

## *Ecological Archives E087-097-A2*

**Spencer R. Hall, Alan J. Tessier, Meghan A. Duffy, Marianne Huebner, and Carla E. Cáceres. 2006. Warmer does not have to mean sicker: temperature and predators can jointly drive timing of epidemics. *Ecology* 87:1684–1695.**

Appendix B. Experimental and statistical methods.

In this Appendix, we provide details on the experiments used to parameterize the  $S-I-Z$  model with predation. We also describe the statistical methods, based on maximum likelihood, used to estimate these parameters.

### Experiments

We conducted several experiments to parameterize a more realistic version of a model describing the daphniid-fungus system. First, we examined temperature-dependent transmission rate of the parasite with two laboratory experiments, and we looked for qualitatively analogous results from a field experiment. Then we quantified the virulence effects of the parasite on host survival and reproduction. Finally, we describe how we estimated spore production and release from dead hosts.

#### *Transmission rate experiments*

We conducted two replicate experiments in the laboratory to estimate temperature-dependent transmission rate of the parasite. We raised hosts for the experiment from our standard clone of *D. dentifera*. Experimental animals were born within two days of each other and were raised until 7–9 days old. Prior to the experiment, we placed six (first experiment) or five (second experiment) animals in 100 mL beakers with filtered lake water and excess food ( $> 2.5$  mg dry weight/L of *Ankistrodesmus*). We acclimated animals to their designated experimental temperatures (10, 15, 20, or 25°C) for 24 hours (10°C animals first spend eight hours at 15°C). Cross-factored with the temperature treatment, we exposed animals to four densities of laboratory-reared spores (25, 75, 150, and 500 spores per mL), eight replicates of each temperature × spore treatment, for 20 hours. After exposure to spores and every four to six days afterwards, animals were placed in fresh water. Each day, we feed animals to excess ( $> 2.5$  mg dry/L). We monitored development of infection until we reliably diagnosed hosts (at 8, 10, 16, and 30+ days for the 25, 20, 15, and 10°C treatments, respectively). We discounted animals that died before diagnosis (which rarely exceeded one animal per beaker).

To qualitatively confirm these laboratory experiments, we conducted an analogous field experiment. We placed 35 daphniid hosts, collected from Lawrence Lake (Barry County, Michigan, USA), into each one-gallon plastic jugs filled with lake water on 12 July 2004. We added algal food to these jugs, inoculated them with spores (at 25, 100, or 500 spores/mL), and incubated them at one of three different depths of Lawrence Lake as a proxy for temperature (2 m corresponding to  $26.2 \pm 0.2^\circ\text{C}$ , 4.5 m to  $19.3 \pm 0.2^\circ\text{C}$ , and 6 m to  $13.2 \pm 0.2^\circ\text{C}$ ; mean  $\pm 1$  SE over three sampling dates, each 4 days apart). We replicated each of the nine spore × temperature treatments five times. We fed animals in these jugs every four days but did not clear spores from the jugs. We diagnosed animals for infection after 8, 12, and 17 days for the 2, 4.5, and 6 meter treatments.

#### *Virulence experiments*

We estimated the virulent effects of the parasite on the survival ( $v$ ) and fecundity ( $b_I$ ) of its host by tracking

the progression of infection in four to six animals at three different temperatures (15, 20, and 25°C). We infected eight-day old animals, and then monitored them daily for production of young and survival while feeding them to excess with *Ankistrodesmus* and periodically changing the water in which they lived. We estimated  $\nu$  as the reciprocal of number of days that the infected *Daphnia* survived. To estimate  $b_I$ , we summed production of young by each animal, calculated its logarithm, and divided by number of days in which young were produced. This procedure yielded estimates of instantaneous birth rate for each animal.

### *Spore production and release rates*

To quantify spore production and release rates from hosts, we conducted a simple experiment. Fifteen heavily-infected animals were placed into 1.5 mL centrifuge tubes after having had their guts cleared with yeast. These tubes were incubated at 30°C until the daphniid hosts died (within four-five hours). Once dead, the animals were rinsed and placed in tubes filled with 1.5 mL of filtered water. Tubes were mixed gently and sampled repeatedly through time (1.5  $\mu$ L per sample); we estimated density of spores released using a haemocytometer. We ran this experiment four times, three times at 10°C and once at 25°C.

### Statistics

To estimate parameters and temperature scaling coefficients, we fit simpler forms of the host-parasite model (Eq. 1) to our experimental data. These simpler models were formed around the biology, design, and time-scale of the experiments. For instance, to estimate transmission rate, we used a simplified model to calculate anticipated prevalence of infection:

$$\frac{dS}{dt} = -\beta(T)SZ = -\beta_{20} \exp \left[ \rho T_A \left( \frac{1}{T_R} - \frac{1}{T} \right) \right] SZ \quad (\text{B.1a})$$

$$\frac{dI}{dt} = \beta(T)SZ = \beta_{20} \exp \left[ \rho T_A \left( \frac{1}{T_R} - \frac{1}{T} \right) \right] SZ \quad (\text{B.1b})$$

where we assumed that spores did not change density in the beaker during the 20 hour exposure period (i.e.,  $dZ/dt = 0$ ). This simpler model derives from the full model (Eq. 1), except now only one thing happens through time: susceptible hosts ( $S$ ) become infected ( $I$ ) by contacting spores at rate  $\beta$  times the Arrhenius function. This formulation represents transmission rate ( $\beta$ ) as a function of temperature ( $T$ ) following the Arrhenius model (Eq. 2) modified for traits of the parasite, where  $\rho$  is the parasite scaling factor, and  $\beta_{20}$  is transmission rate at reference temperature ( $T_R$ ) of 20°C. By integrating the equation from conditions at the start of the experiment forward 20 hours, we could estimate the predicted prevalence of infection,  $I/N$ . (where  $N = S + I$ ), given values of the two parameters,  $\beta_{20}$  and  $\rho$ . We assumed that the Arrhenius temperature ( $T_A$ ) of the host was 6400 (after Kooijman 1993:47).

To find the best maximum likelihood estimates (MLE) the parameters  $\beta$  and  $\rho$ , we assumed that error in prevalence of infection was distributed binomially. The binomial error distribution applies to situations in which only two outcomes (i.e., infected or not-infected) occur in trials repeated  $N$  times (where  $N$  is the number of hosts in each beaker). If  $p$  is the predicted prevalence (probability) of infection of a host, then  $I$  hosts become infected among all  $N$  hosts within a beaker with probability (Hilborn and Mangel 1997:64):

$$p(I, N) = \binom{N}{I} p^I (1-p)^{N-I} \quad (\text{B.2})$$

When infection prevalence  $p$  is calculated by integrating the skeleton model (Eq. A.1), this binomial distribution (Eq. B.2) provides the likelihood of the outcome observed in each beaker, given the data and prevalence predicted by the parameters. Over the entire experiment, one can then sum the negative log-likelihood of the results from each beaker; the MLE of the parameters ( $\beta_{20}$  and  $\rho$ ) minimize the summed negative log likelihood of the experiment (Hilborn and Mangel 1997). These MLE parameters were located using a standard search algorithm (Nelder-Mead downhill simplex) as implemented by Matlab 5.3 (Mathworks, Inc. 1999). We then calculated confidence intervals around each parameter using the likelihood profile method (Hilborn and Mangel 1997:162–167). Finally, we calculated a coefficient of determination ( $R^2$ ) following the standard formula found in general statistics books (Legendre and Legendre 1998:525):

$$R_2 = \frac{\sum_{i=1}^n (\hat{y}_i - \bar{y})^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \quad (\text{B.3})$$

where  $y_i$  are the observed values,  $\hat{y}_i$  are the values predicted by the best-fitting model, and  $\bar{y}$  is the mean value of  $n$  data points.

The procedure to estimate temperature-dependent virulence mortality ( $v$ ) and maximal birth rate of infected hosts ( $b_I$ ) was similar but simpler. To the experimental data, we fit temperature-dependent functions comprised of the parameter of interest times the Arrhenius function (Eq. 3), equipped with the additional scaling parameter ( $\rho$ ), by adding an error term,  $\varepsilon$ :

$$v(T) = v_{20} \exp \left[ \rho T_A \left( \frac{1}{T_R} - \frac{1}{T} \right) \right] \varepsilon \quad (\text{B.4a})$$

$$b_I(T) = b_{I,20} \exp \left[ \rho T_A \left( \frac{1}{T_R} - \frac{1}{T} \right) \right] \varepsilon \quad (\text{B.4b})$$

For each function, this error term proved to be best described as log-normally (multiplicatively) distributed (as determined by comparing the distributions of errors after fits with both log-normal and normal distributions). We also profiled 95% confidence intervals (Hilborn and Mangel 1997) for each estimated parameter ( $v_{20}$  and its  $\rho$  or  $b_{I,20}$  and its  $\rho$ ). Finally, we calculated  $R^2$  for each dataset following standard methods (Eq. B.3; Legendre and Legendre 1998).

To data generated from each of the four spore-release experiments, we fit a simple, nonlinear time series model (NLTSM, Turchin 2003):

$$Z_t = F(Z_{t-1}, \varepsilon_t) \quad (\text{B.5})$$

where  $Z_t$  is the density of spores per mL at time  $t$ ,  $Z_{t-1}$  is that density at one time step beforehand,  $\varepsilon_t$  is environmental variation at  $t$ , and  $F$  is a function. This function  $F$  involves integrating the differential

equation:

$$\frac{dZ}{dt} = rZ \left( 1 - \frac{Z}{\sigma I/V} \right) \quad (\text{B.6})$$

where  $I$  is the number of infected hosts, and  $V$  is the volume of water in the vial (1.5 mL). This differential equation model (Eq. B.6) closely resembles the familiar logistic model of population growth. When integrated, it represents change in spore concentration in the vial as a sigmoidal function which increases until all spores contained in the hosts ( $\sigma I/V$ ) are released. By integrating this model (Eq. B.6) between each time step of the data, we fit the NLTSM (Eq. B.5) to the time series of observed spore release (Fig. B1). We assumed that errors ( $\varepsilon_i$ ) between predicted and observed spore density at each time point were generated by normally-distributed (additive), or in one case, log-normally distributed (multiplicative) environmental noise (Hilborn and Mangel 1997, Turchin 2003). We determined which noise distribution to use by visually inspecting residuals for outliers and patterns and by creating normal quantile plots. Then, this noise distribution provided the log likelihood function we minimized to find best fitting (MLE) parameter values (using the Nelder-Mead downhill simplex method as implemented in Matlab 5.3; Mathworks, Inc. 1999). Once we found the MLE parameter values, we calculated a coefficient of prediction,  $R^2_{Pre}$ , following Turchin (2003:195):

$$R^2_{Pre} = 1 - \frac{\sum_{t=1}^n (Z_t^* - Z_t)^2}{\sum_{t=1}^n (\bar{Z} - Z_t)^2} \quad (\text{B.7})$$

where  $\bar{Z}$  is the mean spore concentration,  $Z_t^*$  is the predicted data point generated using the NLTSM (Eqs. B.5 and B.6),  $Z_t$  is the actual data point of the time series, and  $n$  is the number of datapoints used to estimate the parameters. This coefficient of prediction, described by Turchin (2003) for NLTSMs, closely resembles the familiar coefficient of determination ( $R^2$ ).

The one-step-ahead method provided strong fits to the data ( $R^2_{Pre} \geq 0.86$ ; Table B1, Fig. B1). Spore concentration per host ( $\sigma$ ) varied widely among experiments (Table B1), however, and we used the average of these values for the modeling results we presented in the text. The MLE spore release rates among 10°C treatments were quite similar, however, and the 25°C release rate was higher. This difference in release rates between temperatures looks interesting. Of course, without replication at 25°C and other temperatures, this result will await further exploration to see if release rate indeed scales predictably with temperature. These results do suggest, however, that one could model more realism in this host-parasite system by incorporating an equation that captured temperature-dependent dynamics of spores contained in the sediments to which dead hosts sink (and the resulting time lag of spore release).

#### LITERATURE CITED

Hilborn, R., and M. Mangel. 1997. *The ecological detective: confronting models with data*. Princeton University Press, Princeton, New Jersey, USA.

Legendre, P., and L. Legendre. 1998. *Numerical Ecology*. Elsevier, New York, New York, USA.

MathWorks, Inc. 1999. *Matlab 5.3: the language of technical computing*. The MathWorks, Inc., Natick, Massachusetts, USA.

Turchin, P. 2003. Complex population dynamics: a theoretical/empirical synthesis. Princeton University Press, Princeton, New Jersey, USA.

TABLE B1. Fits of transmission rate ( $\beta_{20}$ ), birth rate of infected hosts ( $b_{I,20}$ ), and parasite-induced mortality (virulence,  $v_{20}$ ), estimated at the reference temperature of 20°C, to experimental data with daphniid hosts and fungal parasites. Underlying biological models assume rates scale with temperature following a modified Arrhenius function (Eq. 3) which incorporates a scaling factor for parasites ( $\rho$ ).

Experiment		Parameter value		Parasite scaling, $\rho$		Diagnostics <sup>e</sup>			
Par. <sup>a</sup>	Ver. <sup>b</sup>	MLE <sup>c</sup>	Prof. CI <sup>d</sup>	MLE <sup>c</sup>	Prof. CI <sup>d</sup>	NLL	<i>n</i>	<i>R</i> <sup>2</sup>	LF
$\beta_{20}$	1A	3.00	2.53, 3.51	3.04	2.52, 3.59	133.63	123	0.67	Bi
$\beta_{20}$	1B	3.20	2.70, 3.75	2.69	2.11, 3.28	128.55	96	0.64	Bi
$\beta_{20}$	2A	6.87	5.93, 7.93	2.47	2.11, 2.84	195.22	128	0.54	Bi
$\beta_{20}$	2B	8.66	7.46, 10.01	1.00	0.51, 1.49	150.94	96	0.27	Bi
$b_{I,20}$	–	0.21	0.19, 0.25	1.14	0.62, 1.65	-0.41	13	0.72	LN
$v_{20}$	–	0.049	0.046, 0.054	0.97	0.63, 1.31	-4.7	13	0.87	LN

<sup>a</sup> Units of parameters:  $\beta_{20}$  L·day<sup>-1</sup>·spore<sup>-1</sup> · 10<sup>-6</sup>;  $b_{I,20}$  day<sup>-1</sup>;  $v_{20}$  day<sup>-1</sup>.

<sup>b</sup> Number (“No.,”) and version (“Ver.,”) of experiment. Version A: all four temperature treatments (25, 20, 15, and 10°C); Version B: only the three warmest treatments (25, 20, and 15°C).

<sup>c</sup> Maximum likelihood estimates (MLE) of the parameters.

<sup>d</sup> Confidence intervals (95%) estimated using the likelihood profile method of Hilborn and Mangel (1997).

<sup>e</sup> NLL: negative log-likelihood of the models fit to datasets with *n* experimental units. LF: likelihood function, where “Bi” and “LN” indicate binomially- and log-normally distributed errors, respectively, and *R*<sup>2</sup> is the coefficient of prediction.

TABLE B2. Results from fits of a nonlinear time series model (Eqs. B.5 and B.6) describing rates of release of fungal spores from dead daphniid hosts to data from laboratory experiments. This model has two parameters to estimate, release rate of spores from dead hosts ( $\gamma$ , day<sup>-1</sup>), and concentration of spores released per dead host ( $\sigma$ , spores·host<sup>-1</sup> × 10<sup>4</sup>).

		Parameters <sup>b</sup>		Diagnostics <sup>c</sup>			
Temp. <sup>a</sup>	Num. <sup>a</sup>	Release rate ( $\gamma$ )	Spore conc. ( $\sigma$ )	NLL	<i>n</i>	<i>R</i> <sub>Pred</sub> <sup>2</sup>	LF
10°C	1	0.58	4.39	30.07	10	0.86	Norm.

10°C	2	0.60	6.22	-8.01	15	0.94	LN
10°C	3	0.59	11.86	41.65	13	0.98	Norm.
25°C	–	1.02	2.67	21.72	11	0.97	Norm.

<sup>a</sup> Temperature (“Temp.”) and number (“Num.”) of experiment.

<sup>b</sup> Maximum-likelihood estimates of the parameters.

<sup>c</sup> NLL: negative log-likelihood of the models fit to truncated time series with  $n$  points.  $R_{Pred}^2$ : Coefficient of prediction, following Turchin (2003). LF: likelihood function, where “Norm.” and “LN” indicate normally and log-normally distributed errors, respectively.

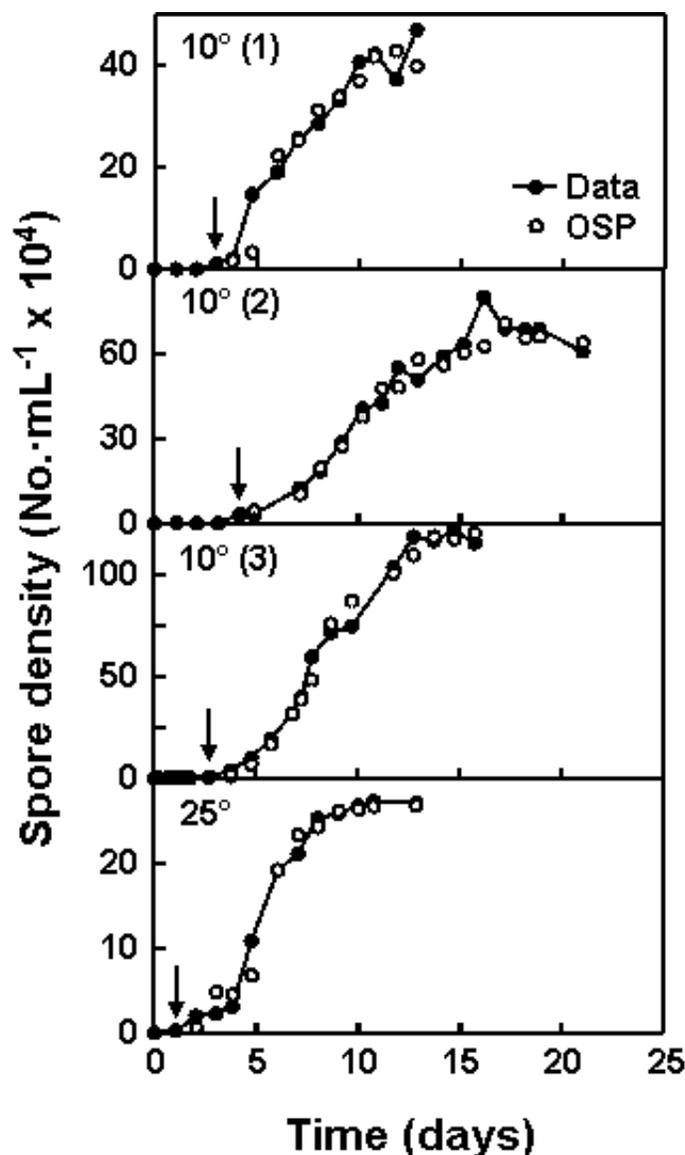


FIG. B1. Release of fungal spores from dead, infected *Daphnia* at two different temperatures (three replicates at 10°C, one replicate at 25°C). The spore-release model (Eqs. B.5 and B.6) was fit to data once time reached the arrows to minimize errors introduced early in the time

series by very low observed counts of spores. We present one-step-ahead prediction (OSP) errors with the time series data.

---

[\[Back to E087-097\]](#)