Supporting Information for

Cold tolerance strategy and lower temperature thresholds of *Lycorma delicatula* egg masses

Anna Turbelin[1], Brent Sinclair[2], John Rost[3], Amanda D. Roe[1]

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Supporting Text

Annual differences in fall acclimatization temperature

We compared the minimum, maximum and average daily temperatures in Blue Marsh Lake Pennsylvania from September, October, and November between 2021 and 2022. We used a Kruskal-Wallis chi-squared test in R (version 3.6.2, R Core Team 2022) to evaluate whether the acclimatization temperatures in each month were significantly different between years. Temperature data were acquired online in March 2024 at https://www.weather.gov/wrh from NOAA. We selected the Blue Marsh Lake weather station as it was the closest to our collection sites.

Maximum and minimum daily temperatures in Blue Marsh Lake Pennsylvania between 2021 and 2022 were not significantly different in September (maximum daily temperature Kruskal-Wallis chi-squared = 0.19, df = 1, p = 0.66; minimum daily temperature Kruskal-Wallis chi-squared = 2.11, df = 1, p = 0.15) and November (maximum daily temperature Kruskal-Wallis chi-squared = 2.65, df = 1, p = 0.10; minimum daily temperature Kruskal-Wallis chi-squared = 0.06, df = 1, p = 0.81). However, egg masses experiences significantly lower temperatures in 2022 compared to 2021 in October (maximum daily temperature Kruskal-Wallis chi-squared = 15.97, df = 1, $p < 0.00$; minimum daily temperature Kruskal-Wallis chi-squared = 21.98, df = 1, $p < 0.00$) with the average minimum daily temperature ~ 6 °C colder in 2022 than in 2021.

Handling effects on survival

In 2021, to assess the impact of egg mass manipulation on hatch rate prior to experimental treatment we removed individual eggs from egg masses (20 eggs/egg mass, n=10 masses) and compared the hatch rate of individual eggs to the remaining egg mass. First, we removed the waxy covering on each egg mass with a paint brush and removed individual eggs from the host bark taking care not to disrupt the chorion. We removed ~50% of eggs from the host bark and the remaining eggs were left on the bark. We placed individual eggs into petri dishes lined with filter paper moistened with distilled water. Manipulations of the eggs were conducted in January and February, so eggs and remaining masses were returned to +6 °C to complete diapause. Once diapause requirements were met, individual eggs and their associated egg masses were placed

in a chamber (PGC-6L4X, Percival Scientific, Inc., Perry, Iowa) set to 25 °C with 16:8h (Light:Dark) and ≥65% relative humidity to initiate hatch. We compared the hatch rate of individual eggs to the remaining undisturbed egg mass. Hatch was considered successful if a nymph fully emerged.

We used a binomial Generalized Linear Model with logit link with the function `glm()` from the `MASS` package (Venables and Ripley, 2002) in R (version 3.6.2, R Core Team 2022) to test for differences in hatch rates between disturbed and undisturbed egg masses. The probability of hatch (πi) was calculated using the following equation:

$$\pi i = \frac{e^{\eta i}}{1 + e^{\eta i}} \quad \text{where} \quad \eta i = \beta 0 + \beta i \quad \text{(Eq 1.)}$$

With β0 the intercept and βi the coefficient associated with disturbed ('removed') or intact egg masses, so for disturbed egg masses $\eta i = -0.31425 - 1.10960$ and for intact egg masses $\eta i = -0.31425$ (see **Table S1**).

We found that removing individual eggs from an egg mass negatively impacted the hatch rate of the eggs ($p < 0.0001$) (**Fig. S3, Table S1**). Intact egg masses had a 42% likelihood of hatching whilst individual eggs had a 19% likelihood of hatching. Hatch rates for intact egg masses ranged between 0% and 100% with a mean and median hatch rate of 40% and 42%, respectively. Hatch rates for removed eggs ranged between 0% and 69% with a mean and median hatch rate of 20% and 9% respectively. These results guided the downstream design of 2023 experiments.

Our results align with another study, which showed that removing the wax layer directly impacted hatch success in egg masses (Nakashita et al. 2022). Indeed, Nakashita et al. (2022) found that in natural winter conditions the hatch rate of egg masses with an intact wax coating was significantly higher than those lacking wax. Both our study and Nakashita et al. (2022) suggest that the wax layer serves a protective function for eggs in the mass. This is further supported by a study from Powel et al. (2023) which describes the hydrophobic properties of the wax, which may prevent water or ice contact during winter. Therefore, we recommend that future studies use whole egg masses with an intact waxy coating if hatch rate is used to assess survival.

Lower Lethal Temperature – YR1

We conducted a preliminary lower lethal limit experiment on whole egg masses (n = 7 per temperature) for a single exposure temperature of one hour. Between the 1st and 7th March 2022, we exposed seven egg masses to one of five temperatures (-5, -10, -15, -20, and -25 °C) for 1h. We placed egg masses individually into 15 mL Falcon tubes sealed with parafilm and placed in an airtight bag before submerging them in the bath of a refrigerated circulator (A45HC, Thermo Fisher Scientific, Newington, USA). We equilibrated the egg masses at +6 °C for 10 min, then cooled them at 0.5 °C/min to 0 °C, then 0.1 °C/min to the treatment temperature where they were held for 1 h before being rewarmed at 0.5 °C/min to +6 °C. After completing the cold exposure, we stored egg masses at 6 °C to complete chill requirement before assessing survival. Once the chilling requirement was met, we exposed egg masses to 25°C at 16:8h (Light:Dark) and ≥65% relative humidity in a Percival environmental chamber (PGC-6L4X, Percival Scientific, Inc., Perry, Iowa) to initiate hatch. Hatch was initiated on the 11th and 28th March 2022. We considered the hatch successful if a *L. delicatula* nymph emerged from the egg. Eggs that had not hatched after four weeks were considered dead.

In our preliminary experiment, 21 out of 35 egg masses hatched, ranging between four and seven per treatment (out of seven egg masses per treatment). Hatch rate per egg mass ranged between 0% and 100%. We found that all egg masses held at -25 °C for 1h failed to hatch (**Fig. S5**). Eggs hatched in all other treatments (**Table S2**), with mean hatch rate ranging from 25% (±11%) to 55% (±15%). Although the mean hatch rate at -20 °C was 25% (±11%), we observed hatch in five out of seven egg masses (**Table S2**; **Fig. S5**), with three showing hatch rates >40%.

Lower Lethal Temperature modelling

To determine which model to use to estimate the lower thermal limits (LLT_{90}, LLT_{50}, and LLT_{10}) at different exposure durations, we first compared the performance of the probit and logit link functions using the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) values derived from the respective models. Second, to select the fixed variables for inclusion in our final model we ran a binomial GLM with a probit link function considering temperature ('TreatmentTemp'), treatment duration ('TreatmentTime') and egg mass classification ('EggMassClass', categorized as either 'single' or 'clustered'). We used the `anova()` function from the `stats` package (version 3.6.2), and performed a chi-square test to evaluate the significance of the model terms and interaction between terms. We also calculated the chance of

survival in clustered and single egg masses for each temperature / time treatment using equation Eq.1 and used the `anova()` function to test for significance.

Overall, we found that models with a probit link had a slightly lower AIC and BIC than with a logit link and provided a better fit (**Table S5**). Our findings also show that both the interaction between temperature and time, and egg mass classification significantly influenced lower lethal temperature estimation at the 5% significance level (**Table S6** and **S7**). Therefore, both terms were included in our model and incorporated into function `dose.adj()` to estimate the lower lethal temperatures. This function is adapted from the `dose.p()` function from the MASS package (Venables and Ripley, 2002). Further investigation of the effect of clustered egg masses showed that the probability of an egg surviving treatment from a clustered egg mass was generally higher than from a single egg mass (**Table S8**). However, it was not the case for all temperature / time treatments and the difference was significant in 10 out of 17 treatments which had both single and clustered egg masses. We opted to include egg mass classification in our model and used the mean between the temperature estimated for clustered and single egg masses as the lower lethal temperatures for which 10% (LLT10), 50% (LLT50) and 90% (LLT90) of the eggs die.

We ran the model with 1) the raw data and 2) a dataset bounded with a theoretical LLT based on the lowest supercooling point measured (-27.7 °C) and an upper LLT0 of 10 °C based on the highest temperature reported to satisfy the egg mass chill requirement (Keena and Nielsen 2021). For the dataset with a theoretical LLT100 we added the number of eggs classified as dead equal to the mean number of eggs within a temperature/time treatment to a new temperature category of -27.7 °C for each treatment time. For example, the average total number of eggs per temperature treatment exposed to one hour was 344 so we added 344 eggs classified as dead in our theoretical dataset of one hour exposure to -27.7 °C. A similar approach was used for the LLT0 but classified as alive in our dataset.

When estimating the lower lethal limits with the raw survival data, we found that the LLT90 ranged between -17.4 °C and -26 °C across the different exposure times. The LLT50 ranged between 7.9 °C and -10.3 °C and LLT10 ranged between 5.4 °C and 33.1 °C (**Supplementary Table S19**). We introduced theoretical bounds (LLT, LLT0) to these calculations, the LLT90 increased by 2 to 8 °C across the different exposure times, whilst the LLT50 and LLT10 decreased by 1 to 23 °C. We were not able to identify the temperatures at which 0% and 100% of eggs hatch which affected the generalized linear model results and our ability to estimate lower lethal temperatures for this

species, as illustrated with our unrealistically high results for the LLT10 (**Supplementary Table S19**, no theoretical bounds). While temperature treatments could be conducted that would result in 0% hatch, the same cannot be said for 100% hatch. No studies to date have shown complete egg mass hatch, therefore egg hatch success is likely determined by a range of factors, including but not limited to temperature.

Supplementary Tables:

Table S1. Binomial generalized linear model summary output showing that removing individual eggs from egg masses negatively impacted hatch rates.

Generalized linear model binomial (probit link)

Formula: Survival ~ Fitness ~ Type

Observations: 1429

AIC	BIC	deviance	residual deviance
1759.38	1769.91	1834.9	1755.4

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.0472	-1.0472	-0.6569	1.3134	1.8109

Coefficients:

	Estimate	Std. Error	z value	p
(Intercept)	-0.31425	0.06661	-4.718	2.38e-06 ***
TypeRemoved	-1.1096	0.13076	-8.486	< 2e-16 ***

Number of Fisher Scoring iterations: 4

Table S2. Number of egg masses per treatment in YR1 (2021-2022) and YR2 (2022-2023), including the number of egg masses with eggs that hatched and the number of egg masses where no eggs hatched. Hatch percentage represents the proportion of egg masses with hatch within a treatment. Mean hatch rate represents the average number of eggs that hatched per egg mass within a treatment.

Year (YR)	Treatment Temperature (°C)	Treatment Time	Total # egg masses	# egg masses with hatch	hatch %	Mean hatch rate ±SE	Median hatch rate
1	-5	1 hour	7	5	71%	0.55 ±0.15	0.58
1	-10	1 hour	7	4	57%	0.45 ±0.16	0.62
1	-15	1 hour	7	7	100%	0.50 ±0.12	0.46
1	-20	1 hour	7	5	71%	0.25 ±0.11	0.04
1	-25	1 hour	7	0	0%	-	-
2	-5	1 hour	10	7	70%	0.39 ±0.11	0.48
2	-10	1 hour	13	10	77%	0.33 ±0.08	0.25
2	-15	1 hour	12	7	58%	0.26 ±0.08	0.26
2	-20	1 hour	10	2	20%	0.06 ±0.05	-
2	-22.5	1 hour	10	0	0%	-	-
2	-25	1 hour	12	2	17%	0.09 ±0.08	-
2	-5	12 hours	15	8	53%	0.16 ±0.07	0.01
2	-10	12 hours	11	7	64%	0.26 ±0.09	0.15
2	-15	12 hours	11	7	64%	0.34 ±0.11	0.28
2	-20	12 hours	11	1	9%	0.00 ±0.00	-
2	-22.5	12 hours	10	0	0%	-	-
2	-25	12 hours	11	0	0%	-	-
2	0	10 days	10	6	60%	0.23 ±0.08	0.16
2	-5	10 days	11	7	64%	0.27 ±0.08	0.27
2	-10	10 days	13	13	100%	0.47 ±1.1	0.50
2	-15	10 days	12	3	25%	0.09 ±0.06	-
2	-20	10 days	10	1	10%	0.07 ±0.07	-
2	0	15 days	19	14	74%	0.38 ±0.08	0.32
2	-5	15 days	10	8	80%	0.26 ±0.08	0.19
2	-10	15 days	13	8	62%	0.33 ±0.10	0.17
2	-15	15 days	11	1	9%	0.01 ±0.01	-
2	-20	15 days	12	0	0%	-	-
2	6	No exposure	22	18	82%	0.44 ±0.08	0.49

Table S3. Results from Kruskal-Wallis rank sum test when comparing egg mass hatch rate from each temperature/time treatment to hatch rate of no exposure egg masses. Values below 0.01 are bolded.

Treatment Temperature (°C)	Treatment Time (hour)	X^2	p	df
0	240	3.0362	0.0814	1
0	360	0.4854	0.4860	1
-5	1	0.4017	0.5262	1
-5	12	6.0592	0.0138	1
-5	240	1.9740	0.1600	1
-5	360	1.2595	0.2618	1
-10	1	1.3589	0.2437	1
-10	12	1.8667	0.1719	1
-10	240	0.0748	0.7845	1
-10	360	0.6833	0.4085	1
-15	1	2.8016	0.0942	1
-15	12	0.6529	0.4191	1
-15	240	10.1791	**0.0014**	1
-15	360	13.3721	**0.0003**	1
-20	1	10.7615	**0.0010**	1
-20	12	14.8651	**0.0001**	1
-20	240	10.7857	**0.0010**	1
-20	360	16.9188	**0.0000**	1
-22.5	1	14.6212	**0.0001**	1
-22.5	12	14.6212	**0.0001**	1
-25	1	9.8564	**0.0017**	1
-25	12	15.7845	**0.0001**	1

Table S4. Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) comparison between binomial generalized linear model with probit or logit links and different fixed variables. The response variable is 'Survival' and fixed variables are temperature (TreatmentTemp), treatment duration (TreatmentTime) and/or egg mass classification ('EggMassClass' – single vs. clustered).

Model Link	Formula	Year	Time	AIC	BIC
probit	Survival ~ TreatmentTemp	1	1h	1,514	1,525
logit	Survival ~ TreatmentTemp	1	1h	1,521	1,531
probit	Survival ~ TreatmentTemp * TreatmentTime	2	all	8,518	8,575
logit	Survival ~ TreatmentTemp * TreatmentTime	2	all	8,551	8,607
probit	Survival ~ TreatmentTemp * TreatmentTime + EggMassClass	2	all	8,466	8,530
logit	Survival ~ TreatmentTemp * TreatmentTime + EggMassClass	2	all	8,501	8,565
probit	Survival ~ TreatmentTemp + TreatmentTime + EggMassClass	2	all	8,478	8,520
logit	Survival ~ TreatmentTemp + TreatmentTime + EggMassClass	2	all	8,513	8,555
probit	Survival ~ TreatmentTemp + EggMassClass	2	1h	2,164	2,181
logit	Survival ~ TreatmentTemp + EggMassClass	2	1h	2,174	2191
probit	Survival ~ TreatmentTemp	2	1h	2,210	2,221
logit	Survival ~ TreatmentTemp	2	1h	2,212	2,224
probit	Survival ~ TreatmentTemp + EggMassClass	2	12h	1,631	1,648
logit	Survival ~ TreatmentTemp + EggMassClass	2	12h	1,642	1,659
probit	Survival ~ TreatmentTemp	2	12h	1,637	1,649
logit	Survival ~ TreatmentTemp	2	12h	1,649	1,661
probit	Survival ~ TreatmentTemp + EggMassClass	2	240h	2,287	2,304
logit	Survival ~ TreatmentTemp + EggMassClass	2	240h	2,291	2,308
probit	Survival ~ TreatmentTemp	2	240h	2,320	2,332
logit	Survival ~ TreatmentTemp	2	240h	2,325	2,336
probit	Survival ~ TreatmentTemp + EggMassClass	2	360h	2,335	2,352
logit	Survival ~ TreatmentTemp + EggMassClass	2	360h	2,349	2,366
probit	Survival ~ TreatmentTemp	2	360h	2,351	2,362
logit	Survival ~ TreatmentTemp	2	360h	2,365	2,376

Table S5. Binomial generalized linear model summary output showing the effect of temperature, time of exposure and the interaction between temperature and time of exposure on *L. delicatula* hatch. The response variable is 'Survival' and fixed variables are temperature (TreatmentTemp), treatment duration (TreatmentTime) and egg mass classification ('EggMassClass' – single vs. clustered). Significance. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Generalized linear model binomial (probit link)

Formula: Survival ~ TreatmentTemp * TreatmentTime + EggMassClass

Observations: 8911

AIC	BIC	deviance	residual deviance
8465.9	8529.71	9472.3	8447.9

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.2920	-0.7727	-0.4803	-0.2705	2.3680

Coefficients:

	Estimate Std.	Error z	value	p
(Intercept)	0.529685	0.074976	7.065	0.000 ***
TreatmentTemp	0.072721	0.00455	15.984	< 2e-16 ***
TreatmentTime12	-0.798844	0.100229	-7.97	0.000 ***
TreatmentTime240	-0.421831	0.088923	-4.744	0.000 ***
TreatmentTime360	-0.486317	0.084161	-5.778	0.000 ***
EggMassClass single	-0.261606	0.035715	-7.325	0.000 ***
TreatmentTemp:TreatmentTime12	-0.021953	0.006655	-3.298	0.001 ***
TreatmentTemp:TreatmentTime240	-0.023173	0.006747	-3.435	0.001 ***
TreatmentTemp:TreatmentTime360	-0.005126	0.006457	-0.794	0.427

Number of Fisher Scoring iterations: 6

Table S6. ANOVA Chi squared test results on generalized linear model output to assess the effect of temperature (TreatmentTemp), duration of exposure (TreatmentTime), the interaction between temperature and time of exposureand and egg mass classification ('EggMassClass' – single vs. clustered) on *L. delicatula* hatch. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	Deviance Resid.	Df Resid.	Dev	p
NULL			8910	9472.3	
TreatmentTemp	1	820.68	8909	8651.6	< 2.2e-16 ***
TreatmentTime	3	129.26	8906	8522.4	< 2.2e-16 ***
EggMassClass	1	56.89	8905	8465.5	4.605e-14 ***
TreatmentTemp:TreatmentTime	3	17.64	8902	8447.9	0.0005215 ***

Table S7. Probability of survival of individual eggs in an egg mass for clustered egg masses and single egg masses for each temperature / time treatment. Also shown is the p-value from the analysis of deviance (anova) Chi-squared test. Values bolded represent the higher probability of survival between clustered and single egg masses.

Treatment Time (hours)	Treatment Temperature (°C)	Probability of survival in clustered egg masses	Probability of survival in single egg masses	p
No exposure	6	**65.8%**	38.4%	0.000
360	0	**44.6%**	11.9%	0.000
1	-5	**59.2%**	42.1%	0.026
12	-5	**14.7%**	13.8%	0.756
240	-5	**37.1%**	35.5%	0.813
1	-10	34.2%	**38.5%**	0.372
12	-10	0.0%	**37.9%**	0.000
240	-10	**53.8%**	42.2%	0.012
360	-10	**51.6%**	29.2%	0.000
1	-15	**31.4%**	28.3%	0.493
12	-15	**63.6%**	34.0%	0.000
240	-15	**22.7%**	2.2%	0.000
360	-15	0.0%	**4.8%**	0.025
12	-20	0.0%	0.3%	0.530
360	-20	0.0%	0.0%	1.000
1	-25	**37.9%**	0.8%	0.000
12	-25	0.0%	0.0%	1.000

Table S8. Binomial generalized linear mixed model summary output showing the effect of temperature when exposed for 1 hour on *L. delicatula* hatch. The model includes egg mass classification ('EggMassClass' – single vs. clustered) as a fixed effect. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Generalized linear model binomial (probit link)

Formula: Survival ~ TreatmentTemp + EggMassClass

Observations: 2305

AIC	BIC	deviance	residual deviance
2163.73	2180.95	2485.7	2157.7

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.4057	-0.8483	-0.3995	-0.3324	2.4181

Coefficients:

	Estimate	Std. Error	z value	p
(Intercept)	0.689636	0.086379	7.984	1.42e-15 ***
TreatmentTemp	0.072774	0.004592	15.849	< 2e-16 ***
EggMassClass single	-0.479935	0.070053	-6.851	7.33e-12 ***

Number of Fisher Scoring iterations: 5

Table S9. Binomial generalized linear mixed model summary output showing the effect of temperature when exposed for 12 hours on *L. delicatula* hatch. The model includes egg mass classification ('EggMassClass' – single vs. clustered) as a fixed effect. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Generalized linear model binomial (probit link)

Formula: Survival ~ TreatmentTemp + EggMassClass

Observations: 2324

AIC	BIC	deviance	residual deviance
1630.79	1648.04	1768.7	1624.8

Deviance Residuals:

Min	1Q	Median	3Q	Max
-0.8316	-0.6610	-0.3823	-0.2764	2.3032

Coefficients:

	Estimate	Std. Error	z value	p
(Intercept)	-0.47316	0.078569	-6.022	1.72e-09 ***
TreatmentTemp	0.061707	0.005224	11.813	< 2e-16 ***
EggMassClass single	0.23506	0.08278	2.84	0.00452 **

Number of Fisher Scoring iterations: 6

Table S10. Binomial generalized linear mixed model summary output showing the effect of temperature when exposed for 10 days on *L. delicatula* hatch. The model includes egg mass classification ('EggMassClass' – single vs. clustered) as a fixed effect. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Generalized linear model binomial (probit link)

Formula: Survival ~ TreatmentTemp + EggMassClass

Observations: 2000

AIC	BIC	deviance	residual deviance
2286.93	2303.73	2406.8	2280.9

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.1996	-0.8995	-0.6121	1.2970	2.0878

Coefficients:

	Estimate	Std. Error	z value	p
(Intercept)	0.291889	0.087911	3.32	0.000899 ***
TreatmentTemp	0.051847	0.005085	10.196	< 2e-16 ***
EggMassClass single	-0.46508	0.07757	-5.996	2.03e-09 ***

Number of Fisher Scoring iterations: 4

Table S11. Binomial generalized linear mixed model summary output showing the effect of temperature when exposed for 15 days on *L. delicatula* hatch. The model includes egg mass classification ('EggMassClass' – single vs. clustered) as a fixed effect. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Generalized linear model binomial (probit link)

Formula: Survival ~ TreatmentTemp + EggMassClass

Observations: 2282

AIC	BIC	deviance	residual deviance
2334.76	2351.96	2606.4	2328.8

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.2077	-0.8254	-0.4800	1.1474	2.1062

Coefficients:

	Estimate	Std. Error	z value	p
(Intercept)	0.044514	0.054072	0.823	0.41
TreatmentTemp	0.067553	0.004671	14.463	< 2e-16 ***
EggMassClass single	-0.26402	0.062536	-4.222	2.42e-05 ***

Number of Fisher Scoring iterations: 5

Table S12. Binomial generalized linear mixed model summary output showing the effect of temperature, time of exposure and the interaction between temperature and time of exposure on *L. delicatula* hatch. The response variable is 'Survival' and fixed variables are temperature (TreatmentTemp), treatment duration (TreatmentTime) and egg mass classification ('EggMassClass' – single vs. clustered). Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Generalized linear model binomial (probit link)

Formula: Survival ~ TreatmentTemp * TreatmentTime + EggMassClass

Observations:

AIC	BIC	deviance	residual deviance
9043.36	9109.66	14066.3	9025.4

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.3831	-0.6770	-0.2566	0.4857	2.6191

Coefficients:

	Estimate Std.	Error z	value	p
(Intercept)	0.780516	0.05766	13.537	< 2e-16 ***
TreatmentTemp	0.09726	0.003288	29.584	< 2e-16 ***
TreatmentTime12	-0.466029	0.066885	-6.968	3.22e-12 ***
TreatmentTime240	-0.369369	0.062714	-5.89	3.87e-09 ***
TreatmentTime360	-0.510881	0.061175	-8.351	< 2e-16 ***
EggMassClass single	-0.116831	0.03522	-3.317	0.000909 ***
TreatmentTemp:TreatmentTime12	0.004962	0.004837	1.026	0.304962
TreatmentTemp:TreatmentTime240	-0.00503	0.004742	-1.061	0.288782
TreatmentTemp:TreatmentTime360	0.00678	0.004913	1.38	0.167602

Number of Fisher Scoring iterations: 7

Table S13. ANOVA Chi squared test results on generalized linear model output to assess the effect of temperature (TreatmentTemp), duration of exposure (TreatmentTime), the interaction between temperature and time of exposure and and egg mass classification ('EggMassClass' – single vs. clustered) on *L. delicatula* hatch. Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

	Df	Deviance Resid.	Df Resid.	Dev	p
NULL			11690	14066.3	
TreatmentTemp	1	4830.6	11689	9235.6	< 2.2e-16 ***
TreatmentTime	3	191	11686	9044.6	< 2.2e-16 ***
EggMassClass	1	13	11685	9031.6	0.0003125 ***
TreatmentTemp:TreatmentTime	3	6.2	11682	9025.4	0.100155

Table S14. Binomial generalized linear mixed model summary output showing the effect of temperature when exposed for 1 hour on *L. delicatula* hatch. The model includes egg mass classification ('EggMassClass' – single vs. clustered) as a fixed effect. Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Generalized linear model binomial (probit link)

Formula: Survival ~ TreatmentTemp + EggMassClass

Observations: 2985

AIC	BIC	deviance	residual deviance
2246.05	2264.06	3602.6	2240.1

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.5767	-0.6464	-0.3276	0.3414	2.6390

Coefficients:

	Estimate	Std. Error	z value	p
(Intercept)	1.051065	0.075341	13.951	< 2e-16 ***
TreatmentTemp	0.098682	0.003311	29.808	< 2e-16 ***
EggMassClass single	-0.45409	0.070708	-6.422	1.34e-10 ***

Number of Fisher Scoring iterations: 6

Table S15. Binomial generalized linear mixed model summary output showing the effect of temperature when exposed for 12 hours on *L. delicatula* hatch. The model includes egg mass classification ('EggMassClass' – single vs. clustered) as a fixed effect. Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Generalized linear model binomial (probit link)

Formula: Survival ~ TreatmentTemp + EggMassClass

Observations: 3004

AIC	BIC	deviance	residual deviance
1748.01	1766.03	3098.8	1742.0

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.0963	-0.5116	-0.2298	-0.1143	2.4790

Coefficients:

	Estimate	Std. Error	z value	p
(Intercept)	-0.1169	0.071536	-1.634	0.102
TreatmentTemp	0.104035	0.003626	28.691	< 2e-16 ***
EggMassClass single	0.515715	0.077761	6.632	3.31e-11 ***

Number of Fisher Scoring iterations: 7

Table S16. Binomial generalized linear mixed model summary output showing the effect of temperature when exposed for 10 days on *L. delicatula* hatch. The model includes egg mass classification ('EggMassClass' – single vs. clustered) as a fixed effect. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Generalized linear model binomial (probit link)

Formula: Survival ~ TreatmentTemp + EggMassClass

Observations: 2720

AIC	BIC	deviance	residual deviance
2452.72	2470.45	3505.7	2446.7

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.3776	-0.7391	-0.3111	0.4875	2.4707

Coefficients:

	Estimate	Std. Error	z value	p
(Intercept)	0.767979	0.079713	9.634	< 2e-16 ***
TreatmentTemp	0.096263	0.003617	26.613	< 2e-16 ***
EggMassClass single	-0.51484	0.07896	-6.52	7.02e-11 ***

Number of Fisher Scoring iterations: 6

Table S17. Binomial generalized linear mixed model summary output showing the effect of temperature when exposed for 15 days on *L. delicatula* hatch. The model includes egg mass classification ('EggMassClass' – single vs. clustered) as a fixed effect. Signif. Codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Generalized linear model binomial (probit link)

Formula: Survival ~ TreatmentTemp + EggMassClass

Observations:

AIC	BIC	deviance	residual deviance
2480.18	2498.18	3715.3	2474.2

Deviance Residuals:

Min	1Q	Median	3Q	Max
-1.3355	-0.6559	-0.2394	0.4857	2.2319

Coefficients:

	Estimate	Std. Error	z value	p
(Intercept)	0.227732	0.052648	4.326	1.52e-05 ***
TreatmentTemp	0.104237	0.003663	28.455	< 2e-16 ***
EggMassClass single	-0.05032	0.06145	-0.819	0.413

Number of Fisher Scoring iterations: 7

Table S18. Lower lethal temperature (LLT90, LLT50, LLT10) of *L. delicatula* for each treatment in year 1 and 2 estimated using a binomial Generalized Linear Model (probit link) without and with added theoretical upper and lower bounds as LLT10 of 10 °C and LLT of -27.7 °C. Shown are three LLT predictions estimated by 1) breaking down our dataset into subsets by treatment duration and applying a binomial Generalized Linear Model (probit link) to each of the subsets with temperature (TreatmentTemp) and egg mass classification (EggMassClass) as fixed variables and survival as the response variable and running the `dose.p()` function in R; 2) applying a binomial Generalized Linear Model (probit link) to the whole dataset with temperature (TreatmentTemp), duration of exposure (TreatmentTime) and egg mass classification (EggMassClass) as fixed variables and survival as the response variable and adjusting the `dose.p()` function to account for the effect of time and clustered egg masses; 3) is the same as 2), but egg masses are treated individually (i.e 'single') and 4) is the average between outputs in 2) and 3).

Year (YR)	Treatment Time	LT_{90} ±SE	LT_{50} ±SE	LT_{10} ±SE	LT_{90} ±SE [Theoretical bounds]	LT_{50} ±SE [Theoretical bounds]	LT_{10} ±SE [Theoretical bounds]
1) GLM formula for each treatment time: Survival ~ TreatmentTemp + EggMassClass							
1	1h	-26.0 ±0.9	-10.3 ±0.5	5.4 ± 1.4	-23.3 ±0.5	-10.8 ±0.4	1.8 ±07
2	1h	-27.1 ±1.2	-9.5 ±0.9	10.3 ±1.6	-23.6 ±0.7	-10.7 ±0.6	2.3 ±0.8
2	12h	-13.1 ±1.1	7.7 ±1.7	30.5 ±2.8	-11.2 ±0.7	1.1 ±0.7	13.4 ±1.0
2	10 days	-30.3 ±2.3	-5.6 ±1.4	23.7 ±2.4	–21.3 ±0.8	-8.0 ±0.7	5.3 ±0.9
2	15 days	-19.6 ±1.2	-0.7 ±0.8	18.3 ±2.1	-14.5 ±0.6	-2.2 ±0.5	10.1 ±0.7
2) GLM formula: Survival ~ TreatmentTemp * TreatmentTime + EggMassClass (EggMassClass = 'clustered')							
2	1h	-24.9 ± 0.9	-7.3 ± 0.7	10.3 ± 1.6	-21.2 ± 0.5	-8 ± 0.4	5.2 ± 0.7
2	12h	-19.9 ± 0.7	5.3 ± 1.3	30.5 ± 2.8	-15.6 ± 0.4	-3.1 ± 0.5	9.5 ± 0.9
2	10 days	-28 ± 1.1	-2.2 ± 0.9	23.7 ± 2.4	-18.4 ± 0.5	-4.5 ± 0.5	9.4 ± 0.8
2	15 days	-19.6 ± 0.7	-0.6 ± 1	18.3 ± 2.1	-14.9 ± 0.4	-2.6 ± 0.5	9.7 ± 0.9
3) GLM formula: Survival ~ TreatmentTemp * TreatmentTime + EggMassClass (EggMassClass = 'single')							
2	1h	-21.3 ± 0.7	-3.7 ± 0.8	13.9 ± 1.8	-21.2 ± 0.5	-8 ± 0.4	5.2 ± 0.7
2	12h	-14.8 ± 0.6	10.5 ± 1.6	35.7 ± 3.1	-14.5 ± 0.4	-1.9 ± 0.5	10.6 ± 0.9
2	10 days	-22.8 ± 0.8	3.1 ± 1.2	29 ± 2.7	-17.1 ± 0.4	-3.2 ± 0.5	10.7 ± 0.9
2	15 days	-15.7 ± 0.6	3.2 ± 1.2	22.2 ± 2.3	-13.8 ± 0.4	-1.5 ± 0.6	10.8 ± 0.9
4) GLM formula: Survival ~ TreatmentTemp * TreatmentTime + EggMassClass (mean)							
2	1h	-23.1 ± 0.8	-5.5 ± 0.8	12.1 ± 1.7	-21.2 ± 0.5	-8.0 ± 0.4	5.2 ± 0.7
2	12h	-17.4 ± 0.6	7.9 ± 1.5	33.1 ± 3.0	-15.0 ± 0.4	-2.5 ± 0.5	10.0 ± 0.9
2	10 days	-25.4 ± 0.9	0.5 ± 1.1	26.3 ± 2.6	-17.7 ± 0.5	-3.8 ± 0.5	10.1 ± 0.9
2	15 days	-17.7 ± 0.6	1.3 ± 1.1	20.3 ± 2.2	-14.3 ± 0.4	-2.0 ± 0.5	10.3 ± 0.9

Supplementary Figures:

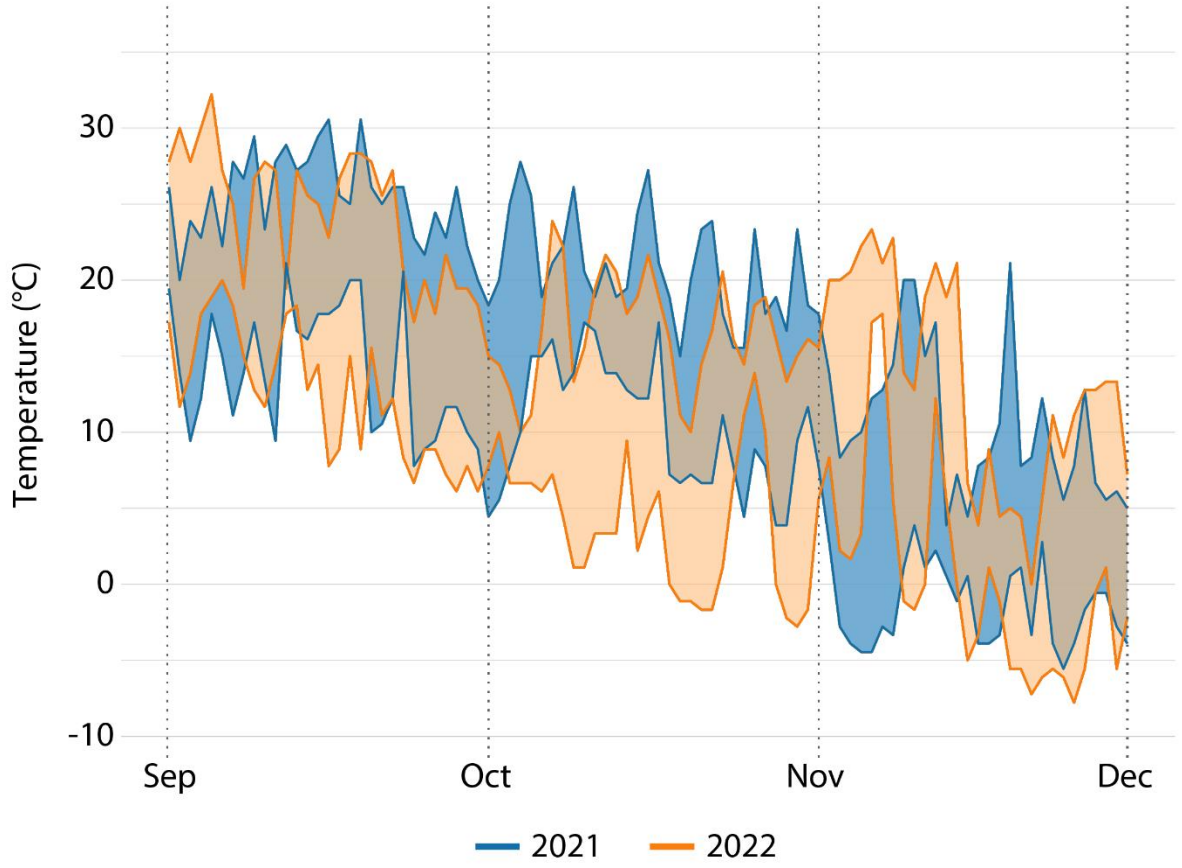

Figure S1: Minimum and maximum daily temperature between 01 September and 30 November for the years 2021 (blue) and 2022 (orange) in Blue Marsh Lake Pennsylvania. Data from the National Weather service NOAA (https://www.weather.gov/wrh/Climate?wfo=phi) accessed January 2024.

Figure S2: Spotted lanternfly (*L. delicatula*) egg masses. Eggs laid in linear rows and then coated with waxy covering by females.

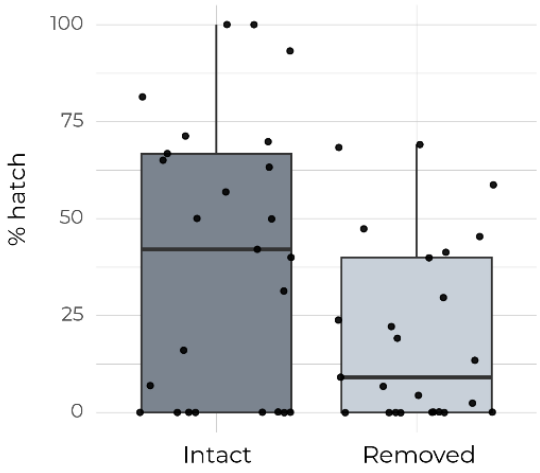

Figure S3. Percentage of hatch for intact egg masses of *Lycorma delicatula* and eggs removed from 25 egg masses. The horizontal black bar represents the 50th percentile.

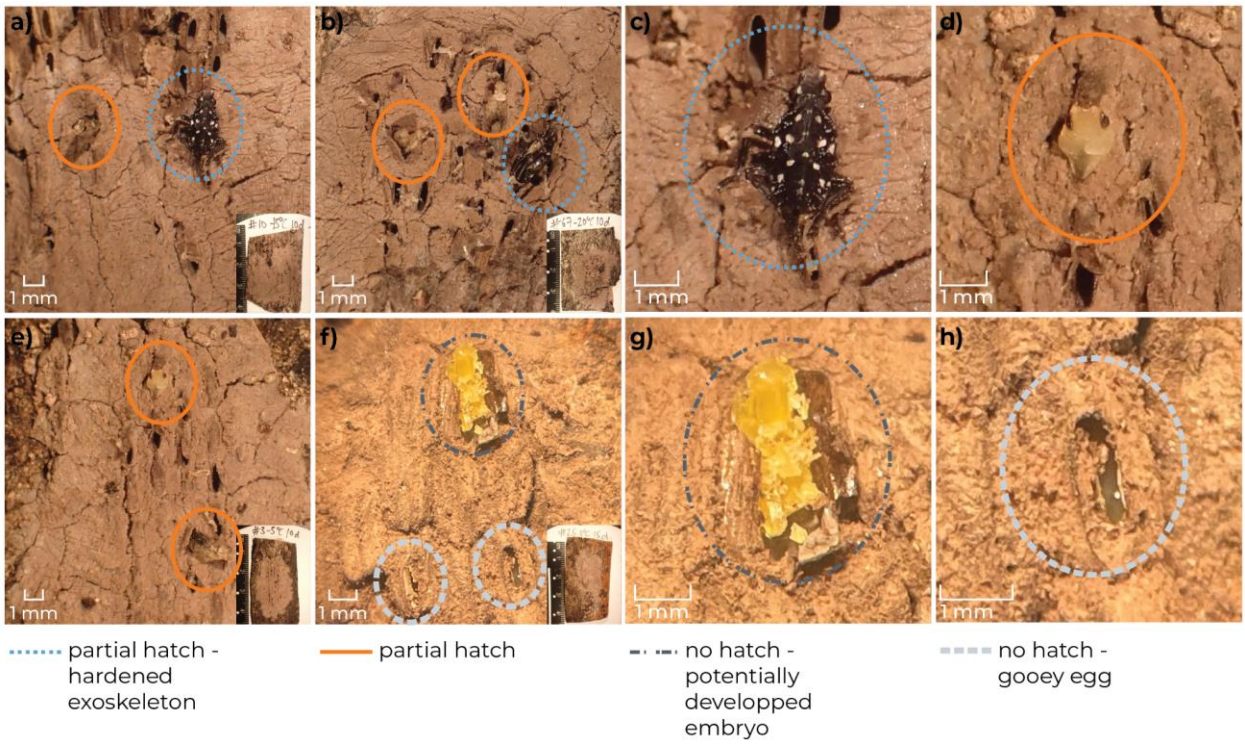

Figure S4: Spotted lanternfly (*L. delicatula*) egg masses showing partial hatch with hardened exoskeleton (blue dashed oval, a-c) and without (orange oval, a,b & e) and content of eggs with no hatch from the same egg mass showing a potentially developed embryo (dark grey dashed oval, f-d) and undeveloped gooey egg (light grey dashed oval with thicker line, f & h). Inserts show the whole egg mass, egg mass number, treatment, and scale.

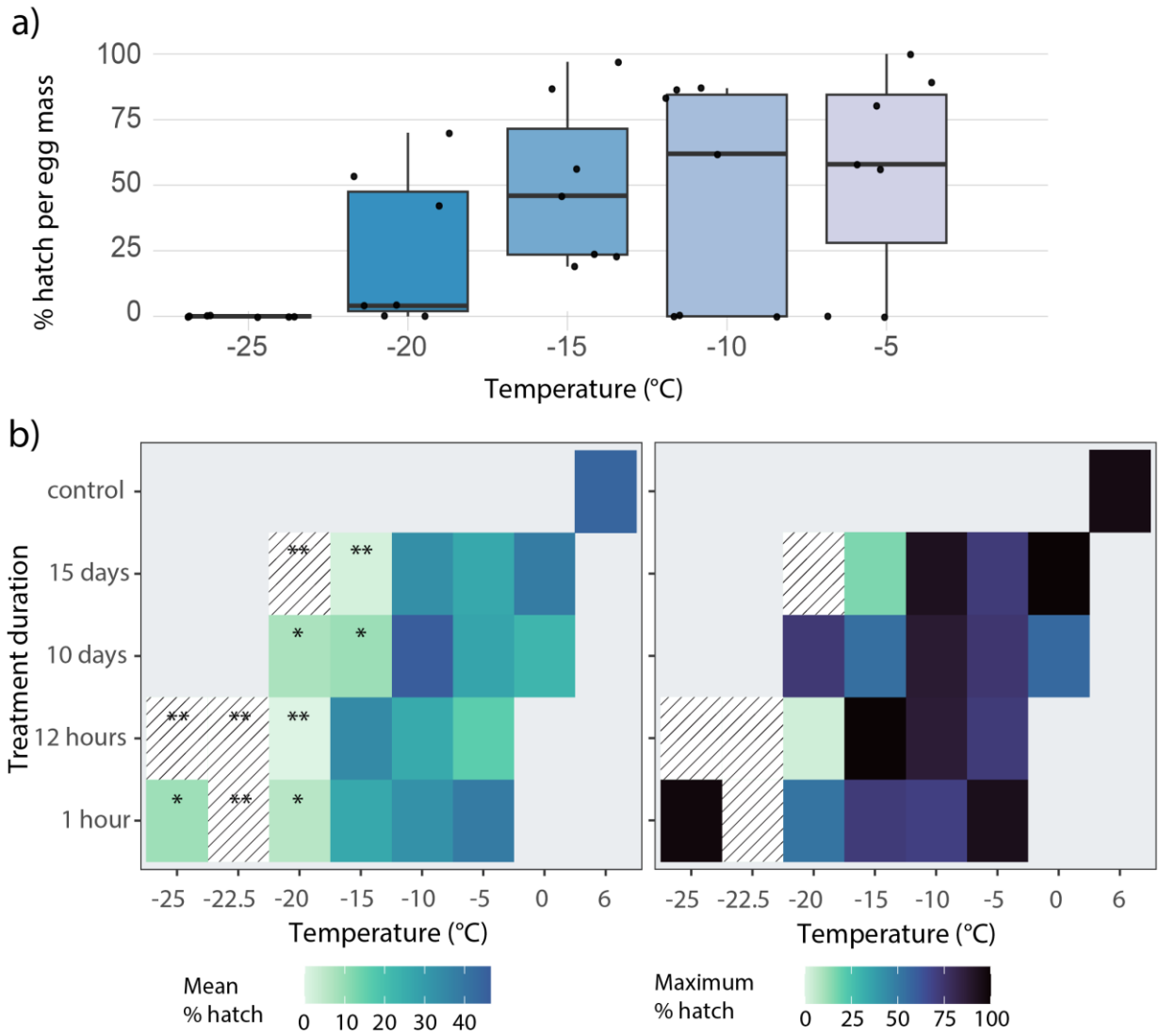

Figure S5: Percentage of hatch of *Lycorma delicatula* egg masses exposed to different temperatures (0, -5, -10, -15, -20, -22.5, -25 °C) and durations (1 hour, 12 hours, 10 days, 15 days) (n=10 egg masses per treatment) and no exposure conditions (+6.0 °C, n=15 egg masses) with a) percentage hatch per temperature treatment after one hour exposure of egg masses collected in 2021, b) mean percentage hatch and maximum percentage hatch per egg mass of egg masses collected in 2022. The asterisks in (b) are significant p-values from Kruskal-Wallis rank sum test when comparing treatments to 'no exposure' egg masses, with **p <0.001 and *p <0.01. Maximum percentage hatch refers to the highest percentage hatch for individual egg masses within a treatment. Treatments shown with hash marks indicate those where no hatch occurred.

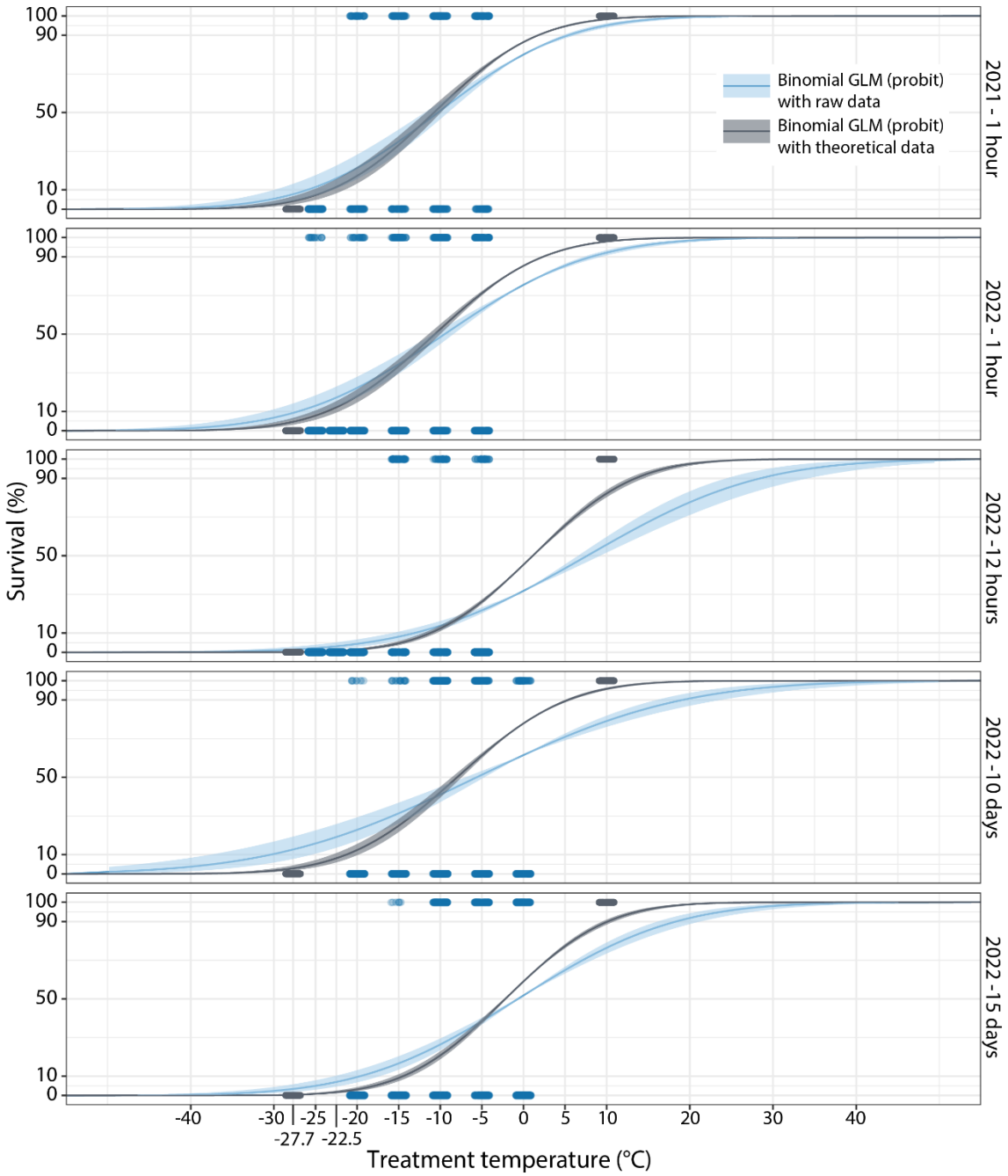

Figure S6: Binomial generalized linear model (GLM) fit by maximum likelihood curves with 95% confidence interval for each treatment time exposure of *Lycorma delicatula* egg masses collected in 2021 and 2022. Blue dots represent eggs exposed to the different time / temperature treatments and grey dots are theoretical eggs that either hatched (100% survival) or died (0% survival). The curves show the likelihood of survival for each time treatment given the temperature exposure based on the raw data (blue) and theoretical data with added upper and lower bound with LLT of -27.7 °C and LLT_{10} of 10 °C (grey). For example, an egg has close to 50% chances of surviving 1 hour exposure at -10 °C or 10 days at -5 °C.

Figure S7: Spotted lanternfly (*L. delicatula*) egg mass. New eggs were overlaid on top of old egg masses. We show egg masses a) before and b) after removing the wax layer.

Legend for Dataset S1 to S2

Dataset S1. Field and laboratory temperature exposure of *Lycorma delicatula* egg masses used in YR2 experiments. Climate data for Blue Marsh Lake Pennsylvania from NOAA (2023) online https://www.weather.gov/wrh

Dataset S2. Field temperature data from Blue Marsh Lake Pennsylvania where *Lycorma delicatula* egg masses were collected in YR1 (November 2021) and YR2 (December 2022). Climate data from NOAA (2023) online https://www.weather.gov/wrh

Dataset S3. Handling effects on survival. Results from testing the effect of removing eggs from egg masses and rearing individuals; includes hatch success of each treatment.

Dataset S4. Supercooling point (SCP) measurements. Results from exposing sets of eggs to decreasing temperatures until all eggs experience a SCP, eggs are returned to +6 °C to complete chill requirement and then eggs are warmed and checked for survival.

Dataset S5. Lower Lethal Limits assessment. Results from exposing groups of egg masses to set temperatures to identify lower lethal limits and hatch success assessment.

Dataset S6. Adjusted and Homogenized Canadian Climate Data (AHCCD) for stations near which L. delicatula was observed. Data from Environment and Climate Change Canada (Available online: https://climatedata.ca/download/#ahccd-download)

SI References

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