

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

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SUMMARY

16 Several hypotheses have been proposed for explaining animal aggregation, including
17 energy or water conservation. However, the latter physiological hypotheses have not
18 been well investigated. Here, we report the effects of aggregation on metabolic ($\dot{V}\text{CO}_2$)
19 and evaporative water-loss rates ($\dot{V}\text{H}_2\text{O}$) of the gregarious caterpillar *Eutricha*
20 *capensis*, by comparing individuals and groups of individuals ($n=10-100$). Contrary to
21 findings from previous physiological studies, we did not find an advantage to
22 aggregation: unexpectedly, $\dot{V}\text{CO}_2$ and $\dot{V}\text{H}_2\text{O}$ did not decrease with increasing group
23 size. $\dot{V}\text{CO}_2$ and $\dot{V}\text{H}_2\text{O}$ generally remained constant or increased in larger groups
24 relative to individuals. The amount of water lost per unit of CO_2 exchanged ($\dot{V}\text{H}_2\text{O}$:
25 $\dot{V}\text{CO}_2$ ratio) showed a marked increase in grouped caterpillars, particularly in larger
26 groups. Other benefits of aggregation (e.g. reduced predation or increased growth
27 rates) likely outweigh these potential costs, because individuals of *E. capensis*
28 aggregate voluntarily despite no obvious energetic or hygric advantage, and other
29 potentially confounding group effects (e.g. increased thermoregulatory advantage or
30 whole-animal activity) are inconsequential. The results of this study provide an
31 important exception to physiological studies reporting enhanced energy or water
32 conservation in animal groups.

33 Keywords: Grouping, respiratory metabolism, desiccation, scaling

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INTRODUCTION

36 Aggregation of individuals within species is a common biological phenomenon. The reasons proposed
37 for aggregation are wide-ranging including a reduction in predation risk (e.g. Ruxton and Sherratt,
38 2006), increased sexual signalling or mating success (e.g. Sullivan, 1981), enhanced foraging success,
39 increased growth rates (e.g. Knapp and Casey, 1986) and improved energetic or hygric efficiency (e.g.
40 Benoit et al., 2007; Killen et al., 2012).

41 However, for terrestrial animals, scaling of energetic and/or hygric efficiency with
42 experimental manipulation of group size (i.e. number of individuals), termed herein as the ‘resource-
43 conservation hypothesis’, has only been examined in a handful of studies and species to date, with
44 most reporting marked benefits of aggregation (e.g. Benoit et al., 2007; Waters et al., 2010;
45 Modlmeier et al., 2013). These studies have mainly focused on Hymenoptera and other highly social
46 insects (e.g. Cao and Dornhaus, 2008; Waters et al., 2010; Modlmeier et al., 2013) and their generality
47 is therefore unclear. Based on metabolic scaling theories, varying group size could alter metabolic or
48 hygric efficiency during inactivity in at least four possible ways. First, increasing group size may
49 change the surface area-to-volume relationship and thereby influence physiological rates in a
50 predictable, geometric manner. One general geometric prediction is that metabolic rate should scale as
51 $m^{0.67}$, where m = body mass, which is unlikely to change with variation in aggregation size. However,
52 for evaporative water loss rates the geometric expectation of changing group size is less clear and
53 depends, at least partly, on the physical arrangement of the aggregation (see Material and Methods,
54 Fig. 1). Second, the metabolic theory of ecology (MTE) predicts a $m^{0.75}$ scaling relationship for
55 metabolic rate irrespective of group size (both within and between individuals), unless the
56 assumptions underlying the MTE are violated in some way (reviewed in Sibly et al., 2012). Third,
57 variation in group size may have no effect, or be balanced by increases in some rates and reductions in
58 others, resulting in isometric scaling ($m^{1.0}$) across groups varying in mass. Finally, increasing group
59 size could entail metabolic or hygric costs, resulting in scaling of rates greater than isometry ($m^{>1.0}$).
60 Two general predictions can be made for the resource-conservation hypothesis of grouped individuals.
61 First, grouped animals should have lower rates per individual than individuals measured in isolation,
62 and second, that as groups get larger the benefits should increase (i.e. rates should be reduced even
63 further when calculated on a per capita basis).

64 Here we examine the impacts of group size on metabolic and water-loss rates ($\dot{V}\text{CO}_2$ and
65 $\dot{V}\text{H}_2\text{O}$) in an insect species that aggregates voluntarily in nature (Fig. 2*a,b*). Using Cape Lappet moth
66 caterpillars (*Eutricha capensis*, Linnaeus 1767) collected during an outbreak, we measured $\dot{V}\text{CO}_2$ and
67 $\dot{V}\text{H}_2\text{O}$ across a range of group sizes. Using an experimental approach, we tested the resource-
68 conservation hypothesis and the two general predictions which expect different effects of group size
69 on energetic or hygric efficiency, while attempting to eliminate temperature and activity as potential
70 confounding factors.

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MATERIAL AND METHODS

73 Mid-developmental stage (4th or 5th instar) Cape Lappet moth caterpillars ($n=212$) were collected from
74 a home garden in Stellenbosch, Western Cape, South Africa. At the start of laboratory rearing,
75 caterpillars had a mean mass of 0.6 g (total group mass 136.7 g). During the experiments caterpillars
76 grew nine-fold to 5.4 ± 0.4 g before pupating after a period of *c.* two months. During rearing animals
77 were maintained at a mean temperature of $20.3\pm 0.03^\circ\text{C}$ and were kept in the dark to avoid the
78 potential confounding effects of diurnal photoperiod fluctuations. Caterpillars were fed *Acacia*
79 *saligna* leaves and given water *ad libitum*.

80 Rates of CO_2 and H_2O release ($\dot{V}\text{CO}_2$ and $\dot{V}\text{H}_2\text{O}$ respectively) by caterpillars in groups of
81 varying size ($n=1, 10, 15, 25, 50, 100$) were estimated using flow-through respirometry. A calibrated
82 infra-red $\text{CO}_2/\text{H}_2\text{O}$ analyser (Li-7000, LiCor, Lincoln, NE, USA) was set up as follows: an aquarium
83 pump (Hailea, China) fed atmospheric air into scrubber columns containing soda lime (MERCCK,
84 Gauteng, RSA) and silica gel/Drierite (ratio 1:1) (WA Hammond Drierite Company Ltd, Ohio, USA)
85 respectively to remove CO_2 and H_2O vapour from the airstream. This airstream was controlled at a
86 constant flow rate of $250\text{ ml}\cdot\text{min}^{-1}$ by a flow control valve (Model 840, Side-Trak, Sierra Instruments,
87 Monterey, CA, USA) connected to a mass flow control unit (Sable Systems, MFC-2, Las Vegas, NV,
88 USA). Thereafter, air was fed through the zero channel of the $\text{CO}_2/\text{H}_2\text{O}$ analyzer and through a
89 custom-built cuvette (each designed to accommodate different caterpillar group sizes), which was
90 placed in a cooler box to minimize disturbance. Cuvettes had a wooden dowel suspended inside to
91 allow the caterpillars to aggregate as in their natural environment. Only hydrophobic Bev-A-Line
92 tubing was used for plumbing throughout the whole system, as this tubing minimizes water vapour
93 adsorbance. Calibration span gas concentrations varied among group sizes to ensure that $\dot{V}\text{CO}_2$ and
94 $\dot{V}\text{H}_2\text{O}$ were recorded accurately within the analyser's measurement range. For all trials, baseline
95 recordings were undertaken with a cuvette containing only the dowel. Thereafter animals were
96 introduced and allowed to settle before recordings began. Caterpillars were counted and a group mass
97 was measured (± 0.1 mg) with an electronic microbalance (MS104S, Mettler Toledo, Greifensee,
98 Switzerland) prior to and after each trial. For each group size, the smallest possible cuvette was used
99 to minimize analyser response times. The time constant for the largest cuvette was calculated to be 6.6
100 min ($1650\text{ ml}/250\text{ ml}\cdot\text{min}^{-1}$), therefore taking 33 min ($6.6*5$) for 99% of CO_2 to be read by the
101 analyser. In all cases, the durations of data used for analysis greatly exceeded the maximum time
102 constant (mean selected data periods were 288 min and 278 min for $\dot{V}\text{CO}_2$ and $\dot{V}\text{H}_2\text{O}$, respectively).

103 Each recording was performed overnight at a mean temperature of $20.2\pm 0.3^\circ\text{C}$. The activity
104 of individual caterpillars was recorded using an infrared activity detector (AD2, Sable Systems, Las
105 Vegas, Nevada, USA). Activity of groups ($n= 10, 15, 25$ and 50) was monitored using a webcam
106 (Logitech QuickCam Pro 9000) with an imaging frequency of 30 sec, which was subsequently
107 converted into a video (Yawcam version 0.3.9). A thermocouple (T-type, 36 standard wire gauge) was

108 attached to the dowel inside the cuvette to record the temperature inside the aggregated group (T_{AGG}).
109 A second thermocouple was secured against the outside of the cuvette to measure ambient chamber
110 temperature (T_a). Thermocouples were connected to a datalogger (TC-08, Pico Technology,
111 Cambridgeshire, UK) and recorded at 1 Hz sampling frequency with PicoLogger software.

112 Respirometry data were extracted using ExpeData (version 1.1.25, Sable Systems, Las
113 Vegas, Nevada, USA). Only periods of resting $\dot{V}CO_2$ and $\dot{V}H_2O$ (confirmed with activity detection
114 and video analysis, Fig. 2c) were used for analyses. Data were corrected for baseline drift at STP and
115 converted to ml/h for $\dot{V}CO_2$ and mg/h for $\dot{V}H_2O$.

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

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

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

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

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

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

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

$$\frac{A_{c,exp}}{A_c} = \eta.$$

136 For a group of n caterpillars, the expected $\dot{V}H_2O$ for the group is given by

$$\dot{V}H_2O_{\text{total}} = \eta \times N \times \dot{V}H_2O_{\text{individual}}$$

137 where $\dot{V}H_2O_{\text{individual}}$ is the $\dot{V}H_2O$ of an individual caterpillar with its surface area fully exposed. The
138 expected $\dot{V}H_2O$ therefore requires the calculation of η , which is the sum of the exposed arcs (shown in
139 green in Fig. 1b) divided by the sum of the cylinder circumferences. The total exposed arc length can
140 then be calculated as the sum of n half-circle arcs (shown in blue) and n smaller arcs (shown in red) of
141 which the combined length of the latter is equal to the circumference of a single cylinder end (since
142 $N \times \theta = 360^\circ$) (Fig. 1c). The value of η can therefore be calculated for individuals as

$$\eta = \frac{\frac{1}{2}N \times 2\pi r + 2\pi r}{N \times 2\pi r} = \frac{1}{2} + \frac{1}{N}$$

143 For $n = 1$, the whole caterpillar surface area is exposed and therefore, $\eta = 1$. Consequently, the
144 normalised $\dot{V}H_2O$ is expected to decrease as group size increase (Fig. 1d).

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Statistical analyses

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

161 The temperature inside the respirometry cuvette was estimated and compared between
162 grouped and individual caterpillars to ensure the temperature remained constant across all trials (T_{AGG}
163 20.5 ± 2.1 ; T_{IND} 19.8 ± 1.2 °C; Mann-Whitney $U_{33} = 129.5$, $p > 0.44$). Furthermore, these temperature
164 estimates likely approximate the body temperature of individuals, but owing to potential aggregation-
165 related heating may not necessarily approximate a group's body temperature. Therefore, we also
166 estimated differences between cuvette air temperature during measurement and the inside of the

167 group's core temperature and compared these between grouped and isolated individuals, assuming a
168 zero difference between air and body temperature of singletons (t -test, $t_{30}=-1.73$, $p>0.09$).

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RESULTS AND DISCUSSION

171 At rest, $\dot{V}\text{CO}_2$ did not decrease significantly as group size increased (Fig. 2d) and there were no
172 statistically significant differences between groups ($\dot{V}\text{CO}_2$: $H_{5,34}=9.04$, $p>0.10$). At rest, $\dot{V}\text{H}_2\text{O}$
173 increased as group size increased (Fig. 2e) and there was a significant effect of group size ($\dot{V}\text{H}_2\text{O}$:
174 $H_{5,36}=14.96$, $p<0.05$) suggesting a hygric penalty to increasing group size.

175 The ratio of $\dot{V}\text{H}_2\text{O}$ to $\dot{V}\text{CO}_2$, indicating the hygric cost of gas exchange, did not decrease as
176 group size increased, as predicted by the resource conservation hypothesis (Fig. 3). By contrast, there
177 was a non-significant positive trend suggesting that aggregated caterpillars lost more water per ml
178 CO_2 exchanged than did smaller groups or solitary individuals ($H_{5,33}=9.93$, $p>0.07$). Therefore, all of
179 our measurements of the above physiological parameters contradict the resource conservation
180 hypothesis.

181 In insects, benefits of aggregation have been relatively well established and include reduced
182 predation risk and increased mating success (e.g. Sullivan, 1981; Ruxton and Sherratt, 2006). From a
183 physiological perspective, reported benefits have mainly involved energetic, hygric or thermal
184 advantages. Several previous physiological studies have reported marked, group-related reductions in
185 rates of resource loss or consumption (so-called 'group effects'), by using indirect calorimetric or
186 gravimetric approaches (e.g. Bartholomew et al., 1988; Benoit et al., 2007; Waters et al., 2010). At
187 low ambient temperatures, groups of insects may show elevated body temperatures, which can
188 provide growth and development advantages that would not be present in solitary, more ectothermic
189 individuals (Knapp and Casey, 1986). The results of our study on Cape Lappet Moth caterpillars are
190 unique because they suggest no obvious physiological benefit to aggregation, because $\dot{V}\text{CO}_2$, $\dot{V}\text{H}_2\text{O}$,
191 and $\dot{V}\text{H}_2\text{O}:\dot{V}\text{CO}_2$ did not decrease with an increasing group size, while $\dot{V}\text{H}_2\text{O}$ even showed a
192 significant increase. Furthermore, in the case of $\dot{V}\text{H}_2\text{O}$ the changes in rates with increasing group size
193 are in the opposite direction to what might be expected based on changes in surface area/volume
194 relationships (Fig. 1d).

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

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

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

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222

AUTHOR CONTRIBUTIONS

223 R.S., B.G., L.B., C.V.D. gathered the data; all authors analysed and interpreted the data; all authors
224 contributed to writing the paper.

225

COMPETING INTERESTS

226 No competing interests declared.

227

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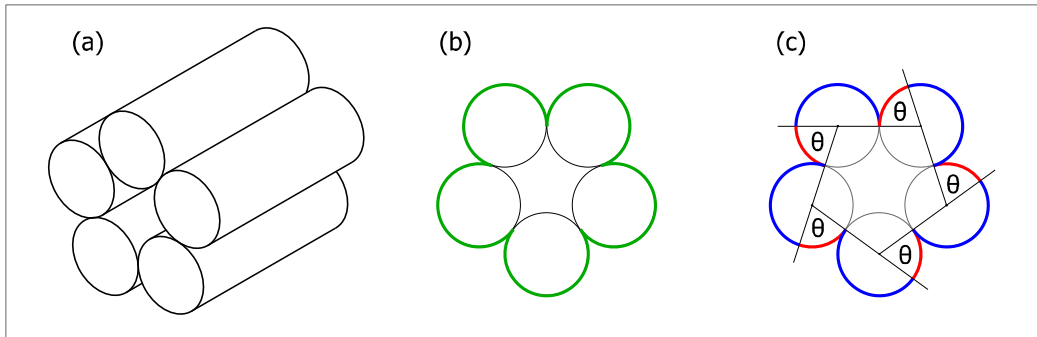
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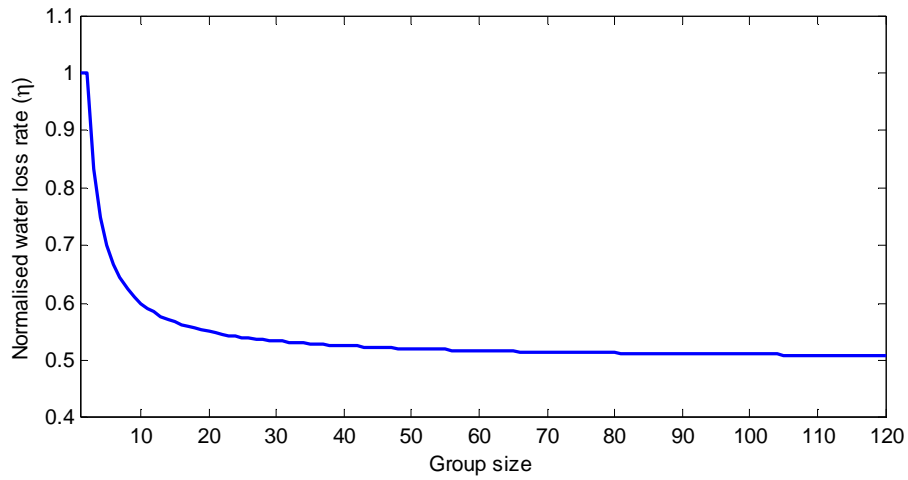
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262 **FIGURES**

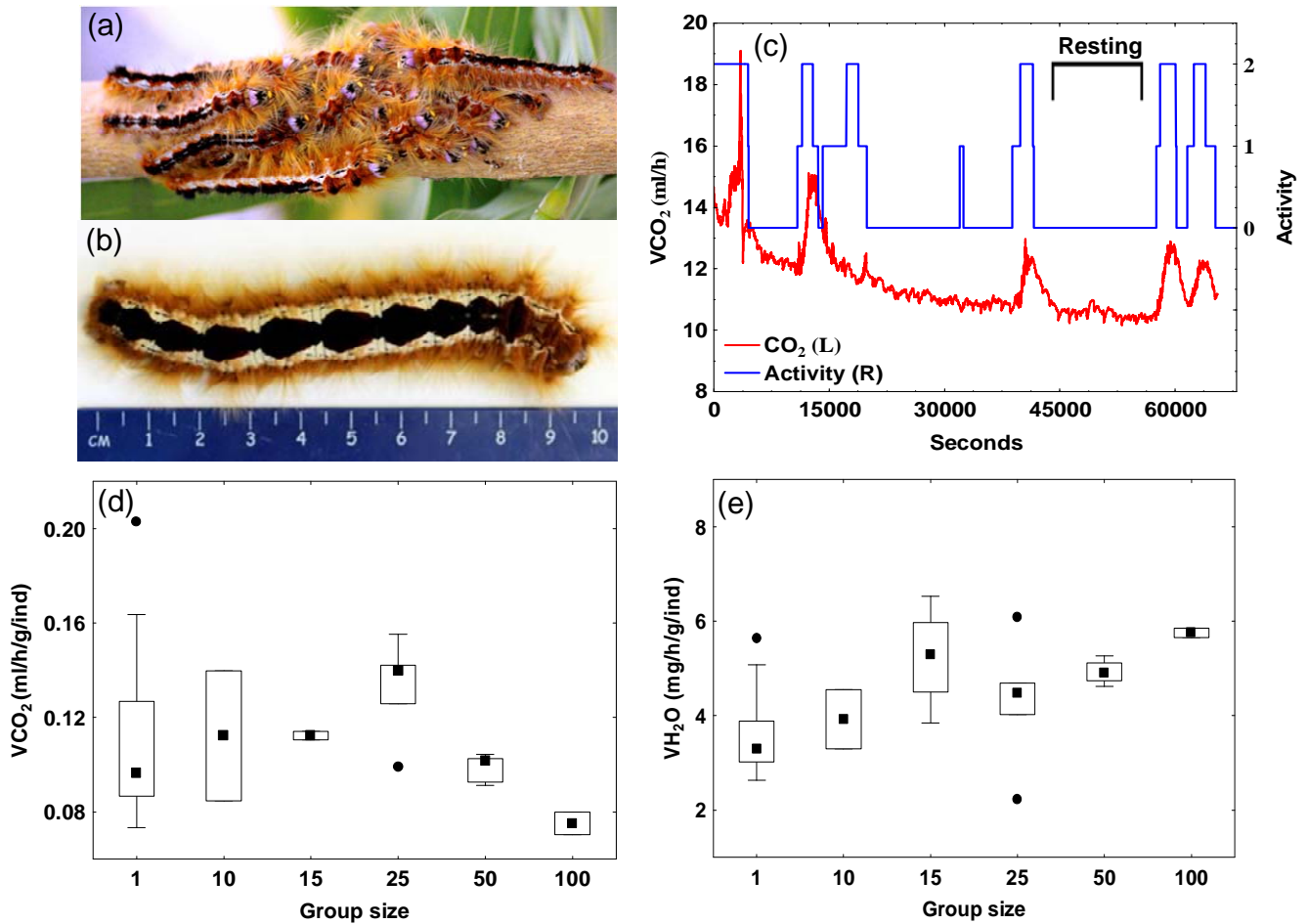


264 (d)

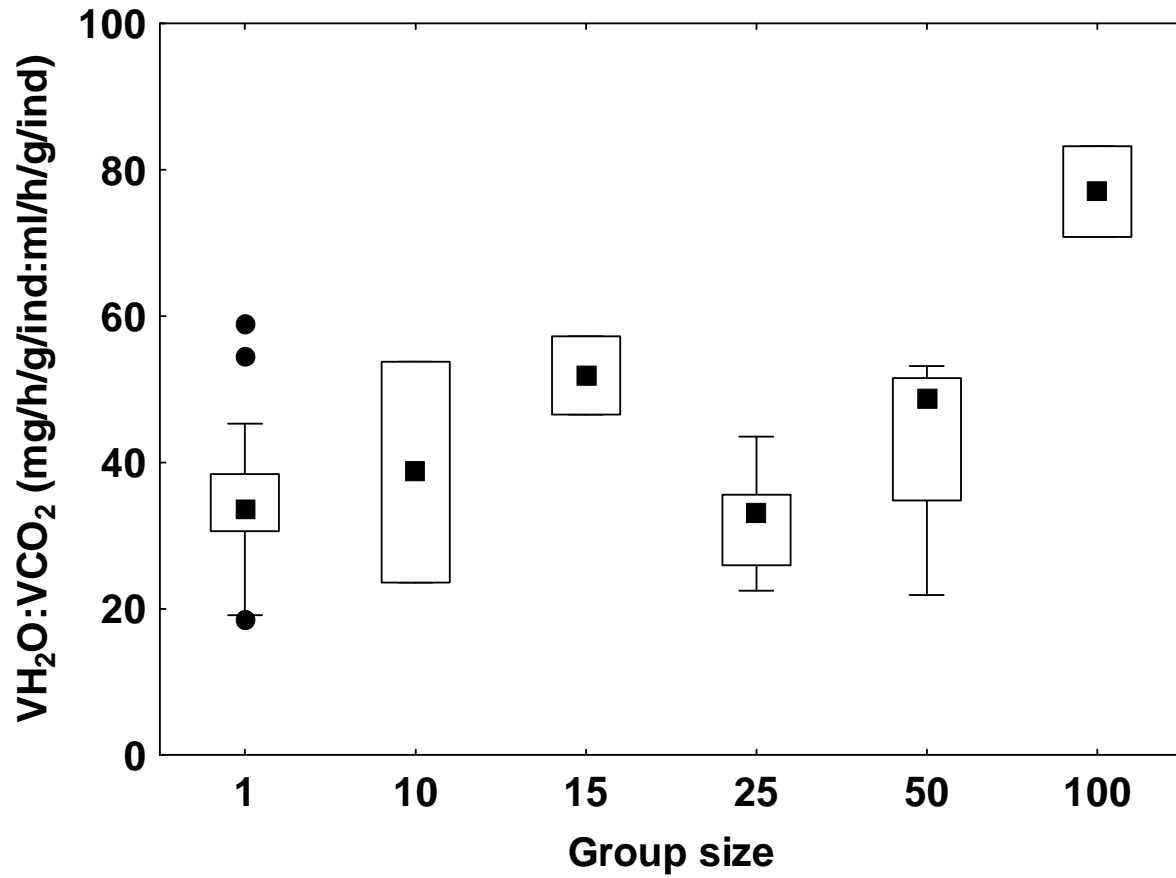


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266 **Fig. 1.** Model of caterpillars stacked in a cylindrical configuration (a), view of cylinder ends
267 with exposed sections coloured green (b) and diagram of cylinder ends used in calculation of
268 normalised exposed area (c). Normalised water loss rate (η) as a function of group size (d).



270 **Fig. 2.** Rates of metabolism and water loss for (a) aggregated and (b) individual *Eutricha*
 271 *capensis*. (c) Metabolic rate recorded as $\dot{V}CO_2$ (red line, left axis) for a group of 50
 272 caterpillars matches activity patterns (blue line, right axis) recorded with a webcam. Activity
 273 was scored as 2=high activity (majority of individuals moving), 1=low activity (one or two
 274 individuals moving) and 0=no activity (and see Online Supplementary Movie S1). Period of
 275 rest where data were extracted is indicated. Metabolic rate measured as $\dot{V}CO_2$ (d) and water
 276 loss rate $\dot{V}H_2O$ (e) did not decrease as group size increased, as predicted by the resource
 277 conservation hypothesis. There was no significant difference between groups for $\dot{V}CO_2$
 278 ($\dot{V}CO_2$: $H_{5,34} = 9.04$, $p > 0.10$). For $\dot{V}H_2O$, there was a significant increase with group size
 279 ($\dot{V}H_2O$: $H_{5,36} = 14.96$, $p < 0.05$). Box plots represent median (squares) with 25 – 75 percentiles
 280 and whiskers (errors) are non-outlier range (minimum and maximum). Circles denote
 281 outliers.



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