Immune priming in *Armadillidium vulgare* against *Salmonella enterica*: direct or indirect costs on life history traits?

Cybèle Prigot-Maurice¹*, Charlotte Depeux¹*, Hélène Paulhac¹, Christine Braquart-Varnier¹#, Sophie Beltran-Bech¹#

¹: Université de Poitiers, Laboratoire Écologie et Biologie des Interactions, UMR CNRS 7267, 3 rue Jacques Fort, TSA 51106, F-86073 POITIERS Cedex 9, France.

* Co-first authors
# Co-last authors

Corresponding author: Cybèle Prigot-Maurice (cybele.prigot@gmail.com)

**Supplementary material**

*Figure S1.* Body weight of females before reproduction according to their priming treatment. NI: never-injected, control females; NP: non-primed females; LBP: females primed with sterile LB broth, SAP: females primed with $10^3$ living *S. enterica*. NP, LBP and SAP females received the LD$_{50}$ injection. Mean ± SE: Control = 0.15g ± 0.008, NP = 0.12g ± 0.01, LBP = 0.14g ± 0.007, SAP = 0.12g ± 0.007).
**Figure S2.** Number of offspring in the first clutch according to the females’ treatment. NI: never-injected, control females; NP: non-primed females; LBP: females primed with sterile LB broth, SAP: females primed with $10^3$ living *S. enterica*. NP, LBP and SAP females received the LD$_{50}$ injection.

**Figure S3.** Total number of offspring (first and second clutches included) according to the females’ treatment. NI: never-injected, control females; NP: non-primed females; LBP: females primed with sterile LB broth, SAP: females primed with $10^3$ living *S. enterica*. NP, LBP and SAP females received the LD$_{50}$ injection.
**Table S1.** Pairwise comparisons (Tukey adjustment) of survival rates according to females’ treatments (NP: non-primed females; LBP: females primed with sterile LB broth; SAP: females primed with $10^3$ *S. enterica* in the priming procedure; Control: never-injected females).

|                      | estimate | SE  | p-value |
|----------------------|----------|-----|---------|
| **SURVIVAL RATES**   |          |     |         |
| **(frequency of living females)** |          |     |         |
| 22 days after LD$_{50}$ |          |     |         |
| NP vs. LPB           | 0.914    | 0.494 | 0.064   |
| NP vs. SAP           | 0.912    | 0.495 | 0.065   |
| LBP vs. SAP          | 0.578    | 0.578 | 0.99    |
| **During the reproductive period** |          |     |         |
| NP vs. LPB           | -0.33    | 0.627 | 0.952   |
| NP vs. SAP           | 0.318    | 0.627 | 0.957   |
| LBP vs. SAP          | 0.01     | 0.535 | 1.00    |
| Control vs. NP       | 0.01     | 0.671 | 1.00    |
| Control vs. LBP      | -0.31    | 0.586 | 0.951   |
| Control vs. SAP      | -0.299   | 0.586 | 0.956   |

**Table S2.** Pairwise comparisons (Tukey adjustment) of body weight of females before reproduction, according to the treatments (NP: non-primed females; LBP: females primed with sterile LB Broth; SAP: females primed with $10^3$ *S. enterica* during the priming procedure; Control: never-injected females).

|                      | estimate | SE  | p-value |
|----------------------|----------|-----|---------|
| **WEIGHT OF FEMALES** |          |     |         |
| NP vs. LPB           | 0.01     | 0.012 | 0.76    |
| NP vs. SAP           | -0.0007  | 0.012 | 0.99    |
| LBP vs. SAP          | 0.01     | 0.011 | 0.76    |
| Control vs. NP       | 0.03     | 0.012 | **0.09** |
| Control vs. LBP      | 0.01     | 0.012 | 0.46    |
| Control vs. SAP      | 0.02     | 0.012 | **0.07** |
**Table S3.** Pairwise comparisons (Tukey adjustment) of the number of offspring in the first clutch according to females’ treatments (NP: non-primed females; LBP: females primed with sterile LB Broth; SAP: females primed with $10^3$ S. enterica during the priming procedure; Control: never-injected females).

|                | estimate | SE  | p-value |
|----------------|----------|-----|---------|
| NP vs. LBP     | -14.37   | 15.4| 0.78    |
| NP vs. SAP     | -2.21    | 14.8| 0.99    |
| LBP vs. SAP    | 12.16    | 14.8| 0.84    |
| Control vs. NP | 39.38    | 16.4| 0.08    |
| Control vs. LBP| 25.01    | 15.9| 0.40    |
| Control vs. SAP| 37.18    | 15.7| 0.09    |

**Table S4.** Average marginal effects of the interaction between the probability to produce a second clutch and the body weight of females by treatment (NP: non-primed, Control females; LBP: females primed with sterile LB Broth; SAP: females primed with $10^3$ S. enterica in the priming procedure; Control: never-injected females).

**MEAN WEIGHT = 0.06 g**

| Treatment | Predicted probability of second clutch | 95% CI       |
|-----------|----------------------------------------|--------------|
| Control   | 0.70                                   | [0.16, 0.96] |
| NP        | 0.79                                   | [0.35, 0.96] |
| LBP       | 0.76                                   | [0.18, 0.98] |
| SAP       | 0.71                                   | [0.26, 0.94] |

**MEAN WEIGHT = 0.20 g**

| Treatment | Predicted probability of second clutch | 95% CI       |
|-----------|----------------------------------------|--------------|
| Control   | 0.35                                   | [0.11, 0.70] |
| NP        | 0.26                                   | [0.05, 0.71] |
| LBP       | 0.32                                   | [0.07, 0.76] |
| SAP       | 0.27                                   | [0.06, 0.71] |
Table S5. Pairwise comparisons (Tukey adjustment) of the total number of offspring according to females’ treatments (NP: non-primed, Control females; LBP: females primed with sterile LB Broth; SAP: females primed with $10^3 S. enterica$ in the priming procedure; Control: never-injected females).

|                  | estimate | SE  | p-value |
|------------------|----------|-----|---------|
| NP vs. LPB       | -35.5    | 27.2| 0.56    |
| NP vs. SAP       | 5.5      | 26.2| 0.99    |
| LBP vs. SAP      | 41.0     | 26.1| 0.40    |
| Control vs. NP   | 56.7     | 28.7| 0.20    |
| Control vs. LBP  | 21.2     | 28.0| 0.87    |
| Control vs. SAP  | 62.2     | 27.4| **0.11**|

Table S6. Statistical results of haemocyte parameters and senescence biomarkers analysis (generalized linear mixed effect models).

**HAEMOCYTE CONCENTRATION**

|                  | $X^2$  | df | p-value |
|------------------|-------|----|---------|
| Treatment        | 2.77  | 3  | 0.42    |
| Total number of offspring | 0.00  | 1  | 0.99    |
| Number of clutches        | 0.16  | 1  | 0.68    |
| Treatment*Total number of offspring | 1.56  | 3  | 0.66    |
| Treatment*Number of clutches        | 0.52  | 3  | 0.91    |

**HAEMOCYTE VIABILITY**

|                  | $X^2$  | df | p-value |
|------------------|-------|----|---------|
| Treatment        | 1.04  | 3  | 0.79    |
| Total number of offspring | 1.15  | 1  | 0.28    |
| Number of clutches        | 0.11  | 1  | 0.73    |
| Treatment*Total number of offspring | 0.67  | 3  | 0.87    |
| Treatment*Number of clutches        | 0.91  | 3  | 0.82    |

**SIZE OF VIVABLE HAEMOCYTES**

|                  | $X^2$  | df | p-value |
|------------------|-------|----|---------|
| Treatment        | 2.60  | 3  | 0.55    |
| Total number of offspring | 0.64  | 1  | 0.64    |
| Number of clutches        | **12.99** | 1 | **0.003** |
| Treatment*Total number of offspring | 2.71  | 3  | 0.43    |
| Treatment*Number of clutches        | 3.62  | 3  | 0.30    |

**β-GALACTOSIDASE ACTIVITY**

|                  | $X^2$  | df | p-value |
|------------------|-------|----|---------|
| Treatment        | 7.10  | 3  | 0.06    |
| Total number of offspring | 0.46  | 1  | 0.49    |
| Number of clutches        | 0.08  | 1  | 0.76    |
| Treatment*Total number of offspring | 2.42  | 3  | 0.48    |
| Treatment*Number of clutches        | 2.24  | 3  | 0.52    |