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Enhanced locomotor performance on familiar surfaces is uncoupled from morphological plasticity in *Anolis* lizards

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Abstract

The radiation of *Anolis* lizards in the Caribbean is associated with a diversification of the functional match between morphology, habitat use, and locomotor performance. It has been hypothesized that the microhabitat a lizard is reared in can achieve a similar fit of form and function within a species. This predicts that plasticity in the locomotor apparatus is accompanied by changes in perching behavior or improved locomotor performance. To test this, we raised juveniles of two species (*Anolis sagrei* and *Anolis carolinensis*) on either broad or narrow surfaces and examined perching behavior and locomotor performance as well as the shape of the pectoral and pelvic girdles, limb length, and thickness of the long bones. Perching behavior was not affected by the habitat surface experienced during ontogeny. However, individuals raised on broad surfaces showed better locomotor performance on broad surfaces, and the magnitude of the effect was as large as the difference between the two species. Both species showed modifications of pectoral and pelvic shape, but only *A. carolinensis* developed longer limbs on broad surfaces. However, these morphological adjustments induced by physical activity did not explain why lizards raised on broad surfaces performed better. Thus, it appears that early-life experiences can affect both the morphology of the locomotor apparatus and locomotor performance in *Anolis* lizards, without the two being functionally connected.

KEYWORDS
behavior, limb morphology, lizards, locomotion, phenotypic integration, plasticity

1 | INTRODUCTION

The morphology, performance, and behavior of animals are often strongly correlated with the habitat in which they occur. This fit of form and function typically arises from natural selection. However, experiences during ontogeny can mold the phenotype in ways that promote the fit between the individual and its environment beyond that achieved by natural selection. Such plasticity often relies on general properties of development, which is readily seen from how mechanical or physiological stress shapes bones and tissues. In turn, these modifications of skeletal structures (i.e., form) may influence how individuals interact with and perform in their environment (i.e., function). Alternatively, experiences during ontogeny may shape form and function simultaneously and independently. Addressing the extent to which plasticity of form and function are phenotypically integrated has implications for the targets of natural selection and therefore which traits are likely to diverge during local adaptation.

Anolis lizards are a prime example of the repeated evolution of the fit between morphology and ecology (Larson & Losos, 1996; Losos, 1990a; Williams, 1983). A defining feature of anole

"ecomorphs" is the relative length of their limbs, which correlates with the structural habitat in which they live (e.g., perch diameter; Losos, 1990a). There is evidence that these species differences are adaptive. Species with longer hindlimbs are more efficient in running on broad surfaces, whereas shorter limbs provide stability and support on narrow branches (Losos & Sinervo, 1989). Studies of anoles transplanted to islands (Losos, Warheit, & Schoener, 1997) or colonizing new habitats (e.g., urban environments; Kolbe, Battles, & Avilés-Rodríguez, 2016; Winchell, Reynolds, Prado-Irwin, Puente-Rolon, & Revell, 2016) suggest that changes in morphology and behavior that are concordant with such functional demands can evolve within a few generations. While ecomorphs also differ in the shape of pectoral (shoulder) and pelvic (hip) girdles (Herrel, Vanhooydonck, Porck, & Irschick, 2008; Tinius & Russell, 2014; Tinius, Russell, Jamniczky, & Anderson, 2018), it is not known to what extent this variation is functionally related to locomotion.

It is currently unknown if any environmentally induced differences in skeletal morphology are accompanied by changes in perching behavior and locomotor performance. Therefore, we studied both morphological plasticity of the locomotor apparatus, perching behavior, and locomotor performance in two different *Anolis* species (*Anolis sagrei* and *Anolis carolinensis*) reared in two different structural habitats. We predicted that, for both species, lizards reared on broad surfaces would develop longer limbs, modify girdle shapes and run faster on broad surfaces compared to lizards reared on narrow surfaces. In contrast, lizards reared on broad surfaces would perform less well on narrow dowels, and be more reluctant to use narrow perch sites, than lizards raised on narrow surfaces. Further, we predicted that any treatment-induced effects on locomotor performance would be partly explained by a corresponding change in skeletal morphology (limbs or girdles). While previous results suggest that the morphological plasticity should be consistent for the two species (Kolbe & Losos, 2005; Langford et al., 2014; Losos et al., 2000), *A. sagrei* and *A. carolinensis* belong to different ecomorphs (trunk-ground and trunk-crown, respectively). Therefore, this allowed us to contrast the magnitude of the plastic response to evolved differences between two relatively distantly related species (~45 my divergence time; Poe et al., 2017; Roman-Palacios, Tavera, & Castaneda, 2018; Zheng & Wiens, 2016). Our results demonstrate an important role of the ontogenetic environment for individual differences in both skeletal morphology and locomotor performance. However, improved locomotor performance did not appear to be caused by the plasticity in skeletal morphology. We discuss the implications of these results for the evolution of functional integration of morphology and performance in *Anolis* lizards.

2 | MATERIALS AND METHODS

2.1 | Study species and housing

Twenty-four female and eight male adult lizards of each of the two species, *A. sagrei* and *A. carolinensis*, were collected in Palm Coast,

Florida in April 2016 and brought to the animal facility at Lund University (range of snout-vent length (SVL) of *A. carolinensis* males, 5.6–5.7 cm, females, 4.5–5.0 cm; *A. sagrei* males, 5.8–7.2 cm, females, 4.3–4.9 cm). We collected tail tissue for parentage analysis (see below). All animals were housed in 80-litre cages (Wham Crystal box with mesh on top, 590 × 390 × 415 mm), with one male and three females per cage. Males were swapped between cages twice during the course of the experiment to increase the spread of parentage among experimental animals. Cages were enriched with twigs, hiding areas, basking spots, and a water bowl. Plastic cups with moist vermiculite were provided for oviposition. Adult lizards were kept at a light cycle of 12 L:12 D and given access to basking lights (60 W) for 10 hr per day and a UV light (EXO-TERRA 10.0 UVB fluorescent tube) for 6 hr per day. Mealworms and crickets were provided *ad libitum*. Eggs were collected every second day and incubated at 26°C in individual small plastic containers filled two-thirds with moist vermiculite (5:1 vermiculite:water volume ratio) and sealed with clingfilm.

Hatchlings (at most 24 hr after hatching) were alternately assigned to experimental treatments that consisted of 80-litre cages (see above) with different interior fittings. The narrow treatment consisted of an arrangement of six unpainted wooden dowels, three 0.4 cm and three 0.8 cm in diameter (Figure S1A). The broad treatment contained two unpainted wooden planks (4 × 8 × 50 cm; Figure S1B). Both dowels and planks were angled at approximately 40°. Each cage contained a water bowl, and light conditions were identical to the adult set-up described above. Cage walls were coated with Fluon® to ensure that juvenile lizards used the experimental interior fittings. Groups of six juveniles were housed in a single cage since prior observations indicated that grouping lizards encourages locomotor activity and the development of a natural behavioral repertoire. To minimize negative effects due to social hierarchies (e.g., "runting"), we grouped lizards of roughly the same age together (±3 days). Juvenile lizards were fed daily with different size classes of crickets according to their gape size, and crickets were dusted with vitamins and calcium (Zoo Med Reptivite™ Reptile Vitamins with D3) twice a week to promote normal bone growth. The structural habitat in this setup reflects biologically relevant conditions (range of natural perch diameters of *A. carolinensis* juveniles and *A. sagrei* juveniles, 0.3–4.4 cm and 0.3 to >10.8 cm, respectively; Schoener, 1968). Although the diameter of perch sites was comparable to previous studies testing the effect of structural habitat on limb plasticity in *Anolis* lizards (Table S1), our setup provided more total surface area and greater cage size than in previous studies, and treatment commenced immediately upon hatching.

Most lizards in our experiment reached sexual maturity at the age of 5 months, at which time growth typically begins to slow down (O'Bryant & Wade, 2001), and we, therefore, chose this as the endpoint of the experiment. Before lizards were killed (through a blow to the back of the head followed by neck dislocation and destruction of the brain), we conducted behavioral experiments and took morphological measurements (see below). Immediately after lizards were killed, we sexed them and collected tail tissue for parentage analyses (see below). Sex was determined based on

external features and confirmed by inspecting internal anatomy *post mortem*.

We obtained a total of 169 eggs for *A. sagrei* and 101 for *A. carolinensis*. Five eggs of *A. sagrei* and six of *A. carolinensis* failed to hatch and the rest was raised to 5 months of age at which we successfully collected data for 147 individuals of *A. sagrei* ($N = 76$ broad treatment, $N = 71$ narrow treatment) and 83 of *A. carolinensis* ($N = 40$ broad treatment, $N = 43$ narrow treatment).

2.2 | Parentage analysis

We assigned maternity and paternity using microsatellite markers. We extracted DNA from tail tissue of experimental animals and their putative dams and sires using the DNeasy Blood and Tissue Kit (Qiagen, Inc.), following the manufacturer's instructions. We genotyped all individuals of *A. sagrei* at 12, and all *A. carolinensis* at eight polymorphic microsatellite loci (Bardeleben, Palchevskiy, Calsbeek, & Wayne, 2004; Wordley, Slate, & Stapley, 2011). Multiplexed polymerase chain reactions (PCRs) were carried out in a total volume of $10\,\mu l$ reaction mix containing $1\,\mu l$ of genomic DNA, $5\,\mu l$ of GoTaq® Green Master Mix (Promega), $0.1–0.3\,\mu l$ of each primer (10 mM) and PCR grade H_2O. PCR conditions for each reaction are specified in Table S2. The 5′-end of each forward primer was labeled with a fluorescent dye (either 6-FAM, VIC, NED, or PET). PCR products were genotyped on an ABI3730 Genetic Analyzer (Life Technology, Inc.), and fragment sizes were binned and verified by eye using Geneious 8.1.7 software (https://www.geneious.com). We used the software CERVUS (Version 3.0.7; Kalinowski, Taper, & Marshall, 2007) for assigning maternity and paternity to all experimental individuals. We selected the sire and dam with the highest likelihood as each offspring's parents at a minimum confidence level of 80%.

2.3 | Morphology

When lizards reached 5 months of age, we measured SVL (in mm to the closest 0.01 mm) using a digital caliper. Killed lizards were fixed in 4% paraformaldehyde in a standardized body posture with limbs stretched out. We counted lamellae on the longest digits of both forelimb and hindlimb (third digit on the forefoot and fourth digit on hindfoot). Since measurements on right and left were highly correlated (lamellae$_{hindfoot}$, Pearson's product-moment correlation $r_P = .91$; lamellae$_{forefoot}$, $r_P = .94$; both $p < .001$), we used the mean of these measurements in statistical analyses.

To obtain a multivariate measure of skeletal morphology, we subjected all lizards to micro-computed tomography (micro-CT) scanning and recorded linear measurements of limb bone length and thickness as well as geometric morphometric variation in girdle shape (Figure 1). Regarding limb morphology, this methodology has several advantages over traditional methods, in particular, since it enhances the accuracy of the length measurement and allows quantification of bone diameter and cortical thickness. In general, increased mechanical load on long bones induces increased mid-diaphysis thickness (bone diameter) and increased cortical thickness (Gardinier, Rostami, Juliano, & Zhang, 2018; Wallace et al., 2007).

Micro-CT scans were obtained using a GE phoenix |tome|x m system (source voltage 100 kV; source current 200 μA; isometric voxel size 55–75 μm). Reconstructed image stacks (software GE phoenix datos|x CT) were further processed using VGStudio MAX software (version 3.2) by applying manual thresholding to extract surface models of skeletal structures. Linear measurements were directly obtained using the VGStudio MAX software. For each lizard, we measured the maximum length of humerus, femur, ulna, tibia, and the individual phalangeal elements (including the claw) of the longest digit of both fore- and hindlimb (in mm to the closest 0.01 mm). This was achieved by placing one landmark each on the proximal and on the distal end of the bone and extracting the distance between these two points in three-dimensional space (Figure 1). A small number ($N = 5$; 0.15%) of individual measurements were missing due to fractured bones, and we imputed these missing values using the "pcaMethods" package (Stacklies, Redestig, Scholz, Walther, & Selbig, 2007). Individual phalangeal elements were added up to capture fore- and hindfoot length. Similarly, the resulting foot length measurements were summed up with long bone measures (humerus, ulna, femur, and tibia) to capture total fore- and hindlimb length. This total limb length corresponds to the measures taken with calipers that typically are reported in previous studies of *Anolis* limb length (Kolbe & Losos, 2005; Langford et al., 2014; Losos et al., 2000; Winchell, Maayan, Fredette, & Revell, 2018).
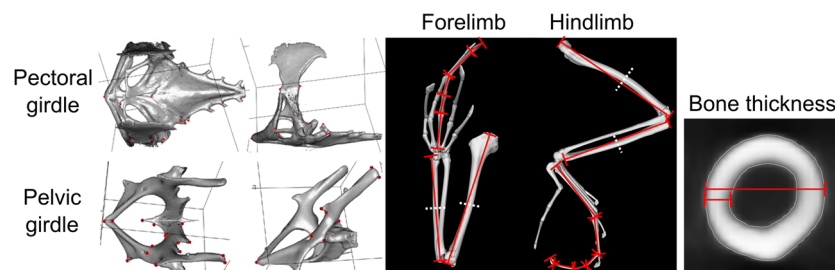

FIGURE 1 Skeletal morphology of the locomotor apparatus that was quantified in this study. The shape of pectoral and pelvic girdles was captured using each 18 three-dimensional landmarks (red dots) on prominent features of the structures (Table S4). The length of individual bone elements of the fore- and hindlimbs were measured as indicated by red lines, and bone thickness was quantified as bone diameter and cortical thickness in virtual cross-sections of the four long bones (marked as dashed white lines in fore- and hindlimbs) [Color figure can be viewed at wileyonlinelibrary.com]

To obtain linear measures of the long bone thicknesses, the midpoint between proximal and distal end of each bone was determined and the image plane perpendicular to the longitudinal bone axis was selected. In this virtual transverse cross-section of the long bones, total bone diameter and cortical thickness (calculated from the difference of total bone diameter and bone cavity, divided by two) were measured to the closest µm by averaging two measurements taken on orthogonal axes of the bone to adjust for the slight deviation of the shape from a perfectly symmetrical ring (Figure 1). All linear measurements were collected for one side (left or right) of each lizard. We took multiple measurements on a subset of individuals to determine measurement errors, which turned out to be negligible ($r_P > .97$, $p < .001$, $N = 10$; Table S3).

To obtain geometric morphometric shape data of the girdles, meshes of segmented pectoral and pelvic girdles were exported as .stl files, which were converted into .ply format using the software MeshLab (version 2016.12). For each pectoral and pelvic girdle, we placed 18 landmarks on anatomical informative features (Table S4 and Figure 1) using the R package "geomorph" (version 3.1.3). To obtain shape variable from the recorded landmarks, a "generalized Procrustes analysis" was performed.

Our phenotyping produced a large number of variables capturing skeletal morphology (132 variables). We performed statistical tests both on total forelimb and hindlimb length as response variable since this is a common practice (e.g., Kolbe & Losos, 2005; Kolbe et al., 2016; Losos, Schoener, Warheit, & Creer, 2001; Spezzano & Jayne, 2004), as well as the first and second principal components (PCs) of pectoral shape, pelvic shape, and bone thickness. These PCs were calculated on centered and scaled values using the R function "prcomp" (Table S5). In addition to this PC-based approach, shape variation in girdles was also analysed using the function "procD.lm" in the R package "geomorph".

For consistency, all morphological variables were scored by the same person (SVL, lamellae count, linear measurements: N.F.; landmarks: I.S.C.J.) and blindly with respect to treatment groups.

2.4 | Perching behavior

We tested the perching behavior of *A. sagrei* and *A. carolinensis* by scoring the propensity of lizards to climb a challengingly narrow dowel. While this does not test for habitat preference *per se,* it assesses an important aspect of how *Anolis* lizards interact with their environment. Locomotion on narrow dowels presents a challenge for *Anolis* lizards, with species not specialized in maneuvering narrow surfaces frequently losing balance or even falling off (Losos & Sinervo, 1989). The rationale of this behavioral assay was to test whether lizards that were reared in the narrow treatment show greater confidence with perching and basking on narrow surfaces.

The experimental setup consisted of a wooden dowel (52 cm), gradually narrowing towards the tip (diameter: 2 cm at base, 0.5 mm at tip), that was placed at a 45° angle with the tip 5 cm under a heat lamp. During a trial, a lizard was placed at the base of the dowel and we recorded the position it would choose to bask. To increase the motivation of the lizard to move towards the narrow tip of the dowel, and thus closer to the heat lamp, the experiment was performed at 16°C, and lizards were acclimatized to this temperature 30 min before the trial. Temperatures were measured at the tip of the dowel directly under the lamp (26°C) and at 5 (21°C), 10 (20°C), and 20 cm (18°C) distance from the tip of the dowel. This means that the lizard could only approach the preferred body temperature range by climbing to the very top of the dowel (preferred body temperature of both species is around 33°C; Corn, 1971). All experiments were performed in the early afternoon between 1 and 3 p.m. Each lizard was tested three sequential times, with each trial lasting for 1 min. During this trial period, we recorded positions (i.e., the distance away from the tip of the dowel) where the lizard was resting for >5 s, and we calculated two different measures: the average position as the mean of all positions, and the average end position of three trials (i.e., the position closest to the tip in each trial). A small number ($N = 3$; 0.43%) of individual measurements were missing, and we imputed these missing values using the "pcaMethods" package (Stacklies et al., 2007). A subset of lizards was also tested at 4 months of age to determine individual consistency (Table S3).

Complementary to the dowel assay, we used another perch choice assay to assess whether lizards show perching site preference, similarly to a previous study (Langford et al., 2014). While the assays are intended to capture similar aspects of *Anolis* biology, this second assay makes it possible to test if lizards raised on broad surfaces actively avoid narrow perch sites if given a choice. We used 454-litre cages (Zoo Med ReptiBreeze Open Air Black Aluminum Screen Cage, $61 \times 61 \times 122$ cm) that were equipped with a tree-like wooden construction that provided narrow dowels on one side, and broad dowels on the other side (left and right sides of the "tree" were randomized between trials; Figure S1C). The walls of the cage were covered with Fluon®-coated plastic foil to constrain lizards to the experimental structures provided. Two heat lamps were placed on top of the cage above the narrow and broad structures, respectively. Ambient temperature was 27°C and humidity was 53%. At 8 a.m., we introduced six juveniles (three of each treatment group) into each experimental cage. Lizards were marked on their back with Sharpie for individual identification. After 1 hr of acclimation, we recorded the perching position of each lizard every hour for 8 hr and compared the number of times lizards were perching on a narrow or broad dowel. Social hierarchy within the experimental group is likely to affect the perch height (more dominant individuals usually basking higher up), but this should not affect the narrow/broad perch choice (left or right side of the "tree") since equal opportunities for basking on narrow and broad surfaces were provided at each height. All trials on perching behavior were conducted by the same person (N.F.) and blindly with respect to the treatment of each individual. Logistical constraints prevented the number of trials that could be conducted, and we, therefore, chose to do this experiment only with a subset of individuals of the species with the largest overall sample size (i.e., *A. sagrei*, $N = 65$) at the age of 5 months.

2.5 | Locomotor performance

We followed previous studies (Kolbe et al., 2016; Losos & Irschick, 1996) and measured locomotor performance of both species using two different setups, a narrow track consisting of a 0.8 cm diameter-wide dowel suspended in mid-air, and a flat track (15 cm broad). To avoid confusion with the broad and narrow treatments, we refer to lizards moving on the narrow track as "climbing" and lizards moving on the broad track as "running." Both tracks were made of unpainted wood (the same material as the lizards were raised on), 1.2 m in length, and oriented at 37° angle to encourage lizards to run rather than jump (Losos & Irschick, 1996).

All trials were conducted blindly with respect to the treatment group of the lizard and by the same person (N.F.), as a second person (K.L.M.) allocated lizards from both a broad and a narrow treatment cage into individual cages (150 × 150 × 200 cm) the evening before the performance trials. This procedure standardized the handling and ensured that lizards could be placed on the running track swiftly. All trials were conducted in the mornings between 10 a.m. and noon at 27.0°C (standard deviation [SD] 0.99°C) with 53% humidity (SD 0.07%). All lizards were tested on both the climbing and running tracks on consecutive days with the order of tracks randomized between groups of lizards. Each lizard was tested three times successively on the same track and we only used the fastest of the runs for each individual per track for the analysis. Lizards were filmed in a dorsal view using a GoPro camera (Hero3+, Silver, CA) at 30 frames/s. The camera was arranged on a tripod so that the lens and the racetrack were in parallel planes to allow for accurate analysis of videos. At the beginning of each trial, we placed the lizard at the start of the track (i.e., the first interval line) and encouraged it to run by gently tapping it on the base of its tail with a paintbrush. By using ImageJ (Schneider, Rasband, & Eliceiri, 2012), we calculated total race time (over 1.2 m), maximum speed across 10 cm, and quantified pauses and distance to first pause. We determined the number of pauses over the total length of the track (i.e., start line to finishing line). Following (Kolbe et al., 2016), we defined a pause as any instance where a lizard remained in the same position for more than one frame. We scored distance to first pause as the length a lizard achieved from the start line to its first pause. A small number (N = 22; 0.89%) of individual measurements were missing, and we imputed these missing values using the "pcaMethods" package (Stacklies et al., 2007). A subset of lizards was also tested at 4 months of age to determine the individual consistency of performance throughout ontogeny (Table S3). This revealed low repeatability of the distance to the first pause, which therefore was not considered further.

2.6 | Statistical analysis

We conducted all statistical analyses in R (version 3.6.1; R Core Team, 2017) using linear mixed-effect models fit by maximum likelihood in the "lme4" package (Bates, Mächler, Bolker, & Walker, 2015). Degrees of freedom for mixed-effects models were estimated using the Satterthwaite's approximation. The statistical analyses had two main goals. First, to test whether the treatment had an effect on

skeletal morphology, performance and perching behavior, and second, to test whether any of the morphological variables explained variation in performance and perching behavior better than treatment alone. The latter would be expected if the environmentally induced morphological variation would be functionally relevant.

To assess the effect of treatment on morphology, performance and perching behavior, we fitted models with the main effects of sex, log-transformed SVL, species, treatment, and an interaction between species and treatment as predictor variables. The cage ID a lizard was reared in was included as random effect in all models to control for shared environmental effects. If the interaction term was highly nonsignificant ($p > .2$) it was dropped and results for main effects are reported for the reduced model. If the interaction term was significant ($p < .05$) we applied a post hoc test by calculating estimated marginal means using the R package "emmeans" (version 1.4.3.01) to determine the differential response of each species to the treatment. We calculated standardized effect sizes according to Cohen's d statistics (Cohen, 1988) using the R package "compute.es" (version 0.2-4).

To assess whether morphological variation explained variation in locomotor performance and perching behavior (in addition to the treatment effect), we adopted a model selection approach using the Akaike information criterion (AIC; Burnham & Anderson, 2002). We refitted models with the main effects of sex, log-transformed SVL, species, and combinations of treatment and one of the morphological features that were significantly affected by the treatment. The model resulting in the lowest AIC score was considered the one that captures the most relevant effects, and we report estimates from these models. Since some morphological features differ substantially between species, we confirmed the results separately for each species.

3 | RESULTS

3.1 | Parentage analysis

We assigned putative dams and sires for 132 out of 147 A. sagrei and 78 out of 83 A. carolinensis at a confidence level of 80% using microsatellite markers. Missing data for A. sagrei were caused by failure to collect tail tissue for two experimental cages. We confirmed that experimental lizards are indeed offspring of a large number of parents, and that relatedness was spread evenly across experimental animals (Table S6). We did not further consider dams and sires in our statistical model to avoid over-parameterizing (cage ID being an important random effect), but we confirmed that results remain qualitatively the same when replacing the random effect of cage ID with random effects of dams and sires.

3.2 | Morphology

At the end of the experiment, A. carolinensis males reached an average SVL of 4.75 cm (±0.06 standard error) and females 3.83 cm (±0.03), while A. sagrei males measured on average 4.40 cm (±0.07) and females

3.84 cm (±0.03) SVL. We found a significant species difference in body size (log-transformed SVL) with *A. carolinensis* being larger at 5 months of age than *A. sagrei* (Table S7). Lizards raised on narrow surfaces were marginally smaller than lizards raised on broad surfaces and males were larger than females, in particular for *A. sagrei* (Table S7).

There was a strong effect of species, sex, and SVL, and an interaction of species and treatment on the total length of both fore- and hindlimbs, and a treatment effect on forelimbs (Table 1, Figures 2 and 3). A *post hoc* test revealed that, when controlling for body size, *A. carolinensis* lizards, but not *A. sagrei* lizards had significantly longer fore- and hindlimbs when raised in the broad treatment group (*A. carolinensis* forelimbs: $t = 3.30$, $df = 40.9$, $p = .002$; *A. sagrei* forelimbs: $t = -0.58$, $df = 38.1$, $p = .569$; *A. carolinensis* hindlimbs: $t = 2.71$, $df = 40.4$, $p = .010$; *A. sagrei* hindlimbs: $t = -0.66$, $df = 38.5$, $p = .514$). Examination of individual limb elements demonstrated that the effect in *A. carolinensis* (and the lack of an effect in *A. sagrei*) was consistent across individual limb elements (Figure 2).

In addition to limb length, we also examined if treatment affects the number of lamellae and the first two PCs capturing bone thickness, and the shape of pectoral and pelvic girdles. The first PC of bone thickness represents mostly variation in bone diameter *per se* (72.2%), while the second PCs reflects the difference between bone diameter and cortical thickness (16.3%; Table S5). The first and second PCs of the 18 landmarks (each with an *X*, *Y*, and *Z* component) of each pectoral and pelvic girdle capture less variation (pectoral: PC1, 24.8%; PC2, 20.2%; pelvic: PC1, 24.3%; PC2, 14.1%). All six PCs show a strong effect of species, and both PC2s of pectoral and pelvic shape were strongly affected by the treatment, while both PC1s show a marginally significant treatment effect (Table 1, Figures 2 and 3). This treatment effect on girdle shape was supported by an

equivalent analysis performed in the geometric morphometric R package "geomorph" (Table S8). The PCs of bone thickness and the number of lamellae differed between species, and in some instances between sexes, but were not affected by the treatment (Table 1).

3.3 | Perching behavior

Our assay testing the propensity of lizards to climb a gradually narrowing dowel revealed that *A. carolinensis* lizards consistently went further (mean distance to the tip of all positions occupied across three trials: 6.35 cm) than did *A. sagrei* lizards (10.60 cm; Table 2, Figures 2 and 3). There was no significant effect of sex, SVL or treatment (Table 2). Similarly, the average end position of the three trials also showed a statistically significant effect of species, but none of the other predictors (Table 2).

In the perch choice experiment, which was conducted only in *A. sagrei*, large lizards perched significantly less frequently on narrow perches ($\chi^2 = 4.51$, $p = .03$), but there was no effect of sex or treatment (Table S9).

3.4 | Locomotor performance

The climbing and running performance of lizards showed a strong difference between the two species in almost all disciplines, except for the maximum velocity on the running track and the distance to the first pause on the climbing track (Table 3). On the running track, *A. sagrei* lizards had consistently shorter total race times, higher maximum velocity, and took fewer pauses than *A. carolinensis* (Table 3, Figures 2 and 3). In contrast, on the climbing track, *A. carolinensis* lizards had shorter total race times and took fewer and later pauses

TABLE 1 Output from linear mixed models examining the effect of species, sex, body size, and treatment on morphology

	log(SVL)	Sex	Species	Treatment	Species × Treatment
log(forelimb)	$F_{(1, 224)} = 1808.23$ $p < .001$	$F_{(1, 224)} = 33.58$ $p < .001$	$F_{(1, 224)} = 954.73$ $p < .001$	$F_{(1, 224)} = 5.25$ $p = .023$	$F_{(1, 224)} = 9.00$ $p = .003$
log(hindlimb)	$F_{(1, 212.42)} = 1507.63$ $p < .001$	$F_{(1, 222.34)} = 36.41$ $p < .001$	$F_{(1, 42.34)} = 1443.76$ $p < .001$	$F_{(1, 39.91)} = 3.11$ $p = .085$	$F_{(1, 39.02)} = 6.60$ $p = .014$
PC1$_{pectoral}$	$F_{(1, 216.65)} = 0.05$ $p = .831$	$F_{(1, 224.79)} = 1.92$ $p = .167$	$F_{(1, 45.19)} = 35.54$ $p < .001$	$F_{(1, 41.90)} = 3.29$ $p = .077$	NS
PC2$_{pectoral}$	$F_{(1, 217.89)} = 5.45$ $p = .021$	$F_{(1, 225.00)} = 0.47$ $p = .495$	$F_{(1, 44.75)} = 10.40$ $p = .002$	$F_{(1, 41.21)} = 9.07$ $p = .004$	NS
PC1$_{pelvic}$	$F_{(1, 213.51)} = 0.60$ $p = .441$	$F_{(1, 223.06)} = 5.25$ $p = .023$	$F_{(1, 44.57)} = 82.50$ $p < .001$	$F_{(1, 41.85)} = 3.79$ $p = .058$	NS
PC2$_{pelvic}$	$F_{(1, 211.34)} = 3.35$ $p = .069$	$F_{(1, 221.64)} = 1.28$ $p = .259$	$F_{(1, 42.42)} = 110.37$ $p < .001$	$F_{(1, 40.02)} = 11.51$ $p = .002$	NS
PC1$_{bone\_thick}$	$F_{(1, 202.03)} = 275.03$ $p < .001$	$F_{(1, 210.12)} = 0.11$ $p = .737$	$F_{(1, 41.98)} = 13.71$ $p < .001$	$F_{(1, 40.68)} = 0.05$ $p = .826$	NS
PC2$_{bone\_thick}$	$F_{(1, 205.42)} = 6.20$ $p = .014$	$F_{(1, 215.41)} = 27.07$ $p < .001$	$F_{(1, 40.65)} = 27.05$ $p < .001$	$F_{(1, 38.96)} = 1.86$ $p = .180$	NS
Lamellae	$F_{(1, 214.71)} = 2.36$ $p = .126$	$F_{(1, 223.90)} = 67.90$ $p < .001$	$F_{(1, 44.76)} = 1019.50$ $p < .001$	$F_{(1, 41.84)} = 0.04$ $p = .837$	NS

Note: Significant effects are highlighted in bold. Cage ID a lizard was reared in was included as random effect. "NS" denotes a highly nonsignificant interaction term ($p > .2$) that was dropped from the model. N = 230.
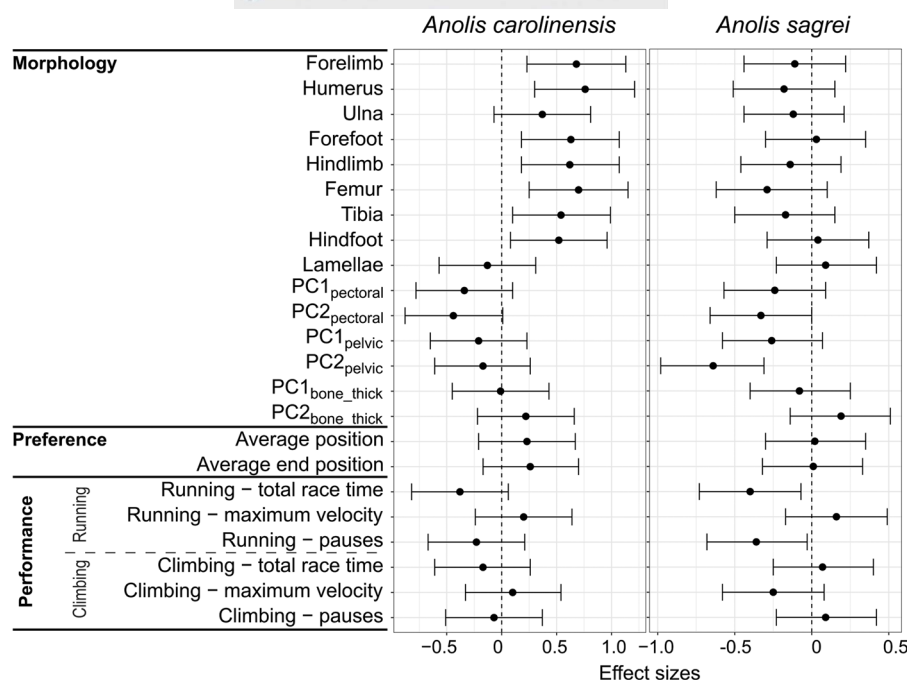Abbreviation: SVL, snout-vent length.

FIGURE 2 Effect sizes of all measured variables in response to the treatment. Effect sizes were obtained from linear mixed models that were fitted separately for the two species, and that take sex, log-transformed snout-vent length, and rearing cage into account. Resulting values with 95% confidence intervals are plotted and positive values indicate a positive effect of the broad treatment compared with the narrow treatment group. In general, the magnitude of the plastic response is larger in *Anolis carolinensis* than in *Anolis sagrei*

than *A. sagrei* (Table 3, Figures 2 and 3). In both species, lizards that were raised in the broad treatment significantly outperformed lizards raised in the narrow treatment on the broad running track, but there was no significant effect of treatment on performance on the climbing track (Table 3, Figures 2 and 3). Model selection suggested that the additional effect of morphological characters were weak at best and only treatment is a significant effect in models that also include morphological variables (i.e., the confidence intervals of the model estimates for morphological variables consistently included zero; Tables S10 and S11).

4 | DISCUSSION

Anolis ecomorphs are characterized by a functional integration of skeletal morphology, habitat use and locomotor performance that match their structural habitat (Losos, 1990a; Williams, 1983). It has been hypothesized that this fit of form and function can also be induced by direct environmental effects on skeletal morphology (Losos et al., 2000; Losos et al., 2001; West-Eberhard, 2003, p. 594). Our experiments demonstrate that the structural habitat experienced in ontogeny indeed has a substantial effect on adult running performance. However, this response appears functionally decoupled from habitat-induced variation in limbs and girdles. In contrast, perching behavior was not affected by rearing conditions. Here we discuss these results in light of previous research on the relationships between limb length, perching behavior, and locomotor performance in *Anolis*.

Biomechanical considerations predict that longer limbs should be more efficient for running on broad surfaces whereas shorter limbs should help to maintain stability on narrow surfaces (Losos, 1990b; McElroy & Reilly, 2009; Vanhooydonck, Herrel, Van Damme, &

Irschick, 2006). The correlation between limb length and locomotor performance across *Anolis* species support this prediction, and there is a match between a species' morphology and the habitat in which it is found (Williams, 1972, 1983). Our experiment in *A. carolinensis* and *A. sagrei* (the former a trunk-crown, the latter a trunk-ground ecomorph) revealed species differences in relative limb length, perching behavior, and locomotor performance that are consistent with this pattern. Our assays thus captured important features of *Anolis* biology.

Sprint speed and climbing agility in lizards are widely considered important aspects of fitness. The key prediction in our experiment was that lizards of both species raised on broad surfaces would grow longer limbs and, consequently, run faster and more efficiently on broad surfaces than lizards raised on narrow surfaces. A within-species correlation between limb length and sprint speed has recently been demonstrated in *A. cristatellus* (Winchell et al., 2018). On the basis of locomotion assays on different *Anolis* species (Losos & Sinervo, 1989), we expected the structural environment experienced during ontogeny to have a weaker effect on locomotor performance on narrow surfaces (i.e., climbing) than on broad surfaces. *A. carolinensis* raised on broad surfaces indeed developed longer limbs. In addition, we found that the shapes of both pectoral and pelvic girdles were significantly affected by rearing habitat. These effects on skeletal morphology were paralleled by improved running performance on broad surfaces, but lizards raised on narrow surfaces did not significantly improve locomotor performance on narrow surfaces. Importantly, plastic responses in skeletal morphology caused by the structure of the habitat appeared decoupled from the locomotor performance. Variation in limb length and the shapes of pectoral and pelvic girdles, all of which affected by the treatment, did not explain variation in performance. Thus, in our experiment, the structural habitat-induced morphological changes in the loco-motor apparatus that appears relatively unimportant for running
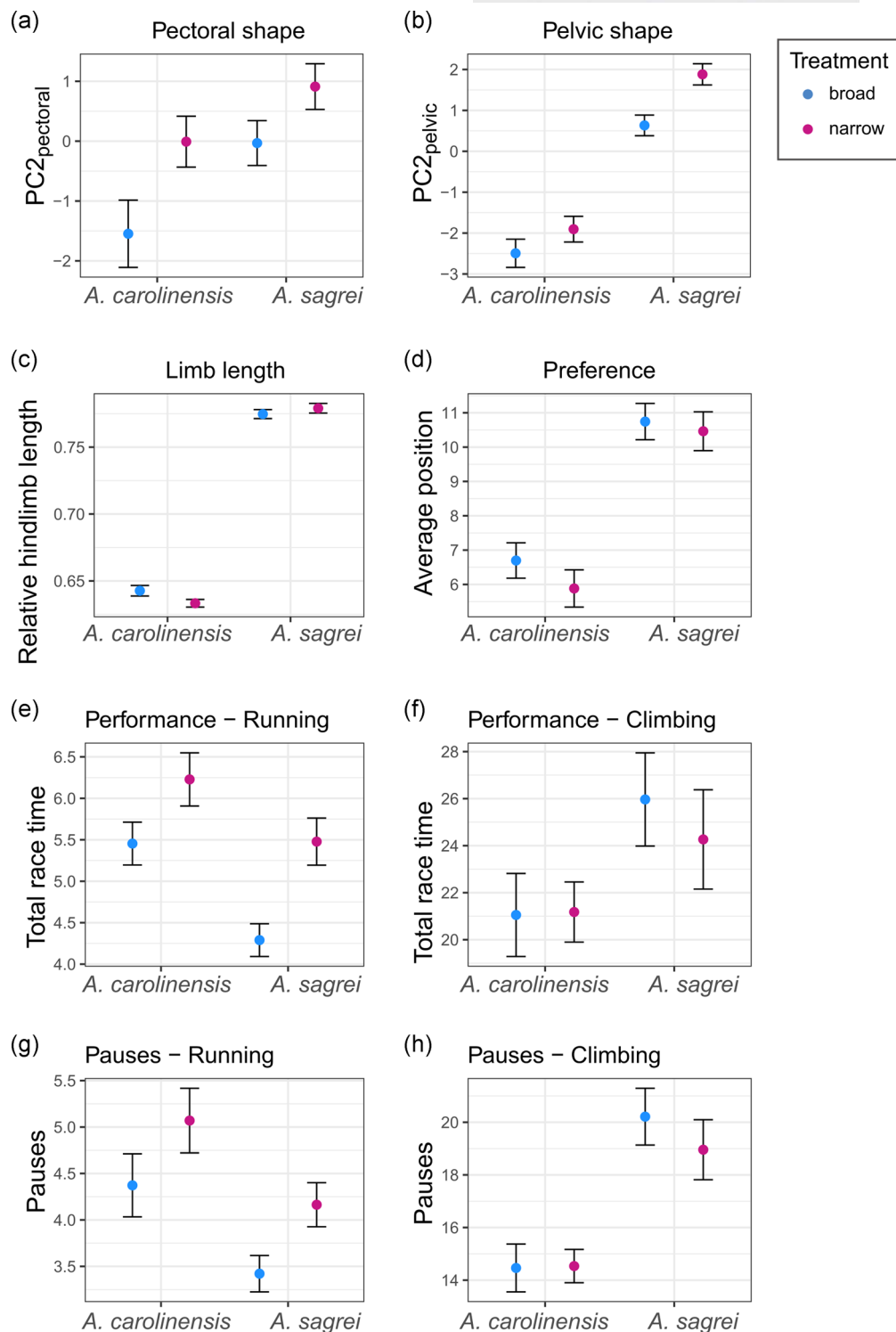
FIGURE 3 Treatment-induced differences in morphology, perching site preference, and locomotor performance for *Anolis carolinensis* and *Anolis sagrei*. Second principal component of the (a) pectoral and (b) pelvic girdle. (c) Relative hindlimb length, (d) average position in perching site choice experiment, (e, f) total race time and (g, h) number of pauses in running and climbing tasks are plotted for *A. carolinensis* and *A. sagrei* lizards of both treatments. Dots indicate the mean of the raw data and error bars show standard errors [Color figure can be viewed at wileyonlinelibrary.com]

performance within species. This decoupling of form and function is particularly evident in *A. sagrei*, which ran faster on the broad track if raised on broad surfaces, but only exhibited developmental plasticity in the shape of the pectoral and pelvic girdles, but not limb length.

The lack of a treatment effect on limb length in *A. sagrei* was surprising since two previous studies found that juveniles of this species raised on broad surfaces grew longer limbs than juveniles raised on narrow dowels (Langford et al., 2014; Losos et al., 2000). The differences in the structural habitat that these studies provided for the juvenile

TABLE 2 Output from linear mixed models examining the effect of species, sex, body size, and treatment on the propensity of lizards to bask on a challengingly narrow dowel

	log(SVL)	Sex	Species	Treatment	Species × Treatment
Average distance from tip	$F_{(1, 206.25)} = 0.50$ $p = .482$	$F_{(1, 215.52)} = 0.02$ $p = .894$	$F_{(1, 43.01)} = 33.38$ $p < .001$	$F_{(1, 41.35)} = 0.27$ $p = .609$	NS
Average end position of three trials	$F_{(1, 207.89)} = 1.49$ $p = .223$	$F_{(1, 217.53)} = 0.19$ $p = .663$	$F_{(1, 43.28)} = 23.49$ $p < .001$	$F_{(1, 41.36)} = 0.22$ $p = .638$	NS

Note: Significant effects are highlighted in bold. Cage ID a lizard was reared in was included as random effect. "NS" denotes a highly nonsignificant interaction term ($p > .2$) that was dropped from the model. $N = 230$.
Abbreviation: SVL, snout-vent length.

lizards compared with our setup (see Section 2.1) appear unlikely to explain the differences in the observed outcome. Previous studies did not examine locomotor performance, however, so it is not known if plasticity had functional consequences. Limb length is a crude measure that may not capture functionally important morphological changes caused by mechanical stress. Since the two species differ in locomotion and perching posture, mechanical stress may be distributed differently across the body in *A. sagrei* compared with *A. carolinensis*. Indeed, the effects on the pelvis appeared stronger in *A. sagrei* than in *A. carolinensis*. While in previous studies treatment commenced for juveniles that had reached an average SVL of 33.1 mm (Losos et al., 2000) and 23.4 mm (Langford et al., 2014), our experiment started immediately upon hatching (average SVL 16.9 mm). It is, therefore, possible that the effects on limb length in previous studies were caused by compensation for an unfit phenotype at the onset of the experiment. Exposing a juvenile lizard to an experimental condition that differs from the structural habitat it has experienced previously in the wild might well have different effects than experiencing the same experimental condition continuously from hatching onwards. This explanation seems particularly likely in light of research on mouse skeletal development that suggests that the process of bone remodeling is greatest early in life (Gardinier et al., 2018). More detailed studies of musculoskeletal anatomy including its impact on locomotor performance would be useful to understand the functional consequences of plasticity, and how it may affect the targets of natural selection.

Improved performance in a familiar environment is not exclusively relying on morphological modification; it also arises from behavioral and physiological plasticity. For example, lizards receiving endurance training develop an increased endurance capacity (Husak, Keith, & Wittry, 2015). Therefore, we may also expect locomotor performance and perching behavior to be affected by ontogenetic experience in *Anolis* lizards. Although *A. carolinensis* reared on narrow dowels appeared more comfortable on narrow surfaces, the effect was small (effect size 0.23, 95% confidence interval included zero). Previous research on wild lizards have yielded conflicting results. While one study found that *Anolis* lizards in cities do not avoid structures on which they perform poorly (Kolbe et al., 2016), other studies suggest they may do so (Irschick & Losos, 1999; see also Winchell et al., 2018). Fitness differences can be substantial if individuals fail to adjust their behavior or habitat preference to match their morphology. However, since habitat-induced plasticity in limb length does not appear to improve locomotor performance, other characters, including behavior, may be the first to change when lizards colonize a new habitat. Indeed, *A. sagrei* lizards on Caribbean islands have been observed to perch preferentially higher up in the tree canopy after ground-dwelling predators were introduced (Losos, Schoener, & Spiller, 2004), and communities of *Anolis* lizard species usually show a predictable pattern of habitat use (Losos, 1990a). The extent to which such patterns are caused by behavioral plasticity,

TABLE 3 Output from linear mixed models examining the effect of species, sex, treatment, and body size on locomotor performance

	log(SVL)	Sex	Species	Treatment	Species × Treatment
Running track					
log(TotalRaceTime)	$F_{(1, 212.90)} = 3.80$ $p = .053$	$F_{(1, 222.38)} = 0.63$ $p = .428$	$F_{(1, 45.42)} = 14.41$ $p < .001$	$F_{(1, 42.81)} = 8.20$ $p = .006$	NS
log(VelocityMax)	$F_{(1, 213.20)} = 7.23$ $p = .008$	$F_{(1, 224.20)} = 0.21$ $p = .644$	$F_{(1, 38.20)} = 63.08$ $p < .001$	$F_{(1, 35.49)} = 1.56$ $p = .219$	NS
Pauses	$F_{(1, 214.90)} = 7.65$ $p = .006$	$F_{(1, 23.93)} = 0.17$ $p = .680$	$F_{(1, 45.39)} = 13.86$ $p < .001$	$F_{(1, 42.43)} = 4.78$ $p = .034$	NS
Climbing track					
log(TotalRaceTime)	$F_{(1, 214.10)} = 3.54$ $p = .061$	$F_{(1, 223.85)} = 1.35$ $p = .247$	$F_{(1, 42.50)} = 4.23$ $p = .046$	$F_{(1, 39.69)} = 0.01$ $p = .926$	NS
log(VelocityMax)	$F_{(1, 217.88)} = 1.36$ $p = .245$	$F_{(1, 225.00)} = 0.01$ $p = .943$	$F_{(1, 45.49)} = 2.33$ $p = .134$	$F_{(1, 41.93)} = 1.44$ $p = .237$	NS
Pauses	$F_{(1, 209.88)} = 0.00$ $p = .967$	$F_{(1, 219.75)} = 1.32$ $p = .251$	$F_{(1, 43.71)} = 13.86$ $p < .001$	$F_{(1, 41.53)} = 0.24$ $p = .628$	NS

Note: Significant effects are highlighted in bold. Cage ID a lizard was reared in was included as random effect. "NS" denotes a highly nonsignificant interaction term ($p > .2$) that was dropped from the model. $N = 230$.
Abbreviation: SVL, snout-vent length.

exclusion through competition or differential survival is poorly understood, although all three may very well contribute.

In contrast to our results on *Anolis* lizards, garden skinks (*Lampropholis delicata*) reared in enclosures with high densities of an invasive weed modified their limb length, perching behavior, and locomotor performance in parallel (Downes & Hoefer, 2007). A similar integration between morphology, habitat preference and performance has been found to be organized by incubation temperature in the skink *Carlia longipes* (Goodman, Schwarzkopf, & Krockenberger, 2013). These responses are expected for populations that have encountered variable environments in the past (i.e., plasticity is adaptive), but the history of selection in these species remains unknown. Our results do not support the hypothesis that habitat-induced plasticity in skeletal morphology has been a direct target of selection for matching locomotor performance to local conditions in *Anolis*. With the obvious caveat that the locomotor and perching assays do not fully capture functional variation that cause differential survival in the wild, it thus appears that environmentally induced skeletal morphology *per se* is unlikely to be a target of natural selection.

5 | CONCLUSIONS

Lizards growing up on broad surfaces showed improved running performance in adulthood and significant modification of their skeletal morphology. However, there was no evidence that plasticity in morphology was an important contributor to improved locomotor performance. Although biologically relevant differences may be difficult to detect in laboratory trials, we suggest that direct selection on environmentally induced changes in limb length is likely to be weak, and that there are more important morphological and behavioral changes that maintain the fit of form and function in the early stages of population divergence and local adaptation.

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AUTHOR CONTRIBUTIONS

N.F. and T.U. conceived the study. N.F., K.L.M., I.S.C.J., and T.U. designed the methodology. N.F., K.L.M., and I.S.C.J. collected the data.

N.F., K.L.M., I.S.C.J., and T.U. analysed the data, interpreted the results and wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA AVAILABILITY

The full data set used in statistical analyses is accessible as the Supplementary Material.

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