A direct physiological trade-off between personal and social immunity

Cotter, S. C., Littlefair, J. E., Grantham, P. J., & Kilner, R. M. (2013). A direct physiological trade-off between personal and social immunity. Journal of Animal Ecology, 82(4), 846-853. DOI: 10.1111/1365-2656.12047

Published in:
Journal of Animal Ecology

Document Version:
Early version, also known as pre-print

Title: A direct physiological trade-off between personal and social immunity

Authors: S.C. Cotter[1,2], J.E. Littlefair[1,3], P.J. Grantham[1] & R.M. Kilner[1]

Addresses:

1. Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, UK
2. School of Biological Sciences, Queen's University Belfast, MBC, 97 Lisburn Rd, Belfast, BT9 7BL, UK
3. Current address: The School of Biological and Chemical Sciences, Queen Mary, University of London, Mile End Road, London E1 4NS, UK

Corresponding author: Dr. Sheena Cotter, School of Biological Sciences, Queen's University Belfast, MBC, 97 Lisburn Rd, Belfast, BT9 7BL, UK. Tel: +44(0)28 9097 2691, Fax: +44(0)28 9097 5877. Email: s.cotter@qub.ac.uk

Keywords: antibacterial, ecological immunology, insect, lysozyme, *Nicrophorus*, wounding

Running title: Immune system trade-offs

Abstract

1. Recent work shows that organisms possess two strategies of immune response: personal immunity, which defends an individual, and social immunity, which protects other individuals, such as kin. However, it is unclear how individuals divide their limited resources between protecting themselves and protecting others.

2. Here, with experiments on female burying beetles, we challenged the personal immune system and measured subsequent investment in social immunity (antibacterial activity of the anal exudates).

3. Our results show that increased investment in one aspect of personal immunity (wound repair) causes a temporary decrease in one aspect of the social immune response.

4. Our experiments further show that by balancing investment in personal and social immunity in this way during one breeding attempt, females are able to defend their subsequent lifetime reproductive success.

5. We discuss the nature of the physiological trade-off between personal and social immunity in species that differ in the degree of eusocality and coloniality, and suggest that it may also vary within species in relation to age and partner contributions to social immunity.

Introduction

Parasites are ubiquitous and can threaten the survival prospects of their hosts, dramatically impacting upon their fitness (Thomas, Guegan & Renaud 2009). In response to this threat, organisms have developed highly effective immune responses that recognise invaders and act to eliminate them. Organisms can defend their fitness from attack by parasites and pathogens with two different strategies of immune response: personal and social (Cremer, Armitage & Schmid-Hempel 2007; Cotter & Kilner 2010a). The well-characterized personal immune system comprises an innate cell-based and humoral response that can phagocytose parasites or inhibit their growth. In addition, vertebrates have an acquired response with immunological memory (Janeway *et al.* 2001). This personal immune response is typically deployed internally (but see Martin-Vivaldi *et al.* 2010) and mainly serves to defend an individual's survival, whereas the more recently identified social immune responses are typically deployed externally and have evolved mainly to protect the fitness of others. Social immune responses (*sensu* Cotter & Kilner 2010a) range from antibodies provided in mammalian milk that protect newborn offspring, to antimicrobial metapleural gland secretions in ants that can provide benefits to the whole colony (Cotter & Kilner 2010a).

Mounting an immune response can be costly in terms of energetic expenditure e.g. (Long 1977; Ots *et al.* 2001; Freitak *et al.* 2003) or in terms of the availability of specific nutrients such as protein e.g. (Beisel 1977; Povey *et al.* 2009; Cotter *et al.* 2011). How, then, do individuals allocate their limited resources between personal and social immunity? Is there a direct trade-off between the two strategies of immune response, as has been suggested for honeybees, who appear to possess fewer genes for personal immunity than non-social insects, but bear many genes for colony level immune function (Evans *et al.* 2006; Wilson-Rich *et al.*

2009)? Or, in the face of an immune challenge, are both personal and social immune responses maintained at a cost to current or future reproduction?

To answer these questions, we focused on the sub-social burying beetle *Nicrophorus vespilloides*, which rears its offspring on carrion (Pukowski 1933). Biparental care is typical, both parents care for the developing larvae, protecting them from predators and competitors and regurgitating pre-digested meat for them (Pukowski 1933), but in some cases more than two adults may breed on a single carcass (Muller *et al.* 2007). Burying beetles fight for access to carcasses and so are particularly prone to wounding (Trumbo & Wilson 1993; Steiger *et al.* 2012). An open wound is susceptible to infection and so rapid wound healing is an early line of defence for the immune system. In insects this involves cell migration and adhesion (Fauvarque & Williams 2011), rapid clotting, which involves the immune enzyme phenoloxidase (Bidla *et al.* 2005; Haine, Rolff & Siva-Jothy 2007) and localised upregulation of phenoloxidases and lysozymes which show antimicrobial activity (Haine, Rolff & Siva-Jothy 2007). Sterile wounding stimulates these responses but they are enhanced by the presence of PAMPs (pathogen associated molecular patterns) (Haine, Rolff & Siva-Jothy 2007). Any pathogens that manage to invade the body further stimulate antimicrobial peptide (AMP) production, phagocytosis and nodulation of microparasites, and encapsulation of macroparasites (Hoffmann, Dimarcq & Bulet 1992 and references therein).

N. vespilloides show the typical insect haemolymph personal immune responses of constitutive phenoloxidase activity (S. Cotter unpublished data), an enzyme involved in wound healing and the melanisation of encapsulated invaders, and AMPs produced after bacterial challenge (Cotter, Ward & Kilner 2011; Vogel, Badapanda & Vilcinskas 2011). However, during reproduction, adults also invest heavily in lysozyme-like antibacterial activity in their anal exudates, which are smeared on the carcass to protect it from microbes

(Cotter & Kilner 2010b). This is a social immune response because it benefits others, namely the burying beetle larvae, whose growth rate and survival prospects are severely reduced if reared on a carcass that has been heavily compromised by microbial infestation (Rozen, Engelmoer & Smiseth 2008). This social immune response is phenotypically plastic, it is produced only during reproduction (Cotter & Kilner 2010b), it is tailored to the perceived state of the carcass and is costly to upregulate (Cotter *et al.* 2010). It is likely, therefore, to compete for limiting resources with the internally deployed personal immune responses.

We challenged the personal immune system of female burying beetles during reproduction and measured their subsequent investment in the social immune response. We also quantified the lifetime reproductive success (LRS) of our challenged individuals to assess whether females choose to maintain investment in both forms of immunity at a cost to future reproduction, or whether the costs of a personal immune response are paid by a reduction in the social immune response.

Methods

Nicrophorus vespilloides colony

The burying beetle colony was established in 2005 and a pedigreed, outbred population maintained as described previously (Cotter & Kilner 2010b). Briefly, adult beetles were maintained in individual containers and fed twice-weekly on minced beef. For breeding, a female was placed with a non-sibling male in breeding chambers comprising clear plastic boxes measuring 17×12×6cm containing a 2cm depth of moistened compost. A freshly defrosted mouse carcass was weighed and placed in each breeding chamber. The breeding chambers were stored in a dark cupboard in order to simulate underground conditions.

111 Carcass preparation, mating and egg laying occur during the first 3 days, eggs hatch in ~3
112 days and larvae disperse from the carcass after ~5 days of feeding, making the time from egg
113 laying to dispersal ~8 days at 21°C. After breeding, adults were transferred back to individual
114 containers and dispersed larvae were placed in 25-cell petri dishes, covered with moist soil
115 and left to pupate. Field-caught beetles were bred into the population every summer to
116 maintain the genetic variability in the population.

117

118 **Experiment 1: Characterising the antibacterial activity of exudates throughout a**
119 **breeding bout**

120 We have previously shown that the antibacterial activity of beetle exudates is
121 phenotypically plastic and is only switched on when beetles are presented with a carcass
122 (Cotter & Kilner 2010b). Here we wanted to assess in more detail how activity levels change
123 across the 8 days of the breeding bout in unmanipulated females. To do this, 80 young virgin
124 females (Mean age in days (SE) = 25.26 (1.45)) were paired as described above. After 2 days
125 the male was removed to ensure that the female was mated but that his presence did not
126 interfere with her investment in exudate antibacterial activity later in the breeding cycle as
127 has been shown previously (Cotter & Kilner 2010b).

128 Of the 80 pairs set up, 54 bred successfully. Anal exudate was collected from these
129 females on days 0, 2 (male removed), 4, 6 and 8 of the breeding bout using capillary tubes,
130 blown into eppendorf 1.5ml reaction tubes and stored at -20°C until they could be subjected
131 to further testing (Cotter & Kilner 2010b). Burying beetles readily produce an anal exudate
132 when handled, and gentle tapping of the abdomen is generally sufficient to encourage beetles
133 to produce enough exudate for collection and analysis. However, in some cases a beetle

cannot be coerced to produce an exudate sample. Therefore, we did not successfully collect exudate from every female at every sampling point resulting in 195 samples in total out of a possible 270. All females produced at least 1 exudate sample, with the median number of samples produced per beetle being 3.

Experiment 2: Testing for a trade-off between personal and social immunity

Young virgin females (mean age (SE) = 25.89 (± 0.35) days) were randomly assigned to one of three experimental groups: bacteria-challenged (n=30), sterile-challenged (n=31) and controls (unchallenged; n=34). The treatments were designed to challenge two components of the personal defence system independently: wound healing in response to piercing of the cuticle (sterile-challenged and bacteria-challenged treatments) and increased antibacterial activity in the haemolymph caused by injection of bacteria (bacteria-challenged treatment only). Beetles in the challenged treatment groups were pierced with a needle either dipped in ethanol that had been allowed to air dry (sterile-challenged) or a solution of *Micrococcus lysodeikticus* (bacteria-challenged), which was made up of 50mg of lyophilised cells (Sigma) in 100 ul of sterile water. Piercing was carried out in such a way as to minimise haemolymph loss, just the tip of the needle pierced the cuticle, and in the majority of cases there was no bleeding at all. *M. lysodeikticus* is a common soil bacterium that beetles should encounter in their natural environment. It is not pathogenic to the burying beetles but causes upregulation of the antibacterial response in the haemolymph (Mean diameter of zone of bacterial inhibition on test plates in mm: sterile challenged = 6.84 ± 0.86, bacteria- challenged = 10.00 ± 0.68; $F_{1,28} = 8.15$, $P = 0.008$; (Cotter, Ward & Kilner 2011)). Haemolymph collected from

156 unchallenged beetles has no measurable antibacterial activity (mean diameter of clear zone in
157 mm = 0.)

158 Directly after the immune challenge treatment, each female was paired with a non-sibling
159 male (day 0) as described above The males were removed before exudate collection on day
160 2 so that we could focus on the female response without any confounding effects of partner
161 compensation (Cotter & Kilner 2010b). At this point all beetles had prepared the carcass
162 ready for the arrival of larvae.

163 Anal exudate was collected from the females on days 2, 4, and 6 of the breeding bout (we
164 chose these sampling points after analysing the data from experiment 1 and finding that this
165 was when social immune activity peaked). Samples were collected with capillary tubes,
166 blown into eppendorf containers and stored at -20°C until they could be subjected to further
167 testing. A random subset of the beetles was then repeatedly bred without further immune
168 challenge, using the same protocol as above, until death, with a new virgin male for each
169 breeding attempt (bacteria-challenged (n=16), sterile-challenged (n=16) and controls (n=17)).
170 Females were returned to their own containers and allowed to rest for 3 days between each
171 breeding bout. The exudates were collected on days 2, 4, and 6 of each breeding bout. For
172 each brood, the number and weight of larvae dispersing from the carcass were recorded in
173 order to ascertain the lifetime reproductive success (LRS) of each female. All larvae from all
174 treatments dispersed by day 8 of each breeding bout, indicating that females were not slowing
175 reproduction in response to the immune challenge. As for experiment 1, not all females gave
176 exudate samples at every time point. In total, 388 samples were collected out of a possible
177 646, but these were equally distributed across the treatment groups (Control = 140, sterile =
178 127, Bacteria = 121). The success rate varied with brood with 45% of the possible samples

collected in broods 1 and 4 and 78% and 86% of samples collected in broods 2 and 3 respectively.

Analysing the lytic activity of anal exudates

The antibacterial activity of the exudates was analysed using a lytic zone assay against *Micrococcus lysodeikticus* (Cotter & Kilner 2010b). In brief, agar plates were prepared with 0.75g of freeze-dried *M. lysodeikticus*, 1.5g agar, 100ml distilled water and 50ml 0.2M potassium phosphate buffer (pH 6.4). Holes were punched in the set agar and 1µl of defrosted exudate was pipetted into each hole, 2 replicates per sample, with each replicate on a different plate. The plates were incubated overnight at 33°C and photographed the following day. The diameter of the clear zones around each hole, indicating lysis of bacterial cells, was measured using Image J software (http://rsbweb.nih.gov/ij/). The diameter of the clear zone is indicative of the concentration of lysozymes in the sample.

Statistical analyses

As females were sampled repeatedly, data were analysed using linear mixed effects Restricted Estimate Maximum Likelihood (REML) models, including female ID as a random effect. All interactions were considered and final models were determined using stepwise deletion; the p-values of the retained terms were determined by dropping individual terms from the minimum adequate model. Exudate antibacterial activity was measured as the diameter of the clear zone around the sample. These data were log-transformed prior to

analysis to approximate normality. The term Day had unequal variances and so these were allowed to vary in the model.

For experiment 2, exudate antibacterial activity was analysed separately for the first brood, directly after the immune challenge, using data from all 95 females. For the assessment of antibacterial activity over future breeding bouts and the reproductive output by brood data, we restricted this analysis to the random subset of females that had been bred repeatedly (n=49). We excluded data after brood 4 to avoid biasing the dataset, due to a large reduction in successful broods after this point (brood 5 onwards, n<15). However, data for LRS included all broods produced by repeatedly-bred females.

The number of broods females achieved over their lifetime was analysed with GLM using Poisson errors. Data were not overdispersed; estimating the dispersion parameter gave values <1 and did not change the significance of the results. Where included, the terms Day and Brood were coded as factors. All data were analysed in Genstat 13 (VSN International, Hemel Hempstead, UK) and the assumptions of the models were tested by visual inspection of the diagnostic plots produced by Genstat.

Results

Experiment 1: Characterising the antibacterial activity of exudates throughout a breeding bout

The antibacterial activity of the exudates increased to day 4 then decreased again to day 8 (REML, day: $F_{4,56} = 41.57$, $P < 0.001$; Figure 1). Exudate activity was not significantly affected by carcass weight (REML, carcass weight: $F_{1,123} = 0.05$, $P = 0.818$) nor by the

number, nor weight of larvae that the females produced (REML, number of larvae: $F_{1,68}$ = 1.37, P = 0.245; weight of larvae: $F_{1,73}$ = 1.72, P = 0.194).

Experiment 2: Testing for a trade-off between personal and social immunity

Wounding negatively affected the upregulation of exudate antibacterial activity over the course of the first brood, with the control group producing exudates with much higher antibacterial activity than either the sterile-challenged or bacteria-challenged females (REML, treatment*day: $F_{4,78}$ = 2.80, P = 0.032; Figure 2). The identity of the female from which the exudates were collected explained a small amount of the variance (Variance Component = 0.093 $\pm$ 0.034), however, neither the weight of the carcass, nor the weight of the brood reared on that carcass, had any effect on the antibacterial activity of the exudates (REML, carcass weight: $F_{1,60}$ = 2.96, P = 0.091, brood weight: $F_{1,53}$ = 0.16, P = 0.691).

We then examined if this trade-off between personal and social immunity continued over the subsequent broods (reared with no further challenge to the mother's personal immune system). We found that antibacterial activity of the anal exudates increased in all females from brood 1 to brood 2, but that the increase was far greater when females were immune-challenged due to their much lower levels of investment in social immunity in brood 1 (brood*treatment: $F_{6,290}$ = 6.20, P < 0.001; Figure 3). By broods 3 and 4, antibacterial levels in all 3 treatment groups tended to fall off slightly and the differences between the groups disappeared (Figure 3). There was also an interaction between day and brood number, suggesting that the pattern of upregulation over days 2-6 changed with different broods (brood*day: $F_{6,268}$ = 2.75, P = 0.013; Figure 4), however the 3-way interaction was not significant (brood*day*treatment, $F_{12,252}$ = 1.23, P = 0.264). The identity of the female from

246 whom the exudates were collected explained only a small amount of the variance as can be
247 seen by the marginal estimated variance component (VC = 0.011 $\pm$ 0.005).

248 To test whether the differences between the treatments in broods 2-4 were significant, we
249 reanalysed the results from broods 2-4 only. In this case the interaction between brood
250 number and treatment was no longer significant (brood*treatment: $F_{4,230} = 0.64$, P = 0.635;
251 Figure 3), suggesting that the level of antibacterial activity in the exudates differed between
252 the treatment groups only in the first brood directly after the immune challenge. However the
253 interaction between day and brood number was still significant (brood*day: $F_{4,207} = 4.09$, P =
254 0.003; Figure 4). Again the variance component estimated for individual females was small
255 (VC = 0.013 $\pm$ 0.006).

256 To test for a correlation between exudate lytic activity and fecundity, we had to look at
257 each day separately as there were up to 3 exudate measures per female per breeding bout but
258 only one measure of brood weight. Interestingly, if we just consider those broods that were
259 successful, there was a significant negative effect of brood weight on lytic activity,
260 independent of treatment, on day 4 only (REML: Day 4, brood weight, $F_{2,68} = 5.23$, P =
261 0.025; treatment, $F_{2,68} = 4.53$, P = 0.014; treatment* brood weight, $F_{2,69} = 0.11$, P = 0.90;
262 Days 2 and 6, $F < 0.19$, $P > 0.66$) indicating a possible trade-off between the size of the brood
263 and the maximal amount of lytic activity a female can produce.

264

265 **The effect of treatment on fecundity**

266 The immune challenge treatments did not affect the total number of broods attempted, nor
267 the number of those broods that successfully produced offspring (GLM, total broods: χ^2_2 =
268 0.58, P = 0.74; successful broods: $\chi^2_2 = 0.49$, P = 0.78; Table 1). Moreover, immune

challenge did not affect the beetles' lifetime reproductive success (REML, total weight of offspring, treatment effect: $F_{2,41} = 0.29$, $P = 0.75$). However, females confronted with a bacterial immune challenge differed from the other two treatments in the way they invested in each brood (REML, treatment*brood: $F_{6,448} = 9.60$, $P < 0.001$; Figure 5). The bacteria-challenge treatment caused females to increase their investment in the first brood relative to the other two groups, with this brood attaining a greater mass, but investment fell sharply thereafter, with very low brood weights by the 4th breeding attempt. By contrast, the other two treatment groups slightly increased their investment in their brood the second time they bred, but gradually reduced their investment levels when raising broods 3 and 4 (Figure 5). The variance component estimated for females was high (VC = 0.657 $\pm$ 0.168), suggesting that females consistently produced either larger or smaller broods.

Discussion

Our study reveals a trade-off between one aspect of the burying beetle's personal immune defence and one component of its social immune response. By inflicting wounds on female burying beetles, and thus challenging the personal immune system prior to reproduction, we caused a down-regulation in the antibacterial activity of their anal exudates (Figure 2). Down-regulation was only temporary, however, because by the next breeding bout the social immune response was restored to control levels (Figure 3).

To our knowledge, this is the first study to find a direct physiological trade-off between these two strategies of immune response, experimentally induced by increased investment in personal immunity. Recently reported experiments on the congeneric burying beetle *N. orbicollis* similarly sought evidence of a trade-off between the social immune response and

292 personal immunity, but found none (Steiger *et al.* 2011). Perhaps this is because these
293 experiments focused on different components of the personal immune system (in a different
294 species). Or perhaps the data conceal a trade-off which might have been detected had they
295 included an unchallenged treatment group for comparison (Steiger *et al.* 2011). Whilst a
296 negative correlation between traits can indicate a trade-off, a positive correlation between
297 traits does not necessarily mean that the two traits do not trade-off. If there is more variation
298 between individuals in the levels of resources than in how those resources are allocated
299 between traits, this can generate a positive correlation between traits despite an underlying
300 trade-off (van Noordwijk & de Jong 1986). It is possible that challenged individuals
301 upregulated their personal immune response and downregulated their social immune response
302 whilst still maintaining a positive correlation between the two. Studies with other insect
303 species have yielded results that are more consistent with our own. For example, honeybees
304 appear to possess fewer genes for personal immunity than non-social insects, but bear many
305 genes for colony-level immune function (Evans *et al.* 2006; Wilson-Rich *et al.* 2009).
306 Similarly, certain resins have antimicrobial properties and so are collected by ants and bees
307 for use in the nest. In wood ants, the presence of the resin decreases both the bacterial and
308 fungal load in nest material, and this resulted in lower lytic and AMP activity in worker
309 haemolymph (Castella, Chapuisat & Christe 2008). In honeybees, the presence of this resin
310 also decreases bacterial load and so decreases expression of genes connected with personal
311 immunity ((Castella, Chapuisat & Christe 2008; Simone, Evans & Spivak 2009). In both of
312 these cases it may have been the reduction in pathogens that led to the decrease in the
313 immune response.

314 Interestingly, the direct bacterial challenge to the haemolymph did not cause further down-
315 regulation of social immunity, even though wounding alone is known to elicit a weaker AMP

316 –based personal immune response than the bacterial challenge (Cotter, Ward & Kilner 2011).
317 One possible explanation is that any form of antibacterial immune upregulation (whether
318 through wounding alone or through bacterial injection) is sufficient to trigger a down-
319 regulation in the antibacterial activity of the anal exudates, and that down-regulation of the
320 social immunity in this way is an all or nothing response. Alternatively, it is possible that the
321 separate components of the personal immune system exhibit different trade-offs with the
322 separate components of the social immune system. Or perhaps wounding alone is a good
323 general indicator of the risk of infection for species that live in microbe-rich environments,
324 such as burying beetles (Plaistow *et al.* 2003), and so is the sole trigger for down-regulation
325 of the social immune response. In addition, it is worth noting that we used dead bacteria for
326 our immune challenge to avoid confounding the effects of immune upregulation and the
327 illness induced by an actively replicating parasite. However, a next step would be to consider
328 the effects of live pathogenic challenge on social immunity. Due to the additional costs
329 associated with fighting a live infection we might expect a more dramatic reduction of social
330 immunity during live infection, and possibly stronger effects on female fecundity. It would
331 also be interesting to test whether or not this trade-off is apparent in both directions, in other
332 words does stimulating the social immune response reduce an individual's capacity to defend
333 themselves against parasites?

334 Although we have evidence that the concentration of lysozyme activity decreased in
335 immune-challenged individuals, we do not know how the quantity of exudates produced by
336 the females changes. Although we found no consistent patterns in the amounts of exudate we
337 were able to collect from females in the different treatment groups (Cotter pers obs), it is
338 possible that females compensate for decreased lytic concentration of the exudates by
339 producing a greater quantity of exudates, but this has yet to be tested.

Whatever the precise details of the trade-off between personal and social immunity, we found no evidence that mounting a personal immune response of any sort impaired lifetime reproductive success. Instead, by down-regulating expression of their social immune response, and thus moderating its considerable effect on their future fecundity (Cotter *et al.* 2010), challenged females were able to defend their lifetime reproductive success. However, we did find that females with large broods produced a lower peak lytic activity (day 4). Lysozyme activity peaks at the time the young larvae are at their most demanding, perhaps the costs of caring for a large number of larvae limits the lytic activity that females can produce. To test this hypothesis brood size would have to be experimentally manipulated and the effects on lytic activity measured.

Our findings contrast with a recent study on carpenter ants, which found that workers challenged with lipopolysaccharides or heat-killed bacteria showed an *increased* social immune response, with raised antimicrobial activity in the regurgitates they passed to nestmates (Hamilton, Lejeune & Rosengaus 2011). Perhaps the difference in results can be attributed to the sterility of the carpenter ant workers and the greater threat of disease that results from colonial living with genetically similar individuals (Cremer, Armitage & Schmid-Hempel 2007). For sterile workers of eusocial species, a personal immune challenge potentially represents a threat to the colony as a whole and defending inclusive fitness far outweighs the benefits of defending personal lifespan. Under these conditions, it is not surprising that a personal immune challenge upregulated social immune defences. In contrast, burying beetles, which can reproduce and are not colonial, gain more by down-regulating their social immune response when their personal immunity is challenged because heavy investment in social immunity compromises their lifetime reproductive success (Cotter *et al.* 2010).

Although the beetle larvae did not appear to suffer from downregulation of social immunity in our lab setting, it is likely that under more natural microbial conditions this would result in an inferior carcass, something that has been shown to reduce offspring quality (Rozen, Engelmoer & Smiseth 2008). So why might females effectively choose to sacrifice the survival of their current brood in favour of mounting a personal immune response? One possibility is that under conditions of biparental care, males may compensate for the reduced social immunity of their partner, thus mitigating its impact. We have shown previously that females bear the greater burden of the social immune response, but that males will increase their antibacterial output if experimentally widowed (Cotter & Kilner 2010b). The mechanism underpinning this flexible response to widowing may involve the perceived level of microbial activity on the carcass. Reduced activity by the female would then cause bacteria on the carcass to multiply which could in turn cause the male to produce more antimicrobial exudates.

A second possibility is linked to the residual reproductive value (RRV) of our experimental females, which were all young virgins. We have shown previously that young females have a high RRV and so should prioritise their future fecundity over their current brood, all else being equal (Ward, Cotter & Kilner 2009). However, we have also shown that a bacterial challenge to the immune system causes young females to behave as if their RRV is very low, perhaps because they perceive a greater risk of death (Cotter, Ward & Kilner 2011). This might explain why the bacteria-challenged females increased their immediate reproductive output compared to either controls or sterile-challenged females (Fig. 5, and see Cotter, Ward & Kilner 2011); unlike females in the other two treatments, the bacteria-challenged females prioritized their current brood over their future fecundity.

In conclusion, we provide the first evidence for a direct physiological trade-off between components of personal and social immunity, which allows females to defend their lifetime reproductive success when immune challenged in their first breeding attempt. In future work it would be interesting to compare the nature of this trade-off across species with varying degrees of coloniality and eusociality. Further studies are also required to assess how the trade-off is affected by the presence of a partner and whether it changes with age at first reproduction.

Acknowledgements

This work was supported by The Leverhulme Trust (PLP／2006／0033 to RMK), The Newton Trust, a Natural Environment Research Council Fellowship to SCC (NE/H014225/2), the Natural Environment Research Council (NE/H019731/1),and the Department of Zoology, University of Cambridge, UK. We are very grateful to Per Smiseth and Allen Moore for their logistical help and support in getting this project started. Author contributions: SCC designed the experiments, collected and analysed the data, and co-wrote the paper; JEL and PJG designed the experiments and collected the data; RMK co-wrote the paper.

References

Beisel, W.R. (1977) Magnitude of host nutritional responses to infection. *American Journal of Clinical Nutrition,* **30,** 1236-1247.

Bidla, G., Lindgren, M., Theopold, U. & Dushay, M.S. (2005) Hemolymph coagulation and phenoloxidase in Drosophila larvae. *Developmental and Comparative Immunology*, **29,** 669-679.

Castella, G., Chapuisat, M. & Christe, P. (2008) Prophylaxis with resin in wood ants. *Animal Behaviour*, **75,** 1591-1596.

Cotter, S.C. & Kilner, R.M. (2010a) Personal immunity versus social immunity. *Behavioral Ecology*, **21,** 663-668.

Cotter, S.C. & Kilner, R.M. (2010b) Sexual division of antibacterial resource defence in breeding burying beetles, *Nicrophorus vespilloides*. *Journal of Animal Ecology*, **79,** 35-43.

Cotter, S.C., Raubenheimer, D., Simpson, S.J. & Wilson, K. (2011) Macronutrient balance mediates trade-offs between immune function and life history traits. *Functional ecology*, **25,** 186-198.

Cotter, S.C., Topham, E., Price, A.J.P. & Kilner, R.M. (2010) Fitness costs associated with mounting a social immune response. *Ecology Letters*, **13,** 1114-1123.

Cotter, S.C., Ward, R.J.S. & Kilner, R.M. (2011) Age-specific reproductive investment in female burying beetles: independent effects of state and risk of death. *Functional ecology*, **25,** 652-660.

Cremer, S., Armitage, S.A.O. & Schmid-Hempel, P. (2007) Social immunity. *Current Biology*, **17,** R693-R702.

Evans, J.D., Aronstein, K., Chen, Y.P., Hetru, C., Imler, J.L., Jiang, H., Kanost, M., Thompson, G.J., Zou, Z. & Hultmark, D. (2006) Immune pathways and defence mechanisms in honey bees *Apis mellifera*. *Insect Molecular Biology*, **15,** 645-656.

Fauvarque, M.O. & Williams, M.J. (2011) *Drosophila* cellular immunity: a story of migration and adhesion. *Journal of Cell Science,* **124,** 1373-1382.

Freitak, D., Ots, I., Vanatoa, A. & Horak, P. (2003) Immune response is energetically costly in white cabbage butterfly pupae. *Proceedings of the Royal Society of London Series B-Biological Sciences,* **270,** S220-S222.

Haine, E.R., Rolff, J. & Siva-Jothy, M.T. (2007) Functional consequences of blood clotting in insects. *Developmental and Comparative Immunology,* **31,** 456-464.

Hamilton, C., Lejeune, B.T. & Rosengaus, R.B. (2011) Trophallaxis and prophylaxis: social immunity in the carpenter ant *Camponotus pennsylvanicus*. *Biology Letters,* **7,** 89-92.

Hoffmann, J.A., Dimarcq, J.L. & Bulet, P. (1992) Inducible antibacterial peptides of insects. *M S-Medecine Sciences,* **8,** 432-439.

Janeway, C.A., Travers, P., Walport, M. & Shlomchik, M.J. (2001) *Immunobiology* 5th edn. Garland Science, New York and London.

Long, C.L. (1977) Energy-balance and carbohydrate-metabolism in infection and sepsis. *American Journal of Clinical Nutrition,* **30,** 1301-1310.

Martin-Vivaldi, M., Pena, A., Peralta-Sanchez, J.M., Sanchez, L., Ananou, S., Ruiz-Rodriguez, M. & Soler, J.J. (2010) Antimicrobial chemicals in hoopoe preen secretions are produced by symbiotic bacteria. *Proceedings of the Royal Society B-Biological Sciences,* **277,** 123-130.

Muller, J.K., Braunisch, V., Hwang, W.B. & Eggert, A.K. (2007) Alternative tactics and individual reproductive success in natural associations of the burying beetle, *Nicrophorus vespilloides*. *Behavioral Ecology,* **18,** 196-203.

453 Ots, I., Kerimov, A.B., Ivankina, E.V., Ilyina, T.A. & Horak, P. (2001) Immune challenge
454 affects basal metabolic activity in wintering great tits. *Proceedings of the Royal*
455 *Society of London Series B-Biological Sciences,* **268,** 1175-1181.
456 Plaistow, S.J., Outreman, Y., Moret, Y. & Rigaud, T. (2003) Variation in the risk of being
457 wounded: an overlooked factor in studies of invertebrate immune function? *Ecology*
458 *Letters,* **6,** 489-494.
459 Povey, S.R., Cotter, S.C., Simpson, S.J., Lee, K. & Wilson, K. (2009) Can the protein costs
460 of bacterial resistance be offset by altered feeding behaviour? *Journal of Animal*
461 *Ecology,* **78,** 437-446.
462 Pukowski, E. (1933) Okoloische untersuchungen an *Necrophorus*. *Zeitschrift fur*
463 *Morphologie und Oekologie der Tiere,* **27,** 518–586.
464 Rozen, D.E., Engelmoer, D.J.P. & Smiseth, P.T. (2008) Antimicrobial strategies in burying
465 beetles breeding on carrion. *Proceedings of the National Academy of Sciences,* **105,**
466 17890-17895.
467 Simone, M., Evans, J.D. & Spivak, M. (2009) Resin collection and social immunity in honey
468 bees. *Evolution,* **63,** 3016-3022.
469 Steiger, S., Gershman, S.N., Pettinger, A.M., Eggert, A.-K. & Sakaluk, S.K. (2011) Sex
470 differences in immunity and rapid upregulation of immune defence during parental
471 care in the burying beetle, Nicrophorus orbicollis. *Functional Ecology*. **25,** 1368-1378
472 Steiger, S., Gershman, S.N., Pettinger, A.M., Eggert, A.-K. & Sakaluk, S.K. (2012)
473 Dominance status and sex influence nutritional state and immunity in burying beetles
474 *Nicrophorus orbicollis*. *Behavioral Ecology*. 23: 1126-1132.
475 Thomas, F., Guegan, J.F. & Renaud, F. (2009) Ecology and Evolution of Parasitism. Oxford
476 University Press, New York.

Trumbo, S.T. & Wilson, D.S. (1993) Brood discrimination, nest mate discrimination, and determinants of social-behavior in facultatively quasisocial beetles (Nicrophorus spp). *Behavioral Ecology,* **4,** 332-339.

van Noordwijk, A.J. & de Jong, G. (1986) Acquisition and allocation of resources - their influence on variation in life-history tactics. *American Naturalist,* **128,** 137-142.

Vogel, H., Badapanda, C. & Vilcinskas, A. (2011) Identification of immunity-related genes in the burying beetle Nicrophorus vespilloides by suppression subtractive hybridization. *Insect Molecular Biology,* **20,** 787-800.

Ward, R.J.S., Cotter, S.C. & Kilner, R.M. (2009) Current brood size and residual reproductive value predict offspring desertion in the burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology,* **20,** 1274-1281.

Wilson-Rich, N., Spivak, M., Fefferman, N.H. & Starks, P.T. (2009) Genetic, individual, and group facilitation of disease resistance in insect societies. *Annual Review of Entomology,* **54,** 405-423.

Tables

Table 1 – the total number of broods and the number of broods that were successful for each treatment group. Values are means +/- SE.

Treatment	**Total number of broods**	**Number of successful broods**
Control	3.8 ± 0.27	2.0 + 0.24
Sterile - challenged	4.2 ± 0.28	2.1 + 0.30
Bacteria - challenged	4.1 ± 0.41	2.4 + 0.36

Figures legends

Figure 1: Antibacterial activity of the females' anal exudates over the course of the first reproductive bout in unmanipulated females. The open circles are the raw data; the filled circles are the predicted means and SEs from a REML model controlling for female identity.

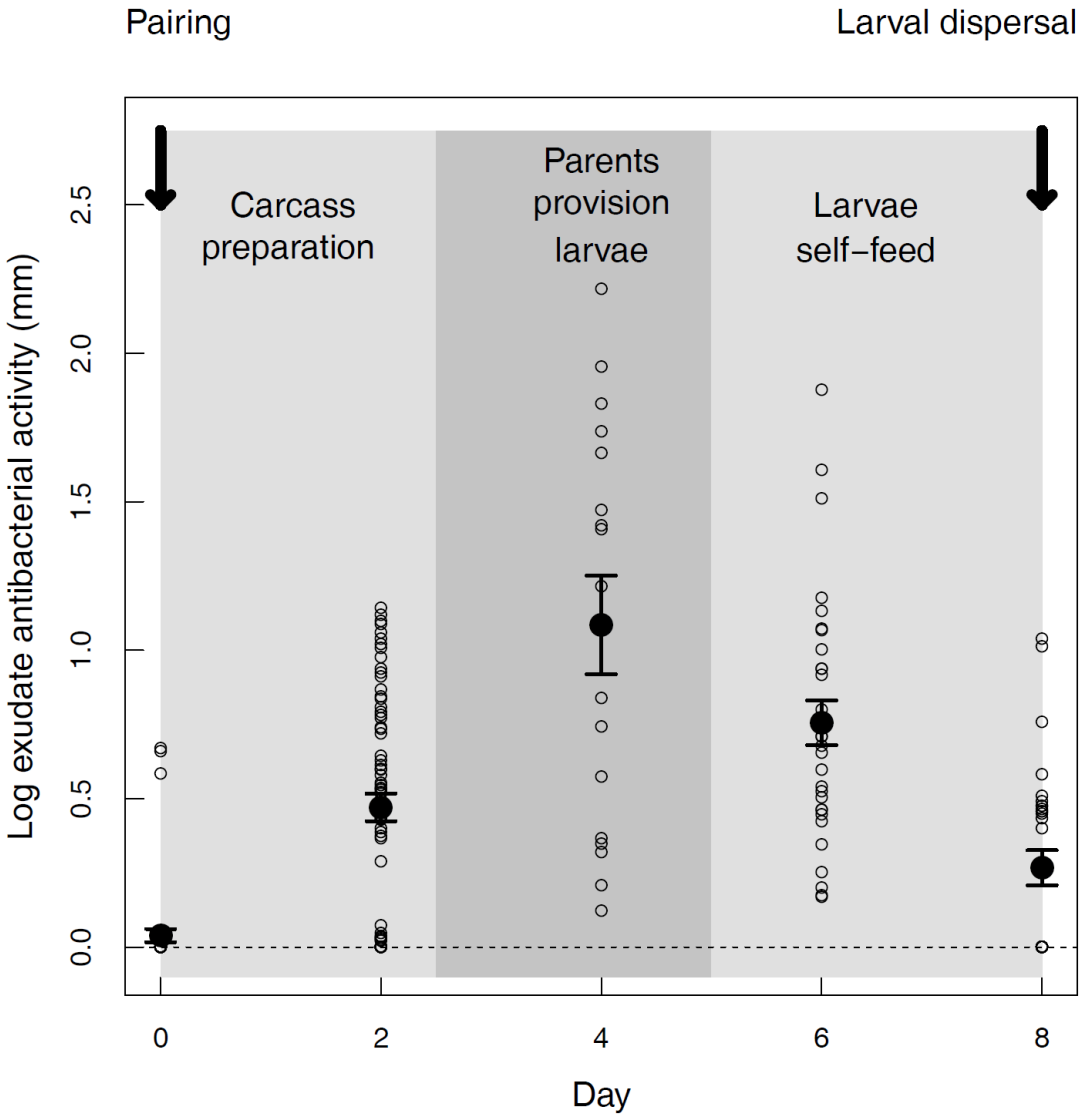
Figure 2: Antibacterial activity of the females' anal exudates over days 2, 4 and 6 of the first reproductive bout in unmanipulated females and those whose immune systems have been challenged by wounding with a sterile or bacteria-dipped needle. The open circles are the raw data; the filled circles are the predicted means and SEs from a REML model controlling for female identity. The data points for each treatment have been offset to improve the clarity of the figure.

Figure 3: Exudate antibacterial activity for each brood in unmanipulated females and those whose immune systems have been challenged by wounding with a sterile or bacteria-dipped needle. Females experienced immune challenge prior to the first brood only and were bred repeatedly until death. The N for each brood are as follows: 1 (49), 2 (45), 3 (41) and 4 (33). The open circles are the raw data; the filled circles are the predicted means and SEs from a REML model controlling for female identity. The data points for each treatment have been offset to improve the clarity of the figure.

Figure 4: Mean exudate antibacterial activity for each brood by the day that the exudate was sampled. The N for each brood are as follows: 1 (49), 2 (45), 3 (41) and 4 (33). The open circles are the raw data, the filled circles are the predicted means and SEs from a REML model controlling for female identity. The data points for each treatment have been offset to improve the clarity of the figure.

Figure 5: Brood weight for each brood in unmanipulated females and those whose immune systems have been challenged by wounding with a sterile or bacteria-dipped needle. Females experienced immune challenge prior to the first brood only and were bred repeatedly until death. The N for each brood are as follows: 1 (49), 2 (45), 3 (41) and 4 (33). The open circles are the raw data; the filled circles are the predicted means and SEs from a REML model controlling for female identity. The data points for each treatment have been offset to improve the clarity of the figure.

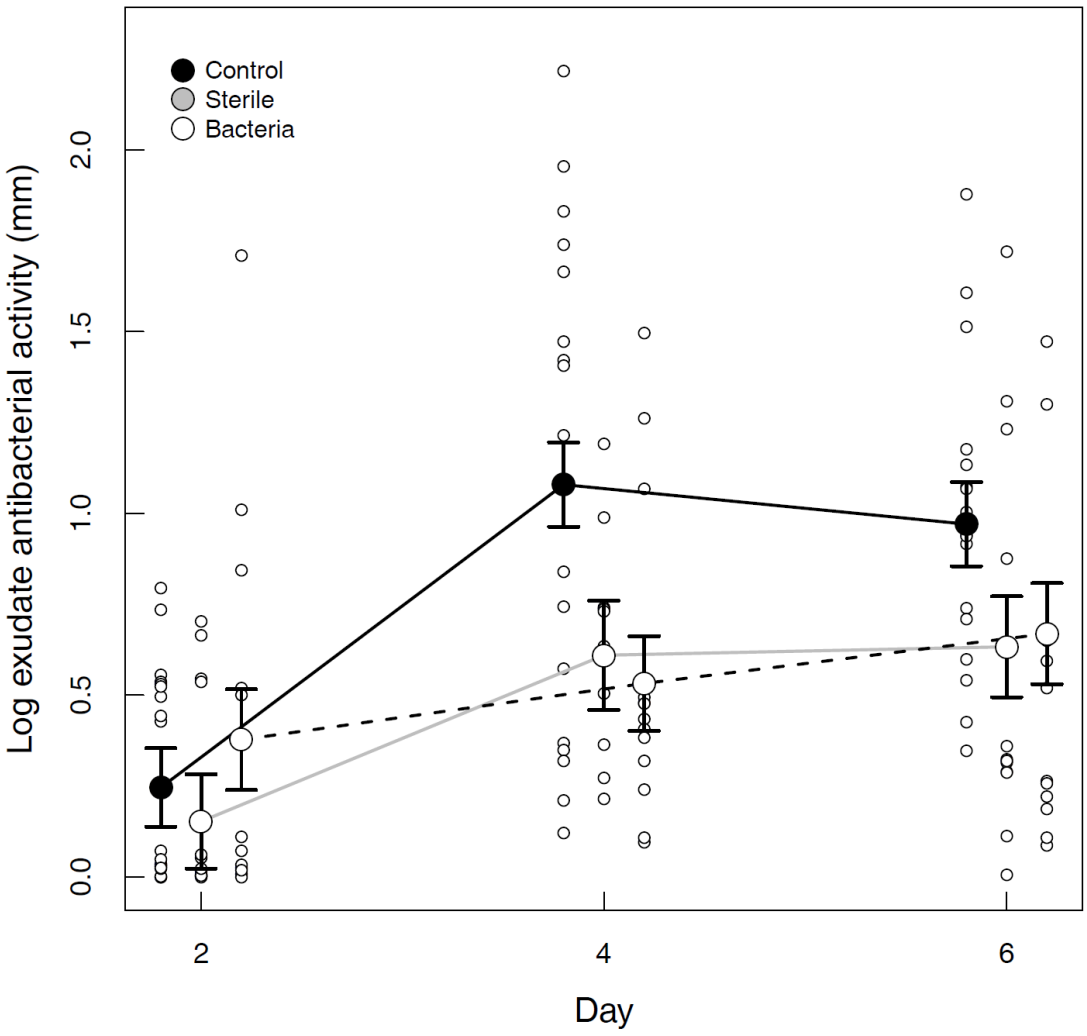
539 Figure 1



540

541

542 Figure 2

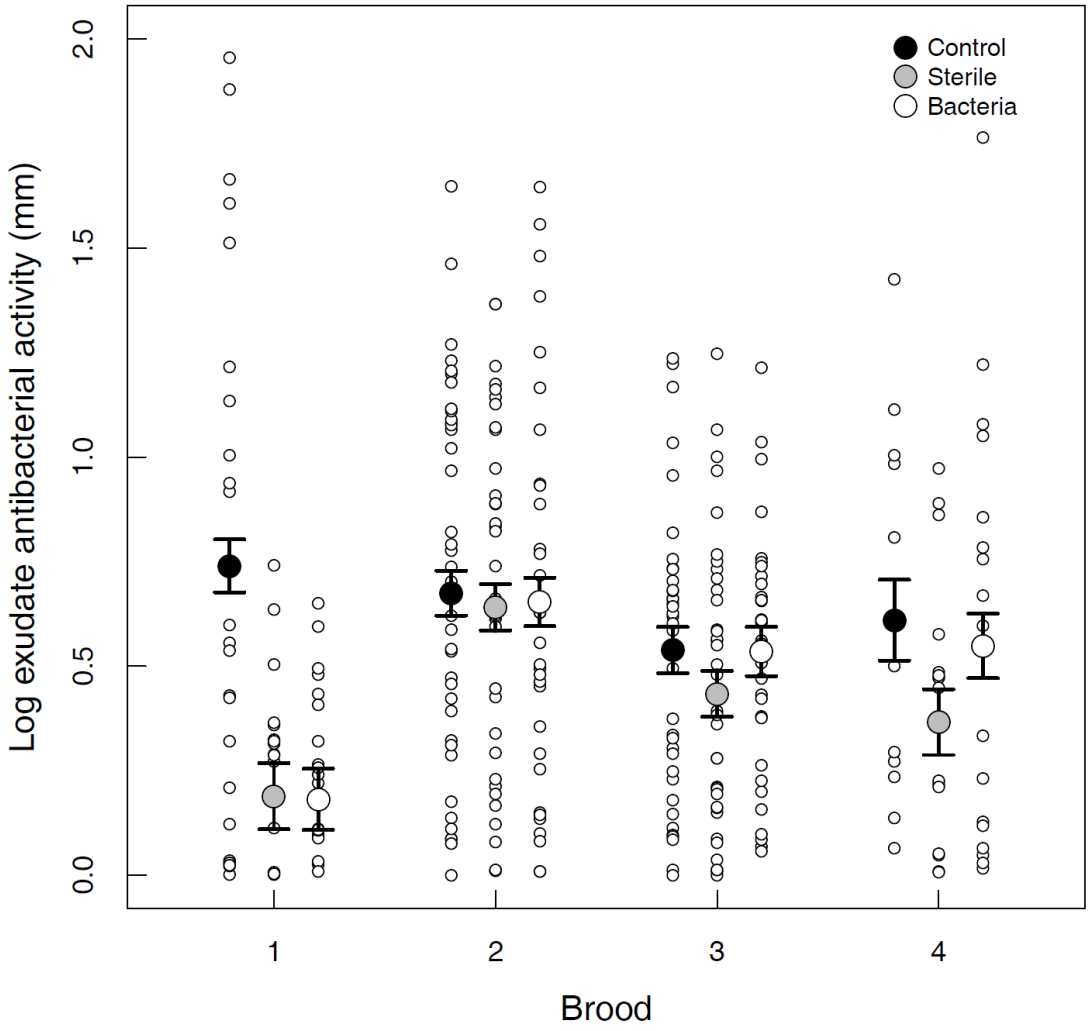


543

544

545

546 Figure 3

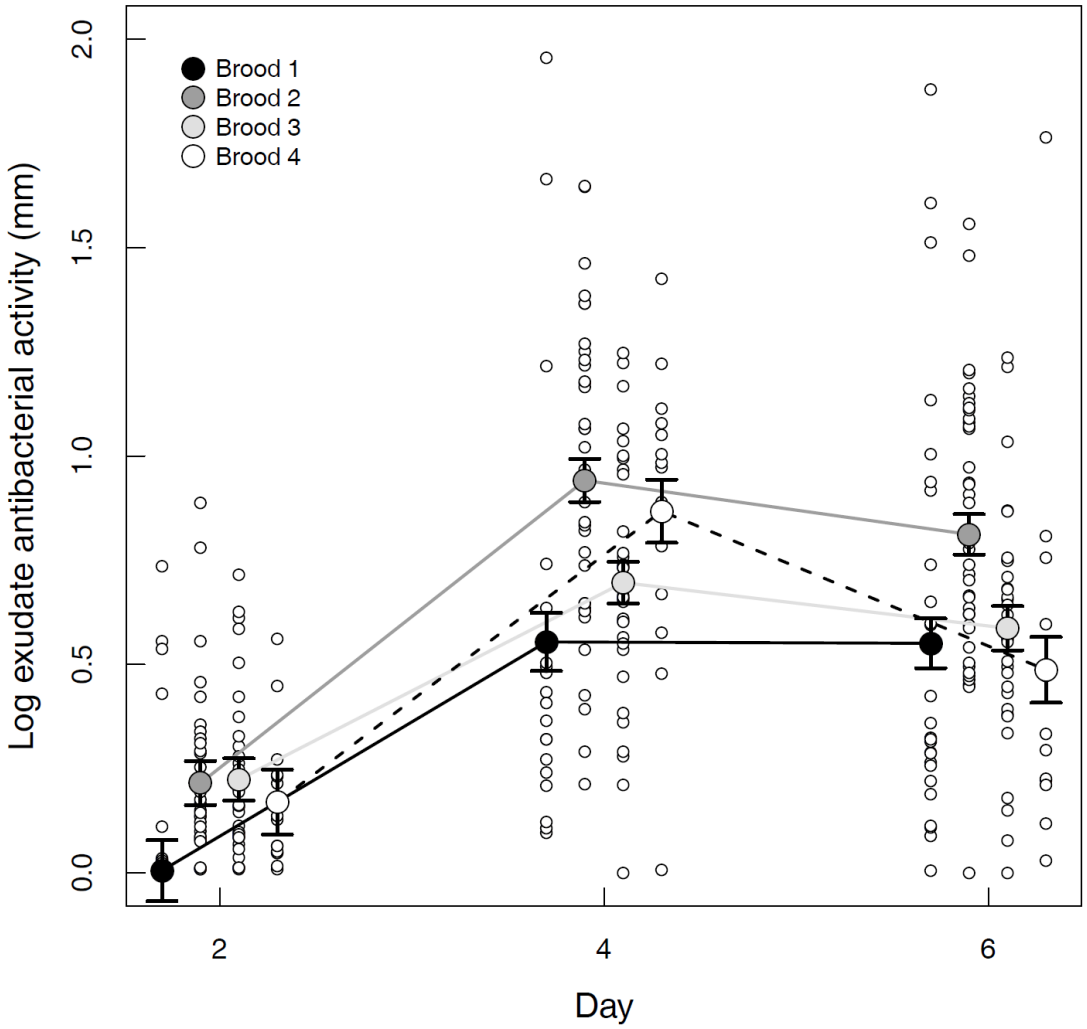


547

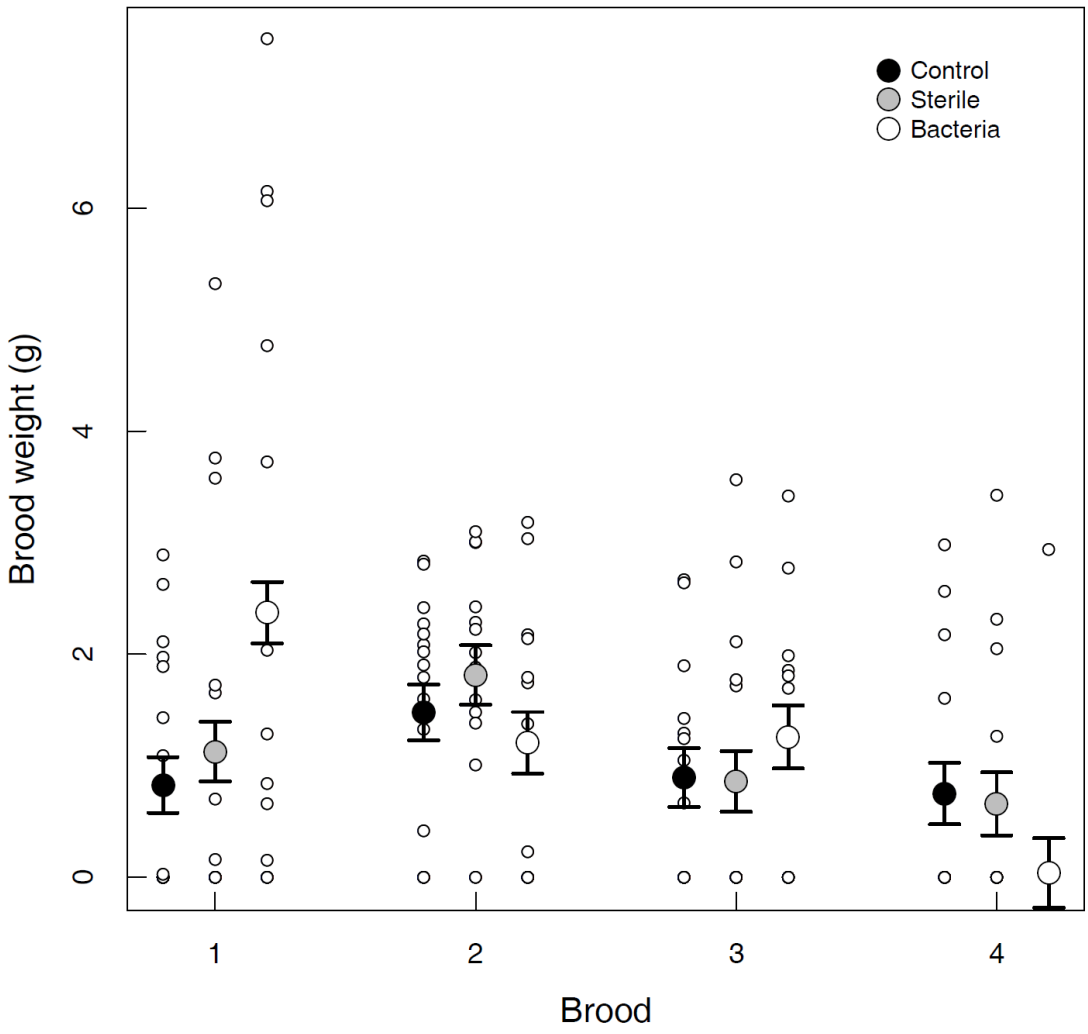
548

549

Figure 4


Brood 1
Brood 2
Brood 3
Brood 4
Log exudate antibacterial activity (mm)
0.0
0.5
1.0
1.5
2.0
2
4
6
Day

554 Figure 5



555