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SHORT COMMUNICATION

High metabolic and water-loss rates in caterpillar aggregations: evidence against the resource-conservation hypothesis

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SUMMARY

Several hypotheses have been proposed for explaining animal aggregation, including energy or water conservation. However, these physiological hypotheses have not been well investigated. Here, we report the effects of aggregation on metabolic ($\dot{V}_{CO_2}$) and evaporative water-loss rates ($\dot{V}_{H_2O}$) of the gregarious caterpillar *Eutricha capensis*, by comparing individuals and groups of individuals (*N*=10–100). Contrary to findings from previous physiological studies, we did not find an advantage to aggregation: unexpectedly, $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$ did not decrease with increasing group size. $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$ generally remained constant or increased in larger groups relative to individuals. The amount of water lost per unit of CO_2 exchanged ($\dot{V}_{H_2O}$:$\dot{V}_{CO_2}$ ratio) showed a marked increase in grouped caterpillars, particularly in larger groups. Other benefits of aggregation (e.g. reduced predation or increased growth rates) likely outweigh these potential costs, because individuals of *E. capensis* aggregate voluntarily despite no obvious energetic or hygric advantage, and other potentially confounding group effects (e.g. increased thermoregulatory advantage or whole-animal activity) are inconsequential. The results of this study provide an important exception to physiological studies reporting enhanced energy or water conservation in animal groups.

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INTRODUCTION

Aggregation of individuals within species is a common biological phenomenon. The reasons proposed for aggregation are wide-ranging, including a reduction in predation risk (e.g. Ruxton and Sherratt, 2006), increased sexual signalling or mating success (e.g. Sullivan, 1981), enhanced foraging success, increased growth rates (e.g. Knapp and Casey, 1986) and improved energetic or hygric efficiency (e.g. Benoit et al., 2007; Killen et al., 2012).

However, for terrestrial animals, scaling of energetic and/or hygric efficiency with experimental manipulation of group size (i.e. number of individuals), termed herein the 'resource-conservation hypothesis', has only been examined in a handful of studies and species to date, with most reporting marked benefits of aggregation (e.g. Benoit et al., 2007; Waters et al., 2010; Modlmeier et al., 2013). These studies have mainly focused on Hymenoptera and other highly social insects (e.g. Cao and Dornhaus, 2008; Waters et al., 2010; Modlmeier et al., 2013) and their generality is therefore unclear. Based on metabolic scaling theories, varying group size could alter metabolic or hygric efficiency during inactivity in at least four possible ways. First, increasing group size may change the surface area-to-volume relationship and thereby influence physiological rates in a predictable, geometric manner. One general geometric prediction is that metabolic rate should scale as $m^{0.67}$, where m=body mass, which is unlikely to change with variation in aggregation size. However, for evaporative water loss rates the geometric expectation of changing group size is less clear and depends, at least partly, on the physical arrangement of the aggregation (see Materials and methods, Fig. 1). Second, the metabolic theory of ecology (MTE) predicts a $m^{0.75}$ scaling relationship for metabolic rate irrespective of group size (both within and between individuals), unless the assumptions underlying the MTE are violated in some way (reviewed in Sibly et al., 2012). Third, variation in group size may have no effect, or be balanced by increases in some rates and reductions in others, resulting in isometric scaling ($m^{1.0}$) across groups varying in mass. Finally, increasing group size could entail metabolic or hygric costs, resulting in scaling of rates greater than isometry ($m^{>1.0}$). Two general predictions can be made for the resource-conservation hypothesis of grouped individuals. First, grouped animals should have lower physiological rates per individual than individuals measured in isolation, and second, as groups get larger the benefits should increase (i.e. rates should be reduced even further when calculated on a *per capita* basis).

Here, we examined the impact of group size on metabolic and water-loss rates ($\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$) in an insect species that aggregates voluntarily in nature (Fig. 2A,B). Using Cape Lappet moth caterpillars (*Eutricha capensis* Linnaeus 1767) collected during an outbreak, we measured $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$ across a range of group sizes. Using an experimental approach, we tested the resource-conservation hypothesis and the two general predictions which expect different

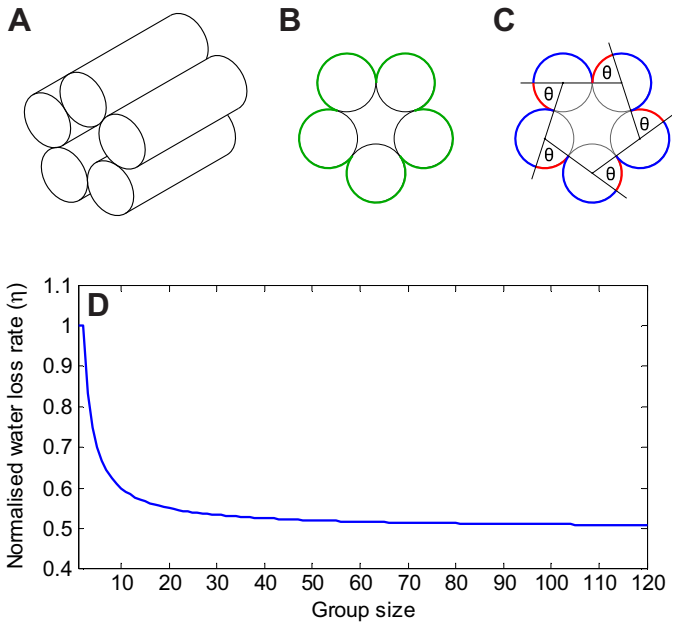

Fig. 1. (A) Model of caterpillars stacked in a cylindrical configuration, (B) view of cylinder ends with exposed sections coloured green and (C) diagram of cylinder ends used in the calculation of normalised exposed area. θ is the angular change in contact point between cylinders (as viewed in an anti-clockwise direction for subsequent adjacent cylinders). (D) Normalised water loss rate (η) as a function of group size.

effects of group size on energetic or hygric efficiency, while attempting to eliminate temperature and activity as potential confounding factors.

MATERIALS AND METHODS

Mid-developmental stage (4th or 5th instar) Cape Lappet moth caterpillars (N=212) were collected from a home garden in Stellenbosch, Western Cape, South Africa. At the start of laboratory rearing, caterpillars had a mean mass of 0.6 g (total group mass 136.7 g). During the experiments, caterpillars grew ninefold to 5.4±0.4 g before pupating after a period of *ca*. 2 months. During rearing, animals were maintained at a mean temperature of 20.3±0.03°C and were kept in the dark to avoid the potential confounding effects of diurnal photoperiod fluctuations. Caterpillars were fed *Acacia saligna* leaves and given water *ad libitum*.

Rates of CO_2 and H_2O release ($\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$, respectively) by caterpillars in groups of varying size (N=1, 10, 15, 25, 50 and 100) were estimated using flow-through respirometry. A calibrated infrared CO_2/H_2O analyser (Li-7000, Li-Cor, Lincoln, NE, USA) was set up as follows: an aquarium pump (Hailea, RaoPing County, GuangDong Province, China) fed atmospheric air into scrubber columns containing soda lime (MERCK, Gauteng, RSA) and silica gel/Drierite (ratio 1:1) (WA Hammond Drierite Company Ltd, Xenia, OH, USA) to remove CO_2 and H_2O vapour, respectively, from the airstream. This airstream was controlled at a constant flow rate of 250 ml min^{-1} by a flow control valve (Model 840, Side-Trak, Sierra Instruments, Monterey, CA, USA) connected to a mass flow control unit (Sable Systems, MFC-2, Las Vegas, NV, USA). Thereafter, air was fed through the zero channel of the CO_2/H_2O analyser and through a custom-built cuvette (each designed to accommodate different caterpillar group sizes), which was placed in a cooler box to minimise disturbance. Cuvettes had a wooden dowel suspended inside to allow the caterpillars to aggregate as in their natural environment. Only hydrophobic Bev-A-Line tubing was used for plumbing throughout the whole system, as this tubing minimises water vapour adsorbance. Calibration span gas concentrations varied among group sizes to ensure that $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$ were recorded accurately within the analyser's measurement range. For all trials, baseline recordings were undertaken with a cuvette containing only the dowel. Thereafter, animals were introduced and allowed to settle before recordings began. Caterpillars were counted and a group mass was measured (±0.1 mg) with an electronic microbalance (MS104S, Mettler Toledo, Greifensee, Switzerland) prior to and after each trial. For each group size, the smallest possible cuvette was used to minimise analyser response times. The time constant for the largest cuvette was calculated to be 6.6 min (1650 ml/250 ml min^{-1}), therefore taking 33 min (6.6×5) for 99% of CO_2 to be read by the analyser. In all cases, the durations of data used for analysis greatly exceeded the maximum time constant (mean selected data periods were 288 min and 278 min for $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$, respectively).

Each recording was performed overnight at a mean temperature of 20.2±0.3°C. The activity of individual caterpillars was recorded using an infrared activity detector (AD2, Sable Systems). Activity of groups (N=10, 15, 25 and 50) was monitored using a webcam (Logitech QuickCam Pro 9000) with an imaging frequency of 30 s, which was subsequently converted into a video (Yawcam version 0.3.9). A thermocouple (T-type, 36 standard wire gauge) was attached to the dowel inside the cuvette to record the temperature inside the aggregated group (T_{Agg}). A second thermocouple was secured against the outside of the cuvette to measure ambient chamber temperature (T_a). Thermocouples were connected to a datalogger (TC-08, Pico Technology, St Neots, UK) and recorded at 1 Hz sampling frequency with PicoLogger software.

Respirometry data were extracted using ExpeData (version 1.1.25, Sable Systems). Only periods of resting $\dot{V}_{CO_2}$ and $\dot{V}_{H_2O}$ (confirmed with activity detection and video analysis, Fig. 2C) were used for analyses. Data were corrected for baseline drift at standard temperature and pressure and converted to ml h^{-1} for $\dot{V}_{CO_2}$ and mg h^{-1} for $\dot{V}_{H_2O}$.

As the analyser's H_2O channel response times were slow for the largest group sizes (N=50 and 100), $\dot{V}_{H_2O}$ was estimated using two different methods. The first method was based on $\dot{V}_{H_2O}$ data obtained from respirometry trials calculated for group sizes of N=25 and smaller. The second method involved determining the $\dot{V}_{H_2O}$ gravimetrically as the difference between mass before and after a respirometry run divided by the duration of the run. There was a strong positive correlation between these two methods of determining $\dot{V}_{H_2O}$ (r^2=0.969) and therefore, to increase the size of the dataset, all analyses were performed using the gravimetric $\dot{V}_{H_2O}$ estimate and included groups of up to N=100 individuals.

Calculation of expected $\dot{V}_{H_2O}$ as a function of group size

To calculate the expected $\dot{V}_{H_2O}$ as a function of group size, we modelled the caterpillars as cylinders with constant length (l) and radius (r) arranged in a cylindrical configuration (Fig. 1A). We did not expect the surfaces on the inside of the cylindrical configuration to contribute to the $\dot{V}_{H_2O}$ of the group of caterpillars and assumed that the combined $\dot{V}_{H_2O}$ is proportional to the exposed surface area of the group of caterpillars. The surface area of a cylinder (excluding the surface area of the ends) is given by the product of the circumference of the circular end and the cylinder length, or:

$$A_c = (2\pi r) \times l\,, \quad (1)$$

where r is the radius and l the length of the cylinder. For cylinders arranged in a cylindrical configuration, the exposed surface area is given by:

$$A_{c,exp} = \eta(2\pi r) \times l\,, \quad (2)$$

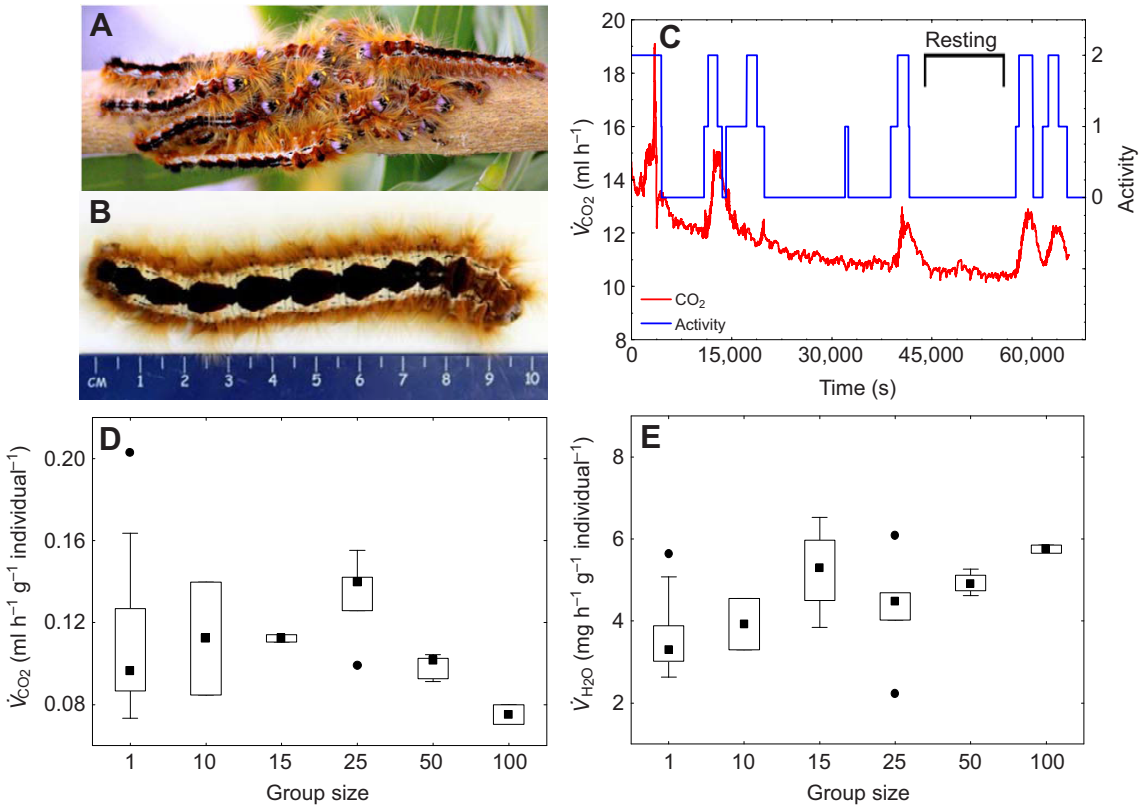

Fig. 2. Rates of metabolism and water loss for (A) aggregated and (B) individual *Eutricha capensis*. (C) Metabolic rate recorded as $\dot{V}_{CO_2}$ (red line, left axis) for a group of 50 caterpillars matches activity patterns (blue line, right axis) recorded with a webcam. Activity was scored as 2=high activity (majority of individuals moving), 1=low activity (one or two individuals moving) and 0=no activity (see also supplementary material Movie 1). The period of rest where data were extracted is indicated. Metabolic rate measured as $\dot{V}_{CO_2}$ (D) and water loss rate $\dot{V}_{H_2O}$ (E) did not decrease as group size increased, as predicted by the resource-conservation hypothesis. There was no significant difference between groups for $\dot{V}_{CO_2}$ ($\dot{V}_{CO_2}$: $H_{5,34}$=9.04, P>0.10). For $\dot{V}_{H_2O}$, there was a significant increase with group size ($\dot{V}_{H_2O}$: $H_{5,36}$=14.96, P<0.05). Box plots represent median (squares) with 25–75 percentiles, and whiskers (errors) are non-outlier range (minimum and maximum). Circles denote outliers.

where η is the fraction of the circumference of the cylinder ends that is exposed. The ratio of the exposed surface area to the total surface area is therefore given by:

$$\frac{A_{c,exp}}{A_c} = \eta . \quad (3)$$

For a group of N caterpillars, the expected $\dot{V}_{H_2O}$ for the group is given by:

$$\dot{V}_{H2O,total} = \eta \times N \times \dot{V}_{H2O,individual} , \quad (4)$$

where $\dot{V}_{H2O,individual}$ is the $\dot{V}_{H_2O}$ of an individual caterpillar with its surface area fully exposed. The expected $\dot{V}_{H_2O}$ therefore requires the calculation of η, which is the sum of the exposed arcs (shown in green in Fig. 1B) divided by the sum of the cylinder circumferences. The total exposed arc length can then be calculated as the sum of N half-circle arcs (shown in blue) and N smaller arcs (shown in red) of which the combined length of the latter is equal to the circumference of a single cylinder end (as $N\times\theta$=360 deg) (Fig. 1C). The value of η can therefore be calculated for individuals as:

$$\eta = \frac{\frac{1}{2}N \times 2\pi r + 2\pi r}{N \times 2\pi r} = \frac{1}{2} + \frac{1}{N} . \quad (5)$$

For N=1, the whole caterpillar surface area is exposed and therefore, η=1. Consequently, the normalised $\dot{V}_{H_2O}$ is expected to decrease as group size increases (Fig. 1D).

Statistical analyses

Data were checked for normality and homogeneity of variance, and where these assumptions were violated, non-parametric tests were used. In preliminary analyses, a Type I general linear model was performed to assess the effects of age (number of days from initiation of laboratory holding) and number of individuals independently of individual mass on $\dot{V}_{CO_2}$. This analysis showed that the number of individuals and start mass had a significant effect (P<0.01) on $\dot{V}_{CO_2}$, whereas age did not (P=0.569). Because age did not have a distinct effect on $\dot{V}_{CO_2}$, it was not incorporated in further analyses. We report $\dot{V}_{CO_2}$ in ml h^{-1} g^{-1} individual^{-1}, which was calculated by dividing the average $\dot{V}_{CO_2}$ recorded during a respirometry run ($\dot{V}_{CO_2}$ divided by group size) by the average mass per individual in the group (using the start mass before respirometry divided by group size). $\dot{V}_{H_2O}$ was calculated in the same way and is presented in mg h^{-1} g^{-1} individual^{-1}. We tested for normality using a Shapiro–Wilk tests after three extreme outliers had been removed (two extremes removed from the $\dot{V}_{H_2O}$ dataset, and one removed from the $\dot{V}_{H_2O}$:$\dot{V}_{CO_2}$ ratio dataset) and found that the data for most groups were not normally distributed. Therefore, a non-parametric approach (Kruskal–Wallis test) was used to compare physiological rates among groups.

The temperature inside the respirometry cuvette was estimated and compared between grouped and individual caterpillars to ensure the temperature remained constant across all trials (T_{Agg} 20.5±2.1°C; T_{Ind} 19.8±1.2°C; Mann–Whitney U_{33}=129.5, P>0.44). Furthermore,

these temperature estimates most likely approximate the body temperature of individuals, but owing to potential aggregation-related heating may not necessarily approximate a group's body temperature. Therefore, we also estimated differences between cuvette air temperature during measurement and the inside of the group's core temperature and compared these between grouped and isolated individuals, assuming a zero difference between air and body temperature of singletons (*t*-test, t_{30}=–1.73, P>0.09).

RESULTS AND DISCUSSION

At rest, $\dot{V}_{CO_2}$ did not decrease significantly as group size increased (Fig. 2D) and there were no statistically significant differences between groups ($\dot{V}_{CO_2}$: $H_{5,34}$=9.04, P>0.10). At rest, $\dot{V}_{H_2O}$ increased as group size increased (Fig. 2E) and there was a significant effect of group size ($\dot{V}_{H_2O}$: $H_{5,36}$=14.96, P<0.05), suggesting a hygric penalty to increasing group size.

The ratio of $\dot{V}_{H_2O}$ to $\dot{V}_{CO_2}$, indicating the hygric cost of gas exchange, did not decrease as group size increased, as predicted by the resource-conservation hypothesis (Fig. 3). By contrast, there was a non-significant positive trend suggesting that aggregated caterpillars lost more water per ml CO_2 exchanged than did smaller groups or solitary individuals ($H_{5,33}$=9.93, P>0.07). Therefore, all of our measurements of the above physiological parameters contradict the resource-conservation hypothesis.

In insects, the benefits of aggregation are relatively well established and include reduced predation risk and increased mating success (e.g. Sullivan, 1981; Ruxton and Sherratt, 2006). From a physiological perspective, reported benefits have mainly involved energetic, hygric or thermal advantages. Several previous physiological studies have reported marked, group-related reductions in rates of resource loss or consumption (so-called 'group effects'), by using indirect calorimetric or gravimetric approaches (e.g. Bartholomew et al., 1988; Benoit et al., 2007; Waters et al., 2010). At low ambient temperatures, groups of insects may show elevated body temperatures, which can provide growth and development advantages that would not be present in solitary, more ectothermic individuals (Knapp and Casey, 1986). The results of our study on Cape Lappet Moth caterpillars are unique because they suggest no obvious physiological benefit to aggregation, as $\dot{V}_{CO_2}$, $\dot{V}_{H_2O}$ and $\dot{V}_{H_2O}$:$\dot{V}_{CO_2}$ did not decrease with increasing group size, while $\dot{V}_{H_2O}$ even showed a significant increase. Furthermore, in the case of $\dot{V}_{H_2O}$, the change in rate with increasing group size is in the opposite direction to what might be expected based on changes in surface area/volume relationships (Fig. 1D).

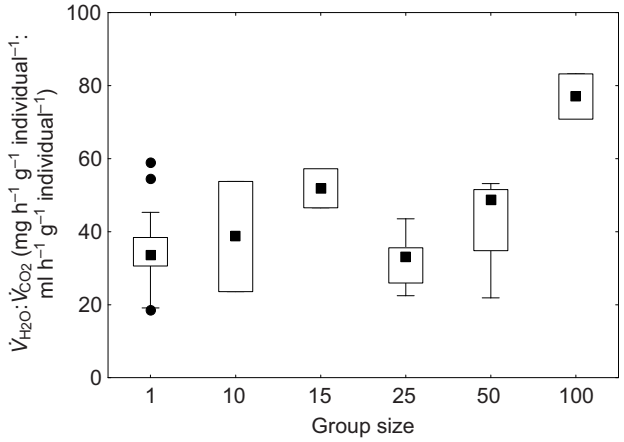

Fig. 3. The ratio between water loss rate ($\dot{V}_{H_2O}$) and metabolic rate ($\dot{V}_{CO_2}$) does not differ among groups of varying size ($H_{5,33}$=9.93, P>0.07). Box plots represent median (squares) with 25–75 percentiles, and whiskers (errors) are non-outlier range (minimum and maximum). Circles denote outliers.

Several potential factors may explain this lack of group-related resource conservation in *E. capensis*. First, increased costs may occur if groups experience temperatures that are elevated above ambient conditions. However, our measurements of the temperature inside and outside aggregations in the laboratory showed this not to be true. Furthermore, an endothermic response seems unlikely given the moderate temperatures experienced during the growing and activity season of *E. capensis*. Most species showing thermal aggregation benefits inhabit Arctic or polar environments where low temperatures may be a limiting factor for population growth.

Second, groups of insects may be more active than solitary individuals, thereby increasing gas-flux rates. However, differential whole-animal activity cannot explain our results because our use of activity detectors and video monitoring ensured that our data only came from resting animals.

Third, aggregation may directly or indirectly increase resting metabolic rates and by association water-loss rates. For example, the immediate presence of other individuals may increase sensory inputs, thus stimulating neural activity, which is known to be energetically expensive (Niven et al., 2007). Alternatively, grouping behaviour may foster higher growth rates, as observed in gypsy moth and eastern tent caterpillars (Knapp and Casey, 1986). Higher costs of biosynthesis may then result in elevated $\dot{V}_{CO_2}$ (and associated $\dot{V}_{H_2O}$). Both of these latter two explanations require further testing. Although we are presently unable to offer a conclusive explanation for the lack of support for the resource-conservation hypothesis – and therefore the relatively high energy and water costs associated with aggregations of *E. capensis* – the frequent occurrence of this aggregation behaviour under natural conditions suggests that it must have some significant counterbalancing benefits. These benefits may include increased growth rates (Knapp and Casey, 1986) or reduced predation risk (Ruxton and Sherratt, 2006). Regardless, our results clearly demonstrate that the resource-conservation hypothesis is not a generally applicable explanation for aggregation behaviour.

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AUTHOR CONTRIBUTIONS

J.S.T. conceived the study; all authors contributed to designing the experiments; R.E.S., B.G., L.B. and C.E.v.D. gathered the data; all authors analysed and interpreted the data; all authors contributed to writing the paper.

COMPETING INTERESTS

No competing interests declared.

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