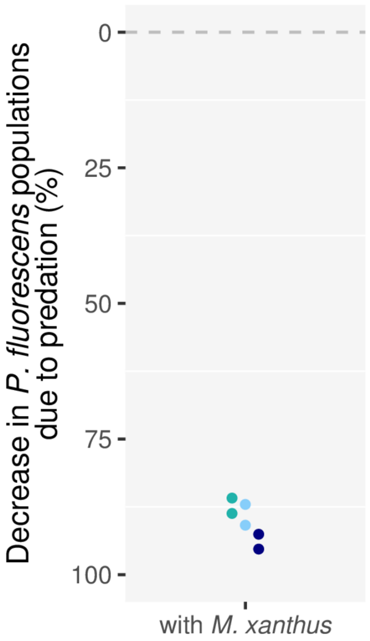
Supporting information for Killer prey: Pre-interaction ecology reverses bacterial predation

Marie Vasse[1*], Francesca Fiegna[2], Ben Kriesel[2], and Gregory J. Velicer[2*]

1. MIVEGEC (UMR 5290 CNRS, IRD, UM), CNRS 34394 Montpellier, France
2. Institute for Integrative Biology, ETH Zürich 8092 Zürich, Switzerland

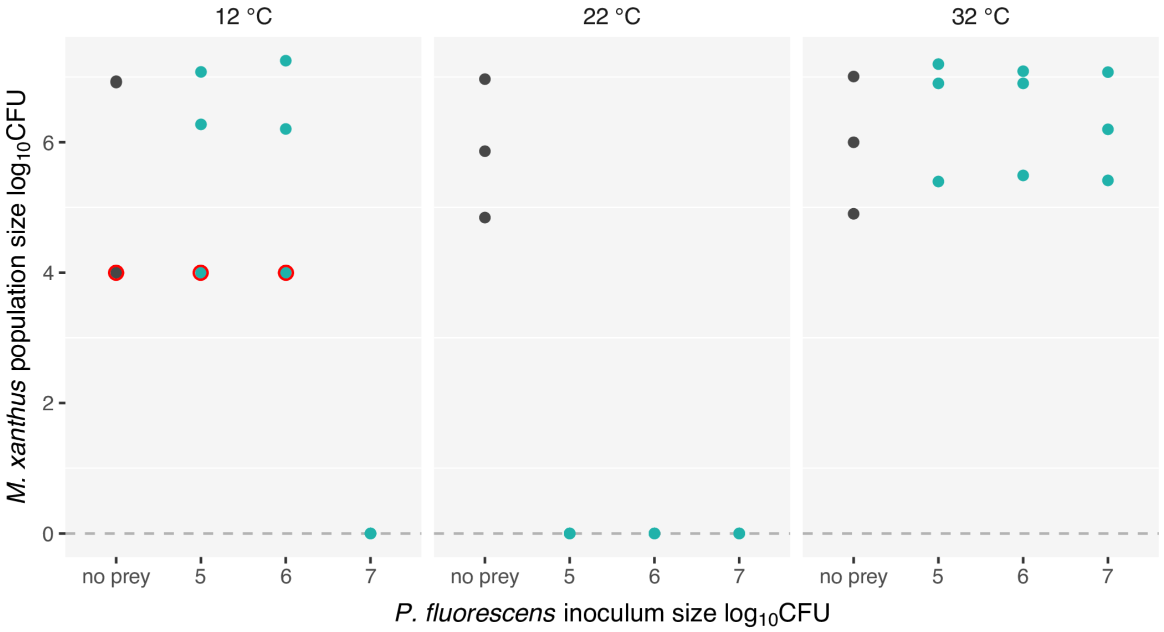
This document contains the Supplementary figures S1-6 and Supplementary tables S1 and S2.

Figure S1.



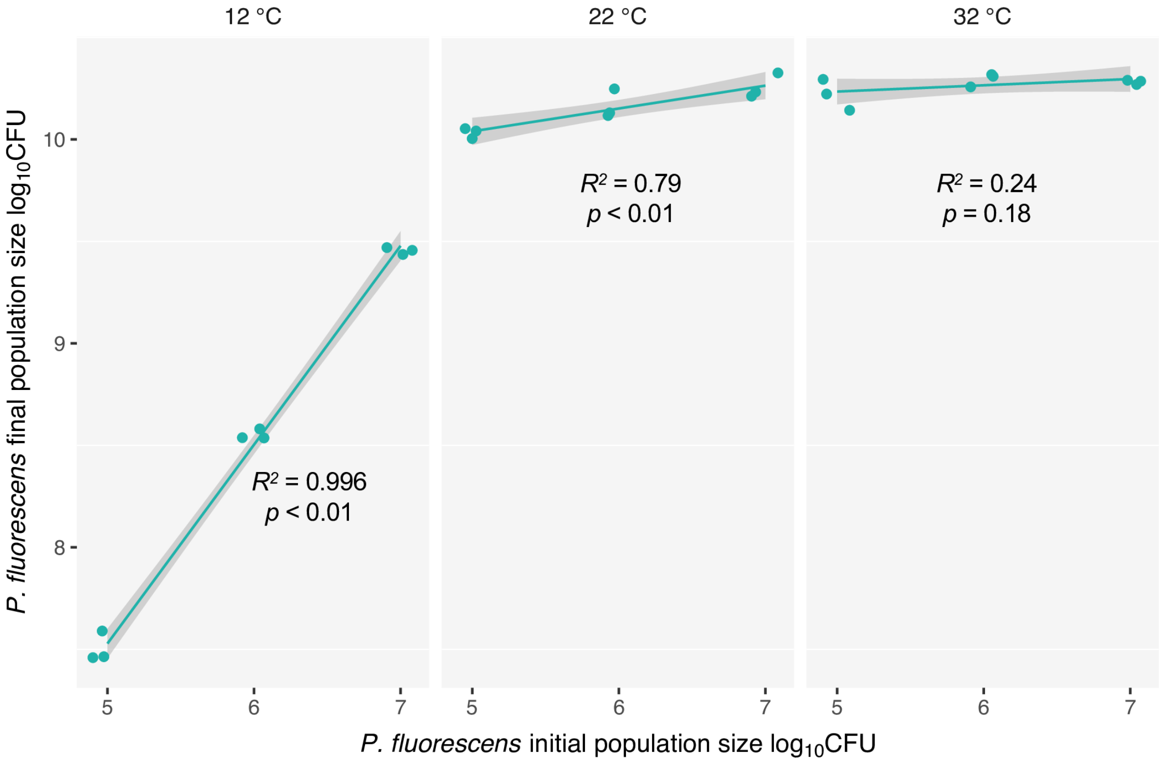
***M. xanthus* kills *P. fluorescens* grown on M9cas agar at 32 °C.** Percentage reduction of *P. fluorescens* population size after four days in the presence of *M. xanthus* relative to in the absence of *M. xanthus*. Colors correspond to three independent replicates each run with two technical replicates.

Figure S2.



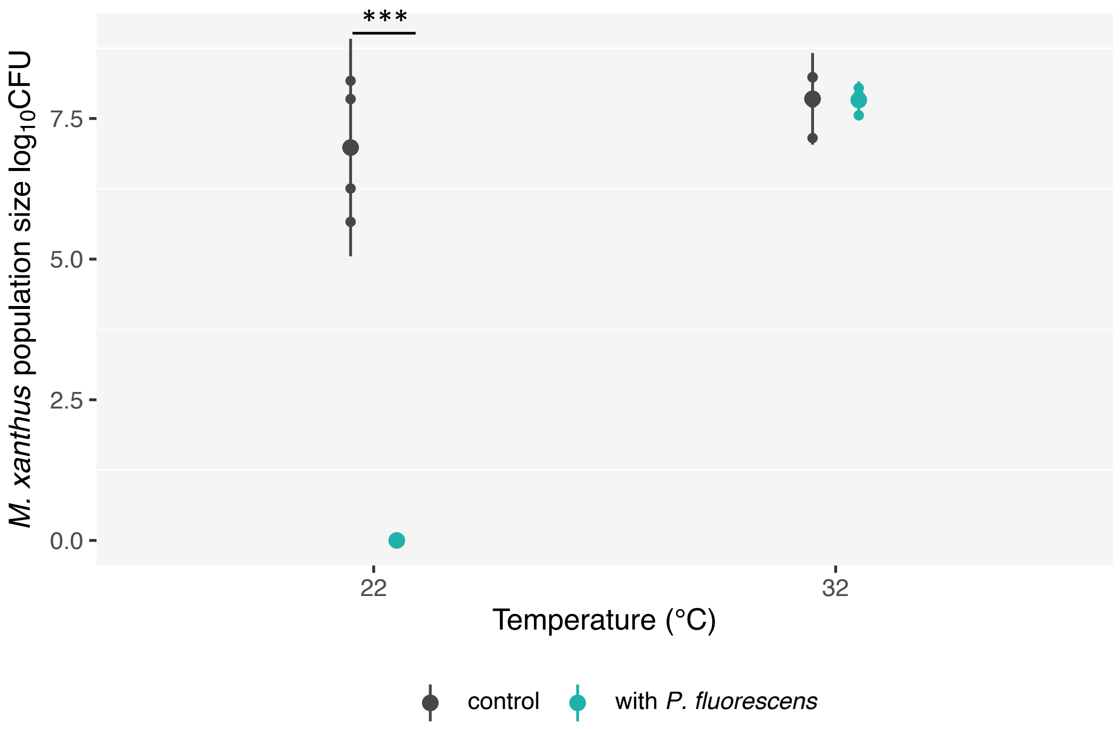
***P. fluorescens* grown at 22 °C kills *M. xanthus* after 30 minutes of interaction.** *M. xanthus* population sizes 30 minutes after inoculation onto *P. fluorescens* lawns grown overnight from one of three inoculum sizes and at one of three temperatures (green dots) or onto bacteria-free control plates that had been incubated overnight at one of the same three temperatures prey (black dots). Means of $\log_{10}$-transformed CFU + 1 values and 95% confidence intervals are shown. Note that, due to a technical issue with dilution-plating for one replicate of the 12 °C treatment, the highest available plated dilution was too low to accurately count colonies; the corresponding plates had more colonies than could feasibly be counted. We therefore attributed counts of 1000 for these plates, which was clearly a substantial underestimate in each case. These underestimated values are identified in the graph with red circles around corresponding data points. Lighter dots are biological replicates (*n* = 3).

Figure S3.



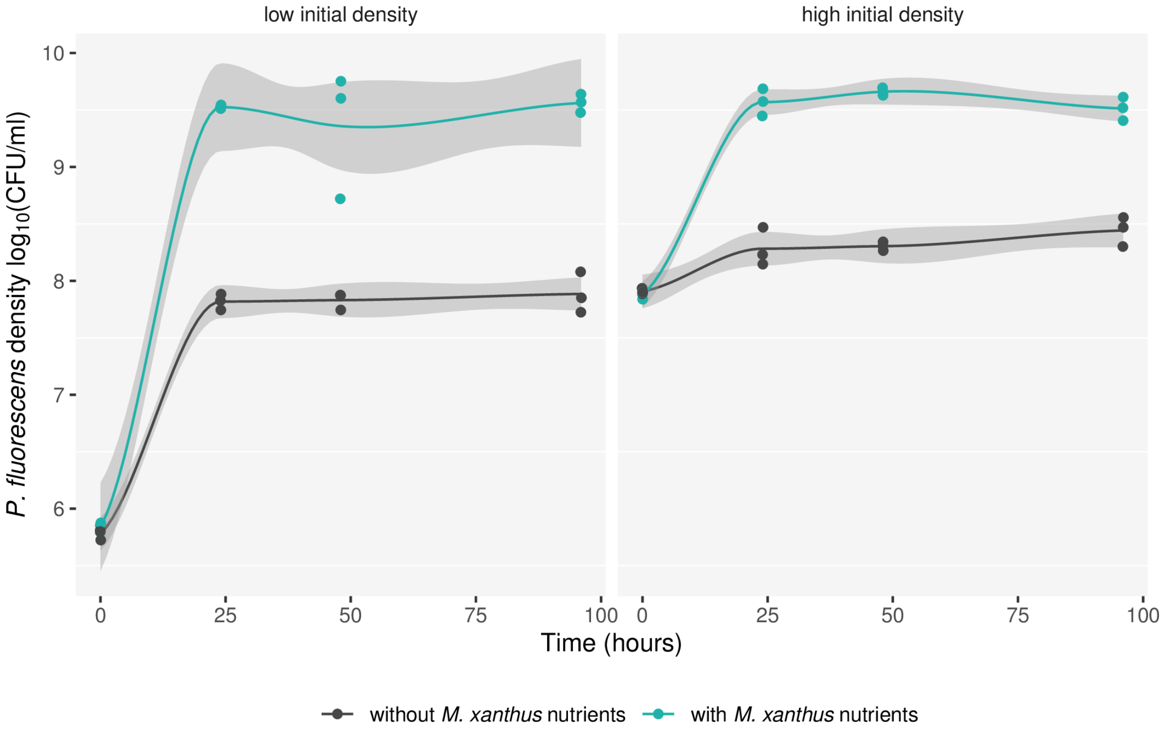
Final *P. fluorescens* population size correlates positively with initial population size after growth at 12 and 22 °C but not at 32 °C. Relationship between *P. fluorescens* initial and final population sizes after 22 hours of growth at different temperatures 12, 22 or 32 °C. Log_{10}-transformed CFU values (n = 3), linear fits and 95% confidence intervals about the linear fits are shown.

Figure S4.



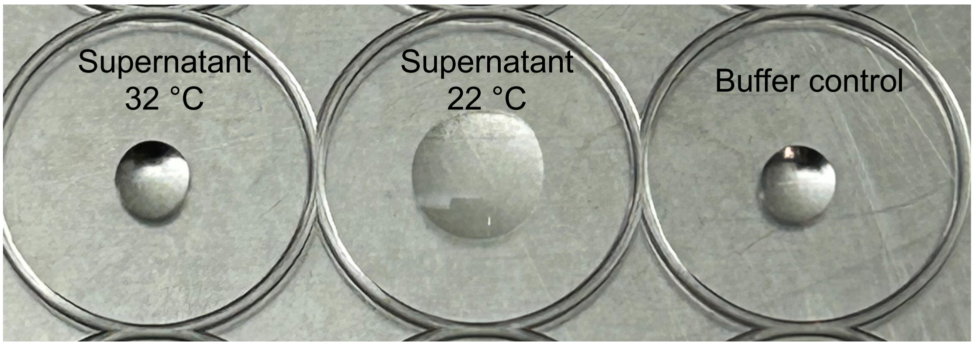
***P. fluorescens* kills *M. xanthus* when both species are pre-grown and interact at 22 °C but not when both are pre-grown and interact at 32 °C.** DK3470 population sizes are shown 24 hours after interaction with *P. fluorescens* when the two species interact at the same temperature at which both had been reared prior to interaction. Means of $\log_{10}$-transformed CFU + 1 values and 95% confidence intervals are shown. Lighter dots are biological replicates (n = 3). *** $p < 0.001$ (Tukey-adjusted contrasts) for the difference between *M. xanthus* population size after interaction with *P. fluorescens* (green dots) *vs* in the control treatment (black dots) when the two species were reared and interacted at 22 °C.

Figure S5.



Reversed predation decomposes *M. xanthus* cells into diffusible nutrients. Remains of *M. xanthus* killed by 22 °C-reared *P. fluorescens* sufficient to fuel large *P. fluorescens* population growth pass through 0.2-μm filters within six hours of inter-species interaction. Estimated densities (log-transformed CFU/ml, *n* = 3) of *P. fluorescens* populations over time inoculated at two initial densities (~10^6 and ~10^8 CFU/ml) into supernatant from 22 °C-reared *P. fluorescens* lawns to which *M. xanthus* cells were either added (and which killed those *M. xanthus* cells, green dots) or not (black dots). Trend lines show local polynomial regression fitting and dark-gray bands represent 95% confidence regions.

Figure S6.



Drop-collapse assay with *P. fluorescens* supernatants. Picture of 20-µl aliquots of supernatants from liquid suspensions of *P. fluorescens* grown on M9cas agar at 32 °C (left) and 22 °C (center), and of M9 buffer control (right). The three replicates of this assay yielded visually indistinguishable results; one replicate is shown here.

Table S1. Statistical analysis of *M. xanthus* swarming data on prey lawns. Linear model and Type III ANOVA for swarming data using prey identity, predator identity, temperature treatment and their interactions as explanatory variables. Posthoc contrasts between temperature treatments are computed for each predator-prey combination.

A. Linear model

```
model <- lm(formula = swarm_diameter ~ prey * predator * temperature)
```

```
Multiple R-squared:  0.9325,     Adjusted R-squared:  0.908
F-statistic: 38.08 on 62 and 171 DF,  p-value: < 2.2e-16
```

Anova Table (Type III tests)
Response: swarm_diameter

	Sum Sq	**Degree of freedom**	**F value**	**Pr(>F)**
(Intercept)	52.926	1	442.719	< 2.2e-16 ***
prey	22.681	6	31.621	< 2.2e-16 ***
predator	8.065	2	33.733	4.478e-13 ***
temperature	0.035	2	0.146	0.864
prey:predator	10.384	12	7.238	1.234e-10 ***
prey:temperature	7.294	12	5.084	3.151e-07 ***
predator:temperature	0.022	4	0.047	0.996
prey:predator:temperature	1.439	24	0.501	0.975
Residuals	20.442	171		

Signif. codes: 0 = ***, 0.001 = **, 0.01 = *, 0.05 = .

B. Tukey-adjusted contrasts on temperature treatment for each predator-prey combination

```
pairs(emmeans::emmeans(model, "temperature", by = c("prey", "predator")))
```

```
prey = NO, predator = GJV1:
 contrast   estimate  SE  df.    t.ratio p.value
 12 - 22    0.1250 0.244 171   0.511  0.8660
 12 - 32    0.1000 0.244 171   0.409  0.9120
 22 - 32   -0.0250 0.244 171  -0.102  0.9943

prey = AG, predator = GJV1:
 contrast   estimate  SE  df.    t.ratio p.value
 12 - 22    0.1625 0.244 171   0.665  0.7843
 12 - 32    0.2875 0.244 171   1.176  0.4691
 22 - 32    0.1250 0.244 171   0.511  0.8660

prey = BB, predator = GJV1:
 contrast   estimate  SE  df.    t.ratio p.value
 12 - 22   -0.1250 0.244 171  -0.511  0.8660
 12 - 32   -0.0125 0.244 171  -0.051  0.9986
 22 - 32    0.1125 0.244 171   0.460  0.8899

prey = EC, predator = GJV1:
 contrast   estimate  SE  df.    t.ratio p.value
 12 - 22   -0.2250 0.244 171  -0.920  0.6282
 12 - 32   -0.3750 0.244 171  -1.534  0.2777
 22 - 32   -0.1500 0.244 171  -0.614  0.8129
```

```
prey = ML, predator = GJV1:
 contrast estimate    SE df.  t.ratio p.value
 12 - 22   -0.2875 0.244 171  -1.176  0.4691
 12 - 32   -0.0750 0.244 171  -0.307  0.9495
 22 - 32    0.2125 0.244 171   0.869  0.6604

prey = PF, predator = GJV1:
 contrast estimate    SE df.  t.ratio p.value
 12 - 22    0.7250 0.244 171   2.965  0.0096
 12 - 32   -1.1875 0.244 171  -4.857  <.0001
 22 - 32   -1.9125 0.244 171  -7.823  <.0001

prey = RV, predator = GJV1:
 contrast estimate    SE df.  t.ratio p.value
 12 - 22    0.0250 0.346 171   0.072  0.9971
 12 - 32    0.0750 0.346 171   0.217  0.9744
 22 - 32    0.0500 0.346 171   0.145  0.9885

prey = NO, predator = A75:
 contrast estimate    SE df.  t.ratio p.value
 12 - 22    0.0000 0.244 171   0.000  1.0000
 12 - 32    0.0875 0.244 171   0.358  0.9319
 22 - 32    0.0875 0.244 171   0.358  0.9319

prey = AG, predator = A75:
 contrast estimate    SE df.  t.ratio p.value
 12 - 22    0.2250 0.244 171   0.920  0.6282
 12 - 32    0.4125 0.244 171   1.687  0.2130
 22 - 32    0.1875 0.244 171   0.767  0.7238

prey = BB, predator = A75:
 contrast estimate    SE df.  t.ratio p.value
 12 - 22    0.0875 0.244 171   0.358  0.9319
 12 - 32    0.0625 0.244 171   0.256  0.9646
 22 - 32   -0.0250 0.244 171  -0.102  0.9943

prey = EC, predator = A75:
 contrast estimate    SE df.  t.ratio p.value
 12 - 22   -0.1375 0.244 171  -0.562  0.8402
 12 - 32   -0.1375 0.244 171  -0.562  0.8402
 22 - 32    0.0000 0.244 171   0.000  1.0000

prey = ML, predator = A75:
 contrast estimate    SE df.  t.ratio p.value
 12 - 22    0.2000 0.244 171   0.818  0.6924
 12 - 32    0.0625 0.244 171   0.256  0.9646
 22 - 32   -0.1375 0.244 171  -0.562  0.8402

prey = PF, predator = A75:
 contrast estimate    SE df.  t.ratio p.value
 12 - 22    0.5125 0.244 171   2.096  0.0936
 12 - 32   -1.8625 0.244 171  -7.618  <.0001
 22 - 32   -2.3750 0.244 171  -9.714  <.0001
```

```
prey = RV, predator = A75:
 contrast   estimate   SE  df.  t.ratio p.value
 12 - 22    0.0250 0.346 171   0.072  0.9971
 12 - 32   -0.0250 0.346 171  -0.072  0.9971
 22 - 32   -0.0500 0.346 171  -0.145  0.9885

prey = NO, predator = SO1:
 contrast   estimate   SE  df.  t.ratio p.value
 12 - 22    0.1125 0.244 171   0.460  0.8899
 12 - 32    0.1125 0.244 171   0.460  0.8899
 22 - 32    0.0000 0.244 171   0.000  1.0000

prey = AG, predator = SO1:
 contrast   estimate   SE  df.  t.ratio p.value
 12 - 22    0.1375 0.244 171   0.562  0.8402
 12 - 32    0.2750 0.244 171   1.125  0.5001
 22 - 32    0.1375 0.244 171   0.562  0.8402

prey = BB, predator = SO1:
 contrast   estimate   SE  df.  t.ratio p.value
 12 - 22    0.1500 0.244 171   0.614  0.8129
 12 - 32    0.1625 0.244 171   0.665  0.7843
 22 - 32    0.0125 0.244 171   0.051  0.9986

prey = EC, predator = SO1:
 contrast   estimate   SE  df.  t.ratio p.value
 12 - 22    0.0000 0.244 171   0.000  1.0000
 12 - 32   -0.1000 0.244 171  -0.409  0.9120
 22 - 32   -0.1000 0.244 171  -0.409  0.9120

prey = ML, predator = SO1:
 contrast   estimate   SE  df.  t.ratio p.value
 12 - 22    0.0125 0.244 171   0.051  0.9986
 12 - 32    0.0625 0.244 171   0.256  0.9646
 22 - 32    0.0500 0.244 171   0.205  0.9772

prey = PF, predator = SO1:
 contrast   estimate   SE  df.  t.ratio p.value
 12 - 22    0.5625 0.244 171   2.301  0.0584
 12 - 32   -0.9375 0.244 171  -3.835  0.0005
 22 - 32   -1.5000 0.244 171  -6.135  <.0001

prey = RV, predator = SO1:
 contrast   estimate   SE  df.  t.ratio p.value
 12 - 22    0.2250 0.346 171   0.651  0.7922
 12 - 32    0.1000 0.346 171   0.289  0.9549
 22 - 32   -0.1250 0.346 171  -0.362  0.9305

P value adjustment: tukey method for comparing a family of 3 estimates
```

Table S2. Statistical analysis of *M. xanthus* growth data. Linear model and Type III ANOVA for *M. xanthus* growth data using *P. fluorescens* inoculum size, temperature treatment, their interaction and time as explanatory variables. Posthoc contrasts between inoculum sizes are computed for each temperature-time combination.

A. Linear model

```
model <- lm(log10(predator_number +1) ~ prey_inoculum_size * rearing_temperature + time)
```

```
Multiple R-squared:  0.9407,                Adjusted R-squared:  0.9286
F-statistic: 77.93 on 12 and 59 DF,  p-value: < 2.2e-16
```

Anova Table (Type III tests)
Response: log10(predator_number +1)

	Sum Sq	**Degree of freedom**	**F value**	**Pr(>F)**
(Intercept)	244.066	1	315.709	< 2.2e-16 ***
prey_inoculum_size	192.800	3	83.131	< 2.2e-16 ***
rearing_temperature	0.049	2	0.032	0.969
time	10.421	1	13.480	0.001
prey_inoculum_size:rearing_temperature	195.261	6	42.096	< 2.2e-16 ***
Residuals	45.611	59		

Signif. codes: 0 = ***, 0.001 = **, 0.01 = *, 0.05 = .

B. Tukey-adjusted contrasts on prey inoculum size for each temperature-time combination

```
pairs(emmeans::emmeans(model, "prey_inoculum_size", by = c("rearing_temperature", "time")))
```

```
temperature = 12, time = 30 min:
 contrast      estimate   SE  df.   t.ratio p.value
 no prey - 5    0.8001 0.508 59    1.576  0.3999
 no prey - 6    0.7414 0.508 59    1.461  0.4675
 no prey - 7    7.0188 0.508 59   13.826  <.0001
 5 - 6         -0.0587 0.508 59   -0.116  0.9994
 5 - 7          6.2187 0.508 59   12.250  <.0001
 6 - 7          6.2773 0.508 59   12.366  <.0001

temperature = 22, time = 30 min:
 contrast      estimate   SE  df.   t.ratio p.value
 no prey - 5    6.9464 0.508 59   13.684  <.0001
 no prey - 6    6.9464 0.508 59   13.684  <.0001
 no prey - 7    6.9464 0.508 59   13.684  <.0001
 5 - 6          0.0000 0.508 59    0.000  1.0000
 5 - 7          0.0000 0.508 59    0.000  1.0000
 6 - 7          0.0000 0.508 59    0.000  1.0000

temperature = 32, time = 30 min:
 contrast      estimate   SE  df.   t.ratio p.value
 no prey - 5    0.4443 0.508 59    0.875  0.8176
 no prey - 6    0.4176 0.508 59    0.823  0.8435
 no prey - 7    0.6910 0.508 59    1.361  0.5284
 5 - 6         -0.0267 0.508 59   -0.053  0.9999
 5 - 7          0.2467 0.508 59    0.486  0.9619
 6 - 7          0.2734 0.508 59    0.539  0.9492
```

```
temperature = 12, time = 7 days:
 contrast     estimate    SE df t.ratio p.value
 no prey - 5    0.8001 0.508 59   1.576  0.3999
 no prey - 6    0.7414 0.508 59   1.461  0.4675
 no prey - 7    7.0188 0.508 59  13.826  <.0001
 5 - 6         -0.0587 0.508 59  -0.116  0.9994
 5 - 7          6.2187 0.508 59  12.250  <.0001
 6 - 7          6.2773 0.508 59  12.366  <.0001

temperature = 22, time = 7 days:
 contrast     estimate    SE df t.ratio p.value
 no prey - 5    6.9464 0.508 59  13.684  <.0001
 no prey - 6    6.9464 0.508 59  13.684  <.0001
 no prey - 7    6.9464 0.508 59  13.684  <.0001
 5 - 6          0.0000 0.508 59   0.000  1.0000
 5 - 7          0.0000 0.508 59   0.000  1.0000
 6 - 7          0.0000 0.508 59   0.000  1.0000

temperature = 32, time = 7 days:
 contrast     estimate    SE df t.ratio p.value
 no prey - 5    0.4443 0.508 59   0.875  0.8176
 no prey - 6    0.4176 0.508 59   0.823  0.8435
 no prey - 7    0.6910 0.508 59   1.361  0.5284
 5 - 6         -0.0267 0.508 59  -0.053  0.9999
 5 - 7          0.2467 0.508 59   0.486  0.9619
 6 - 7          0.2734 0.508 59   0.539  0.9492

P value adjustment: tukey method for comparing a family of 4 estimates
```