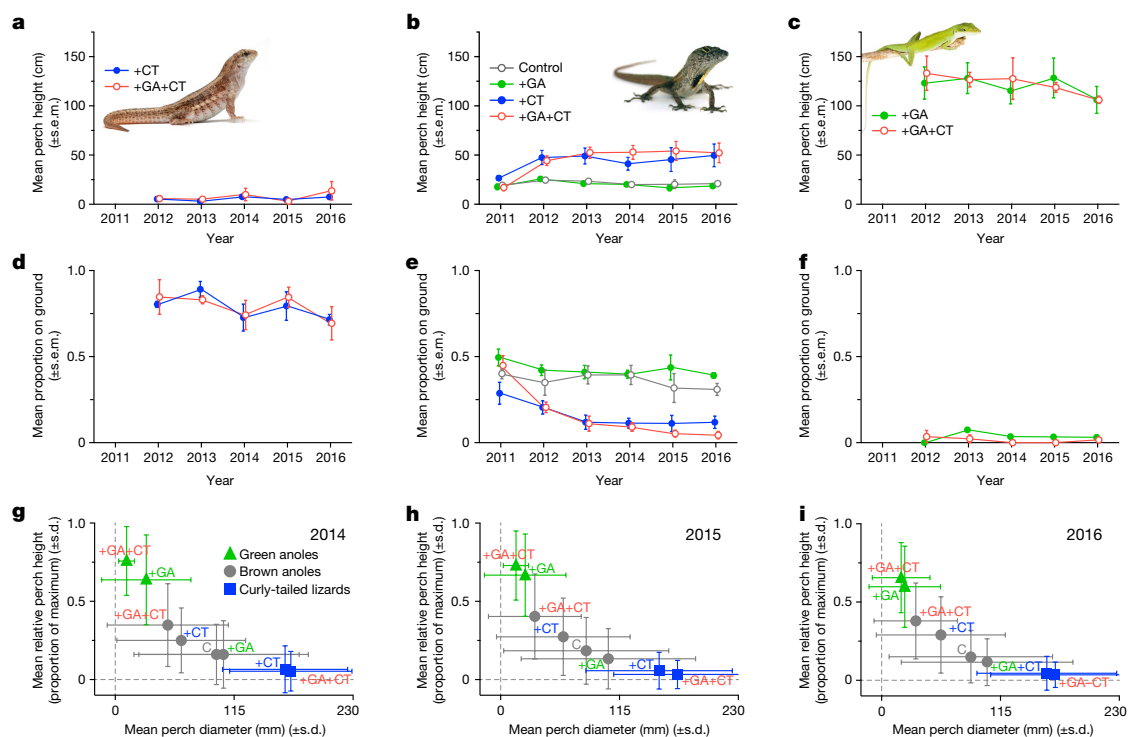


Fig. 3 | Predator-induced collapse of spatial niche structure. **a–c**, Mean perch height by species and treatment for curly-tailed lizards (left), brown anoles (middle), and green anoles (right), showing the rapid doubling of brown-anole perch height on +CT and +GA+CT islands (effect of curly-tailed lizards $t = 2.71$, $P = 0.02$; effect of green anoles $t = -1.79$, $P = 0.10$; interaction $t = 0.31$, $P = 0.76$; all d.f. = 11, $n = 15$ islands), with no significant treatment effects on curly-tailed lizards or green anoles. **d–f**, Mean proportion of individuals on the ground; brown anoles differed significantly by treatment (effect of curly-tailed lizards $t = -3.82$, $P = 0.003$; effect of green anoles $t = 2.13$, $P = 0.056$; interaction $t = -2.88$, $P = 0.015$; all d.f. = 11, $n = 15$ islands). **g–i**, Two axes of the spatial niche—relative perch height (ratio of perch height to maximum available vegetation height) and perch diameter—for each species (symbols) and treatment (letters) from 2014 to 2016. C, control.

Green anoles used the highest and narrowest perches and did not overlap spatially with curly-tailed lizards; brown anoles encroached into green-anole habitat space on +GA+CT islands. In **a–f**, data are mean \pm 1 s.e.m. of island-wide averages for each treatment in each year (curly-tailed lizards, $n = 6, 7$, or 8 islands; green anoles, $n = 7$ or 8 islands; brown anoles, $n = 15$ or 16 islands; underlying data are in Supplementary Data 3). In **g–i**, data were pooled across replicate islands within each treatment in each year to yield treatment-wide means (\pm 1 s.d.) for each habitat axis ($n = 55–1,328$ (2014), $99–1,097$ (2015), and $63–1,172$ (2016) observations per species per treatment). Statistical effect tests for **a–e** are two-sided t -tests from generalized least-squares linear models of island-level means from the terminal census in 2016 (see Methods); full model results are in Extended Data Table 3. No statistical tests were performed on the data in **f–i**. Images are from Dreamstime, Shutterstock, and Alamy.

these samples were collected (it is possible that our mid-experiment sampling missed some window of more-intensive predation at the outset of the study).

Trophic position and length of food chains

Stable-isotope analysis revealed that all three lizard species occupied similar trophic positions (see Methods), which provides additional evidence that anoles were not a major part of the diet of curly-tailed lizards. Although curly-tailed lizards accounted for the single highest trophic position recorded for any lizard individual (4.30) or population (3.66), their population-level trophic position (mean \pm s.e.m., 2.63 ± 0.17) did not differ significantly from that of brown or green anoles (mean \pm s.e.m., 2.78 ± 0.11 and 2.77 ± 0.12 , respectively) (Fig. 5a, b). Indeed, curly-tailed lizards tended to occupy lower trophic positions than anoles within islands—significantly so on three of the eight islands (ANOVA, all $P \leq 0.0073$), with no significant differences on the remainder (all $P > 0.25$). These results are consistent with our finding (Fig. 4a, h, Extended Data Fig. 1m–o) that the primary prey of curly-tailed lizards was cockroaches (which had the lowest $\delta^{15}\text{N}$ values of any consumer measured in this study), and with previous findings that Bahamian curly-tailed lizards have diverse omnivorous diets with “a very low incidence of saurophagy”³⁶.

Our experimental treatments altered the length of food chains, calculated using the consumer species with the highest mean trophic position on each island²⁵. Overall, food-chain length increased with island area (Fig. 5c), which is consistent with previous work on

Bahamian islands³⁰ and with general theoretical expectations^{9–11,26}. After accounting for this effect of island area, we found significant interactive effects of the experimental treatments on food-chain length: adding either green anoles or curly-tailed lizards lengthened food chains relative to control islands, but adding both species reversed this effect (Fig. 5d, Extended Data Fig. 6, Extended Data Table 2). We also found an interaction between the experimental treatments and island area: the overarching positive correlation between food-chain length and island area was not observed across the +GA+CT islands (Fig. 5c, Extended Data Fig. 7, Extended Data Table 2), which suggests that this treatment truncated food chains in part by disrupting the influence of ecosystem size.

Food-chain length can change through three proximate mechanisms: addition or removal of top predators, insertion or deletion of intermediate consumers, and changes in trophic omnivory by top predators (with greater omnivory expected to shorten food chains)^{9–11,25}. The available evidence—although not conclusive—suggests that all three mechanisms may have operated in this study. First, green anoles or curly-tailed lizards had the highest trophic position on five of the eight +GA and +CT islands, which suggests that the addition of these species may have directly lengthened food chains in these two treatments. Second, deletion of intermediate links has been documented in previous studies on Bahamian islands, which have shown that lizards not only reduce the abundance and species richness of intermediate consumers such as spiders^{18,31} but also decrease the slope of the insect species–area relationship⁴⁰. Such effects should be strongest on +GA+CT

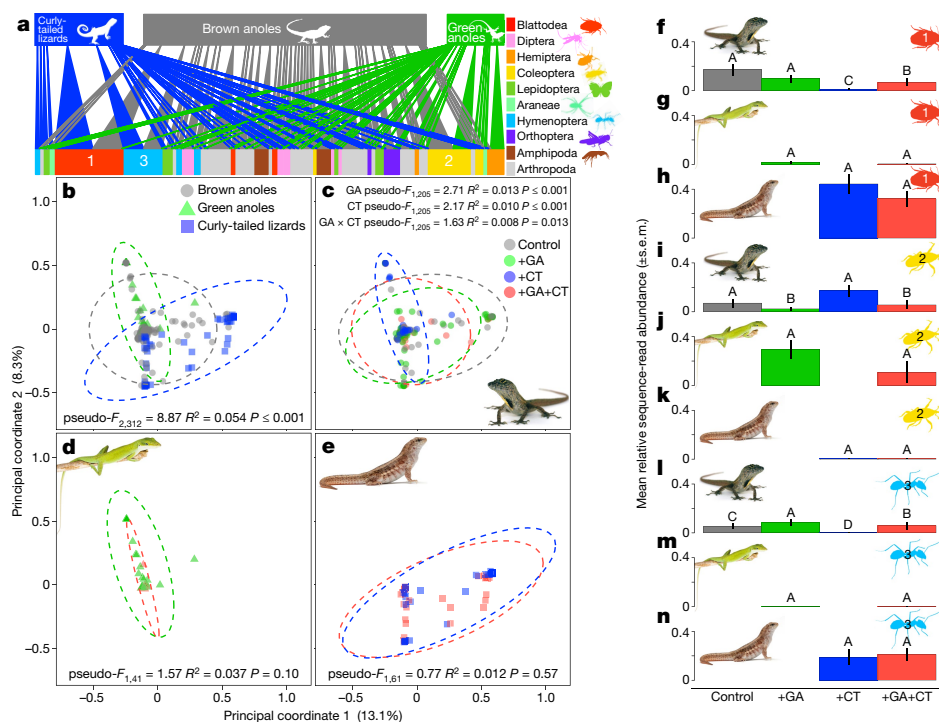


Fig. 4 | Treatment-induced shifts and overlaps in dietary niches suggest competition for food. **a**, Bipartite network showing the top 50 prey taxa (bottom, coloured by taxonomic group) across all lizard species (top), based on DNA metabarcoding of faecal samples from $n = 315$ unique individuals across all treatments. These 50 prey taxa accounted for 82–87% of estimated total diet for each species. The width of connecting lines reflects relative abundance within diets. Numerals indicate the three most-abundant prey taxa (1, ground-dwelling cockroach *H. pabulator*; 2, arboreal beetle *A. floridanus*; 3, ground-nesting ants *Brachymyrmex* spp.). **b–e**, Principal coordinates analyses of Bray–Curtis dietary dissimilarities. Points, individual samples (distance reflects dissimilarity); ellipses, 95% confidence intervals. All plots are based on the same ordination with identical coordinates to facilitate comparison, but confidence intervals were calculated separately for each treatment in **c–e**. **b**, Each species, pooled across treatments, with permutational multivariate ANOVA (PERMANOVA) testing the effect of species identity ($P \leq 0.001$, $n = 315$ samples). **c**, Brown anoles by treatment, with PERMANOVA testing

the independent and interactive effects of green anoles (GA) and curly-tailed lizards (CT) (all $P \leq 0.013$, $n = 209$ samples). **d**, Green anoles by treatment, with PERMANOVA testing the effect of curly-tailed lizards ($P = 0.10$, $n = 43$ samples). **e**, Curly-tailed lizards by treatment, with PERMANOVA testing the effect of green anoles ($P = 0.57$, $n = 63$ samples). **f–n**, Dietary abundance by species and treatment of *H. pabulator* (**f–h**), *A. floridanus* (**i–k**), and *Brachymyrmex* spp. (**l–n**). Bars, mean relative read abundances (± 1 s.e.m.); sample sizes for each species match those in **c–e**. Letters denote statistically significant differences in pairwise two-sided treatment contrasts, based on Wald statistics calculated using DESeq2 (all significant effects $P \leq 0.042$; all non-significant effects $P > 0.066$). All graphs in this figure are based on relative read abundances from rarefied sequence data, but note that the statistical tests for **f–n** were calculated using unrarefied data (see Methods); analyses based on presence–absence data yielded qualitatively equivalent results (Extended Data Fig. 5). Images are from Dreamstime, Shutterstock, and Alamy.

islands, where lizards occupied all habitat strata and anoles were forced into marginal microhabitats that might otherwise be refuges for arthropod prey. Finally, patterns of lizard dietary diversity (a likely proxy for trophic omnivory, see Methods) are at least superficially consistent with the hypothesis that shifts in predator omnivory contributed to the observed variation in food-chain length (Extended Data Fig. 7).

Implications for the biogeography of adaptive radiation

A hallmark of adaptive radiation is the evolution—typically through sustained competitive interactions in the presence of ecological opportunity (vacant niche space)—of differences in resource use that mitigate interspecific competition and facilitate coexistence^{41,42}. In Caribbean *Anolis* lizards, these differences manifest in the partitioning of vegetation strata³⁵, as exemplified by brown and green anoles. By collapsing the spatial dimension of this niche structure—and with it the dietary dimension—curly-tailed lizards increased the ecological similarity of brown and green anoles on +GA+CT islands, blurring the lines between inter- and intra-specific competition and destabilizing coexistence. All else being equal, green anoles might ‘win’ in this scenario, because they are better adapted to arboreal habitats. In our experiment, however, green anoles were colonizers that were introduced at a low initial abundance; accordingly, even if green anoles held a per capita competitive edge over brown anoles, their

populations were nonetheless more prone to extinction owing to the greater initial abundance of brown anoles (a priority effect, in which the order and timing of colonization events influences community assembly⁴³).

A potential eco-evolutionary pathway to coexistence—habitat shift and character displacement, leading to the reestablishment of niche differences—is unlikely in this system given that essentially all of the available microhabitats were occupied by lizards on +GA+CT islands (that is, there was no vacant niche space). If our islands had taller vegetation, green anoles might have been able to establish by shifting upwards and adapting morphologically. For example, when brown anoles invaded Floridian islands occupied by green anoles, the green anoles moved higher and evolved larger toepads⁴⁴. Indeed, brown and green anoles do co-occur with curly-tailed lizards on many larger Caribbean islands that contain bigger trees. However, green anoles already occupied the treetops on our +GA islands (Fig. 3) and had no other refuge when predators forced brown anoles to become arboreal on +GA+CT islands. Our results suggest that predation interacts with habitat architecture to limit the number of *Anolis* species that can coexist on an island. For a given vegetation structure, adding predators should reduce the likelihood that an additional species can establish—and for a given predation regime, vegetation structure should govern whether the expected anole species richness is zero, one, two, or more.

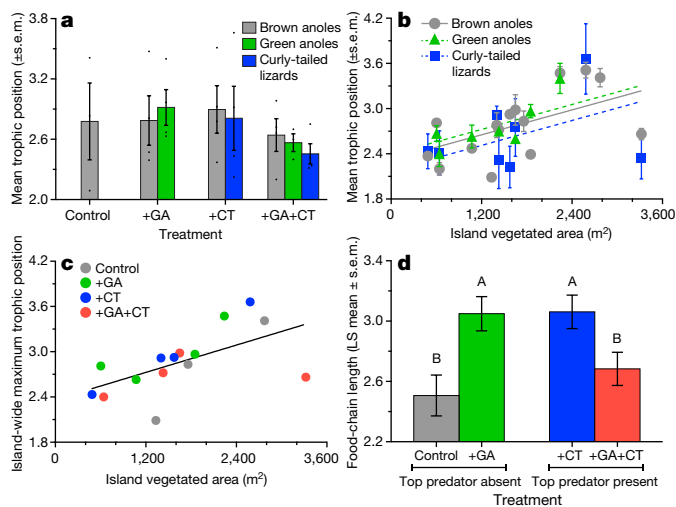


Fig. 5 | Trophic position and food-chain length. **a**, Trophic position of each lizard species by experimental treatment, not accounting for effects of island area. Data are mean ± 1 s.e.m. of island-wide averages in each treatment ($n = 3$ control islands; $n = 4$ islands for all other treatments). Dots, values for each population. **b**, Mean trophic position (± 1 s.e.m.) of each lizard population on each island, showing an influence of ecosystem size but no effect of species identity (analysis of covariance (ANCOVA) effect tests: island area $F_{1,26} = 9.73$, $P = 0.0044$; species $F_{2,26} = 0.65$, $P = 0.53$; $n = 30$ populations). **c**, Increase in food-chain length with island area; points, $n = 15$ islands, coloured by experimental treatment (linear regression $r = 0.59$, $F_{1,13} = 6.82$, $P = 0.022$). **d**, Mean food-chain length (± 1 s.e.m.) in each experimental treatment, after accounting for effects of island area and all first-order interactions (Extended Data Table 2a). Data are least-squares (LS) means ± 1 s.e.m. from the green anole \times curly-tailed lizard interaction term in the generalized least-squares linear model (whole model, adjusted $R^2 = 0.73$, $F_{6,8} = 7.35$, $P = 0.0064$; green anole \times curly-tailed lizard interaction $t = -3.86$, d.f. = 8, $P = 0.0048$; $n = 15$ islands). Letters denote statistically significant differences ($P \leq 0.05$) between treatments in pairwise two-sided t -tests. The full model (Extended Data Table 2a) also included significant interactions between island area and treatments, which reflects the lack of increase in food-chain length with ecosystem size on +GA+CT islands (c; see also Extended Data Fig. 7). Results in this figure are based only on lizards; qualitatively equivalent analyses that include the dominant spider species, *Metepseira datona* (Araneidae), are shown in Extended Data Fig. 6 and Extended Data Table 2b.

The Caribbean *Anolis* radiation occurred on islands much larger than those in this study, and the role of predation (or its absence) in promoting eco-morphological differentiation is not known³⁵. Although predators can theoretically promote adaptive divergence through ‘apparent competition’⁴⁵, our results suggest that they might instead constrain it by foreclosing ecological opportunities and intensifying actual competition within refuges (‘apparent predation’?). In this regard, our findings may not be scalable, because vast islands present more ecological opportunities than do tiny cays. However, in *Anolis* and many other adaptively radiating lineages, differentiated species subsequently disperse across hundreds of smaller satellite ecosystems (such as our experimental islands), with the interplay of colonization and extirpation creating mosaics of species occupancy⁴⁶. Our study shows that predation risk can shape the biogeographical assortment of niche specialists by modifying competitive interactions between new arrivals and residents. Similarly, our results highlight a non-consumptive pathway by which introduced predators can cause extinction. Scores of endemic cichlid fishes (which, like anoles, exhibited clear niche structure) disappeared from the Lake Victoria basin after the introduction of Nile perch (which, like curly-tailed lizards, acted as intraguild predators). Predator-free refuges were crucial in enabling the persistence of many cichlid species⁴⁷, but intensified competition within these refuges may also have contributed to species loss.

Discussion

We found that top predators impeded the coexistence of two *Anolis* lizard species that occupied divergent niches in the absence of higher predators. In the presence of predators, niche dimensionality was reduced and green anoles did not increase from low initial abundance, which indicates unstable coexistence (an inference that is underscored by the extinction of two of the four green-anole populations within six years; Fig. 2). Direct predation presumably influenced demographic trends to some extent, but multiple lines of evidence suggest that consumptive effects were weak, and that non-consumptive effects were important. Faecal DNA analysis, isotopic data, field observations (Extended Data Fig. 1d–f), previous work³⁶, and comparison of brown-anole trajectories on +CT and +GA islands (Fig. 2d) all point to relatively infrequent consumption. By contrast, the behavioural responses of brown anoles to curly-tailed lizards were immediate and persistent. Overlap in resource use between brown and green anoles, which had strong demographic effects even in the absence of predators (Fig. 2d), was greater where predators were present (Figs. 3, 4). Collectively, our results align fully with the refuge-competition model, although further work would be required to quantify each direct and indirect interaction shown in Extended Data Fig. 2e (for example, by establishing whether anole offspring production was reduced in the presence of predators, as the model implies).

Trophic omnivory was also important in several ways. First, because curly-tailed lizards can subsist on a diet of arthropod prey (Fig. 4), their populations could increase even as anole populations declined (Fig. 2), which ensured continuous predation risk. Second, because curly-tailed lizards exploited the arthropod prey base, they competed with brown anoles for food (Fig. 4, Extended Data Fig. 1d, Supplementary Video 1) and probably further intensified competition between brown and green anoles for the remaining arthropods. Third, because curly-tailed lizards were trophic omnivores, they occupied relatively low trophic positions, which enabled food chains to be shorter on +GA+CT islands than on +GA islands despite the addition of a top predator (Fig. 5). Indeed, trophic omnivory by all lizard species may have driven the observed variation in food-chain length (Extended Data Fig. 7). Theory predicts that omnivory should be greater, and food chains shorter, when habitats are strongly coupled by consumers²⁹. This can occur when predators are mobile²⁹ and/or when refuges are eliminated⁴⁸. On control islands, brown anoles ranged widely and coupled arboreal and terrestrial habitats; on +GA and +CT islands, each lizard species was more compartmentalized within its respective habitat; and on +GA+CT islands, refuges were eliminated and ecosystems effectively compressed (regardless of island area) by the saturation of all habitats with lizards (Figs. 3, 4).

Predator-mediated coexistence has remained an influential idea in ecology for over 50 years^{13–15,19,20}; however, it does not emerge easily in theoretical models without particular conditions—for example, trade-offs between competitive ability and vulnerability to predation^{6,7,16,17,49,50}. Such a trade-off seemed plausible in our system: we expected predation to be strongest on brown anoles, which are numerically and competitively³² dominant on these islands. Yet we found that consumption was similarly infrequent for both anole species by the middle of the experimental period, in part because the surviving brown anoles became arboreal to escape predation. Thus, rather than increasing resource availability for green anoles by depressing the abundance of brown anoles (Extended Data Fig. 2d), predators instead reduced it by transforming the behaviour of brown anoles (Extended Data Fig. 2e). In this way, risk destabilized coexistence. This outcome may predictably arise when top predators are trophic generalists, and when their prey occupy discrete spatial niches—conditions that apply to many potential predator-introduction scenarios in islands and lakes worldwide.

Online content

Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at <https://doi.org/10.1038/s41586-019-1264-6>.

Received: 2 October 2018; Accepted: 1 May 2019;
Published online 5 June 2019.

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Acknowledgements This work is a product of US National Science Foundation grant DEB-1457697 to R.M.P. and R.D.H.B. T.W.S., D.A.S., and J.B.L. acknowledge NSF DEB-1314691. Support was provided by the Princeton Environmental Institute (R.M.P.), a Canada Research Chair (R.D.H.B.), and a Vanier Canada Scholarship (C.C.Y.X.). Research was conducted under permits from The Bahamas Environment, Science & Technology Commission, the Bahamas Department of Agriculture, and Princeton University's Institutional Animal Care and Use Committee (protocols 1922-13, 1922-F16). We thank S. Lubin-Gray, L. Hanna, the Chamberlain family, A. Askary, P. Chen, N. and A. Dappen, T. Ingram, O. Lapedra, N. Losin, L. Revell, C. Tarnita, J. Ware, and L. Wyman.

Reviewer information *Nature* thanks Raymond Huey, Oswald Schmitz and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Author contributions R.M.P. and J.B.L. conceived the study. R.M.P. designed the research. R.M.P., T.M.P., and R.D.H.B. coordinated the study. R.M.P., T.R.K., T.M.P., T.J.T., J.J.K., T.W.S., D.A.S., J.B.L., and R.D.H.B. implemented the experiment. T.R.K. conducted DNA-metabarcoding analyses. K.F.-D. conducted stable-isotope analyses. C.C.Y.X. conducted qPCR analysis. D.A.E. contributed insect taxonomic identifications. R.M.P., T.R.K., T.M.P., T.J.T., C.C.Y.X., T.C.C., J.H.D., K.M.G., N.A.M.i't V., J.E.W., J.J.K., D.A.S., J.B.L., and R.D.H.B. collected data. R.M.P., T.R.K., and M.C.H. conducted statistical analyses. R.M.P. and T.R.K. wrote the paper with T.M.P., T.J.T., K.F.-D., C.C.Y.X., and R.D.H.B. All authors contributed to revisions.

Competing interests The authors declare no competing interests.

Additional information

Extended data is available for this paper at <https://doi.org/10.1038/s41586-019-1264-6>.

Supplementary information is available for this paper at <https://doi.org/10.1038/s41586-019-1264-6>.

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METHODS

The sample size of islands used in the experiment was set at $n = 4$ per treatment combination ($n = 16$ islands in total), which was the maximum number of islands that was deemed suitable for inclusion according to the criteria described below; no statistical methods were used to predetermine sample size. The allocation of experimental treatments and introduced lizards to islands was randomized, as described below. Investigators were blind to the allocation of islands to treatments during the initial population census in 2011; blinding was impossible in subsequent surveys owing to the unique suite of lizard species in each treatment. Investigators were blind to the experimental treatment of tissue samples analysed for trophic position and of faecal samples analysed for diet composition during laboratory analysis. Descriptive statistics are given as mean \pm 1 s.e.m., unless otherwise specified.

Study site and experimental setup. The experimental islands are located near Staniel Cay in the Exuma chain of Grand Bahama Bank (26° 10' 12" N, 76° 26' 24" W), where small islands have been used for experimental research since 1977 (ref. ⁵¹). All three focal lizard species are native to the broader study area, but differ in their occurrence across individual islands owing to natural colonization and extirpation dynamics (with brown anoles being the most widespread); our study simply mimicked these natural processes in a controlled fashion. In 2010, we surveyed 25 islands for possible inclusion in the study and selected 16 on the basis of the following criteria: presence of brown anoles; presumed absence of any other lizard or top-predator species; presence of trees >2-m tall, deemed necessary to support green anoles³² (see Fig. 1e, f and Extended Data Fig. 1p, q); and sufficiently small area that an observer could survey the entire island within 90 min. Four islands were excluded because we did not detect brown anoles, two because the vegetation was too short, and three because the islands were too large. We conducted a census of brown-anole populations on the remaining 16 islands in May 2011 (see 'Lizard censuses'), during which no other lizard or top-predator species were detected.

After this initial census, we randomly assigned experimental treatments to islands. Islands were first stratified by size (vegetated area) such that two replicates of each treatment would be assigned to the eight smallest and eight largest islands. We quantified island area by manually delineating the perimeter of the vegetation on each island in Google Earth Pro, to exclude the rim of bare rock that surrounds each island (Fig. 1f), and then calculating the area of the resulting polygon. Green anoles were collected from Staniel Cay, on which brown anoles occur but curly-tailed lizards do not; thus, all anoles of both species were ecologically (but not evolutionarily) naive to the top predator before the experiment. Curly-tailed lizards were collected from a nearby large island, Thomas Cay, on which both brown and green anoles occur. These lizards were randomly assigned to islands and released in groups of 10 or 11 green anoles (5:5 sex ratio, with the 11th individual, where present, being a juvenile) and 5–7 curly-tailed lizards (unsexed). These numbers were chosen to simulate colonization by relatively small founder populations and to avoid unduly depleting source populations.

Lizard censuses. We conducted censuses of all lizard populations annually in late April and/or early May from 2011 to 2016. In addition, we assayed population persistence (verifying whether populations were still present or had gone extinct) in January 2016, January 2017, and April 2017. For all censuses, we used a mark-re-sight procedure that was developed specifically for Caribbean *Anolis*⁵² and that has been used for decades in this study system^{33,51} and others^{53,54}. During each census, islands were comprehensively searched by teams of 3–6 observers on 3 consecutive days. All lizards were marked with a day-specific colour of non-toxic, water-soluble paint, which was applied gently from a short distance with squirt guns (blue, red, and yellow paint were used on days 1, 2, and 3, respectively; see Extended Data Fig. 1). In the vast majority of cases, we were able to confirm that lizards had been marked (scoring a value of 1 for that day); when uncertain, we followed a previous study⁵⁵ in estimating the likelihood of a successful mark (0.25, 0.50, or 0.75). We followed previously described methods⁵⁴ in estimating population sizes from these data as the mean of the three possible Chapman estimates, a variant of the Lincoln index adjusted to reduce bias at small sample sizes,

$$n = \frac{(M + 1) \times (C + 1)}{(R + 1)}$$

in which M and C are the numbers of individuals marked on the first and second visits, respectively, and R is the number of previously marked individuals re-sighted on the second visit. These estimates were almost identical to those generated using a more-complex multivariate contingency-table approach⁵² (linear regression $r = 0.99$, slope = 1.09, $n = 175$). In just 1 of the 176 censuses conducted across all lizard species, islands, and years, we were unable to obtain a valid population estimate owing to scarcity of re-sighted individuals (namely for brown anoles on island 922 in 2016); this point was omitted from analysis. In the few cases in which the estimated population size for green anoles or curly-tailed lizards was less than the minimum number of individuals known to be alive (that is, the total number

of unique individuals marked across all 3 days of the census), we used the latter number in lieu of the former.

We analysed the main effects of green anoles and/or curly-tailed lizards (along with their interactive effect, for brown anoles) on the population size of each species in 2016 (the year of the terminal population census) using generalized least-squares linear models fit using restricted maximum likelihood in the nlme package⁵⁶ in R version 3.4.0⁵⁷. Full model results are in Extended Data Table 3a–c. We present analyses of the 2016 end-point data because populations of green anoles and curly-tailed lizards were introduced at low initial abundances and grew during the experiment, stabilizing from 2015–2016 (Fig. 2). Analysing the full time series, using mixed-effects models to account for the repeated measures⁵⁸, yielded equivalent inferences. We plotted standardized residuals of these models against fitted values and experimental treatments to check for heterogeneities of variance, and we inspected histograms of residuals to assess normality⁵⁸; no violations of model assumptions were detected. For curly-tailed lizards and green anoles, which were introduced in standardized numbers in 2011, we analysed absolute population-size estimates. For brown anoles, which had variable population sizes across islands at the outset of the experiment, we analysed the change in population size on each island relative to the 2011 pre-manipulation value using the ratio n_{year}/n_{2011} . Analysing relative densities in the same fashion yielded statistically equivalent results.

Green-anole populations had gone extinct on island 926 (the largest +GA+CT island at 3,320 m²) by May 2015, and on island 312 (the smallest +GA+CT island at 640 m²) by January 2017 (after the final annual census in April 2016). These extirpations were verified in subsequent surveys (in January 2016 and April 2016 for island 926, and in April 2017 for island 312), and no replacements were added. Island 926 was therefore excluded from graphical presentation as of 2015, and from the statistical analyses of the 2016 data (except for the analysis of the size of green-anole population, in which island 926 was retained with a value of 0). Curly-tailed lizards had disappeared from one island (204, the smallest overall at 487 m²) by April 2012, one year after the initial introduction (whereupon an additional 6 individuals were added to maintain the treatment) and again as of April 2016, the final census. Other than these events, however, the density of curly-tailed lizards on this small island was similar to the densities on other islands, and brown anoles on this island consistently exhibited the behaviours associated with the presence of curly-tailed lizards. We therefore retained this island in analyses; its exclusion did not qualitatively alter any of our results. In 2015, we found and removed a single female green anole on island 314, a +CT island on which green anoles should not have been present, and the following year we found and removed three more individuals. We do not yet know whether these individuals resulted from natural dispersal or were unwittingly transported by investigators. In any case, because this event occurred in the penultimate year of the study and involved a very small number of individuals (which were removed), we retained island 314 in analyses; and again, excluding it did not qualitatively alter our results. Population-size estimates for each species on each island in each year, along with island areas, are given in Supplementary Data 3.

Habitat use. During censuses, we recorded the following data for each lizard: species, sex or age (male, female, or juvenile for anoles; adult or juvenile for curly-tailed lizards), perch height (estimated visually, typically to the nearest 5 cm), and perch diameter (typically estimated to the nearest 0.5 cm). Our dataset comprises a total of 20,937 observations across all species, islands, and years. For all lizards on the ground or other exceptionally large perches, we assigned a standard maximum perch diameter of 20 cm (perch diameters were analysed descriptively only, for illustration in Fig. 3g–i). From 2014–2016, we also recorded the maximum vegetation height within a 1-m radius of the lizard, which enabled us to calculate relative perch height (ratio of perch height to the maximum available vegetation height at that location) (Fig. 3g–i, Extended Data Fig. 3). We analysed treatment effects on habitat use using generalized least-squares linear models of island-level means from 2016, as described for the analysis of population sizes in 'Lizard censuses'. Observations of individuals marked on successive survey days were included in calculating the island-level means of each habitat-use metric in each year; however, because islands were used as the units of analysis, these records were not treated as statistically independent in hypothesis testing (thus, there was no pseudo-replication). We did not analyse the proportion of green anoles on the ground, because most values were 0 (Fig. 3f). Violations of model assumptions were detected in the analyses of perch heights of brown anoles and curly-tailed lizards, and in the analysis of the proportion of brown anoles on the ground; these violations arose from uneven residual spread across treatments and were resolved by including the relevant treatment factor as a variance covariate using the varIdent variance structure⁵⁸ in the nlme package (model specifications and results are in Extended Data Table 3d–k). The inclusion of variance covariates did not alter the statistical significance of any variable in these models but did substantially improve the model fits. Mean habitat-use data for each lizard species on each island in each year are given in Supplementary Data 3.

DNA-metabarcoding analysis of diet. Our analysis of lizard diets generally followed previously described procedures³⁸ and are summarized below. During three field trips (May 2013, December 2013, and May 2014), we captured individuals of all 3 lizard species on 15 of the 16 islands and held them individually in disposable plastic containers while checking regularly for faecal samples. Thus, all samples were fresh (maximally 8-h old³⁸). Whole faecal pellets were preserved in stabilizing buffer, frozen, and transported to Princeton University, where they were stored (-80°C) and later extracted in small batches (~ 15), using blanks to monitor for cross-contamination. Following previously described methods³⁸, we used arthropod-specific primers to amplify a ~ 109 -bp (modal length; range, 109–117 bp in our local reference library) target sequence of arthropod mitochondrial 16S DNA that could be pooled for DNA metabarcoding as single-end 170-bp reads on an Illumina HiSeq 2500 Rapid Flowcell. All PCRs were conducted with a no-template negative control (substituting molecular-grade water for a sample) and a positive control (comprising DNA extracted from arthropods during the construction of our local DNA reference library). The results of all PCR reactions were visualized using gel electrophoresis to confirm the presence of a PCR product in the positive control and the absence of a PCR product in the negative control, and to evaluate whether PCR products were present in each sample and extraction blank. We only sequenced samples when there was evidence of positive amplification of the sample and successful positive and negative controls. We sequenced PCR products from 368 samples (including up to 5 replicate faecal extracts obtained from 45 individuals that yielded more than one faecal sample), all 42 extraction blanks, and a subset of controls (5 positive and 4 negative).

Illumina sequencing data were demultiplexed according to the sample of origin. We first investigated the sequencing depth obtained across runs from each control and blank sample. The positive controls consistently yielded a large volume of data (range, 524,735 to 2,970,409 sequences per reaction; median, 1,023,593), as did most of the faecal samples (range, 2,099 to 2,009,727; median, 245,603). These sequencing depths were typically far greater than those obtained from extraction blanks (the products of the DNA-extraction protocol without a faecal sample; range, 771 to 29,579; median, 4,840) and negative controls (the products of PCR amplification with molecular-grade water substituted for a DNA extract; range, 1,061 to 34,008; median, 5,530). Thus, although our controls indicate that the dataset may include sources of error that are common in DNA-metabarcoding studies (for example, potential contamination and/or tag-jumping during sequence analysis), these errant sequences appeared at exceedingly low levels compared to positive controls and samples (~ 200 -fold and ~ 50 -fold differences in median sequencing depth, respectively). Accordingly, these potential sources of error are unlikely to systematically bias our comparative results.

For the DNA sequences obtained from faecal samples, we filtered data to include only high-quality sequences (≥ 108 bp, no ambiguous base calls, > 35 mean Illumina fastq quality over the length of the sequence). We tallied counts of identical sequences and filtered out putative errors by eliminating singletons (sequences represented only once in any sample in the raw data) and by removing the less-abundant sequence of pairs from the same sample that differed by only 1 bp and in which the rarer sequence in the pair accounted for $< 25\%$ of abundance. Sequences were identified using the *obitools* package⁵⁹ and combined into molecular operational taxonomic units (mOTUs) using the UCLUST algorithm⁶⁰. Identifications were based on two reference libraries: a local library comprising DNA sequences from arthropods collected on and around the study islands, and a global library of arthropod 16S sequences from the European Nucleotide Archive (release 131). The local reference library comprised 381 arthropod specimens sequenced at COI (to facilitate taxonomic identifications) and 16S (for matching with dietary data). From these, we obtained 113 unique local reference sequences that fully overlapped the target DNA-metabarcoding region. The species-level (or finest possible) identification of dietary mOTUs was accepted if any sequence variant in the mOTU perfectly matched a local reference sequence; otherwise the most-abundant sequence variant in the mOTU was used for identifications, accepting up to species-level identification if a reference sequence matched at $\geq 99\%$ and assigning genus- to order-level identifications if a match of $\geq 95\%$ was found. If the most-abundant sequence variant in an mOTU had $< 80\%$ identity with the nearest reference sequence, or was identified as human DNA, then the mOTU was discarded as a putative chimaera or contaminant.

The resulting dataset comprised 1,661 dietary mOTUs (108,610,656 Illumina sequences) across 368 samples ($n = 248$ brown anoles, 49 green anoles, and 71 curly-tailed lizards); 813 of these mOTUs were retained after rarefying to the minimum sequence depth of any sample after all filtering steps were completed ($n = 1,142$ sequences per sample). We provide unrarefied and rarefied count data from all 368 samples in Supplementary Data 1 and 2, respectively. To avoid pseudo-replication in analysis, we reduced the dataset to include only the 315 samples obtained from separate individuals. For each of the 45 individuals that were sampled multiple times, we retained for analysis the sample that yielded the largest number of DNA sequences; of the 813 mOTUs in the total dataset, 35 were detected

only in this subset of replicate samples and were therefore excluded from analyses. Thus, analyses were based on a rarefied dataset comprising 778 mOTUs and 315 samples ($n = 209$ brown anoles, 43 green anoles, and 63 curly-tailed lizards).

The rarefied dataset was used to calculate relative sequence-read abundances (RRA)⁶¹ by dividing the rarefied number of reads assigned to each mOTU by 1,142, the rarefied number of reads per sample. We used RRA for our primary diet analyses, which included: depiction of the bipartite network (Fig. 4a), using the R package *bipartite*⁶²; calculation of Bray–Curtis compositional dissimilarities (Extended Data Table 1), principal coordinates analyses (Fig. 4b–e), and PERMANOVA tests of dietary dissimilarity, all using the R package *vegan*⁶³; and indicator-species analyses (Supplementary Table 1), which tested for significant differences in the RRA of arthropod mOTUs across lizard species and treatments, based on 999 permutations using the R package *indicspecies*⁶⁴. We used the unrarefied dataset (Supplementary Data 1) only for the comparisons of cockroach (*H. pabulator*), beetle (*A. floridanus*), and ant (*Brachymyrmex* spp.) abundances using DESeq2 (Fig. 4f–n); this statistical approach tests for differential representation of sequences in count data from high-throughput sequencing assays, based on a negative binomial distribution, and requires unrarefied data⁶⁵. We first fit a DESeq2 model to the entire dataset for each lizard species, including all arthropod mOTUs present in at least one sample, using the unrarefied read counts. Then, for the aforementioned cockroach, beetle, and ant mOTUs, we tested for significant treatment effects by evaluating the \log_2 -fold change in sequence counts within samples, based on the fit of the Wald statistic to the regression.

RRA is widely used in metabarcoding studies as a proxy for quantitative consumption patterns⁶¹, but it can be prone to errors that arise from differential digestion of tissues, variation in DNA quantity across prey species and life stages, and laboratory procedures (for example, differential PCR amplification of different prey species). We therefore tested whether our RRA-based results were reproduced in analyses based on the frequency with which a prey taxon was present in or absent from samples (that is, frequency of occurrence). We converted our rarefied sequence data (Supplementary Data 2) to a presence–absence matrix, using a threshold of 1% RRA to conclude that an mOTU was truly present in a sample. Such a threshold—which translates to > 11 sequences in any cell of the rarefied dataset in Supplementary Data 2—is commonly used when converting sequence-count data into presence–absence matrices with the aim of mitigating biases that can arise from low-abundance reads, sequencing errors, or contaminants⁶¹. This conversion to presence–absence data eliminated the rarest taxa in the dataset, reducing the total number of mOTUs from 778 to 240 for this analysis. We used this presence–absence dataset to repeat the main analyses presented in Fig. 4 and Extended Data Table 1, including the bipartite network of the top-50 most-frequently detected mOTUs, ordinations and PERMANOVA statistical tests by species and treatment, and comparisons of the frequency of occurrence of the cockroach, beetle, and ant species in all samples from each lizard species in each treatment (where frequency of occurrence is the number of samples in which each taxon was present divided by the total number of samples obtained from that treatment; we did not calculate average frequencies of occurrence for individual islands within treatments owing to the low number of samples obtained for some species on several islands). These results were qualitatively equivalent to those based on RRA (compare Fig. 4 and Extended Data Fig. 5). Frequencies of occurrence of each mOTU are also presented descriptively for each species and treatment in Supplementary Table 1. We base our inferences primarily on analyses of RRA data, which recent studies⁶¹ suggest are likely to be more robust in contexts such as ours (that is, a comparative study design, medium-to-large sample sizes, and many more than 3 prey mOTUs per sample).

We also probed the sensitivity of our results to temporal variation, given that our samples were collected over three expeditions at two different times of year and were subsequently pooled for analysis. In a previous descriptive study of brown-anole populations from these islands³⁸, we found that dietary species richness did not differ across seasons, and that compositional dissimilarity across seasons was mild. To further test whether differences in prey availability and island-specific sampling intensities across periods might have influenced the results presented here, we separately analysed the data from the two best-sampled seasons, December 2013 ($n = 131$ samples; range, 6–97 per species) and May 2014 ($n = 129$ samples; range, 13–88 per species). For each of these subsets, we repeated the analyses in Fig. 4b, c and found qualitatively identical results to those based on the full dataset. In December 2013, lizard species had significantly different diet compositions (PERMANOVA, pseudo- $F_{2,128} = 4.18$, $R^2 = 0.06$, $P < 0.001$), and the diets of brown anoles showed significant independent and interactive responses to the treatments (effect of green anoles, pseudo- $F_{1,93} = 2.19$, $R^2 = 0.02$, $P = 0.002$; effect of curly-tailed lizards, pseudo- $F_{1,93} = 1.43$, $R^2 = 0.02$, $P = 0.046$; interaction, pseudo- $F_{1,93} = 1.51$, $R^2 = 0.02$, $P = 0.037$). Likewise, in May 2014, diets differed among species (pseudo- $F_{2,126} = 3.34$, $R^2 = 0.05$, $P < 0.001$), and the main and interactive effects of the treatments were significant (effect of green anoles, pseudo- $F_{1,84} = 2.01$, $R^2 = 0.02$, $P = 0.01$; effect of curly-tailed lizards,

pseudo- $F_{1,84} = 2.30$, $R^2 = 0.026$, $P < 0.001$; interaction, pseudo- $F_{1,84} = 1.46$, $R^2 = 0.02$, $P = 0.050$). We also verified that the three most-abundant prey taxa overall (the cockroach, beetle, and ant species in Fig. 4f–n) occurred at similar relative abundances in faecal samples from brown anoles across the three sampling intervals (0.11–0.20, 0.06–0.11, and 0.06–0.09, respectively).

Quantitative PCR assay of intraguild predation. We developed species-specific SYBR Green quantitative PCR (qPCR) assays for brown anoles, green anoles, and curly-tailed lizards. We designed primers based on sequences from locally collected lizards and NCBI GenBank, using oboitools⁵⁹ and Primer3Plus⁶⁶ to identify COI regions (50–100 bp) flanked by potentially suitable priming sites. Primer sequences and amplicon sizes (including primers) are: brown anoles, AS_01_F 5'-GCCTCAGTTGATTTAACCAT-3' and AS_01_R 5'-CCAAGAATAGATGAAACCCC-3', amplifying 59 bp; green anoles, AC_12_for 5'-TTACCCCATCATTTCTTCTTC-3' and AC_12_rev 5'-AGGTTACTTGCTAGTGGTGGG-3' amplifying 95 bp; and curly-tailed lizards, curly_F3 5'-GCTACTAGCATCATCTGGAGTTG-3' and curly_R3 5'-GCTAAGTTCTCGAGTGG-3', amplifying 78 bp.

We tested the species specificity of each assay using DNA extracted from tail samples of brown anoles from all 16 experimental islands, of green anoles from the 8 experimental islands on which they were present, and of curly-tailed lizards from Thomas Cay (the source used for populating experimental islands). No amplification of non-target DNA was detected for any assay. We then applied the qPCR assays to 61 DNA extractions of faecal samples from curly-tailed lizards, which were obtained from 56 unique individuals (with replicate samples from 5 individuals included to test the consistency of results) collected on 7 of the 8 islands on which curly-tailed lizards were present. We performed qPCR reactions on a Stratagene Mx3500P thermocycler (Agilent Technologies). Quadruplicate qPCR reactions were performed in clear optical tube strips at 10 μ l total volume: 5 μ l of 2 \times PowerUp SYBR Green Master Mix (Applied Biosystems), 0.2 μ l (10 μ M) of each primer, 3.6 μ l of Milli-Q H₂O, and 1 μ l of DNA template. A no-template control and a positive control using tissue-derived DNA were included in each qPCR run. Identical thermocycling conditions were used for each assay, except the annealing temperatures and cycle numbers: 95 °C for 10 min followed by 30 (brown and green anoles) or 35 (curly-tailed lizards) three-step cycles of 95 °C for 30 s, 53 °C (brown anoles), 58 °C (green anoles), or 56 °C (curly-tailed lizards) for 1 min, and 72 °C for 1 min. A dissociation curve was included at the end of each assay with the following conditions: 95 °C for 1 min, 53 °C (brown anoles), 58 °C (green anoles), or 56 °C (curly-tailed lizards) for 30 s, and a ramp-up to 95 °C for 30 s. Fluorescence was recorded at the end of each annealing step and on the dissociation-curve ramp. We analysed the data using MxPro software (Mx3005P v.4.01), with the baseline fluorescence threshold determined automatically using the amplification-based threshold option. We considered DNA to be present whenever fluorescence crossed this threshold.

We detected DNA of curly-tailed lizards in 56 of 61 faecal extracts from 51 of 56 individuals, which suggests that PCR inhibition was unlikely to prevent amplification of anole DNA if present in these samples. These 56 samples represented all 7 sampled islands and included 23 individuals from +CT islands and 28 from +GA+CT islands (range, 2–18 unique individuals per island), along with all 5 replicate samples. We detected brown-anole DNA in 3 extracts obtained from 2 of the 51 individual curly-tailed lizards tested, both of which were from island 926 (the largest +GA+CT island). We detected green-anole DNA in 2 replicate faecal samples from 1 of the 28 curly-tailed lizards sampled on islands on which green anoles were present (from 312, the smallest +GA+CT island). All 5 replicate samples were consistently either positive or negative for anole DNA. Data are presented in Supplementary Data 4. All positive DNA amplifications from faecal samples displayed identical peak temperatures in dissociation curves as positive controls, which indicates that the target fragment was correctly amplified. The three curly-tailed lizards that were found to have consumed anoles were of average size (snout–vent length 8.8 ± 1.4 cm, weight 27.2 ± 11.4 g) relative to 55 individuals that we measured (8.2 ± 0.3 cm, 25.3 ± 3.1 g).

Stable-isotope analyses of trophic position and food-chain length. For a subset of the lizards captured to obtain faecal samples for DNA metabarcoding, along with a number of other lizards from which no faecal samples were collected, we amputated the terminal ~1 cm of tail for stable-isotope analysis ($n = 203$ brown anoles, 33 green anoles, and 65 curly-tailed lizards). We also collected 108 individuals of the most-common spider species *M. datona* (Araneidae), along with a small and haphazard assortment of insects ($n = 1$ –4 each of ants, beetles, flies, crickets and cockroaches) and less-common spiders ($n = 5$ and 7 of *Gasteracantha cancriformis* and *Eustala cazieri*, respectively).

In general, we followed methods used in previous isotopic studies at a Bahamian site ~100 km to the south of our islands^{30,67}. To establish an isotopic baseline for each island, we collected foliage from three buttonwood (*Conocarpus erectus*) trees at each of three sites per island, targeting the youngest fully emerged leaves, which were dried and homogenized within each site and then averaged across sites to

obtain a single island-wide value. Buttonwood (a C₃ plant) is the dominant woody species in this low-diversity system^{67,68}, was common on all islands, and is a major food plant of *A. floridanus* (the primary prey of green anoles and of brown anoles on +CT islands) and other insect herbivores^{67,68}. C₄ plants such as grasses are very rare on these islands, as noted in previous studies from nearby Bahamian sites^{30,67}. Lizards and spiders in this system are known to consume marine-derived nutrients, although this pathway is most pronounced on small islands and shoreline habitats^{30,67}, whereas our islands were relatively large and most lizards occurred in the interior. Nonetheless, because our 16 islands differed in size and shoreline:area ratio, we sought to correct for potential marine-derived subsidies. We assumed that our 16 study islands shared a common marine isotopic baseline⁶⁹, which we estimated using average values from 11 macroalgae samples collected from locations across our 13-km string of study islands (Fig. 1d). A previous Bahamian study found that conclusions about trophic position and food-chain length were not sensitive to whether the marine isotopic baseline was estimated using macroalgae averaged across all islands (as we did) or particulate organic matter filtered from seawater at each island³⁰, which provides further justification for our approach. All samples were dried in the field at 60 °C for 48 h and stored in paper envelopes with silica gel desiccant.

We prepared lizard-tail samples for analysis using previously described protocols⁷⁰, which are similar to those used in previous Bahamian studies^{30,67}. We used elemental carbon (C) and nitrogen (N) content to verify that tail tissues (primarily scale keratin and proteinaceous connective material) were comparable among individuals; wt% C:N ratios were consistent (range, 2.8–3.4, except for 6 samples that were not isotopically anomalous and thus retained for analysis). To obtain direct estimates of lipid-free $\delta^{13}\text{C}$ values, arthropod samples (targeting head, thorax, and leg tissue, but including whole bodies for small animals) were placed into glass vials, rinsed three times (30-min sonication at room temperature) in 1:1 chloroform:methanol solution to remove lipids, and then dried. Previous work⁷¹ has shown that a similar extraction method did not affect the $\delta^{15}\text{N}$ values of terrestrial arthropod whole-body samples. Arthropod wt% C:N ratios ranged from 3.1 to 5.3, indicating that tissue samples were a mixture of proteinaceous materials and chitin. Because each spider sample was a unique mixture of these two tissues, we sought to test whether the observed isotopic variation might be an artefact of differences in the relative contributions of protein and chitin to the whole-body samples; there was no strong correlation between spider sample $\delta^{13}\text{C}$ values and C:N ratios ($R^2 = 0.03$, $F_{1,105} = 3.07$, $P = 0.08$), so we assumed that all spider samples were comparable. Approximately 0.8 mg of lizard and arthropod tissues, and 1–3 mg of terrestrial plants and macroalgae, were weighed into tin boats and analysed at the University of California Santa Cruz Stable Isotope Laboratory on a Thermo Finnegan Delta-Plus XP IRMS. Stable-isotope values are reported using the δ notation, referenced to Vienna PeeDee Belemnite for C and air for N.

We followed previous studies^{25,28,30,69} in calculating the trophic position of individual consumers and the food-chain length of islands. We estimated trophic position as

$$\text{TP} = \lambda + (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{basal}}) / \Delta^{15}\text{N}$$

in which λ , the trophic position of the basal resource (primary producers) is equal to 1, and $\Delta^{15}\text{N}$ is the trophic fractionation for $\delta^{15}\text{N}$ (that is, the difference in $\delta^{15}\text{N}$ values with each increment in trophic level). The $\delta^{15}\text{N}_{\text{basal}}$ value was calculated using a two-end-member mixing model to estimate the isotopic baseline of each island, using data from island-specific terrestrial plant samples and multi-island-averaged marine-macroalgae samples:

$$\delta^{15}\text{N}_{\text{basal}} = [\delta^{15}\text{N}_{\text{terrestrial}} \times (1-a)] - (\delta^{15}\text{N}_{\text{marine}} \times a)$$

in which a is the proportion of consumer carbon derived from the marine baseline, calculated as $[(\delta^{13}\text{C}_{\text{consumer}} - \Delta^{13}\text{C}) - \delta^{13}\text{C}_{\text{terrestrial}}] / (\delta^{13}\text{C}_{\text{marine}} - \delta^{13}\text{C}_{\text{terrestrial}})$ (as previously described⁶⁷), and $\Delta^{13}\text{C}$ is the fractionation for $\delta^{13}\text{C}$.

For the analyses reported in the main text, we calculated trophic positions using the standard $\Delta^{15}\text{N} = 3.4\text{‰}$ (as previously described^{30,72}), and we used $\Delta^{13}\text{C} = 3.8\text{‰}$, because this value was determined from previous measurements of herbivorous beetles feeding on buttonwood leaves in the Exuma island chain⁶⁷. In these analyses, the estimated proportional contribution of marine subsidies to all lizard diets was limited (median, $a = 0.1$; interquartile range, 0.01 to 0.17; $n = 301$ individuals) and was slightly higher for curly-tailed lizards (0.14 ± 0.01) than for either anole species (0.09 – 0.10 ± 0.01) (pairwise nonparametric Wilcoxon comparisons, both $P < 0.02$). We verified that the results reported in the main text were robust to the use of other published values for both $\Delta^{13}\text{C}$ (0‰ (refs. 30,69) and 5‰, corresponding to 3.8‰ from plants to insects⁶⁷ plus 1.2‰ from insects to lizards⁷³) and $\Delta^{15}\text{N}$ (a lizard-specific estimate of 0.7‰ (ref. 73) and 2.4‰, corresponding to the standard 3.4‰ minus its 1‰ s.d. (ref. 72)) (Extended Data Table 2). We did not calculate trophic positions for the miscellaneous insects that we collected, given the small sample sizes; however, the exceedingly low raw $\delta^{15}\text{N}$ values of

three cockroaches (the primary prey of curly-tailed lizards and of brown anoles on control islands) were informative for interpreting the relatively low trophic position of curly-tailed lizards. Because trophic position increased with island area (Fig. 5c; see also a previous publication³⁰), we tested for differences among species using ANCOVA on mean population-level estimates of trophic position, with species identity as a categorical factor and island area as a covariate (Fig. 5b). For each of the 12 islands on which at least 2 lizard species were present, we also conducted separate one-way ANOVA to test whether mean individual-level trophic position differed between anoles and curly-tailed lizards within islands.

In calculating island-wide food-chain length, we followed previous studies^{25,28,30,69} in using the mean trophic-position value of the apical consumer population on each island (see 'Curly-tailed lizards as top predators'). Food-chain length was analysed using a generalized least-squares linear model with main effects of island area and the factorial green-anole and curly-tailed-lizard addition treatments, along with all first-order interactions between these variables (Extended Data Table 2). This model structure was selected in light of previous work showing that food-chain length increased with island area in the Bahamas³⁰, and because our primary interest was to understand the effects of the factorial experimental treatments after accounting for any effects of ecosystem size. Previous work in the Bahamas³⁰ has found that lizards and spiders occupied similarly high trophic positions on islands where they co-occurred. We found that the mean trophic position of the most-abundant spider *M. datona* (2.51 ± 0.06 , $n = 108$ individuals) was variable, but on average significantly lower than that of lizards (2.67 ± 0.03 , $n = 301$ individuals) in a linear model of trophic position as a function of consumer type, island area, and the crossed factorial experimental treatments (effect of consumer type $F_{1,31} = 4.32$, $P = 0.046$). Accordingly, and because *M. datona* was sampled from just seven islands, we used only lizards for the analysis of food-chain length in the main text. However, our conclusions did not change when we used values from *M. datona* on the two islands on which it was the apical consumer population (Extended Data Fig. 6). Isotopic and trophic-position data for each individual lizard and *M. datona* spider are presented in Supplementary Data 5.

To explore whether the omnivory mechanism²⁵ might have contributed to the variation in food-chain length across our experimental treatments, we analysed patterns of lizard dietary diversity and richness. We are unable to directly measure trophic omnivory (that is, feeding at multiple trophic levels) because we do not know the trophic level of the vast majority of arthropod prey taxa. We do know, however, that arthropods on these islands span a wide range of trophic levels, with raw $\delta^{15}\text{N}$ values that ranged from 0.56 in one cockroach, through 10.91 in one fly, to 14.39 in one spider (with the maximal recorded trophic position for a spider of 4.85, higher than any individual lizard). Accordingly, given that these lizards are generalized insectivores (Fig. 4), the taxonomic breadth of arthropod prey taxa is almost certainly a positive correlate of true trophic omnivory—the more arthropod species 'sampled' in a diet, the greater the probability that those arthropods span a broad range of trophic levels. Using the rarefied DNA-metabarcoding dataset (Supplementary Data 2), we calculated both dietary species richness (number of prey mOTUs) and dietary Shannon diversity (which accounts for both richness and evenness) for each individual lizard faecal sample and then calculated the mean of each metric for each lizard population. (We used averaged individual-level metrics rather than pooled population-level metrics because the latter would be sensitive to sample size, which varied across islands³⁸.) We analysed mean per-sample dietary diversity and richness of the apical consumer population on each island using the same generalized least-squares linear model structure used to analyse food-chain length (Extended Data Fig. 7a–c). In addition, we analysed dietary diversity and richness of curly-tailed lizards on +CT and +GA+CT islands, using ANCOVA as a function of treatment \times island area, to explore whether greater trophic omnivory by the top predator on +GA+CT islands might explain why food-chain length was shorter in that treatment and did not increase with ecosystem size (Extended Data Fig. 7d–h). Despite our imperfect proxy for trophic omnivory (which reflects only the arthropod component of diet, and not plants or vertebrates), these analyses were consistent with theoretical predictions about the role of trophic omnivory in shaping food-chain length^{9–11}.

Curly-tailed lizards as top predators. We follow previous work²⁵ in defining 'top' and 'apical' predators, trophic position, and food-chain length. Curly-tailed lizards were the top predators in our experiment because they can eat all other terrestrial species resident on these islands³⁶ but cannot be eaten by any of them, with one anomaly noted below (for an analogously omnivorous yet unambiguously top predator, consider brown bears *Ursus arctos*). Top predators can act simultaneously as intraguild predators and competitors, but are not necessarily the apical predators, which are those with the highest mean trophic position²⁵. Curly-tailed lizards were the apical lizard population on 3 of the 4 +CT islands, green anoles were the apical population on 2 of the 4 +GA islands, and brown anoles were the apical population on 6 of the 12 islands on which at least 1 other lizard species was present. Food-chain length is defined as the mean trophic position of the apical predator population, which averaged 2.86 across islands (range, 2.09–3.66) (Extended Data

Table 2); these values are comparable to those in a previous study of food-chain length on nearby Bahamian islands³⁰.

A previous study³⁶ of the stomach contents of 173 curly-tailed lizards found that just 1 individual contained an anole (0.02% of prey items), corresponding to an average of 3% of diet by volume across individuals—compared with 15% for cockroaches, 12% for lepidopterans, 11% for fruits, 8.2% for ants, and 6.4% for beetles. This previous study concluded that curly-tailed lizards are opportunistic omnivores, with lizards constituting only a minor component of diet³⁶. These findings are consistent with our DNA-based diet analyses and anecdotal observations. Cockroaches, notably *H. pabulator*, were by far the dominant arthropod prey of curly-tailed lizards (detected in 97% of samples and accounting for 32–44% of RRA) (Fig. 4h). Ants (collectively 21% of RRA, notably *Brachymyrmex* spp.) (Fig. 4n) and lepidopterans (collectively 8% of RRA, notably *Halysidota* sp. (Erebidae)) were also common (Supplementary Table 1). Curly-tailed lizards will eat table scraps³⁶, and we observed them scavenging dead hermit crabs. In the final year of this study, we observed a solitary instance of a curly-tailed lizard eating a small brown anole (Extended Data Fig. 1e), which we view as corroborating our qPCR data indicating that predation occurred but was relatively infrequent. By contrast, we frequently observed curly-tailed lizards unsuccessfully chasing brown anoles and the latter escaping by ascending trees (Extended Data Fig. 1f); these observations contributed to our impression of a strong landscape of fear. No direct interaction of any kind was ever observed between curly-tailed lizards and green anoles, although our qPCR assay did reveal one instance of green-anole consumption. Green anoles were almost never on the ground on any island (Fig. 3f) and moved by running or hopping between adjacent tree canopies within the upper third of the vegetation, on perches far too thin and flimsy to support curly-tailed lizards (which in 2016 were on the ground in >70% of observations and in the lower two-thirds of the habitat in >99.5% of observations) (Fig. 3i).

These considerations—coupled with our measurements of trophic position and diet composition—support previous arguments³⁶ that the primary interaction between curly-tailed lizards and anoles is competition, along with the omnipresent risk of intraguild predation. In one instance of interference competition, we observed two juvenile curly-tailed lizards feeding on a termite trail (*Parvitermes* (syn. *Nasutitermes*) *brooksi*) chase away an adult male brown anole (Extended Data Fig. 1d, Supplementary Video 1). We hypothesize that the contrast between our results and previous studies^{33,34,74} documenting rapid devastation of brown-anole populations by introduced curly-tailed lizards stems from the roughly order-of-magnitude larger islands used in this study (range, 487–3,320 m², compared with 100–300 m² in previous studies) and the correspondingly greater availability of thick arboreal refuges on our islands (Extended Data Fig. 1p, q). Indeed, one of these previous studies³⁴ showed that the survival of brown anoles on islands with introduced curly-tailed lizards increased as function of vegetation height, which in turn increased as a function of island size.

As with any large-scale, long-term whole-ecosystem manipulation, we were unable to control immigration and emigration of other species, but we do not believe that any island was consistently occupied by predators capable of consuming adult curly-tailed lizards. The one possible exception occurred in 2013 on the smallest island (204), where we found a Bahamian boa (*Chilabothrus strigilatus*) that we were unable to remove (but never saw again). This snake might explain why curly-tailed lizards initially failed to establish on island 204. However, island 204 is just 15–20 m from an adjacent small island and is <100 m from a much larger island (such that the boa could probably emigrate), and the curly-tailed lizard population on island 204 subsequently persisted for several years. On most if not all islands, rodents were at least intermittently present, judging from bite scars on the stems of leguminous *Pithecellobium* plants. Nocturnal rodents may sometimes prey on lizard eggs or sleeping anoles, but we observed their sign on islands of all treatments (including those with the largest and most behaviourally uninhibited anole populations), which gives us no reason to believe that they confounded our experimental treatments or biased our results. Itinerant birds such as gulls (*Leucophaeus atricilla*) and herons (*Butorides virescens*) were observed nesting in May on multiple islands of all treatments; we do not know whether these birds preyed on lizards, but their numbers were small, their presence was seasonal, and they were not systematically associated with our randomly assigned and spatially interspersed treatments in any way that is likely to bias our results.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The datasets that support the findings of this study are provided in Supplementary Data 1–6. Illumina sequence data from the DNA metabarcoding diet analyses are deposited in Dryad (<https://doi.org/10.5061/dryad.2h87r1f>). Arthropod DNA reference sequences and associated specimen information are deposited in the Barcode of Life Data System (dataset DS-BAHARTR2, available via <https://doi.org/10.5883/DS-BAHARTR2>) and in GenBank

- (accessions MK936608–MK936799). Lizard DNA reference sequences are deposited in GenBank (accessions MK867972–MK868027).
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