Supplementary information (SI)

Recurring infection by crayfish plague pathogen only marginally affects survival and growth of marbled crayfish

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S1 Detection of *A. astaci* DNA in Experiments 1 and 2

Total DNA was isolated from the uropods and/or abdominal cuticle of each crayfish (both euthanized and those that died during the experiment) using the modified standard NucleoSpin® Microbial DNA kit protocol (Macherey Nagel, Germany). Cuticle samples were lysed by agitation on a Vortex Mixer (Corning, USA) for 30 min (medium strength), using Macherey Nagel Bead Tubes Type B provided in the kit. To increase the concentration of the final sample, initial 100 μl DNA eluate was used for a second elution from the column. The quality of the isolated DNA was tested by electrophoresis in 1% agarose gel.

The detection of pathogen DNA in the crayfish cuticle was performed by PCR-based assay using the specific primers 42 (5′ GCT TGT GCT GAG GAT GTTCT 3′) and 640 (5′ CTA TCC GAC TCC GCA TTC TG 3′) which amplify the ITS (Internal Transcribed Spacer) region of the *A. astaci* 5.8 rRNA gene, were used (Oidtmann et al., 2006). Amplification success, i.e. presence or absence of fragment of expected size (569 bp) was tested using 2% agarose gel electrophoresis.

S2 Primers used for amplification of innate immunity and metabolic genes

Table S2.1: Primers for the amplification of innate immunity and metabolic genes. ProPO - prophenoloxidase, C/EBP-β - CCAAT/enhancer-binding protein beta, EF1α - Elongation factor 1-α, GAPDH - Glyceraldehyde 3-phosphate dehydrogenase, CS - citrate synthase.

Primer name	**Sequence (5′-3′)**	**Length**	**Tm**	**GC%**	**Prod. Len.**	**Primer efficiency (%)**
ProPO-F	ACTGGCATCTCGTTTACCCC	20	59.75	55	98	96
ProPO-R	GTCGTACCTAGCGACCATCTG	21	60	57.14		
C/EBP-β-F	AGTGGTTGAAAGGCACGACG	20	61.16	55	99	108
C/EBP-β-R	AAACGCCAGCTCCGTACC	18	60.05	61.11		
EF1α-F	CTGGTACTGGAGAGTTTGAAGC	22	58.67	50	99	90
EF1α-R	CAACAACGAGCTGCTTGAC	19	57.56	52.63		
GAPDH-F	GCCACCCAGAAAACTGTTGA	20	58.6	50	100	106
GAPDH-R	CCTTAGCAGCACCAGTGGAA	20	59.96	55		
CS-F	ACCTCACAATTCACAGTGACCA	22	59.83	45.45	102	108
CS-R	CAGCAGCAAATGCGAGGTA	19	58.53	52.63		

S3 Linear multilevel modelling

The technique of multilevel modeling (MLM) was used for data analysis (Monsalves et al., 2020; Peugh, 2010) to provide answers to growth-related research questions: (a) what average growth trajectory best describes the rate of growth over time for all crayfish, (b) what is the variability in growth rates across crayfish, and finally (c) do *A. astaci* infection and food availability explain variability in growth rates?

S3.1 Methods

Linear multilevel modelling (MLM) with two levels (Table S3.1) was used to estimate random and fixed rates of weight and length gain over time, and to assess the influence of predictors, such as food availability and *A. astaci* zoospore concentration, on crayfish growth. Multilevel models are conceptually similar to regressions. However, in ordinary regressions the parameters (intercept and slope) are generally fixed values estimated from the sample (fixed effect), whereas in multilevel models the parameters may vary due to inter-individual differences (random effect).

Table S3.1: Summary of data and variables at two levels in this study design.

	Level	**Subindex**	**Independant variables**	**Dependant variables**
1	timepoint	$t = 0, 1, 2, ..., n^*$	$TIME_{ti}$	Y_{ti}^1 = WEIGHT Y_{ti}^2 = LENGTH
2	individua	$i = 1, 2, ..., m^{**}$	$PLAGUE_i$ $FOOD_i$	

* $n = 9$ (Experiment 1); 6 (Experiment 2)

** $m = 55$ (Experiment 1); 60 (Experiment 2)

MLM framework used in this study is a family of three nested models for Experiment 1 and four nested models for Experiment 2. In the model selection procedure for Experiment 1, we first start with the simplest form of the model with fixed and random intercepts (unconditional means model, Model 1). In each advanced version of the model, a new aspect is added to the model, such as fixed and random slope (Model 2) and conditional growth due to *A. astaci* infection (Model 3). Each new model is compared with the previous model to evaluate the better model fit, i.e., to assess the contribution of the added aspect to the model through ANOVA, using the most common statistics such as the chi-square likelihood ratio test (χ^2), the Akaike information criterion (AIC), and the Bayesian information criterion (BIC). The first two steps in model selection procedure in Experiment 2 were similar to those in Experiment 1. Additionally, in Experiment 2, Model 3 captures conditional growth due to food availability, and Model 4 captures conditional growth due to food availability and *A. astaci* infection.

MLM models were built using the *lme4* package (optimizer: *bobyqa*) in R (Bates et al., 2015). A detailed specification of all data levels, model variables, and model equations is provided below.

Model 1: The simplest model used in this study is *Unconditional means model* (null-model)

$$\text{Level 1}: \quad Y_{ti} = \beta_{0i} + r_{ti} \tag{1}$$

$$\text{Level 2}: \quad \beta_{0i} = \gamma_{00} + u_{0i} \tag{2}$$

where for each crayfish i, the observed size in time t is modeled as the sum of mean size during the growth (β_{0i}) and residual r_{ti} that reflects the differences between observed and predicted size during the growth. Each individual's mean size during the growth (β_{0i}) is modelled as mean size of all crayfish (γ_{00}; fixed effect) and term u_{01} (random effect) that reflects deviations of individual's mean size around grand-mean γ_{00}. Combined equations (1) and (2) yield the following form of the model:

$$Y_{ti} = \gamma_{00} + u_{0i} + r_{ti}\,. \tag{3}$$

The slope of this linear model is zero.

Model 2: Adding the predictor $TIME$ to Model 1 allows us to predict the growth trajectory with a line whose slope can be different from zero:

$$\text{Level 1}: \quad Y_{ti} = \beta_{0i} + \beta_{1i} TIME_{ti} + r_{ti} \tag{4}$$

$$\text{Level 2}: \quad \beta_{0i} = \gamma_{00} + u_{0i} \tag{5}$$

$$\text{Level 2}: \quad \beta_{1i} = \gamma_{10} + u_{1i}\,. \tag{6}$$

The interpretation of model parameters is changed with respect to the Model 1: β_{0i} now reflects initial size of crayfish at time $t = 0$, while intercept γ_{00} represents grand mean of all crayfish at time $t = 0$. New term β_{1i} reflects how each individual's size changes over time and it is modelled as grand-mean rate of growth in time (slope) of all crayfish γ_{10} and residual term u_{1i} that reflects individual crayfish differences in size change around the grand-mean. Combined equations (4), (5) and (6) form the model:

$$Y_{ti} = \gamma_{00} + \gamma_{10} TIME_{ti} + u_{0i} + u_{1i} TIME_{ti} + r_{ti} \tag{7}$$

Parameters β_{0i} and β_{1i} estimate fixed effects, while parameters u_{0i} and u_{1i} estimate random effect.

Model 3A: Model 3A is conditional model where predictor variable $PLAGUE$ is added to Model 2 to explain intercept and slope variance:

$$\text{Level 1}: \quad Y_{ti} = \beta_{0i} + \beta_{1i} TIME_{ti} + r_{ti} \tag{8}$$

$$\text{Level 2}: \quad \beta_{0i} = \gamma_{00} + \gamma_{01} PLAGUE_i + u_{0i} \tag{9}$$

$$\text{Level 2}: \quad \beta_{1i} = \gamma_{10} + \gamma_{11} PLAGUE_i + u_{1i} \tag{10}$$

Adding categorical predictor variable changes interpretation of γ_{00} and γ_{10} parameters. Since R defaults to the dummy coding system of the categorical variable $PLAGUE$, γ_{00} represents the mean size of crayfish in the Control group at time $t = 0$, while $\gamma_{00} + \gamma_{01}^{(7500)}$ and $\gamma_{00} + \gamma_{01}^{(15000)}$ represent mean size of crayfish in two infected groups in Experiment 1 at time $t = 0$. Similarly, γ_{10} represents the the mean slope of size growth in the Control group, while $\gamma_{10} + \gamma_{11}^{(7500)}$ and $\gamma_{10} + \gamma_{11}^{(15000)}$ represent the mean slope of size growth in infected groups, respectively. Equations (8), (9) and (10) yield the following combined model equation:

$$Y_{ti} = \gamma_{00} + \gamma_{01} PLAGUE_i + \gamma_{10} TIME_{ti} + \gamma_{11} PLAGUE_i \cdot TIME_{ti} + u_{0i} + u_{1i} TIME_{ti} + r_{ti}\,, \tag{11}$$

where γ_{00}, γ_{01}, γ_{10} and γ_{11} are fixed effect parameters, while u_{0i} and u_{1i} are random effects parameters that reflect individual crayfish differences.

Model 3B: Model 3B is version of Model 3A in which the 3-level variable $PLAGUE$ is replaced by the 2-level variable $FOOD$.

Model 4: The most complex in this family of models is Model 4, in which the predictor variable $PLAGUE$ is added to Model 3B. Since one of the study objectives was to investigate the interaction between food and infection on the growth of crayfish, the mathematical interaction term between $FOOD$ and $PLAGUE$ is also added to the model equations:

$$\text{Level 1}: \quad Y_{ti} = \beta_{0i} + \beta_{1i} TIME_{ti} + r_{ti} \tag{12}$$

$$\text{Level 2}: \quad \beta_{0i} = \gamma_{00} + \gamma_{01} PLAGUE_i + \gamma_{02} FOOD_i + \gamma_{03} PLAGUE_i \cdot FOOD_i + u_{0i} \tag{13}$$

$$\text{Level 2}: \quad \beta_{1i} = \gamma_{10} + \gamma_{11} PLAGUE_i + \gamma_{12} FOOD_i + \gamma_{13} PLAGUE_i \cdot FOOD_i + u_{1i} \tag{14}$$

The final form of Model 4 is obtained by joining the equations (12), (13) and (14):

$$\begin{aligned} Y_{ti} &= \gamma_{00} + \gamma_{01}PLAGUE_i + \gamma_{02}FOOD_i + \gamma_{10}TIME_{ti} + \\ &+ \gamma_{11}PLAGUE_i \cdot TIME_{ti} + \gamma_{12}FOOD_i \cdot TIME_{ti} + \gamma_{03}PLAGUE_i \cdot FOOD_i + \\ &+ \gamma_{13}PLAGUE_i \cdot FOOD_i \cdot TIME_{ti} + u_{0i} + u_{1i}TIME_{ti} + r_{ti}\,. \end{aligned} \tag{15}$$

The random effects used in Models 1-4 are assumed to be normally distributed and independent from the error distribution. The variance of the r_{ti} is denoted by σ^2, the variance of the u_{0i} is denoted by τ_{00} and the variance of the u_{1i} is denoted by τ_{11}. Finally, nested models used in the data analysis are:

EXPERIMENT 1:	Model 1	Model 2	Model 3A	
EXPERIMENT 2:	Model 1	Model 2	Model 3B	Model 4

S3.2 Results: EXPERIMENT 1

Rate of growth All three nested MLM models - (i) unconditional means model (Model 1, null- model), (ii) linear growth model with random slope and intercept (Model 2, intermediate model), and (iii) conditional growth model (Model 3A, final model) - were built upon the longitudinal data-set on weight and length measured in Experiment 1. The model selection procedure of ANOVA detected an increase in model predictability with each added feature, and identified Model 3 to have the best model structure in terms of the obtained goodness of fit value and the number of model parameters used (Table S3.2, Table S4.2 A).

The assessment of fixed effects in the Model 3 (Table S3.2; Table S4.2 BC) showed that there was no significant difference in weight between the groups at the beginning of the experiment. This is due to γ_{01} levels which are found not to be significant, despite the fact that intercept values for the 7500-group and the 15000-group ($\gamma_{00} + \gamma_{01} = 136.6$ and 160.5 mg, respectively) differed slightly from the mean weight value of the control group ($\gamma_{00} = 155.7$ mg) at the start of experiment (Figure 2B). Weight increased significantly in all groups, but at different rates. Due to significant interaction term (γ_{11}), the growth rate (i.e., the slope coefficient) of the control group $\gamma_{10} = 12.7$ mg/wk was significantly higher than the growth rates in the 7500-group and the 1500-group ($\gamma_{10} + \gamma_{11} = 10.2$ mg/wk and 9.5 mg/wk, respectively), indicating a significant effect of *A. astaci* infection on growth rate. Variance component estimates showed (Table S3.2; Table S4.2 D): significant variance of observed versus predicted growth within crayfish ($\sigma^2 = 313.8$ mg^2), significant variance of weight at start ($\tau_{00} = 2040.0$ mg^2), small - but significant

Table S3.2: Summary of nested multilevel regression models (MLMs) for the crayfish growth in Experiment 1. Model structures are given in SI Section C, values in parentheses represent 95% confidence intervals (CIs), and bold values denote statistical significance of fixed effects at $p < 0.001$. For a detailed statistical analysis of model selection, goodness of fit, and significance of model parameters, see Table S4.2 and Figure S4.2.

		Model 1	Model 2	Model 3
	Parameters	**Value of category (95% CI)**		
Regression coefficients (fixed effects)				
A	Intercept (γ_{00})	**234.8** (221.6, 247.7)	**150.3** (137.5, 163.2)	**155.7** (131.8, 179.6)
B	Time (γ_{10})	-	**10.7** (10.1, 11.2)	**12.7** (11.9, 13.4)
C	Plague (γ_{01})			
	Control	-	-	*Reference A*
	7500	-	-	-19.1 (-50.7, 12.5)
	15000	-	-	4.8 (-26.9, 36.4)
D	Interaction (γ_{11})			
	Time:Control	-	-	*Reference B*
	Time:7500	-	-	**-2.5** (-3.5, -1.5)
	Time:15000	-	-	**-3.2** (-4.2, -2.2)
Variance components (random effects)[a]				
	Residual (σ)	64.9 (60.7, 69.6)	17.7 (16.5, 19.1)	17.7 (16.5, 19.1)
	Intercept ($\sqrt{\tau_{00}}$)	42.6 (33.1, 55.0)	46.5 (38.6, 57.3)	45.2 (37.4, 55.7)
	Slope ($\sqrt{\tau_{11}}$)	-	1.7 (1.3, 2.2)	1.0 (0.5, 1.4)
	Correlation	-	-0.36 (-0.61, -0.04)	-0.58 (-0.85, -0.22)
Model summary				
	ICC[b]	0.30	0.87	0.87
	LogLik[b]	-2636.1	-2130.9	-2109.6
	Deviance	5272.2	4261.7	4219.2
	AIC[b]	5278.2	4273.7	4239.2
	BIC[b]	5290.6	4298.5	4280.6

[a] Variance components are given in the square-rooted form

[b] Intralevel correlation coefficient (ICC), Log-likelihood (LogLik), Akaike information criterion (AIC), Bayesian information criterion (BIC)

slope variance across crayfish ($\tau_{11} = 1.0$ mg^{2}wk^{-2}), and a negative covariance relationship between intercept and slope.

S3.3 Results: EXPERIMENT 2

Rate of growth The set of four nested models - (1) unconditional means model (Model 1, null-model); (2) linear growth model with random slope and intercept (Model 2); (3) linear growth model conditional on food (Model 3B); and (4) linear growth model conditional on food and *A.astaci* infection (Model 4) - were compared by ANOVA to confirm that each additional variable contributed to the predictive ability of the model. Model 4 was identified to have the best model structure in terms of the goodness-of-fit value obtained and the number of model parameters

used (Table S3.3, Table S5.2 A.)

The fixed-effects assessment in the Model 4 (Table S3.3; Table S5.2 BC) showed no significant difference in weight between groups at the beginning of the experiment (see also Figure 3B). This is due to the statistical insignificance of the main effects of food (γ_{01}), infection (γ_{02}), and food:infection interaction (γ_{03}) in the model (Table S3.3). This result is consistent with the randomized design of the groups at the start of experiment, which were characterized by the similar size distribution of crayfish.

The effects of food and *A. astaci* infection became significant when combined with time (Table S3.3; Table S5.2 BC) resulting in the significantly different growth rates (i.e., linear slopes) under different experimental conditions. The effect of food on growth rate was stronger than the effect of *A. astaci* infection: the average growth rate of control (non-infected) crayfish fed five times a week was 30.8 mg/wk (γ_{10}), compared with an average growth rate of 21.0 mg/wk ($\gamma_{10} + \gamma_{12}$) for infected crayfish under the same feeding regime and average growth rate of 2.5 mg/wk for food-restricted control crayfish group ($\gamma_{10} + \gamma_{11}$). The average growth rate of infected food-restricted individuals was 0.2 mg/wk ($\gamma_{10} + \gamma_{11} + \gamma_{12} + \gamma_{13}$). The decrease in growth rate due to infection was higher when food was abundant (i.e., feeding regime five times per week; γ_{12}) than when food was restricted (i.e., feeding once a week; $\gamma_{12} + \gamma_{13}$), due to a significant three-way interaction term between time, food and *A. astaci* infection (γ_{13}). Variance component estimates showed (Table S3.3; Table S5.2 D): significant variance of observed versus predicted growth within crayfish ($\sigma^2 = 509.5$ mg^2), significant variance of weight between crayfish at the start of experiment ($\tau_{00} = 9550.9$ mg^2) and small - but significant - slope variance across crayfish ($\tau_{11} = 6.1$ mg^{2}wk^{-2}).

Table S3.3: Summary of nested multilevel regression models (MLMs) for the crayfish growth in Experiment 2. Model structures are given in SI Section C, values in parentheses represent 95% confidence intervals (CIs) and bold values denote statistical significance of fixed effects at $p < 0.001$. For a detailed statistical analysis of model selection, goodness of fit, and significance of model parameters, see Table S5.2 and Figure S5.2.

		Model 1	**Model 2**	**Model 3**	**Model 4**
	Parameters		**Value of category (95% CI)**		
Regression coefficients (fixed effects)					
A	Intercept (γ_{00})	**349.5** (320.1, 378.5)	**283.8** (258,1, 309,5)	**277.0** (240.9, 313.2)	**276.8** (225.8, 327.7)
B	Time (γ_{10})	-	**14.2** (10.5, 17.9)	**26.5** (24.5, 28.5)	**30.8** (28.9, 32.7)
C	Food (γ_{01})				
	5x wk	-	-	*Reference A*	*Reference A*
	1x wk	-	-	14.3 (-36.9, 65.4)	17.7 (-54.4, 89.7)
D	Plague (γ_{02})				
	Control	-	-	-	*Reference A*
	15000	-	-	-	1.0 (-71.1, 73.1)
E	Time*Food (γ_{11})				
	Time:5x wk	-	-	*Reference B*	*Reference B*
	Time:1x wk	-	-	**-24.8** (-27.7, -21.9)	**-28.3** (-31.0, -25.7)
F	Time*Plague (γ_{12})				
	Time:Control	-	-	-	*Reference B*
	Time:15000	-	-	-	**-9.8** (-12.6, -6.9)
G	Food*Plague (γ_{03})				
	5x wk:Control	-	-	-	*Reference A/C/D*
	5x wk:15000	-	-	-	*Reference A/C/D*
	1x wk:Control	-	-	-	*Reference A/C/D*
	1x wk:15000	-	-	-	-6.6 (-108.6, 95.4)
H	Time*Food*Plague (γ_{13})				
	Time:5x wk:Control	-	-	-	*Reference B/E/F*
	Time:5x wk:15000	-	-	-	*Reference B/E/F*
	Time:1x wk:Control	-	-	-	*Reference B/E/F*
	Time:1x wk:15000	-	-	-	**7.5** (3.4, 11.6)
Variance components (random effects)[a]					
	Residual (σ)	76.0 (69.9, 83.0)	22.2 (20.2, 24.5)	22.6 (20.6, 24.9)	22.6 (20.6, 24.9)
	Intercept ($\sqrt{\tau_{00}}$)	107.8 (88.9, 132.7)	98.5 (82.76, 119.7)	98.0 (82.3, 119.1)	97.7 (82.1, 118.7)
	Slope ($\sqrt{\tau_{11}}$)	-	13.4 (11.1, 16.6)	4.4 (3.4, 5.8)	2.5 (1.3, 3.6)
	Correlation	-	-0.17 (-0.41, 0.10)	-0.27 (-0.53, 0.04)	-0.37 (-0.69, 0.03)
Model summary					
	ICC[b]	0.67	0.95	0.95	0.95
	LogLik[b]	-1923.9	-1678.6	-1628.9	-1609.8
	Deviance	3847.8	3357.2	3257.8	3219.6
	AIC[b]	3853.8	3369.2	3273.8	3243.6
	BIC[b]	3865.1	3391.9	3304.0	3288.9

[a] Variance components are given in the square-rooted form

[b] Intralevel correlation coefficient (ICC), Log-likelihood (LogLik), Akaike information criterion (AIC), Bayesian information criterion (BIC)

S4 Statistical support for EXPERIMENT 1

Table S4.1: Descriptive statistics (panels A/B), results of robust ANOVA (C/D), and post-hoc testing (E/F) for response variables in Experiment 1: **weight**/**length**.

Descriptive statistics

A **Response: WEIGHT (mg)**

	N	dead	alive	min	max	range	median	mean	SE	CI(95%)	var	std	coef.var
Control	15	0	15	179.5	291.8	112.3	233.4	234.1	8.4	18.1	1064.7	32.6	0.1
7500	20	3	17	138.4	231.4	93.0	194.7	187.7	6.4	13.5	688.4	26.2	0.1
15000	20	4	16	92.9	238.9	146.0	180.7	171.8	9.2	19.6	1355.7	36.8	0.2

B **Response: LENGTH (mm)**

	N	dead	alive	min	max	range	median	mean	SE	CI(95%)	var	std	coef.var
Control	15	0	15	5.08	10.39	5.31	8.30	7.96	0.37	0.79	2.01	1.42	0.18
7500	20	3	17	3.74	9.32	5.58	7.67	7.39	0.36	0.76	2.18	1.48	0.20
15000	20	4	16	2.88	8.95	6.07	6.98	6.44	0.46	0.98	3.40	1.84	0.29

Robust ANOVA

A heteroscedastic one-way ANOVA based on trimmed means (20% trimming level)

Mair & Wilcox (2020); Wilcox (2012)

C **Response: WEIGHT (mg)**

```
Test statistic: F = 10.0385
Degrees of freedom 1: 2
Degrees of freedom 2: 17.06
p-value: 0.00131

Explanatory measure of effect size: 0.84
Bootstrap CI: [0.64; 1.11]
```

D **Response: LENGTH (mm)**

```
Test statistic: F = 1.8618
Degrees of freedom 1: 2
Degrees of freedom 2: 17.19
p-value: 0.1854

Explanatory measure of effect size: 0.49
Bootstrap CI: [0.14; 0.92]
```

Robust post-hoc tests

Inference for all pairwise comparisons

Mair & Wilcox (2020); Wilcox (2012)

E **Response: WEIGHT (mg)**

	psihat	ci.lower	ci.upper	p.value
Control vs. 7500	42.74141	9.45827	76.02456	0.00754
Control vs. 15000	57.03778	23.43251	90.64304	0.00122
7500 vs. 15000	14.29636	-12.21025	40.80298	0.17609

F **Response: LENGTH (mm)**

	psihat	ci.lower	ci.upper	p.value
Control vs. 750	0.33616	-1.08058	1.75289	0.53909
Control vs. 15000	1.24299	-0.45725	2.94323	0.21282
7500 vs. 15000	0.90683	-0.67512	2.48878	0.29707

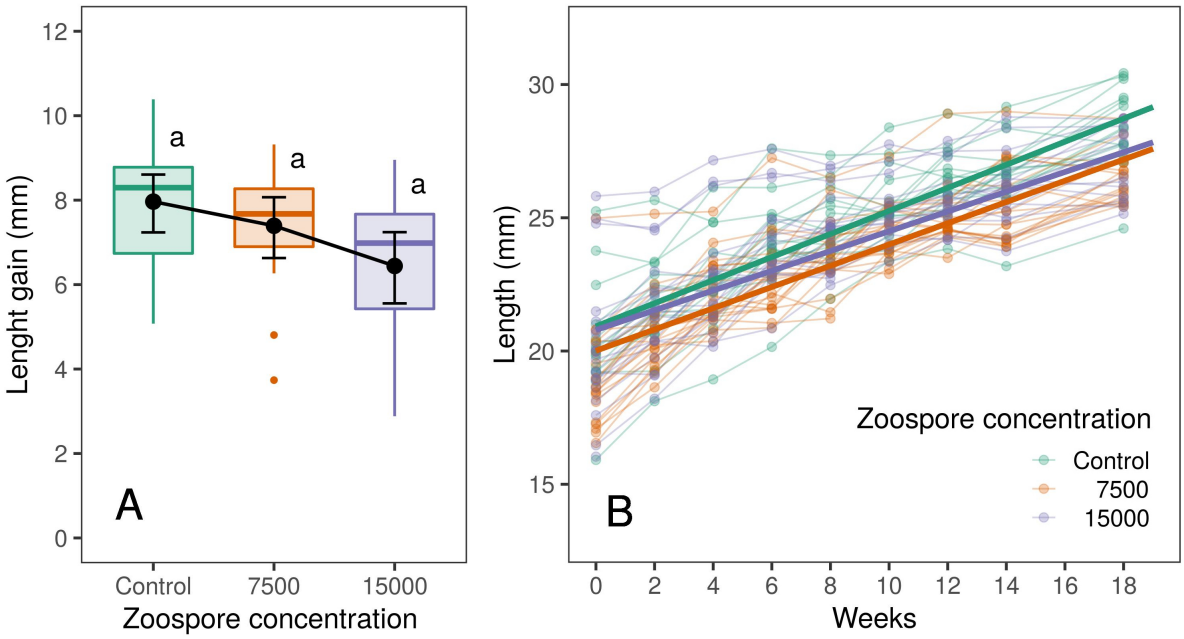
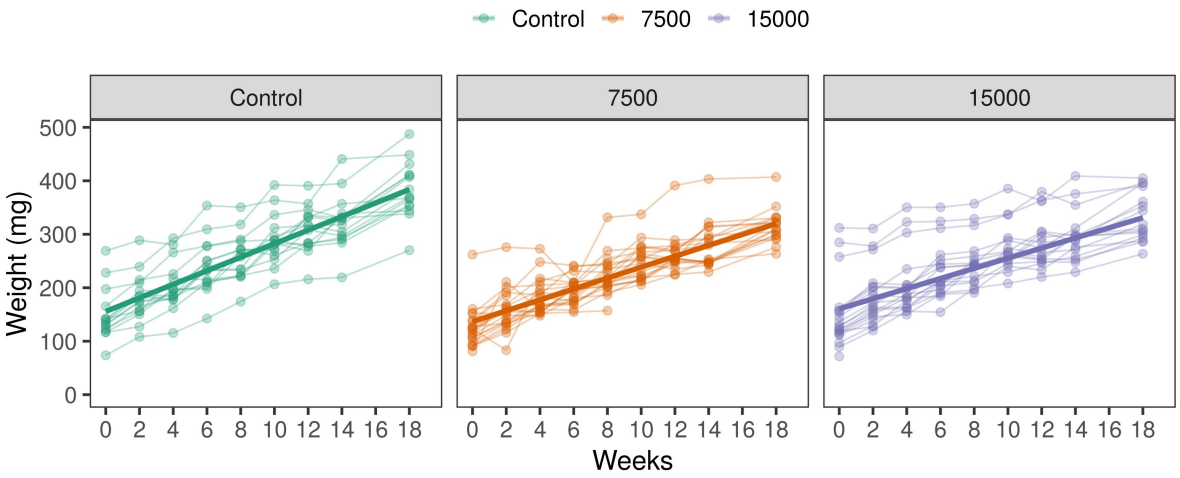
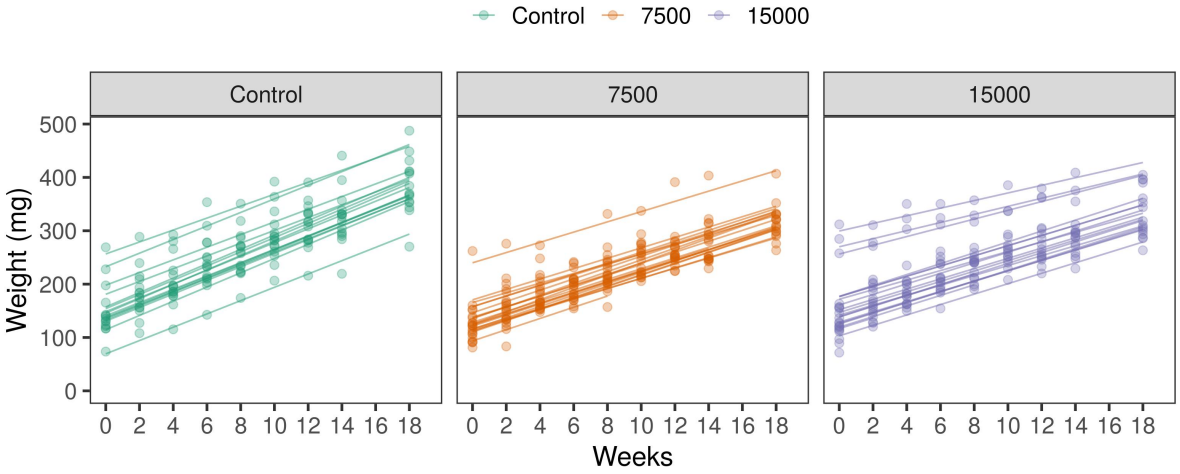


Figure S4.1: Effects of repeated infection of marbled crayfish juveniles using two *A. astaci* zoospore concentrations (7500 and 15000 zoospore/ml) on (A) total **length** gain (total growth), and (B) rate of **length** increment (rate of growth). Significant differences in panel A are marked with different letters, errorbars represent 95% confidence intervals (CIs) around the mean.



(a) Prediction based on fixed effects



(b) Prediction based on fixed and random effects

Figure S4.2: Measured temporal trajectories of **weight** in Experiment 1 and predicted trajectories by final MLM model (Model 3A).

Table S4.2: MLM model selection procedure for response variable **weight** in Experiment 1 (A). Summary statistics for final Model 3A: (B) Analysis of deviance, (C) Analysis of variance, and (D) Summary statistics of random and fixed effects.

Model selection

A

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)	
Model 1	3	5278.2	5290.6	-2636.1	5272.2				
Model 2	6	4273.7	4298.5	-2130.8	4261.7	1010.528	3	< 2.2e-16	***
Model 3	10	4239.2	4280.6	-2109.6	4219.2	42.533	4	1.294e-08	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Model 3

B Analysis of Deviance Table (Type III Wald chisquare tests)

Response: WEIGHT

	Chisq	Df	Pr(>Chisq)	
(Intercept)	168.990	1	< 2.2e-16	***
time	1144.595	1	< 2.2e-16	***
plague	2.911	2	0.2333	
time:plague	42.361	2	6.33e-10	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

C Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)	
time	870413	870413	1	51.152	2773.8720	< 2.2e-16	***
plague	913	457	2	54.626	1.4555	0.2422	
time:plague	13293	6646	2	51.200	21.1806	1.983e-07	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Model 3

D Summary statistics of random and fixed effects

AIC	BIC	logLik	deviance	df.resid
4239.2	4280.6	-2109.6	4219.2	454

Random effects:

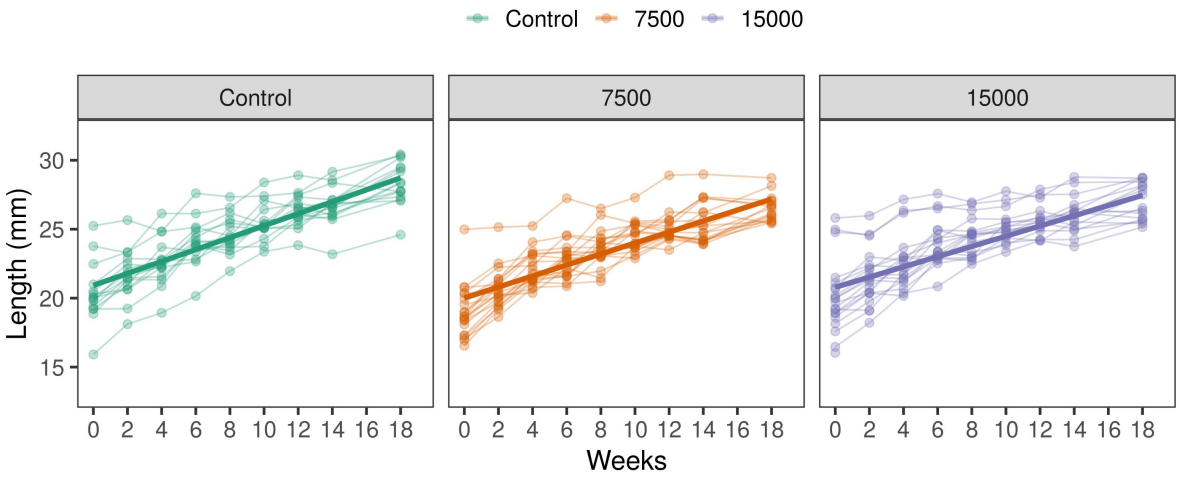
Groups	Name	Variance	Std.Dev.	Corr
id	(Intercept)	2039.9655	45.1660	
	time	0.9672	0.9835	-0.58
Residual		313.7900	17.7141	

Number of obs: 464, groups: id, 55

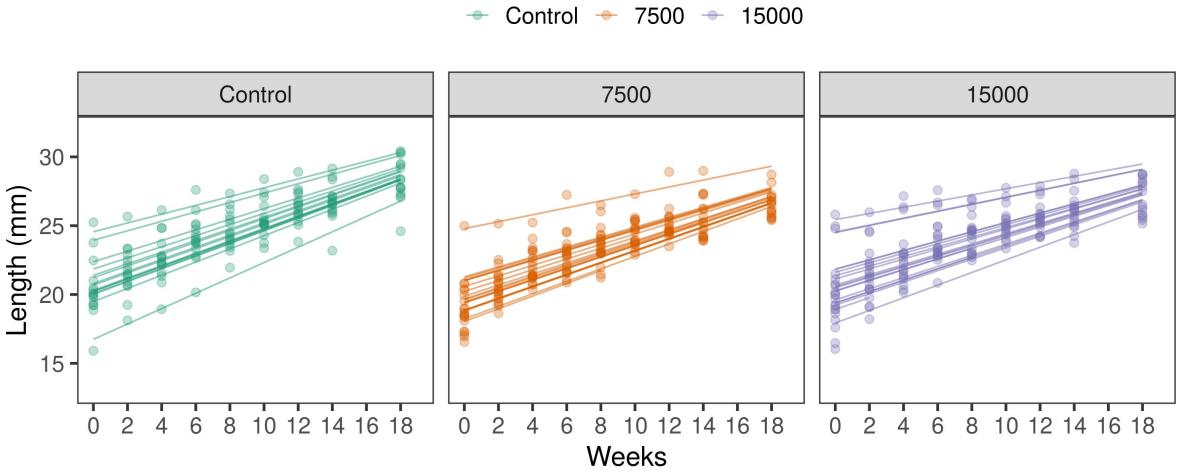
Fixed effects:

	Estimate	Std. Error	df	t value	Pr(>|t|)	
(Intercept)	155.6996	11.9772	54.3607	13.000	< 2e-16	***
time	12.6766	0.3747	48.6045	33.832	< 2e-16	***
plague7500	-19.1193	15.8488	54.4204	-1.206	0.233	
plague15000	4.8047	15.8674	54.6597	0.303	0.763	
time:plague7500	-2.4865	0.5039	50.1858	-4.934	9.25e-06	***
time:plague15000	-3.2045	0.5133	50.6980	-6.243	8.71e-08	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1



(a) Prediction based on fixed effects



(b) Prediction based on fixed and random effects

Figure S4.3: Measured temporal trajectories of **length** in Experiment 1 and predicted trajectories by final MLM model (Model 3A).

Table S4.3: MLM model selection procedure for response variable **length** in Experiment 1 (A). Summary statistics for final Model 3A: (B) Analysis of deviance, (C) Analysis of variance, and (D) Summary statistics of random and fixed effects.

Model selection

A

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)	
Model 1	3	2226.0	2238.4	-1109.98	2220.0				
Model 2	6	1331.4	1356.2	-659.69	1319.4	900.581	3	< 2.2e-16	***
Model 3	10	1312.1	1353.5	-646.06	1292.1	27.261	4	1.761e-05	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Model 3

B Analysis of Deviance Table (Type III Wald chisquare tests)

Response: LENGTH

	Chisq	Df	Pr(>Chisq)	
(Intercept)	1803.4992	1	< 2e-16	***
time	496.8330	1	< 2e-16	***
plague	2.4881	2	0.28821	
time:plague	5.7136	2	0.05745	.

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

C Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)	
time	944.74	944.74	1	51.403	1483.9887	<2e-16	***
plague	1.58	0.79	2	54.135	1.2441	0.2963	
time:plague	3.64	1.82	2	51.486	2.8568	0.0666	.

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Model 3

D Summary statistics of random and fixed effects

AIC	BIC	logLik	deviance	df.resid
1312.1	1353.5	-646.1	1292.1	454

Random effects:

Groups	Name	Variance	Std.Dev.	Corr
id	(Intercept)	3.415762	1.84818	
	time	0.003349	0.05787	-0.99
Residual		0.636623	0.79789	

Number of obs: 464, groups: id, 55

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(>|t|)	
(Intercept)	20.92779	0.49279	53.85014	42.468	<2e-16	***
time	0.43296	0.01942	49.16704	22.290	<2e-16	***
plague7500	-0.91635	0.65206	53.90166	-1.405	0.1657	
plague15000	-0.13912	0.65298	54.18311	-0.213	0.8321	
time:plague7500	-0.03434	0.02590	50.35920	-1.325	0.1910	
time:plague15000	-0.06234	0.02608	51.17560	-2.390	0.0206	*

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

S5 Statistical support for EXPERIMENT 2

Table S5.1: Descriptive statistics (panels A/B), results of robust ANOVA (C/E), and post-hoc testing (D/F) for response variables in Experiment 2: **weight**/**length**.

Descriptive statistics

A **Response: WEIGHT (mg)**

	N dead	alive	min	max	range	median	mean	SE	CI(95%)	var	std	coef.var
5x wk:Control	15	0	15	197.6	347.8	150.2	312.1	300.4	10.4	22.3	1620.1	40.3	0.1
1x wk:Control	15	0	15	-8.2	84.9	93.1	36.7	28.8	7.1	15.3	760.5	27.6	1.0
5x wk:15000	15	4	11	91.2	288.2	197.0	211.2	204.2	16.6	36.9	3022.4	55.0	0.3
1x wk:15000	15	8	7	-20.8	14.4	35.2	-1.6	-2.1	5.0	12.3	177.1	13.3	-6.3

B **Response: LENGTH (mm)**

	N dead	alive	min	max	range	median	mean	SE	CI(95%)	var	std	coef.var
5x wk:Control	15	0	15	3.90	9.96	6.05	8.14	7.76	0.44	0.93	2.85	1.69	0.22
1x wk:Control	15	0	15	-0.20	3.15	3.35	1.20	1.20	0.22	0.47	0.72	0.85	0.71
5x wk:15000	15	4	11	1.99	8.26	6.27	5.13	5.13	0.48	1.06	2.51	1.58	0.31
1x wk:15000	15	8	7	-0.75	1.42	2.17	0.56	0.39	0.29	0.70	0.57	0.75	1.91

Robust ANOVA

A heteroscedastic two-way ANOVA based on trimmed means (20% trimming level)

Mair & Wilcox (2020); Wilcox (2012)

C **Response: WEIGHT (mg)**

	Q.value	p.value
Food	652.2240	0.001
Plague	44.5496	0.001
Plague:Food	11.9241	0.003

E **Response: LENGTH (mm)**

	Q.value	p.value
Food	251.9742	0.001
Plague	24.9497	0.001
Food:Plague	8.3337	0.009

Robust post-hoc tests

Inference for all pairwise comparisons

Hochberg metod was used to correct the p-value

Mair & Wilcox (2020); Wilcox (2012)

D **Response: WEIGHT (mg)**

	psihat	ci.lower	ci.upper	p.value
5x wk:Control vs. 1x wk:Control	279.11111	243.48656	314.73566	0.00000
5x wk:Control vs. 5x wk:15000	97.50159	46.61736	148.38581	0.00023
5x wk:Control vs. 1x wk:15000	310.12444	278.44186	341.80703	0.00000
1x wk:Control vs. 5x wk:15000	-181.60952	-233.11153	-130.10752	0.00000
1x wk:Control vs. 1x wk:15000	31.01333	-2.26759	64.29425	0.01384
5x wk:15000 vs. 1x wk:15000	212.62286	162.28366	262.96205	0.00000

F **Response: LENGTH (mm)**

	psihat	ci.lower	ci.upper	p.value
5x wk:Control vs. 1x wk:Control	6.79288	5.03517	8.55059	0.00000
5x wk:Control vs. 5x wk:15000	2.85387	0.94539	4.76234	0.00103
5x wk:Control vs. 1x wk:15000	7.55621	5.68658	9.42583	0.00000
1x wk:Control vs. 5x wk:15000	-3.93901	-5.23985	-2.63817	0.00001
1x wk:Control vs. 1x wk:15000	0.76333	-0.49053	2.01719	0.07176
5x wk:15000 vs. 1x wk:15000	4.70234	3.20631	6.19837	0.00001

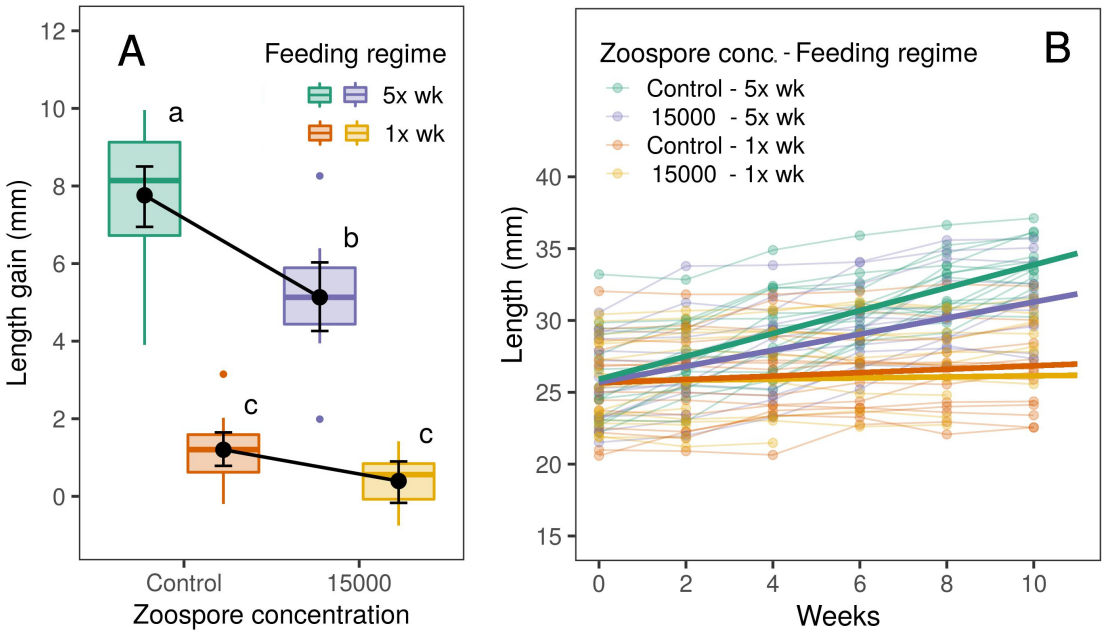
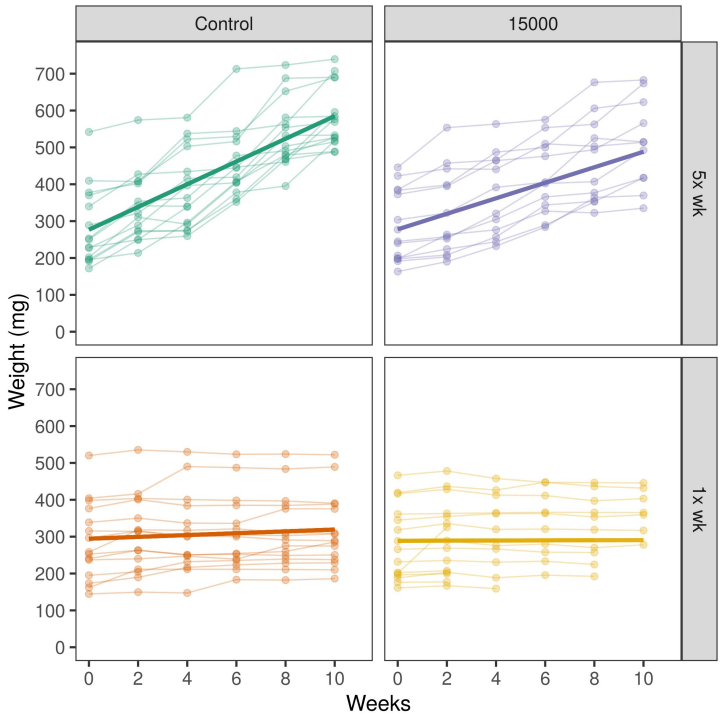
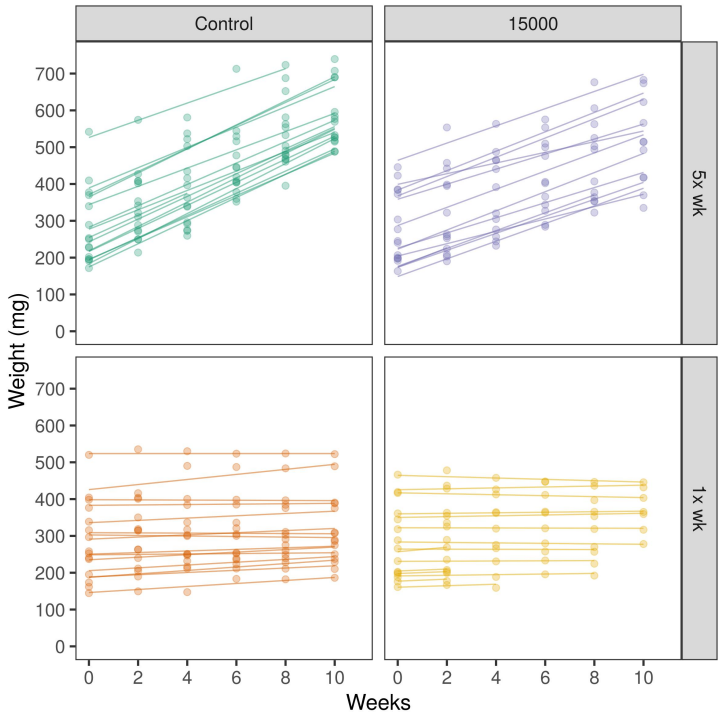


Figure S5.1: Effects of repeated infection of marbled crayfish juveniles with 15000 zoospore/ml of *A. astaci* under two feeding regimes (once a week and five times a week) on: A) total **length** gain, and B) rate of **length** increment. Significant differences in panel A are marked with different letters, errorbars represent 95% confidence intervals (CIs) around the mean.



(a) Prediction based on fixed effects



(b) Prediction based on fixed and random effects

Figure S5.2: Measured temporal trajectories of **weight** in Experiment 2 and predicted trajectories by final MLM model (Model 4).

Table S5.2: MLM model selection procedure for response variable **weight** in Experiment 2 (A). Summary statistics for final Model 4: (B) Analysis of deviance, (C) Analysis of variance, and (D) Summary statistics of random and fixed effects.

Model selection

A

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)	
Model 1	3	3853.8	3865.1	-1923.9	3847.8				
Model 2	6	3369.2	3391.9	-1678.6	3357.2	490.575	3	< 2.2e-16	***
Model 3	8	3273.8	3304.0	-1628.9	3257.8	99.439	2	< 2.2e-16	***
Model 4	12	3243.6	3288.9	-1609.8	3219.6	38.179	4	1.029e-07	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Model 4

B Analysis of Deviance Table (Type III Wald chisquare tests)

Response: WEIGHT

	Chisq	Df	Pr(>Chisq)	
(Intercept)	117.0232	1	< 2.2e-16	***
time	1065.5573	1	< 2.2e-16	***
food	0.2386	1	0.625218	
plague	0.0008	1	0.977626	
time:food	450.3308	1	< 2.2e-16	***
time:plague	47.7715	1	4.789e-12	***
food:plague	0.0166	1	0.897465	
time:food:plague	13.1208	1	0.000292	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

C Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)	
time	355709	355709	1	56.129	698.1474	< 2.2e-16	***
food	161	161	1	60.086	0.3151	0.576641	
plague	4	4	1	60.086	0.0080	0.929223	
time:food	289393	289393	1	56.129	567.9893	< 2.2e-16	***
time:plague	17478	17478	1	56.129	34.3044	2.577e-07	***
food:plague	8	8	1	60.086	0.0166	0.897896	
time:food:plague	6685	6685	1	56.129	13.1208	0.000629	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Model 4

D Summary statistics of random and fixed effects

AIC	BIC	logLik	deviance	df.resid
3243.6	3288.9	-1609.8	3219.6	310

Random effects:

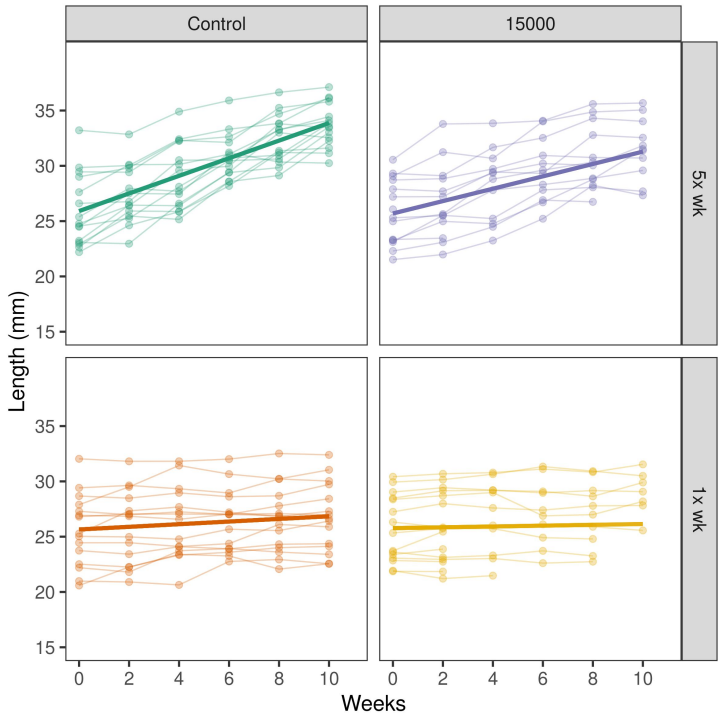
Groups	Name	Variance	Std.Dev.	Corr
id	(Intercept)	9550.866	97.729	
	time	6.093	2.468	-0.37
Residual		509.505	22.572	

Number of obs: 322, groups: id, 60

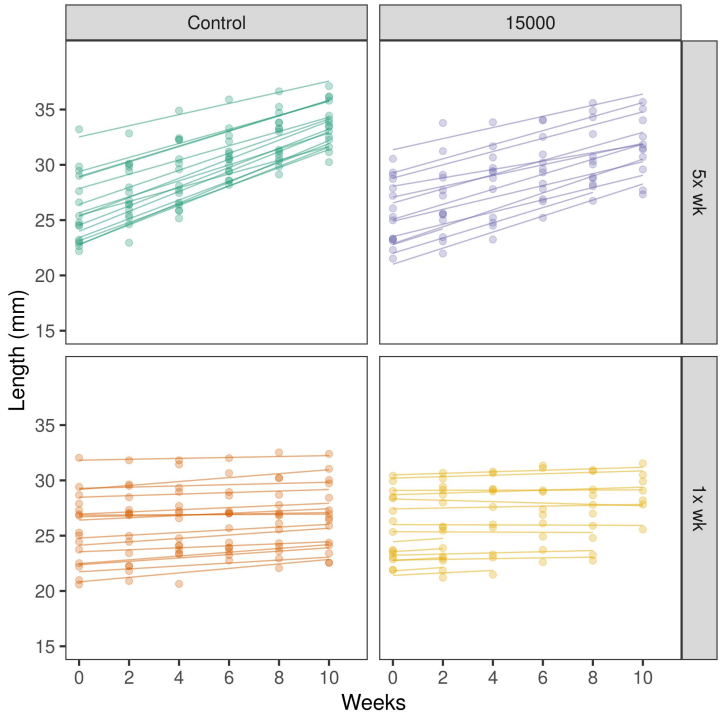
Fixed effects:

	Estimate	Std. Error	df	t value	Pr(>|t|)	
(Intercept)	276.7607	25.5840	59.8902	10.818	1.03e-15	***
time	30.8298	0.9445	50.9893	32.643	< 2e-16	***
food1xwk	17.6732	36.1809	59.8880	0.488	0.627002	
plague15000	1.0157	36.2156	60.1139	0.028	0.977719	
time:food1xwk	-28.3397	1.3355	50.9577	-21.221	< 2e-16	***
time:plague15000	-9.7848	1.4157	53.1784	-6.912	6.16e-09	***
food1xwk:plague15000	-6.5991	51.2102	60.0855	-0.129	0.897896	
time:food1xwk:plague15000	7.4780	2.0645	56.1286	3.622	0.000629	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1



(a) Prediction based on fixed effects



(b) Prediction based on fixed and random effects

Figure S5.3: Measured temporal trajectories of **length** in Experiment 2 and predicted trajectories by final MLM model (Model 4).

Table S5.3: MLM model selection procedure for response variable **length** in Experiment 2 (A). Summary statistics for final Model 4: (B) Analysis of deviance, (C) Analysis of variance, and (D) Summary statistics of random and fixed effects.

Model selection

A

	npar	AIC	BIC	logLik	deviance	Chisq	Df	Pr(>Chisq)	
Model 1	3	1535.9	1547.2	-764.95	1529.90				
Model 2	6	1118.8	1141.5	-553.40	1106.80	423.097	3	< 2.2e-16	***
Model 3	8	1029.1	1059.3	-506.56	1013.12	93.687	2	< 2.2e-16	***
Model 4	12	1007.5	1052.8	-491.74	983.47	29.646	4	5.779e-06	***

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Model 4

B Analysis of Deviance Table (Type III Wald chisquare tests)

Response: LENGTH (mm)

	Chisq	Df	Pr(>Chisq)	
(Intercept)	1095.7032	1	< 2.2e-16	***
time	613.7170	1	< 2.2e-16	***
food	0.0543	1	0.81580	
plague	0.0383	1	0.84484	
time:food	221.8144	1	< 2.2e-16	***
time:plague	24.7473	1	6.536e-07	***
food:plague	0.0463	1	0.82969	
time:food:plague	5.0367	1	0.02482	*

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

C Type III Analysis of Variance Table with Satterthwaite's method

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)	
time	236.777	236.777	1	59.136	477.4969	< 2.2e-16	***
food	0.006	0.006	1	60.066	0.0130	0.90958	
plague	0.002	0.002	1	60.066	0.0038	0.95100	
time:food	148.793	148.793	1	59.136	300.0635	< 2.2e-16	***
time:plague	10.536	10.536	1	59.136	21.2470	2.205e-05	***
food:plague	0.023	0.023	1	60.066	0.0463	0.83041	
time:food:plague	2.498	2.498	1	59.136	5.0367	0.02857	*

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Model 4

D Summary statistics of random and fixed effects

AIC	BIC	logLik	deviance	df.resid
1007.5	1052.8	-491.7	983.5	311

Random effects:

Groups	Name	Variance	Std.Dev.	Corr
id	(Intercept)	8.930896	2.98846	
	time	0.008413	0.09172	-0.63
Residual		0.495870	0.70418	

Number of obs: 323, groups: id, 60

Fixed effects:

	Estimate	Std. Error	df	t value	Pr(>|t|)	
(Intercept)	25.91037	0.78276	59.85996	33.101	< 2e-16	***
time	0.79626	0.03214	52.68150	24.773	< 2e-16	***
food1xwk	-0.25788	1.10699	59.85996	-0.233	0.8166	
plague15000	-0.21686	1.10809	60.09446	-0.196	0.8455	
time:food1xwk	-0.67699	0.04546	52.68150	-14.893	< 2e-16	***
time:plague15000	-0.23713	0.04767	56.05708	-4.975	6.55e-06	***
food1xwk:plague15000	0.33704	1.56688	60.06642	0.215	0.8304	
time:food1xwk:plague15000	0.15530	0.06920	59.13558	2.244	0.0286	*

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table S5.4: Summary statistics for expression level ($\log_2$ (Fold change)) of CS, GAPDH, C/EBP-β and ProPO genes.

CS

A **Kruskal-Wallis rank sum test**

```
Kruskal-Wallis chi-squared = 15.343
df = 3
p-value = 0.001546
```

B **Dunn (1964) Kruskal-Wallis multiple comparison**
p-values adjusted with the Holm method.

	Comparison	Z	P.unadj	P.adj
1	15000.1 - 15000.5	-3.1002304	0.001933702	0.009668508
2	15000.1 - Control.1	-1.0155927	0.309823373	0.619646747
3	15000.5 - Control.1	2.0846377	0.037102201	0.111306603
4	15000.1 - Control.5	-3.2605872	0.001111818	0.006670908
5	15000.5 - Control.5	-0.1603567	0.872600061	0.872600061
6	Control.1 - Control.5	-2.2449944	0.024768490	0.099073960

GAPDH

C **Kruskal-Wallis rank sum test**

```
Kruskal-Wallis chi-squared = 9.72
df = 3
p-value = 0.0211
```

D **Dunn (1964) Kruskal-Wallis multiple comparison**
p-values adjusted with the Holm method.

	Comparison	Z	P.unadj	P.adj
1	15000.1 - 15000.5	-2.67261242	0.007526315	0.03763158
2	15000.1 - Control.1	-1.76392420	0.077744742	0.31097897
3	15000.5 - Control.1	0.90868822	0.363514723	0.72702945
4	15000.1 - Control.5	-2.72606467	0.006409444	0.03845666
5	15000.5 - Control.5	-0.05345225	0.957371576	0.95737158
6	Control.1 - Control.5	-0.96214047	0.335979047	1.00000000

EBP1

E **Kruskal-Wallis rank sum test**

```
Kruskal-Wallis chi-squared = 14.543
df = 3
p-value = 0.002252
```

F **Dunn (1964) Kruskal-Wallis multiple comparison**
p-values adjusted with the Holm method.

	Comparison	Z	P.unadj	P.adj
1	15000.1 - 15000.5	3.260587	0.001111818	0.006670908
2	15000.1 - Control.1	0.641427	0.521245308	1.000000000
3	15000.5 - Control.1	-2.619160	0.008814655	0.035258620
4	15000.1 - Control.5	2.619160	0.008814655	0.044073275
5	15000.5 - Control.5	-0.641427	0.521245308	0.521245308
6	Control.1 - Control.5	1.977733	0.047958814	0.143876442

ProPO

G **Kruskal-Wallis rank sum test**

```
Kruskal-Wallis chi-squared = 3.2971
df = 3
p-value = 0.348
```

Table S5.5: Fisher's exact test (A) and pairwise post-hoc comparisons (B) for the proportion of dead crayfish at the end of Experiment 2.

Mortality

A **Fisher's Exact Test for Count Data**

```
Alternative hypothesis: two.sided
p =  0.0002387243
```

B **Pairwise Fisher test**

	group1	group2	n	p	p.adj	p.adj.signif
1	1500-1	1500-5	30	0.264	0.528	ns
2	1500-1	cont-1	30	0.0022	0.0132	*
3	1500-1	cont-5	30	0.0022	0.0132	*
4	1500-5	cont-1	30	0.0996	0.398	ns
5	1500-5	cont-5	30	0.0996	0.398	ns
6	cont-1	cont-5	30	1	1	ns

References

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models using *lme4*. *Journal of Statistical Software*, 67(1):1–48.

Monsalves, M. J., Bangdiwala, A. S., Thabane, A., and Bangdiwala, S. I. (2020). LEVEL (Logical Explanations & Visualizations of Estimates in Linear mixed models): recommendations for reporting multilevel data and analyses. *BMC Medical Research Methodology*, 20(1):1–9.

Oidtmann, B., Geiger, S., Steinbauer, P., Culas, A., and Hoffmann, R. W. (2006). Detection of *Aphanomyces astaci* in North American crayfish by polymerase chain reaction. *Diseases of Aquatic Organisms*, 72(1):53–64.

Peugh, J. L. (2010). A practical guide to multilevel modeling. *Journal of School Psychology*, 48(1):85–112.