

# **Larger workers outperform smaller workers across resource environments: an evaluation of demographic data using functional linear models**

Running headline: Worker size composition effects in bumblebee colonies

Natalie Z. Kerr<sup>1,2</sup>, Rosemary L. Malfi<sup>3</sup>, Neal M. Williams<sup>4</sup>, Elizabeth E. Crone<sup>1</sup>

<sup>1</sup> [Department of Biology, Tufts University, Medford, Massachusetts 02155, USA](#)

<sup>2</sup> Department of Biology, Duke University, Durham, North Carolina 27710, USA

<sup>3</sup> Department of Biology, University of Massachusetts-Amherst, Amherst, MA 01003, USA

<sup>4</sup> Department of Entomology and Nematology, University of California, Davis, CA 95616, USA

NZK ORCID: 0000-0003-4227-2031

RLM: 0000-0003-0144-5928

NMW: 0000-0003-3053-8445

EEC ORCID: 0000-0002-5287-221X

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\* Corresponding author: Natalie Kerr, [natalie.kerr@duke.edu](mailto:natalie.kerr@duke.edu), [Department of Biology, Duke University, Durham, North Carolina 27710, USA.](#)

Email: [natalie.kerr@duke.edu](mailto:natalie.kerr@duke.edu)

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#### AUTHOR CONTRIBUTIONS

NZK, EEC and NMW conceived the ideas and designed methodology; NZK, RLM, and  
NMW collected the data; NZK and EEC analyzed the data; and NZK, EEC, RLM, and NMW  
wrote the manuscript. All authors contributed critically to the drafts and gave final approval for  
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#### DATA ACCESSIBILITY

All data will be published in Dryad, once manuscript has been accepted in Ecology and  
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48 ABSTRACT

49 1. Behavior and organization of social groups is thought to be vital to the functioning of  
50 societies, yet the contributions of various roles within social groups [towards population growth](#)  
51 [and dynamics](#) have been difficult to quantify. A common approach to quantifying these role-  
52 based contributions is evaluating the [number](#) of individuals conducting certain roles, [which](#)  
53 ignores how [behavior](#) might scale up to effects at the population-level. Manipulative experiments  
54 are another common approach to determine population-level effects, but they often ignore  
55 potential feedbacks associated with these various roles.

56 2. Here, we evaluate the effects of worker size distribution in bumblebee colonies on worker  
57 production [in 24 observational colonies across three environments](#), using functional linear  
58 models. Functional linear models are [an underused](#) correlative technique that has been used to  
59 assess lag effects of environmental drivers on plant performance. We demonstrate potential  
60 applications of this technique [for exploring high-dimensional ecological systems, such as](#) the  
61 contributions of [individuals with different traits](#) to [colony dynamics](#).

62 3. We found that more larger workers had mostly positive effects and more smaller workers  
63 had negative effects on worker production. Most of these effects were only detected under low or  
64 fluctuating resource environments suggesting that the advantage of colonies with larger-bodied  
65 workers becomes more apparent under stressful conditions.

66 4. We [also](#) demonstrate the wider ecological application of functional linear models. We  
67 highlight the advantages and limitations when considering these models, and how they are a  
68 valuable complement to many of these performance-based and manipulative experiments.

69

70 Keywords: *Bombus vosnesenskii*, functional linear models, colony age, egg production, larval  
71 survival, development, callow size

## 72 INTRODUCTION

73           In animal societies, individuals are often observed performing different tasks, such as  
74 guarding nests and burrows (Clutton-Brock *et al.* 2001a), nursing and caring for young  
75 (Wilkinson 1992; Kerth 2008; Sparkman *et al.* 2011), or reproducing (Jarvis 1981; Faulkes &  
76 Bennett 2001). The roles within these social groups are commonly assigned based on the age  
77 (Jarvis 1981; Seeley & Kolmes 1991; Brent *et al.* 2015; Zöttl *et al.* 2016), size (Porter &  
78 Tschinkel 1985; Wenzel 1992; Schwander, Rosset & Chapuisat 2005; Goulson 2009), and/or  
79 status (Frank 1986; Sparkman *et al.* 2011) of individuals. For example, in Meerkats, which are  
80 cooperative breeders, younger non-breeding individuals often stand on ‘sentinel duty’ during  
81 group foraging bouts and care for offspring of the dominant breeding pair (Clutton-Brock *et al.*  
82 2001b; Clutton-Brock *et al.* 2002; Clutton-Brock, Russell & Sharpe 2004). Without the co-  
83 operation of these non-breeders, the survival of individuals within the colonies is likely to  
84 decrease, particularly for the young (Doolan & Macdonald 1999; Russell *et al.* 2007). This social  
85 behavior and organization is often assumed to be vital to the functioning and survival of these  
86 societies.

87           The most common approach to understanding the contribution of roles within social  
88 groups is to observe the behavior and performance of individuals. However, [observing certain](#)  
89 [individuals performing a task does not mean they are better than other individuals at performing](#)  
90 [that task](#). To attempt to tackle the challenges associated with quantifying trait-based  
91 contributions, a few studies have manipulated colonies in the laboratory to evaluate the effects of  
92 the social organization of age- and size- polymorphic species, such as mole rats (Jarvis 1981;  
93 Zöttl *et al.* 2016), ants (Porter & Tschinkel 1985; Billick & Carter 2007), and bumblebees  
94 (Cnaani & Hefetz 1994; Jandt & Dornhaus 2009; Couvillon *et al.* 2010; Jandt & Dornhaus 2011;

Jandt & Dornhaus 2014). In laboratory colonies of a eusocial ant *Pheidole dentata*, larvae gained more mass when reared by older workers, suggesting that older workers contribute more towards worker production in these ant colonies than their younger sisters (Muscedere, Willey & Traniello 2009). However, colonies within these laboratory experiments were not faced with the same external environmental stressors as those in the wild. In the case of bumblebees, larger workers are more susceptible to predators and parasites (Cartar & Dill 1991; Muller, Blackburn & Schmid-Hempel 1996; Malfi & Roulston 2014), despite being better foragers. Therefore, the behaviors of social organism under artificial conditions might not capture all the feedbacks associated with size or age-based roles.

Functional linear models (FLMs) provide an [additional](#) method of inference [about high-dimensional ecological systems using observational data](#). For example, FLMs can evaluate the contributions of age- or size-based roles within societies [to population dynamics](#). These models assume that the effect of a predictor variable (e.g. number of workers) on a response variable (e.g. egg production) is a smooth function of some feature of the predictor variable (e.g. size of workers). Past applications of FLMs in ecology have investigated environmental drivers of plant population dynamics (Teller *et al.* 2016; Tenhumberg *et al.* 2018). These studies evaluated the effects of environmental conditions (e.g. precipitation) on plant performance (e.g. growth) assuming the slope of the effect of environmental conditions and plant performance varies as a smooth function of the time lag between conditions and performance (e.g. precipitation in the past 1, 2, 3... months). For example, the slope of precipitation versus plant growth could go from positive in recent months to zero at longer time lags. This method has potential for wider ecological application to investigate life history phenomena. Here, we explore application of

FLMs to quantifying the relationship between aspects of new worker production as a function of the body size of existing workers in bumblebee colonies.

Bumblebees (*Bombus* spp.) are primitively eusocial insects that form relatively small colonies and have a discrete life cycle lasting only for a single season, which makes them a tractable system for studying trait-based roles within societies. Bumblebees also exhibit worker size polymorphism, where workers within colonies vary up to 10-fold in mass (Goulson 2009). In bumblebee colonies, larger workers are often found foraging and guarding, while smaller workers spend more time in the colony conducting in-nest tasks such as fanning and incubating (Richards 1946; Cumber 1949; Goulson *et al.* 2002; Jandt & Dornhaus 2009; Inoue *et al.* 2010). Many studies have measured the importance of body size in determining how well workers perform various tasks, ranging from foraging and flight dynamics to thermoregulating and undertaking. Most of these have found that larger workers are better at multiple tasks, such as foraging and nursing (Cnaani & Hefetz 1994; Goulson *et al.* 2002; Spaethe & Weidenmüller 2002; Peat & Goulson 2005; Ings 2007; Spaethe *et al.* 2007; Kerr, Crone & Williams 2019), with a few studies concluding either that intermediate-size is better (Jandt & Dornhaus 2014), or that there is no size-based difference in performance (Jandt & Dornhaus 2014). Although these studies demonstrate that body size affects worker performance at certain tasks, they do not demonstrate how their size-based performance at tasks may, in turn, affect colony growth and development.

No studies have found smaller bumblebee workers to be better at performing tasks essential to colony function. However, smaller workers are more resilient to starvation (Couvillon & Dornhaus 2010). Therefore, their value may become more apparent when food resources are limiting. In addition, smaller workers have lower production costs, so they may be

more cost-effective (Kerr, Crone & Williams 2019). Here, we used FLMs to evaluate the contribution of workers of different sizes to worker production in bumblebee colonies under three different environments: a low resource environment; an environment with an early season pulse followed by low resources ('high-low'); and a high resource environment. We looked at five vital rates relating to worker production: (1) number of new eggs laid, (2) development time, (3) larval survival, and (4) mean and (5) variance in worker emergence size, i.e. the size of callow workers. By evaluating the contribution of different-sized workers under different resources environments to worker production, we can assess whether larger workers are more beneficial when resource conditions are more favorable and whether the benefit of small workers to colonies is only seen when resources are low, making both production cost and resistance to starvation a premium.

## MATERIALS AND METHODS

### *Study species and sites*

We hand reared *Bombus vosnesenskii* colonies from wild-caught queens collected at the University of California McLaughlin Reserve (N38 52 25.74, W122 25 56.25) in early spring 2015 and 2016 while they searched for nest sites. These colonies were the basis for two separate studies, both of which are previously published (Kerr, Crone & Williams 2019; Malfi, Crone & Williams 2019). Here, we use previously unpublished data (*Brood mapping*, below) from these studies to investigate effects of worker size on colony growth, so we briefly describe the rearing process.

In 2015 and 2016, we hand-reared colonies in the laboratory in a dark room at 26-28°C for 6 to 9 weeks until their second or first cohort of worker bees eclosed. In 2015, we relocated



seven colonies outside (N38 32 12.21, W121 47 16.95) at the Harry H. Laidlaw Jr. Honey Bee Research Facility (Davis, CA), where the surrounding landscape consisted of agricultural crops, floral research plots, and a 0.2 ha pollinator garden (Fig. S3a). In 2016, we relocated 14 colonies outside in agricultural fields at UC Davis Experimental Farm property (N38 31 32.3, W121 46 56.54). Half of the colonies ( $n = 7$ ) had access to flight cages that provided a pulse of native California wildflower species for ~4 weeks early in the season (“pulse” treatment) and the other half had no supplemental forage (“control” treatment) (Malfi, Crone & Williams 2019). The surrounding landscapes were croplands consisting of mainly non-flowering cereals, corn, and a strip of riparian habitat (Fig. S3b).

In this study, we broadly categorized the resource environments experienced by our experimental colonies in each of these years based on observational differences in the quality and abundance of forage. The 2015 colonies, located next to a pollinator garden at the Honey Bee Research Facility, had the highest resource availability and quality (“high”), colonies in the 2016 pulse treatment had the second highest resource availability and quality (“high-low”), and colonies in the 2016 control treatment had the lowest availability and quality (“low”). These three environments will now be referred to as high, high-low, and low. Note that comparisons between the 2015 colonies and 2016 should be interpreted with the caveat that differences could be due to factors other than nutrition. Based on our observations, the most noticeable differences among treatments were the quality and abundance of floral resources (discussed further in the Discussion).

#### *Brood mapping*

185 Each week, we photographed the brood from multiple angles (above, side, diagonal) to fully  
186 capture all brood cells. We individually numbered each brood cell in the photographs as it  
187 differentiated and tracked the fate of all marked cells throughout colony development (Fig. 1).  
188 We classified each living brood cell into five categories: (1) clump stage, which represents the  
189 egg stage where individual cells have not yet differentiated; (2) pre-differentiated stage, which  
190 represents early larval instars where individual cells have begun differentiating; (3) differentiated  
191 stage, which represents later larval instars where individual brood cells are clearly differentiated;  
192 (4) cocoon stage, where cells had darkened indicating that pupa have spun their cocoons; and (5)  
193 eclosed stage, where the cell has opened and an adult worker emerged (Fig. 2 for stages). We  
194 also had two other categories: (6) dead, where we had observed a dead cell, and (7) unseen,  
195 where the cell could no longer be seen in the brood photos.

196 Some brood clumps did not develop into distinct cells before the end of brood mapping,  
197 while other clumps died before cell partitioning. Rather than exclude these indistinct, dead, or  
198 undeveloped brood clumps in our analyses ( $N_{\text{low}} = 24/115$ ;  $N_{\text{high-low}} = 36/150$ ;  $N_{\text{high}} = 36/163$ ),  
199 which could result in underestimating egg production and overestimating larval survival, we  
200 estimated the number of cells for these clumps. We did this by classifying these indistinct brood  
201 clumps into five size categories (tiny, small, medium, large, extra-large) based on comparisons  
202 with similarly-sized brood clumps that did divide into individual cells and assigning the mean  
203 value of cells for these size categories to indistinct clumps. From the 322 distinct clumps with a  
204 total of 3917 cells with known fates, we estimated 432 cells from 96 indistinct clumps appeared  
205 to have died before differentiating, which comprises of less than 10% of total cells in our larval  
206 survival analyses.

From the brood mapping, we estimated three vital rates: egg production, larval development time, and larval survival. We considered weekly egg production to be the number of newly visible cells in either clump or pre-differentiated stages. We assumed that the number of distinct cells formed by a brood clump represented the total number of eggs laid, i.e. no eggs died before larval cells differentiated. We calculated development time for each cell as the number of days from when it was first seen as an egg (defined as the ‘clump’ stage) to when it was first seen as an eclosed cell. Cells that were not detected in the clump stage or that disappeared from view before visibly eclosing were excluded from our analyses of larval development time. Finally, we classified larval survival as the success of each cell [at surviving to](#) eclosion. We excluded 43 unseen brood cells from our larval analyses because more than 8 days (50% the normal bumblebee development time) passed between photos of them so their fates could not be unambiguously mapped. These represent 10% of 437 unseen cells or 1% of all 4,640 cells mapped across the 21 colonies and three resource environments.

### *Worker surveys*

We conducted weekly night-time surveys to estimate the mean and coefficient of variation (CV) in the size of newly emerged workers (hereafter referred to as “callow size”). We assigned each bee a unique tag using a combination enamel paint and numbered, color-tags or Microsensys radio-frequency identification (RFID) tags (Kerr, Crone & Williams 2019; Malfi, Crone & Williams 2019). For each newly emerged (“callow”) worker, we estimated body size by measuring intertegular (IT) span to the nearest 0.01 mm using digital calipers (Cane 1987; Hagen & Dupont 2013) and wet weight to the nearest 0.01 mg using [an](#) analytical microbalance (Mettler Toledo XS205DU). The size of each worker at initial capture was used to estimate the mean and

CV of callow size. We used these size measurements in combination with presence/absence data to determine the number of workers of each size (now referred to as “worker size composition”) present in each colony for each week of the survey in order to evaluate the effects of worker size composition on aspects of worker production.

### *Functional linear models*

We used functional linear models (FLMs) to estimate how five vital rates varied with worker size composition. FLMs are a type of regression spline that allows a covariate to vary smoothly over a continuous domain (Ramsay & Silverman 2005; Ramsay, Hooker & Graves 2009). Therefore, instead of restricting our predictors (X) to unidimensional space (i.e. simple linear models, such as total worker number predicts number of eggs), we can evaluate the effect of the number of workers on some response variable (e.g. number of eggs) as a continuous function of worker size (i.e. a separate attribute of the predictor variable), such that the smooth function of size-specific slopes versus worker size can be described as:

$$E(Y) = \beta_0 + \sum_{x=1}^{\max(x)} (s_x) W(n_x) \quad (1)$$

where  $E(Y)$  is the expected value of the response variable  $Y$  (e.g. number of eggs);  $\beta_0$  is the intercept;  $W(n_x)$  is the number of workers  $n$  of size  $x$ ; and  $(s_x)$  is the slope of  $Y$  versus the number of workers of each size category  $x$  (c.f. methods in Teller *et al.* 2016). Here, the continuous attribute (i.e. worker size) of the predictor variable (i.e. number of workers) is discretized into many size categories (14 size categories for both low and high-low, and 17 for high resource colonies) to approximate a continuous distribution of sizes (i.e., the worker size composition). The expected value of the response variable is the sum of the product of the size-specific slopes  $(s_x)$  multiplied by the number of workers of size  $x$  (Fig. 3). If the slope of  $Y$  versus the number of

workers of size  $x$  is positive, then more workers of size  $x$  increase values of  $Y$  and vice versa when the slope is negative (Fig. 3).

We parameterized the smooth functions of the size-specific slopes using general additive models (GAMs). We fit our GAMs using the cubic spline basis for all smooth covariates, so that the coefficients will be set to 0 if our covariates have no effects on the response (see Zuur 2012, for an excellent textbook introduction to GAMs). We used worker size composition in the previous week to predict both the number of eggs laid and larval survival in the present time step for our size composition FLMs. For the other three vital rates relating to worker production, we quantified worker size composition as the average number of workers in each size category across their larval development period.

Models were fit separately to data from each study (i.e. low, high-low, and high resource environments), and we included colony ID as a fixed effect (i.e. a different intercept term for each colony) for each model to account for between-colony effects. We used negative binomial GAMs to account for overdispersion for estimating new eggs laid and development time. We offset the number of new eggs laid by the number of days between brood photos. We used binomial and Gaussian-distributed GAMs for larval survival and callow size, respectively. We parameterized the binomial GAMs for estimating larval survival using successes and failures, where the total number of trials was defined as the number of days between brood photos, and the number of successes was defined as the total number of days if the cell survived (i.e., zero failures) and the total number of days minus 1 if the cell died (i.e., one failure). We restricted the number of knots for our smooth terms of the number of workers of size  $j$  to a maximum of five. We also rejected any model structure that did not produce unimodal functions for our smooth term of worker size composition, since GAMs are prone to overfitting, and multimodal functions

generally did not appear to be biologically meaningful. We used likelihood ratio tests to assess the fit of the parametric intercept term and the number of knots for each smooth term in our models given our data. We used cutoff of  $P < 0.05$  for parametric terms and a cutoff of  $P < 0.01$  for smooth terms, since  $P$  values for smooth terms are only approximate and are likely too low (Wood 2017). We ran these general additive models (using `mgcv::gam`; Wood 2004; Wood 2011) in program R (R Core Team 2017); see Appendix S1 for example code for our functional linear models.

To evaluate whether size-specific slopes of worker size differed among treatments, we ran a model with all data combined and evaluated the AIC of the combined model with an AIC of models separated by treatment and by year (Table 1). We repeated all analyses with slopes scaled to size-based worker production costs (see Appendix S2 for methods; Kerr, Crone & Williams 2019 for production costs), rather than numbers of individuals. Because these results were largely parallel (Appendix S2), we do not discuss them further.

Colony size (i.e. number of observed workers) increased with colony age across three resource environments (Fig. S2-4). To avoid potentially confounding effects due to collinearity between colony age and worker number, we ran models separately with colony age and worker size composition as predictors of various measures of worker production success. Results for colony age are described in Appendix S3. Relationships between worker size composition and larval survival and mean callow size were somewhat confounded with colony age effects, and should be interpreted with caution (Table 2, Appendix S4). We found no evidence for potentially confounding relationships of colony age and worker number on mean worker size or CV in worker size across the three resource environments.

## 299 RESULTS

300 Average worker size increased with available ambient resources (likelihood ratio (LR)  
301 test for models with and without treatment;  $\chi^2 = 14701$ ,  $d.f. = 3$ ,  $P \ll 0.001$ ). Worker size was  
302 smallest in the low (mean and SE in IT span:  $3.16 \pm 0.049$ ) and largest in the high resource  
303 environment (IT span:  $3.68 \pm 0.048$ ) (multiple comparison of means between high and low;  
304 estimated difference,  $E = 0.52$ ,  $Z = 7.5$ ,  $P \ll 0.001$ ), with the high-low resource environment  
305 being intermediate (IT span:  $3.31 \pm 0.049$ ) (multiple comparison of means between high-low and  
306 low:  $E = 0.14$ ,  $Z = 2.1$ ,  $P = 0.09$ ; high and high-low:  $E = 0.37$ ,  $Z = 5.4$ ,  $P \ll 0.001$ ). These  
307 results broadly recapitulate results of previous analyses of the separate experiments as reported  
308 by Kerr et al. (2019) and Malfi et al. (2019) for the 2015 and 2016 data, respectively.

309

### 310 *Daily egg production*

311 Worker size composition did not affect egg production in the low resource environment  
312 (Fig. 4a;  $\chi^2 = 6.3E-6$ ,  $e.d.f. = 4.2E-5$ ,  $P = 0.75$ ). More larger workers increased egg production in  
313 both the high-low and high resource environments (Fig. 4b-c;  $\chi^2 = 83.3$ ,  $e.d.f. = 2.8$ ,  $P < 0.001$ ,  
314 and  $\chi^2 = 6.4$ ,  $e.d.f. = 1.3$ ,  $P = 0.01$  for high-low and high (respectively)), but more larger workers  
315 had greater impact on egg production in the high-low resource environment than in the  
316 constantly high resource environment (Table 1). To illustrate these differences for each vital rate,  
317 we plotted the lines predicted by FLMs for workers of different sizes (see egg production  
318 relationships in Fig 5a-c).

319

### 320 *Larval development time*

Larval development time increased with more smaller workers in all three resource environments (Fig. 4d-f; LR test of smooth term vs constant:  $\chi^2 = 124.6$ ,  $e.d.f. = 2.7$ ,  $P < 0.001$ ;  $\chi^2 = 422.8$ ,  $e.d.f. = 2.4$ ,  $P < 0.001$ ;  $\chi^2 = 21.4$ ,  $e.d.f. = 1.9$ ,  $P < 0.001$  for low, high-low and high (respectively)). Worker size composition affected larval development time differently in each environment (Table 1). More larger workers decreased development time in both the high-low and high resource environment (Fig. 4e-f) but not in the low resource environment (Fig. 4d). However, these effects were negligible in the high resource environment compared to the low and high-low resource environments (Fig. 5).

#### *Larval survival*

Larval survival decreased with more smaller workers in the low and high-low resource environments (Fig. 4g-h;  $\chi^2 = 18.9$ ,  $e.d.f. = 2.6$ ,  $P < 0.001$ ;  $\chi^2 = 103.9$ ,  $e.d.f. = 2.6$ ,  $P < 0.001$  for low and high-low (respectively)). The difference between the low and high-low environments was not statistically significant (Table 1). Larval survival slightly decreased with more workers of all sizes in the high resource environment (Fig. 4i;  $\chi^2 = 29.1$ ,  $e.d.f. = 1.7$ ,  $P < 0.001$ ). This effect was negligible (Fig. 5i), and this relationship for high resource colonies (i.e., colonies in 2015) differed significantly from both lower resource environments (i.e., treatments in 2016) (Table 1).

#### *Callow size*

In the low resource environment, mean callow size decreased with more smaller workers (Fig. 4j & 5j;  $F = 3.3$ ,  $e.d.f. = 1.9$ ,  $P = 0.007$ ), but worker size composition was unrelated to CV in callow size (Fig. 4m & 5m;  $F = 2.5E-6$ ,  $e.d.f. = 1.7E-5$ ,  $P = 0.52$ ). In the high-low resource



environment, mean callow size decreased with more smaller workers and increased with more larger workers (Fig. 4k & 5k;  $F = 6.4$ ,  $e.d.f. = 2$ ,  $P < 0.001$ ), whereas more larger workers slightly decreased the CV in callow size (Fig. 4n & 5n; high-low -  $F = 3.8$ ,  $e.d.f. = 3$ ,  $P < 0.001$ ). In the high resource environment, more workers of any size decreased the mean callow size (Fig. 4l & 5l;  $F = 16.5$ ,  $e.d.f. = 1.7$ ,  $P < 0.001$ ), but worker size composition was unrelated to the CV in callow size (Fig. 4o & 5o; high -  $F = 5.2E-6$ ,  $e.d.f. = 4.6E-5$ ,  $P = 0.59$ ). The effect of worker size on mean callow size of new workers did not differ between the lower resource environments (i.e., 2016 treatments), but both differed from the high-resource treatment (i.e., 2015 colonies) (Table 1). The effects of worker size on the CV in callow size differed among all three treatments (Table 1).

## DISCUSSION

Size-based contributions of bumble bee workers to worker production differed among vital rates and resource environments. Despite these differences, we never detected cases where smaller workers outperformed larger workers for vital rates relating to in-nest tasks. Therefore, the fact that smaller workers remain in the nest is likely not due to their superior skill at those in-colony tasks (Jandt & Dornhaus 2014). Instead, colonies with more larger workers often had greater worker production compared to colonies with smaller workers. This pattern is similar to many performance-based (Goulson *et al.* 2002; Spaethe & Weidenmüller 2002; Peat & Goulson 2005; Ings 2007; Kapustjanskij *et al.* 2007; Spaethe *et al.* 2007) and manipulative experiments (Cnaani & Hefetz 1994). However, we found the opposite effect in two cases: more workers of any size slightly decreased both larval survival and mean callow size in the high resource

environment. We discuss each result in turn below, as well as some advantages and limitations of functional linear models.

For two vital rates, larval survival and mean callosity size, both treatments applied in 2016 differed from 2015, and not from each other. Therefore, these differences could be due to other features that differed among the sites where the two experiments were conducted or conditions in the two years. For example, the site of the 2016 experiment was an agricultural field in an agricultural landscape. The field of the experiment was used only for growing flowers to create the “high” resource pulse in the “high-low” treatment. Nevertheless, pesticides and other factors (such as nest temperatures) may have differed between the two landscape contexts. In general, conditions for bumble bees in the 2016 experiment appeared to be more stressful than conditions in the 2015 experiment. Although the results are not uniquely attributable to floral resources, our analyses provide a reasonable test of size-based differences under relatively low to high stress levels.

#### *Functional implications of worker size distribution*

Across social organisms, the number of offspring produced often increases with the number of helpers (Brown *et al.* 1982; Malcolm & Marten 1982; Biedermann & Taborsky 2011; Young *et al.* 2015), particularly when resources are high (Harrington, Mech & Fritts 1983; Doolan & Macdonald 1997). We found a similar per capita effect on colony egg production in both our high-low and high resource treatments, yet FLMs also revealed that in these environments more larger workers increased colony egg production relative to more smaller workers. Laboratory studies of bumblebees have shown that colonies consisting of only larger workers produce more eggs than colonies consisting of only smaller workers (Cnaani & Hefetz

1994). Larger workers are known to return more resources to the colony (Goulson *et al.* 2002; Kerr, Crone & Williams 2019), but they are less resilient against starvation (Couvillon & Dornhaus 2010). This tradeoff might explain why larger workers increased colony egg production only in the high-low and high resource environment **but not in the low resource environment**. The opposite effect has been found in a fire ant, *Solenopsis invicta*, where monomorphic colonies of large workers produced almost no brood compared to monomorphic colonies of small workers (Porter & Tschinkel 1985). However, the size-based roles of workers in these two eusocial insects differs. Larger bumblebees are foragers (Cumber 1949; Goulson *et al.* 2002; Goulson 2009), but smaller fire ant workers do most of the foraging and feeding (Wilson 1978; Cassill & Tschinkel 1999). Larger fire ant workers live longer than smaller workers (Porter & Tschinkel 1985; Calabi & Porter 1989), which is the opposite of bumblebee workers (da Silva-Matos & Garofalo 2000; Kerr, Crone & Williams 2019). Therefore, the general mechanism may be similar, despite contrasting patterns.

The smallest observed workers **had** negative effects on both development time and larval survival in the low and high-low resource environments; note that this worker size was not present in the high resource colonies. In bumblebees, there seems to be a resource-driven trade-off between provisioning for developing larvae and production of new eggs when resources are low. **For example, in the low resource environment, egg-laying did not depend on the number of large workers. In contrast, in higher resource environments, the number of eggs laid increased with more larger workers. This contrast suggests that workers in the low resource environment are allocating more resources to maintaining larval survival and development time, rather than supporting more workers.** Results for small workers in the lower resource environments are similar to those for cooperative breeding species, in which the presence of more helpers often

412 reduces offspring survival when resources are low (Harrington, Mech & Fritts 1983; Woodroffe  
413 & Macdonald 2000). These negative impacts of helpers in cooperative breeding species may be  
414 due to them shifting efforts towards increasing their own survival (Bruitjes, Hekman &  
415 Taborsky 2010), which seems less likely in bumblebees because workers are non-reproductive.  
416 Indeed, bumblebee workers are reported to switch from nursing to foraging tasks when resources  
417 are low (Cartar 1992), indicating that workers overall increase (not decrease) cooperative efforts.  
418 Additionally, bumblebee workers predominantly feed on nectar and larvae predominantly feed  
419 on pollen (Plowright & Pendrel 1977; Goulson 2009), which may reduce competition among  
420 siblings and enhance cooperative behaviors. It would be interesting to monitor foraging behavior  
421 of bumblebee workers during resource dearths, i.e. changes in nectar vs. pollen collection rates,  
422 to better understand their cooperative efforts.

423       Across our three environments, observed average size of all workers decreased in  
424 colonies with less available resources. In the high resource environment, more workers of any  
425 size decreased the size of callow workers. Worker size is known to decrease with colony age  
426 (Couvillon *et al.* 2010), which correlated with colony size. In the low and high-low resource  
427 environments, more smaller workers resulted in callow workers of smaller sizes and more larger  
428 workers resulted in callow workers of larger sizes. Bumblebee workers have been recorded to be  
429 smaller on average in simple, intensively managed landscapes (Persson & Smith 2011).  
430 Laboratory experiments also show that colonies produce smaller workers during food shortages  
431 (Schmid-Hempel & Schmid-Hempel 1998). The correlation between worker size distribution and  
432 callow worker size [in the low and high-low resource environment](#) suggests that stressful resource  
433 conditions may produce a negative feedback loop, where colonies of smaller workers cannot  
434 properly feed and care for brood (Cartar & Dill 1991) causing the emergence of smaller callow

workers. Therefore, the cost and benefits of helpers within social groups may often regulate the traits of individuals (e.g. sex ratios, worker sizes) that are expressed (Griffin, Sheldon & West 2005). Functional linear models are only a correlative technique, so an alternative shared driver could be shifting the size distribution towards smaller workers. For example, lower resources could cause differential mortality of larger workers due to starvation (Couvillon & Dornhaus 2010) and cause larvae to develop into smaller callow workers because of fewer resources brought back by the remaining workers. Laboratory monomorphic colonies consisting of only small or large workers had no difference in the mean and variance in callow size when supplied with abundant resources (Cnaani & Hefetz 1994). If these laboratory colonies had to forage for resources and still produced workers of similar sizes, then we might be able to determine whether a shared driver is most likely causing these effects in our study.

#### *Functional linear models as a statistical approach in ecology*

Previously, FLMs have been used to evaluate the lagged effects of environmental drivers on plant population dynamics (Teller *et al.* 2016; Tenhumberg *et al.* 2018). Here, we extend the use of FLMs to evaluate the size-based contribution of workers in bumblebee colonies. FLMs could be applied to understanding many high-dimensional social systems. For example, they could be used to explore the contributions of trait-based sociality, such as the contributions of age polyethism within social groups of different taxa and levels of sociality, including eusocial honey bees (Seeley & Kolmes 1991), semi-social mole rates (Jarvis 1981; Zöttl *et al.* 2016), and cooperative breeding meerkats (Clutton-Brock *et al.* 2001a) or cichlid fish (Bruitjies & Taborsky 2011). In the African mole rat, larger groups had higher rates of offspring recruitment (Young *et al.* 2015) and cooperative behaviors were found to increase with age (Zöttl *et al.*

2016). Therefore, FLMs might be able to determine how vital rates (e.g. offspring recruitment) differ with the number of helpers of different ages for the African mole rate. FLMs provide an alternative way to study these high-dimensional ecological systems using field observational data, particularly where manipulative experiments may not be possible.

Correlative techniques, such FLMs, provide a valuable complement to many manipulative experiments that aim to test similar hypotheses. However, these separate approaches have their own set of advantages and limitations that need to be considered when making conclusions [from these models](#). For example, FLMs can be data-heavy (e.g., 20-25 independent observations of the signal and response; Teller *et al.* 2016); only inform us about correlations and not causations; and may have collinear predictors that obscure the true driver of these responses. Collinearity is not specific to FLMs but is equally problematic for many simple (e.g. multiple regression) and complex statistical techniques (e.g. structural equation models). To date, only two studies have reported applying functional smoothing approaches to high-dimensional ecological systems by exploring how lagged environmental drivers influence plant performance (Teller *et al.* 2016; Tenhumberg *et al.* 2018). Teller *et al.* (2016) predicted how lagged effects of past precipitation and local competition influenced plant growth and survival; however, they would not be able to parse out the true driver of plant performance if density and precipitation covaried across some gradient. When exploring the trends and collinearity for these several vital rates (Appendix S4), two of four vital rates (Table 2) had confounding effects of colony age and size composition suggesting that either or both might be driving these trends (Table 2). When using simple or complex correlative methods, it is important to explicitly evaluate the collinearity of predictor variables as we have demonstrated here.

481 *Summary*

482       Overall, we found that the advantages and disadvantages of workers of different sizes on  
483 worker production only became apparent when exploring these effects across these three  
484 different resource environments. We also found that bumblebee colonies shifted their worker  
485 size distribution across these resource environments. Among eusocial insects, caste size  
486 polymorphism is hypothesized to be an adaption to expand accessibility of resources, such as  
487 seed size in ants (Davidson 1978; Traniello & Beshers 1991; Retana & Cerdá 1994) and flower  
488 size in bumblebees (Peat, Tucker & Goulson 2005). However, the shift in worker size  
489 distribution across these resource environments could have emerged from the lower tolerance of  
490 larger workers to starvation (Couvillon & Dornhaus 2010). Prior to this study, quantifying the  
491 contribution of individuals in social groups has been challenging. Here, we demonstrate that  
492 functional linear models have the potential [to evaluate observational data](#) for complex, trait-  
493 based life histories of social organisms. [As such, they provide a valuable complement to the](#)  
494 [constraints of experimental work, and a mechanism to focus hypotheses for further experimental](#)  
495 [studies.](#)

496 REFERENCES

- 497 Biedermann, P.H.W. & Taborsky, M. (2011) Larval helpers and age polyethism in ambrosia  
498 beetles. *Proceedings of the National Academy of Sciences of the United States of*  
499 *America*, **108**, 17064-17069.
- 500 Billick, I. & Carter, C. (2007) Testing the importance of the distribution of worker sizes to  
501 colony performance in the ant species *Formica obscuripes* Forel. *Insectes Sociaux*, **54**,  
502 113-117.
- 503 Brent, Lauren J.N. *et al.* (2015) Ecological Knowledge, Leadership, and the Evolution of  
504 Menopause in Killer Whales. *Current Biology*, **25**, 746-750.
- 505 Brown, J.L. *et al.* (1982) Helpers: Effects of Experimental Removal on Reproductive Success.  
506 *Science*, **215**, 421.
- 507 Bruintjes, R., Hekman, R. & Taborsky, M. (2010) Experimental global food reduction raises  
508 resource acquisition costs of brood care helpers and reduces their helping effort.  
509 *Functional Ecology*, **24**, 1054-1063.
- 510 Bruintjes, R. & Taborsky, M. (2011) Size-dependent task specialization in a cooperative cichlid  
511 in response to experimental variation of demand. *Animal Behaviour*, **81**, 387-394.
- 512 Calabi, P. & Porter, S.D. (1989) Worker longevity in the fire ant *Solenopsis invicta*: Ergonomic  
513 considerations of correlations between temperature, size and metabolic rates. *Journal of*  
514 *Insect Physiology*, **35**, 643-649.
- 515 Cane, J.H. (1987) Estimation of Bee Size Using Intertegular Span (Apoidea). **60**, 145-147.
- 516 Cartar, R.V. (1992) Morphological senescence and longevity - an experiment relating wing wear  
517 and life-span in foraging wild bumble bees. *Journal of Animal Ecology*, **61**, 225-231.
- 518 Cartar, R.V. & Dill, L.M. (1991) Costs of energy shortfall for bumble bee colonies: predation,  
519 social parasitism, and brood development. *The Canadian Entomologist*, **123**, 283-293.
- 520 Cassill, D.L. & Tschinkel, W.R. (1999) Task selection by workers of the fire ant *Solenopsis*  
521 *invicta*. *Behavioral Ecology and Sociobiology*, **45**, 301-310.
- 522 Clutton-Brock, T.H. *et al.* (2001a) Cooperation, Control, and Concession in Meerkat Groups.  
523 *Science*, **291**, 478.
- 524 Clutton-Brock, T.H., Russell, A.F. & Sharpe, L.L. (2004) Behavioural tactics of breeders in  
525 cooperative meerkats. *Animal Behaviour*, **68**, 1029-1040.
- 526 Clutton-Brock, T.H. *et al.* (2001b) Effects of Helpers on Juvenile Development and Survival in  
527 Meerkats. *Science*, **293**, 2446.
- 528 Clutton-Brock, T.H. *et al.* (2002) Evolution and development of sex differences in cooperative  
529 behavior in meerkats. *Science*, **297**, 253-256.
- 530 Cnaani, J. & Hefetz, A. (1994) The effect of workers size frequency-distribution on colony  
531 development in *Bombus terrestris*. *Insectes Sociaux*, **41**, 301-307.
- 532 Couvillon, M.J. & Dornhaus, A. (2010) Small worker bumble bees (*Bombus impatiens*) are  
533 hardier against starvation than their larger sisters. **57**, 193-197.



534 Couvillon, M.J. *et al.* (2010) Ontogeny of worker body size distribution in bumble bee (*Bombus*  
535 *impatiens*) colonies. *Ecological Entomology*, **35**, 424-435.

536 Cumber, R.A. (1949) The biology of humble bees, with special reference to the production of the  
537 worker caste. *Transactions of the Royal Entomological Society of London*, **100**, 1-45.

538 da Silva-Matos, E.V. & Garofalo, C.A. (2000) Worker life tables, survivorship, and longevity in  
539 colonies of *Bombus (Fervidobombus) atratus* (Hymenoptera: Apidae). *Revista De*  
540 *Biologia Tropical*, **48**, 657-663.

541 Davidson, D.W. (1978) Size Variability in the Worker Caste of a Social Insect (*Veromessor*  
542 *pergandei* Mayr) as a Function of the Competitive Environment. *The American*  
543 *Naturalist*, **112**, 523-532.

544 Doolan, S.P. & Macdonald, D.W. (1997) Breeding and juvenile survival among slender-tailed  
545 meerkats (*Suricatu suricatta*) in the south-western Kalahari: ecological and social  
546 influences. *Journal of Zoology*, **242**, 309-327.

547 Doolan, S.P. & Macdonald, D.W. (1999) Co-operative Rearing by Slender-tailed Meerkats  
548 (*Suricata suricatta*) in the Southern Kalahari. *Ethology*, **105**, 851-866.

549 Faulkes, C.G. & Bennett, N.C. (2001) Family values: group dynamics and social control of  
550 reproduction in African mole-rats. *Trends in Ecology & Evolution*, **16**, 184-190.

551 Frank, L.G. (1986) Social organization of the spotted hyaena *Crocuta crocuta*. II. Dominance  
552 and reproduction. *Animal Behaviour*, **34**, 1510-1527.

553 Goulson, D. (2009) *Bumblebees: Behaviour, Ecology, and Conservation*, 2 edn. Oxford  
554 University Press.

555 Goulson, D. *et al.* (2002) Can alloethism in workers of the bumblebee, *Bombus terrestris*, be  
556 explained in terms of foraging efficiency? *Animal Behaviour*, **64**, 123-130.

557 Griffin, Ashleigh S., Sheldon, Ben C. & West, Stuart A. (2005) Cooperative Breeders Adjust  
558 Offspring Sex Ratios to Produce Helpful Helpers. *The American Naturalist*, **166**, 628-  
559 632.

560 Hagen, M. & Dupont, Y.L. (2013) Inter-tegular span and head width as estimators of fresh and  
561 dry body mass in bumblebees (*Bombus* spp.). *Insectes Sociaux*, **60**, 251-257.

562 Harrington, F.H., Mech, L.D. & Fritts, S.H. (1983) Pack size and wolf pup survival: their  
563 relationship under varying ecological conditions. *Behavioral Ecology and Sociobiology*,  
564 **13**, 19-26.

565 Ings, T.C. (2007) Body size affects nectar uptake rates in bumblebees (Hymenoptera: Apidae:  
566 *Bombus terrestris*). *Entomologia Generalis*, **30**, 186-186.

567 Inoue, M. *et al.* (2010) Is *Bombus terrestris* (Hymenoptera: Apidae) a stronger competitor  
568 against native Japanese species? A comparison of foraging efficiency. *Applied*  
569 *Entomology and Zoology*, **45**, 71-75.

570 Jandt, J.M. & Dornhaus, A. (2009) Spatial organization and division of labour in the bumblebee  
571 *Bombus impatiens*. *Animal Behaviour*, **77**, 641-651.

572 Jandt, J.M. & Dornhaus, A. (2011) Competition and cooperation: bumblebee spatial organization  
573 and division of labor may affect worker reproduction late in life. *Behavioral Ecology and*  
574 *Sociobiology*, **65**, 2341-2349.

575 Jandt, J.M. & Dornhaus, A. (2014) Bumblebee response thresholds and body size: does worker  
576 diversity increase colony performance? *Animal Behaviour*, **87**, 97-106.

577 Jarvis, J.U.M. (1981) Eusociality in a Mammal: Cooperative Breeding in Naked Mole-Rat  
578 Colonies. *Science*, **212**, 571-573.

579 Kapustjanskij, A. *et al.* (2007) Bigger is better: implications of body size for flight ability under  
580 different light conditions and the evolution of alloethism in bumblebees. *Functional*  
581 *Ecology*, **21**, 1130-1136.

582 Kerr, N.Z., Crone, E.E. & Williams, N.M. (2019) Integrating vital rates explains optimal worker  
583 size for resource return by bumblebee workers. *Functional Ecology*, **33**, 467-478.

584 Kerth, G. (2008) Causes and Consequences of Sociality in Bats. *Bioscience*, **58**, 737-746.

585 Malcolm, J.R. & Marten, K. (1982) Natural selection and the communal rearing of pups in  
586 African wild dogs (*Lycaon pictus*). *Behavioral Ecology and Sociobiology*, **10**, 1-13.

587 Malfi, R.L., Crone, E. & Williams, N. (2019) Demographic benefits of early season resources for  
588 bumble bee (*B. vosnesenskii*) colonies. *Oecologia*, **191**, 377-388.

589 Malfi, R.L. & Roulston, T.H. (2014) Patterns of parasite infection in bumble bees (*Bombus* spp.)  
590 of Northern Virginia. *Ecological Entomology*, **39**, 17-29.

591 Muller, C.B., Blackburn, T.M. & Schmid-Hempel, P. (1996) Field evidence that host selection  
592 by conopid parasitoids is related to host body size. *Insectes Sociaux*, **43**, 227-233.

593 Muscedere, M.L., Willey, T.A. & Traniello, J.F.A. (2009) Age and task efficiency in the ant  
594 *Pheidole dentata*: young minor workers are not specialist nurses. *Animal Behaviour*, **77**,  
595 911-918.

596 Peat, J. & Goulson, D. (2005) Effects of experience and weather on foraging rate and pollen  
597 versus nectar collection in the bumblebee, *Bombus terrestris*. *Behavioral Ecology and*  
598 *Sociobiology*, **58**, 152-156.

599 Peat, J., Tucker, J. & Goulson, D. (2005) Does intraspecific size variation in bumblebees allow  
600 colonies to efficiently exploit different flowers? *Ecological Entomology*, **30**, 176-181.

601 Persson, A.S. & Smith, H.G. (2011) Bumblebee colonies produce larger foragers in complex  
602 landscapes. *Basic and Applied Ecology*, **12**, 695-702.

603 Plowright, R.C. & Pendrel, B.A. (1977) Larval growth in bumble bees (Hymenoptera-Apidae).  
604 *Canadian Entomologist*, **109**, 967-973.

605 Porter, S.D. & Tschinkel, W.R. (1985) Fire ant polymorphism: the ergonomics of brood  
606 production. *Behavioral Ecology and Sociobiology*, **16**, 323-336.

607 R Core Team (2017) R: A language and environment for statistical computing. R Foundation for  
608 Statistical Computing, Vienna, Austria.

609 Ramsay, J.O., Hooker, G. & Graves, S. (2009) *Functional Data Analysis with R and Matlab*.  
610 Springer-Verlag, New York.

611 Ramsay, J.O. & Silverman, B.W. (2005) *Functional Data Analysis*. Springer-Verlag, New York.

612 Retana, J. & Cerdá, X. (1994) Worker Size Polymorphism Conditioning Size Matching in Two  
613 Sympatric Seed-Harvesting Ants. *Oikos*, **71**, 261-266.

- 614 Richards, O.W. (1946) Observations on *Bombus agrorum* (Fabricius) (Hymen., Bomhidae).  
615 *Proceedings of the Royal Entomological Society of London*, **21**, 66-71.
- 616 Russell, A.F. *et al.* (2007) Helpers increase the reproductive potential of offspring in cooperative  
617 meerkats. *Proceedings of the Royal Society B: Biological Sciences*, **274**, 513-520.
- 618 Schmid-Hempel, R. & Schmid-Hempel, P. (1998) Colony performance and immunocompetence  
619 of a social insect, *Bombus terrestris*, in poor and variable environments. *Functional*  
620 *Ecology*, **12**, 22-30.
- 621 Schwander, T., Rosset, H. & Chapuisat, M. (2005) Division of labour and worker size  
622 polymorphism in ant colonies: the impact of social and genetic factors. *Behavioral*  
623 *Ecology and Sociobiology*, **59**, 215-221.
- 624 Seeley, T.D. & Kolmes, S.A. (1991) Age Polyethism for Hive Duties in Honey Bees — Illusion  
625 or Reality? *Ethology*, **87**, 284-297.
- 626 Spaethe, J. *et al.* (2007) Size determines antennal sensitivity and behavioral threshold to odors in  
627 bumblebee workers. *Naturwissenschaften*, **94**, 733-739.
- 628 Spaethe, J. & Weidenmüller, A. (2002) Size variation and foraging rate in bumblebees (*Bombus*  
629 *terrestris*). *Insectes Sociaux*, **49**, 142-146.
- 630 Sparkman, A.M. *et al.* (2011) Helper effects on pup lifetime fitness in the cooperatively breeding  
631 red wolf (*Canis rufus*). *Proc Biol Sci*, **278**, 1381-1389.
- 632 Teller, B.J. *et al.* (2016) Linking demography with drivers: climate and competition. *Methods in*  
633 *Ecology and Evolution*, **7**, 171-183.
- 634 Tenhumberg, B. *et al.* (2018) Time-lagged effects of weather on plant demography: drought and  
635 *Astragalus scaphoides*. *Ecology*, **99**, 915-925.
- 636 Traniello, J.F.A. & Beshers, S.N. (1991) Polymorphism and size-pairing in the harvester ant  
637 *Pogonomyrmex badius*: a test of the ecological release hypothesis. *Insectes Sociaux*, **38**,  
638 121-127.
- 639 Wenzel, J.W. (1992) Extreme queen-worker dimorphism in *Ropalidia ignobilis*, a small-colony  
640 wasp (Hymenoptera: Vespidae). *Insectes Sociaux*, **39**, 31-43.
- 641 Wilkinson, G.S. (1992) Communal nursing in the evening bat, *Nycticeius humeralis*. *Behavioral*  
642 *Ecology and Sociobiology*, **31**, 225-235.
- 643 Wilson, E.O. (1978) Division of Labor in Fire Ants Based on Physical Castes (Hymenoptera:  
644 Formicidae: Solenopsis). *Journal of the Kansas Entomological Society*, **51**, 615-636.
- 645 Wood, S.N. (2004) Stable and Efficient Multiple Smoothing Parameter Estimation for  
646 Generalized Additive Models. *Journal of the American Statistical Association*, **99**, 673-  
647 686.
- 648 Wood, S.N. (2011) Fast stable restricted maximum likelihood and marginal likelihood estimation  
649 of semiparametric generalized linear models. *Journal of the Royal Statistical Society:*  
650 *Series B (Statistical Methodology)*, **73**, 3-36.
- 651 Wood, S.N. (2017) *Generalized Additive Models: An Introduction with R*, 2nd Edition edn.  
652 Chapman and Hall/CRC Press.

- 653 Woodroffe, R. & Macdonald, D.W. (2000) Helpers provide no detectable benefits in the  
654 European badger (*Meles meles*). *Journal of Zoology*, **250**, 113-119.
- 655 Young, A.J. *et al.* (2015) Workforce Effects and the Evolution of Complex Sociality in Wild  
656 Damaraland Mole Rats. *The American Naturalist*, **186**, 302-311.
- 657 Zöttl, M. *et al.* (2016) Differences in cooperative behavior among Damaraland mole rats are  
658 consequences of an age-related polyethism. *Proceedings of the National Academy of*  
659 *Sciences*, **113**, 10382.
- 660 Zuur, A.F. (2012) *A Beginner's Guide to Generalized Additive Models with R*. Highland  
661 Statistics Ltd, Newburgh, UK.

## FIGURE LEGENDS

**Figure 1.** Example of brood mapping photos used to track the fate of individual cells. These mapping photos are aerial photographs for colony 6 in (a) week 5 and (b) week 6 since the first brood photo. Aerial, side, and diagonal photos were taken to capture all cells. Each cell has been individually numbered to track each cell. The larger stand-alone open wax structures are honey pots.

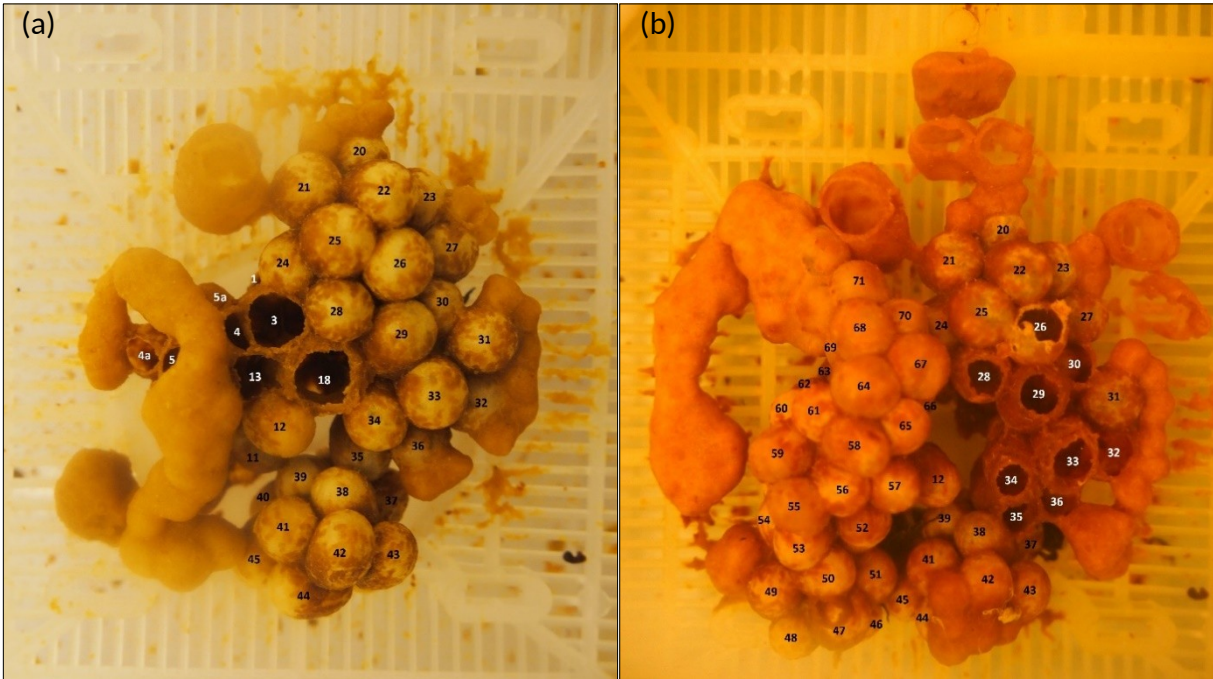
**Figure 2.** Brood mapping photos showing each of the six categories of living or dead stages of cell development. The six stages are: (a) clump stage, which are egg stages; (b) pre-popcorn stages, which represents early larval instars; (c) popcorn stage, which are late instar larvae; (d) cocoon stage; (e) eclosed stage, and (f) a dead cell (dashed circle). These categories assisted with estimating three vital rates relating to worker production: eggs laid, development time, and larval survival.

**Figure 3.** Example of functional linear model results showing the smooth function of the slopes of  $Y$  versus the number of workers as a function of worker size,  $x$ .  $Y$  covariate could be one of the five metrics of worker production: egg production, larval development time, larval survival, and mean and variance in callow size. We illustrate the following examples: (a) no size-based per capita effect, but more workers of any size increases ( $\beta_0 > 0$ ) or decreases ( $\beta_0 < 0$ )  $Y$ ; (b) positive size-based per capita effects on  $Y$ ; (c) negative size-based per capita effects on  $Y$ ; and (d) mixed size-based per capita effects, i.e., more workers of one size have negative effects and more workers of another size have positive effects. The dotted line on each panel represents no per capita effects of workers.

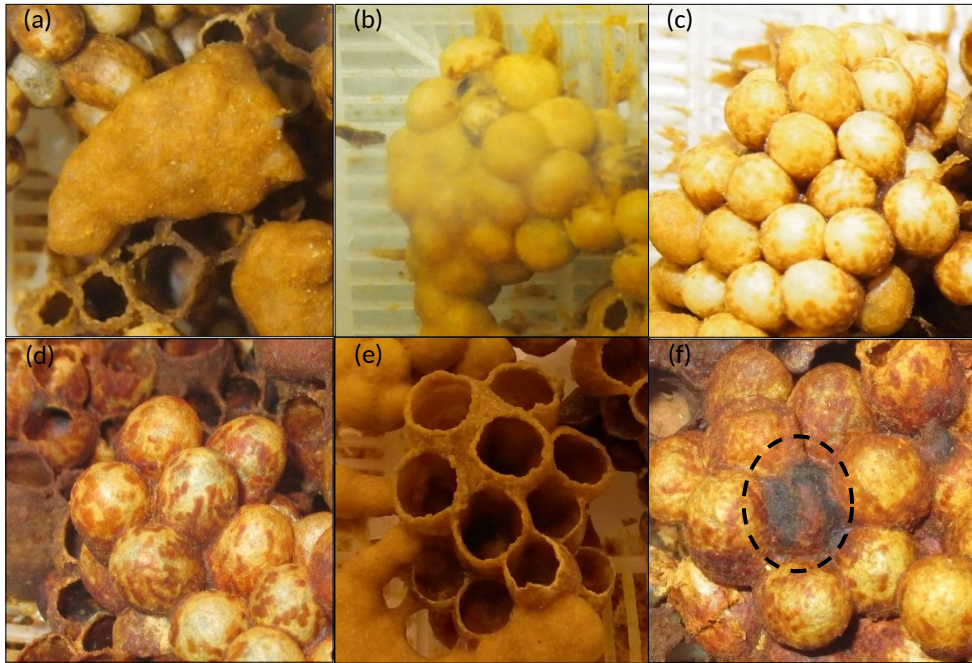
**Figure 4.** Generalized additive model results depicting the smooth function of the size-specific slopes for all five vital rates relating to worker production versus the number of workers of size  $x$  for the low (left), high-low (middle), and high (right) resource environments. Dashed horizontal line at zero represent deviations from mean slope values, i.e. slopes above the line means more workers of size  $x$  have positive impact on  $Y$ . Grey dashed vertical line represents the mean worker size for colonies in each of the resource environments. Plots with a significant smooth term of WSC are labeled with  $P < 0.01$ . Note different scales on the Y-axes in each row.

**Figure 5.** The relationship between number of workers of three observed worker sizes and the five vital rates relating to worker production across the three treatments. Three workers sizes range from the smallest size of 2.5 mm (light grey), intermediate size of 3.5 mm (dark grey), and largest size of 4.5 mm (black line) that are observed in colonies across all three treatments. Each of these lines represents the function defined by  $x = 2.5, 3.5$  and  $4.5$  on the x-axis of figure 4. Parametric intercepts were used from the GAMs, and intercepts were averaged on the link function scale if the model had a significant fixed effect of colony.

FIGURES



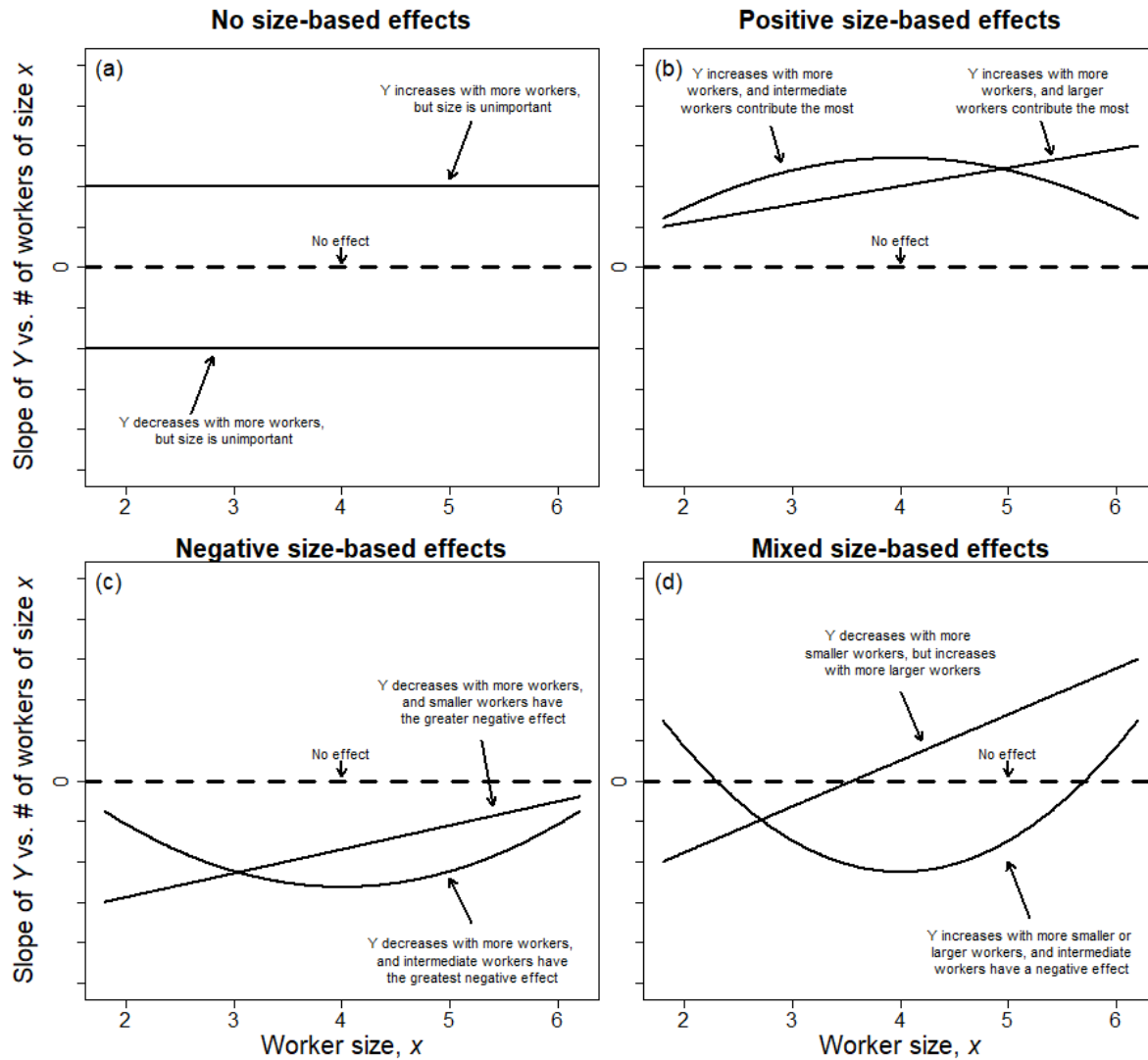
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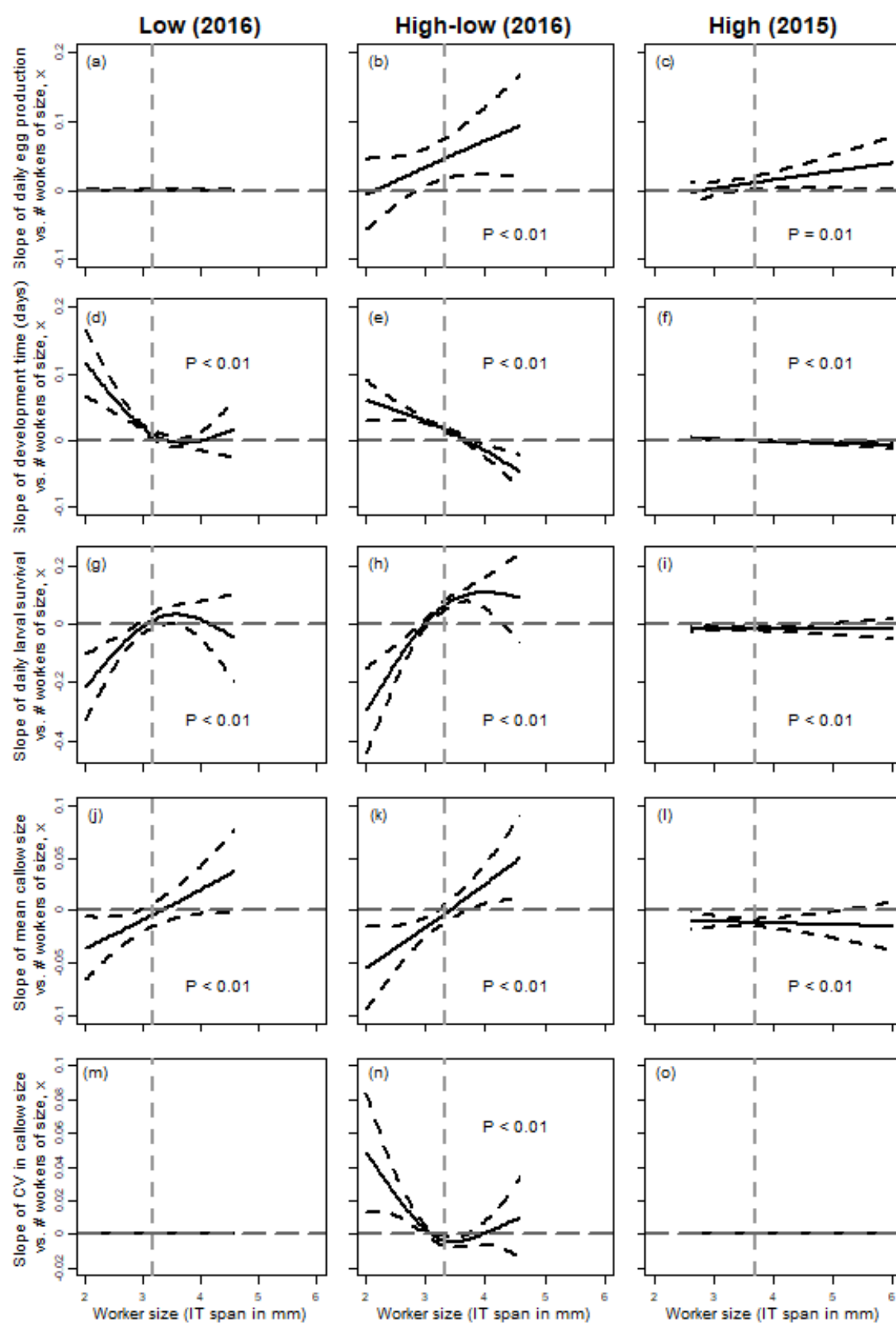
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 716 survival.



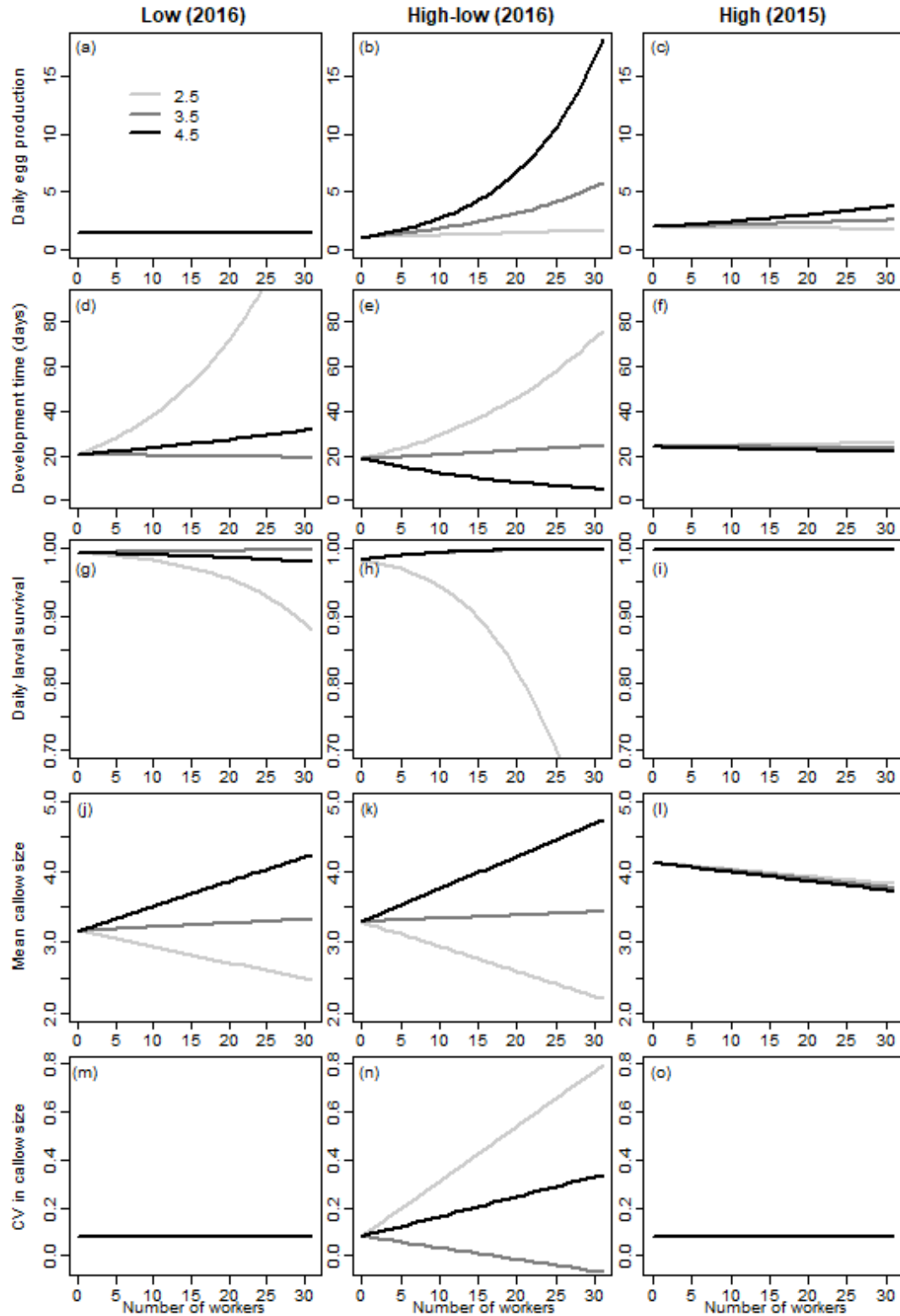


717

718 **Figure 3.** Example of functional linear model results showing the smooth function of the slopes  
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 722 capita effect, but more workers of any size increases ( $\beta_0 > 0$ ) or decreases ( $\beta_0 < 0$ ) Y; (b) positive  
 723 size-based per capita effects on Y; (c) negative size-based per capita effects on Y; and (d) mixed  
 724 size-based per capita effects, i.e., more workers of one size have negative effects and more  
 725 workers of another size have positive effects. The dotted line on each panel represents no per  
 726 capita effects of workers.



728 **Figure 4.** Generalized additive model results depicting the smooth function of the size-specific  
729 slopes for all five vital rates relating to worker production versus the number of workers of size  $x$   
730 for the low (left), high-low (middle), and high (right) resource environments. Dashed horizontal  
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**Figure 5.** The relationship between number of workers of three observed worker sizes and the five vital rates relating to worker production across the three treatments. Three workers sizes

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739 largest size of 4.5 mm (black line) that are observed in colonies across all three treatments. Each  
740 of these lines represents the function defined by  $x = 2.5, 3.5$  and  $4.5$  on the x-axis of figure 4.  
741 Parametric intercepts were used from the GAMs, and intercepts were averaged on the link  
742 function scale if the model had a significant fixed effect of colony.

## 743 TABLES

744 **Table 1.** dAIC values for functional linear models using data combined (i.e., no effect of  
 745 treatment or year) for each daily vital rate.

Vital rates	dAIC (models fit to all data)			dAIC (Pairwise comparisons) <sup>1</sup>		
	<i>Combined</i>	<i>By treatment</i>	<i>By year</i>	<i>Low vs High-low</i>	<i>Low vs High</i>	<i>High-low vs High</i>
Daily egg production	23.1	0	6.4	6.4	15.6	7.9
Development time (days)	352.7	0	48.2	5.2	96.8	272.3
Daily larval survival	24004.1	23.2	0	-23.1	12568.6	17488.6
Mean callow size	10.8	3.1	0	-3.1	7.7	1.25
CV in callow size	41.4	0	11.5	11.5	34.2	40.8
<sup>1</sup> AIC of models fit to data from both groups together, minus AIC of models fit to data from each treatment group separately. Positive values indicate significant differences between groups.						

**Table 2.** Size-specific relationships of the smooth terms of colony age, the number of workers of each size (i.e. worker size composition, WSC), and standardized (“std”) WSC for each of the five vital rates relating to worker production. Relationship descriptions provided are restricted over the observed range of worker body sizes and colony ages including days spent in the laboratory. Since colony age and population size are correlated, we were unable to determine which smooth term was driving these effects if both smooth terms have similar effects. Shaded cells had a significant fixed effect of colony ID on the parametric intercept in the GAM.

Response variable	Resource environment	Sample size	Smooth terms			Confounding effects <sup>2</sup>
			<i>Colony age</i>	<i>WSC</i> <sup>1</sup>	<i>Std WSC</i> <sup>1</sup>	
Egg production	<i>Low</i>	72	Concave	×, ×	×, ×	
	<i>High-low</i>	74	Concave	±, ↑	±, ↑	Possibly
	<i>High</i>	65	Concave	±, ↑	±, ↑	No
Development time	<i>Low</i>	541	Multimodal	±, ↓	±, ↓	Possibly
	<i>High-low</i>	974	Concave	±, ↓	±, ↓	Possibly
	<i>High</i>	1108	Convex	±, ↓	±, ↓	Possibly
Larval survival	<i>Low</i>	3521	Multimodal	±, ↓	±, ↓	Yes
	<i>High-low</i>	6045	Decreases	±, ↑	±, ↓	Yes
	<i>High</i>	5364	Convex	–, ×	–, ↑	Yes
Mean callow size	<i>Low</i>	65	Decreases	±, ↑	±, ↑	Yes
	<i>High-low</i>	59	Multimodal	±, ↑	±, ↑	Yes
	<i>High</i>	57	Multimodal	–, ×	–, ↑	Yes
CV in callow size	<i>Low</i>	65	Concave	×, ×	×, ×	
	<i>High-low</i>	59	Multimodal	±, ↓	±, ↓	No
	<i>High</i>	57	Constant	×, ×	×, ×	

<sup>1</sup>For WSC and std WSC, the first symbol refers to whether the relationship has a positive (+), negative (–), mixed (±), or no (×) per capita effect, and the second symbol refers to whether the relationship increases (↑), decreases (↓), both (↓), or has no effect (×) with worker size. Sample sizes are also provided for each of the five vital rates.

<sup>2</sup>The column “confounding effects” describes whether both colony age and WSC had similar effects on the response variable when both smooth terms are significant.