

SHORT COMMUNICATION



Temperature dependent survival and fecundity of *Lepidelfhax pistiae* Remes Lenicov (Hemiptera: Delphacidae), a potential biological control agent of *Pistia stratiotes* L. (Araceae)

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ABSTRACT

Lepidelfhax pistiae Remes Lenicov (Hemiptera: Delphacidae) is monophagous on *Pistia stratiotes* L. (Araceae), an invasive floating plant in Florida. Temperature studies were conducted to determine the optimal temperature for development and reproduction for this potential biological control agent. Egg development time decreased as temperature increased from 17°C to 30°C. No eggs developed and no nymphs survived at 15°C. Adult females survived the longest at 15°C, indicating that they might be more resilient to cold temperatures. Optimal temperature for nymph development was 25°C with 29% surviving to adulthood in 18.2 ± 0.4 days.

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Lepidelfhax pistiae Remes Lenicov (Hemiptera: Delphacidae) was described in 2013 from specimens collected in northern and central Argentina (Remes Lenicov & Walsh, 2013). After an initial host range test indicated that it was likely specific to *Pistia stratiotes* L. (Araceae), an invasive floating plant in Florida (Cabrera Walsh, Maestro, Sosa, & Tipping, 2014), it was imported into quarantine at the USDA-ARS Invasive Plant Research Laboratory (IPRL) in Fort Lauderdale, FL, for evaluation as a potential biocontrol agent. Additional host range and impact studies confirmed that this species is indeed specific and damaging to *P. stratiotes* (Goode et al., 2019; Remes Lenicov, Defea, Rusconi, & Cabrera Walsh, 2017).

Determining an insect's thermal requirements is essential to optimising its mass rearing, establishing it in the adventive range, and predicting its rate and range of dispersal (May & Coetzee, 2013; Remes Lenicov et al., 2017). Accordingly, *L. pistiae* was tested in a range of constant temperatures to determine the development time of eggs and nymphs, and the longevity and fecundity of adult females. *Lepidelfhax pistiae* has been kept in colony in a quarantine greenhouse at IPRL since 2013 where it has been reared on *P. stratiotes* fertilised with water soluble fertiliser (Peters Professional 24-8-16 Fertilizer,

Everris, Geldermalsen, Netherlands) at a rate of 1 g / 5 L deionised water and kept under natural light conditions, ambient temperatures (18-32°C), and 70-85% RH in the greenhouse year round.

This temperature study was conducted between 2015 and 2019. Trials were conducted concurrently in two environmental chambers (Model E36L, Percival Scientific, Perry IA) for each life stage at each temperature, with ten samples per chamber. Each temperature was repeated at least twice, however some were repeated additional times due to high mortality and loss of individuals. Chambers had a photoperiod of 12L:12D and were set to maintain a constant temperature (15°C, 20°C, 25°C, or 30°C). Additionally, 17°C was tested for eggs only as a tentative threshold for development. *Pistia stratiotes* survives at temperatures of 15-35°C (Neuenschwander, Julien, Center, & Hill, 2009), so these experiments were within reasonable temperatures for this plant species. Temperature sensors (Thermochron iButton DS1921G-F5, Maxim/Dallas iButton Products, San Jose, CA) were kept in all chambers during all trials to monitor and confirm temperature within each chamber. Each sample contained a single *P. stratiotes* rosette placed in a cylindrical 490.3 cm³ polystyrene dish, 6.5 cm in height, with a friction fitting lid. An area of 35.3 cm² of each lid was screened with chiffon fabric. Water fertilised as above was added initially and as needed through the screened lid to minimise disturbance and prevent insect escape.

In the egg development experiment, a parental cohort consisting of three 1-to-2 w old adults (2♂:1♀) was placed on the plants in the containers for 24 h and then removed. Plants were monitored twice daily for F₁ nymph emergence which was recorded, and the nymphs removed as they appeared. The experiment ended once no new nymphs emerged for two consecutive days.

For the nymphal development experiment, a single first or second instar was carefully placed on the plant. These first instars had been collected within 24 h of emergence while second instars were collected within 24 h of the first molt (as indicated by exuviae). Initially, second instars were used because first instars are very delicate and are easily injured, but once a successful protocol was developed for transferring first instars safely, they were then used for all subsequent tests. The samples were then monitored twice daily until the nymphs molted into adults or died. The dates when exuviae appeared were recorded and then removed.

The adult experiment examined female fecundity and longevity using newly molted adults (2♂:1♀) that were placed onto plants in the screened containers. The adults were moved every seven days to new plants in new containers and any mortality was recorded. If a male died, they were not replaced. The adult portion of the experiment ended with the death of the female in each sample group. The previously exposed plants were monitored for emergence of F₁ nymphs, which were counted and removed as they appeared. Previously exposed plants were discarded after no nymphs emerged for two consecutive days or after 30 days if no emergence was observed.

Data were tested for normality and equality of variances using the Shapiro-Wilk test and Levene's test in R (R Core Team, 2019). Egg, nymph, and adult data were not normally distributed and could not be successfully transformed, so the non-parametric Kruskal-Wallis test was used to evaluate differences among temperature treatments, and post-hoc pairwise comparisons were conducted using the Wilcoxon rank sum test with a Bonferroni correction. Generalised additive models were used to analyze egg and adult data only, as nymph data were limited.

The number of days from the time the parental cohort was added to the sample container to emergence of F₁ first instars was influenced by temperature ($\chi^2 = 82.3$, $df = 3$, $p < 0.001$) but not chamber ($\chi^2 = 3.48$, $df = 1$, $p = 0.06$). The pairwise Wilcoxon sign rank test indicated that development time differed significantly between temperatures (all $p < 0.001$) and the time to emergence decreased as temperature increased (Table 1). The generalised additive model found that both the intercept and temperature variable were significant ($p < 0.001$, $adj. R^2 = 0.86$), resulting in the following equation predicting the number of days spent in the egg stage:

$$\text{Days} = (-1.1 * \text{Temperature}) + 40.4$$

Development times for all instars were influenced by temperature ($\chi^2 = 8.7-59.3$, all $p < 0.05$), with 25°C having the shortest duration for all stages (Table 1). Only fourth instar duration was affected by chamber ($\chi^2 = 4.40$, $df = 1$, $p = 0.04$). Post-hoc tests could not be done on nymphs because of their overall low survival. For example, no nymphs developed at 15°C and only one nymph survived to adulthood at 30°C. Although first instar nymphs kept at 20°C had the highest survival rates initially (76% survival to second instar), more nymphs reached the adult stage at 25°C, with 29% developing into adults compared to 25% at 20°C.

Adult female longevity, fecundity, and the number of nymphs emerged per week were influenced primarily by temperature and secondarily by chamber (Table 2). The effect of the environmental chamber was significant in this particular set of experiments because a third chamber was added for one trial at 15°C and one at 20°C, but not for any other temperatures. Temperatures always vary slightly among chambers, but all averaged $\leq \pm 1.4^\circ\text{C}$ from the target temperature in this experiment.

Although adults survived longest at 15°C (3.6 ± 0.3 weeks, pairwise Wilcoxon sign rank test with Bonferroni correction for 15°C versus 25°C and 30°C: $p < 0.01$), no nymphs

Table 1. Mean (\pm SE) duration (days) of each life stage, percent survival of nymphs, and reproductive rate and longevity (weeks) of females in temperature studies. While each trial was started with 20 samples, due to non-experimental mortality and loss of individuals, the number reported and used for analysis was generally less than what was started initially.

Life Stage	Variable	15°C	17°C	20°C	25°C	30°C
Eggs	Duration	0	23.53 \pm 0.31	17.69 \pm 0.57	10.65 \pm 0.22	8.40 \pm 0.17
Nymphs	N	50		80	68	60
1st Instar	Duration	15*		7.87 \pm 0.37	3.76 \pm 0.24	6.15 \pm 0.63
	Survival	2*		76 \pm 0.05	72 \pm 0.05	33 \pm 0.06
2nd Instar	Duration	0		5.53 \pm 0.28	3.60 \pm 0.27	5.09 \pm 0.45
	Survival	0		64 \pm 0.05	51 \pm 0.06	18 \pm 0.05
3rd Instar	Duration	0		6.29 \pm 0.38	3.65 \pm 0.32	4.57 \pm 0.52
	Survival	0		51 \pm 0.06	38 \pm 0.06	12 \pm 0.04
4th Instar	Duration	0		6.11 \pm 0.47	3.76 \pm 0.25	6.80 \pm 0.95
	Survival	0		34 \pm 0.05	25 \pm 0.05	8 \pm 0.04
5th Instar	Duration	0		6.87 \pm 0.77	4.27 \pm 0.27	7*
	Survival	0		19 \pm 0.04	22 \pm 0.05	2*
Total Nymph	Duration	0		32.30 \pm 0.54	18.25 \pm 0.45	22*
	Survival	0		25 \pm 0.05	29 \pm 0.06	2*
Adult Female	N	40		45	84	60
	Longevity	3.6 \pm 0.33		2.82 \pm 0.32	2.32 \pm 0.15	1.23 \pm 0.09
	Reproductive rate	0		53.76 \pm 8.87	88.46 \pm 9.11	15.4 \pm 2.59
	Nymphs/Week	0		14.81 \pm 2.31	34.65 \pm 3.33	10.52 \pm 2.15

*Only one nymph survived, so only one value is given. Only eggs were tested at 17°C.

Table 2. Kruskal-Wallis rank sum test of adult female longevity, total number of nymphs, and number of nymphs per week.

	Variable	χ^2	df	<i>p</i>
Temperature	♀ Longevity	45.31	3	<0.001
	Total Nymphs	74.52	3	<0.001
	Nymphs/Week	69.95	3	<0.001
Chamber	♀ Longevity	5.31	2	0.07
	Total Nymphs	9.32	2	0.009
	Nymphs/Week	12.76	2	0.002

emerged at that temperature in this test or in the egg test. Longevity decreased as the temperature increased with females kept at 30°C having the shortest lifespan (1.23 ± 0.09 weeks, pairwise Wilcoxon sign rank test with Bonferroni correction for 30°C versus all other temperatures: $p < 0.01$). At 30°C, 31.6% of adult females did not survive past one week and of those that did, 41.4% did not produce any offspring at all. In this experiment, plants deteriorated faster at higher temperatures, but all plants were still alive and floating at the end of each experiment.

Generalised additive models found both the intercept and temperature variable significant ($p \ll 0.001$, adj. $R^2 = 0.145$) for female longevity resulting in the equation:

$$\text{Female longevity(weeks)} = (-0.16 * \text{Temperature}) + 6.2$$

Females at 15°C and 30°C produced significantly fewer total nymphs than the females at 20°C and 25°C (pairwise Wilcoxon sign rank test with Bonferroni correction for 15°C versus all other temperatures: $p \ll 0.001$; pairwise Wilcoxon sign rank test with Bonferroni correction for 30°C versus all other temperatures: $p < 0.01$). Nymph emergence per week was similar at 20°C and 30°C (Table 1). However, because of the shorter female lifespan at 30°C, fewer total nymphs were produced. Females kept at 25°C produced more nymphs per week than those kept at other temperatures (pairwise Wilcoxon sign rank test with Bonferroni correction for 25°C versus 20°C and 30°C: $p < 0.05$) and they also had a comparable life span to those kept at 20°C. Overall, the data suggest that, with shorter development time for nymphs, 25°C would be the optimal temperature for mass production of this insect.

In the model for the nymphs emerged per week only the intercept was significant ($p = 0.003$, adj. $R^2 = 0.005$) indicating that temperature did not predict this variable well despite significant differences using Kruskal-Wallis testing. One possible explanation is unaccounted variation in female age that was less responsive to temperature. Female longevity and nymphs emerged per week are both variables that influence total number of nymphs produced. In the model for total nymphs produced, neither variable alone was significant ($p = 0.76$ and $p = 0.65$, respectively) but the model was highly predictive (adj. $R^2 = 0.99$) because of the interaction factor of the two ($p \ll 0.001$):

$$\text{Total nymphs produced} = 0.99 * \text{Nymphs per week} * \text{Female longevity}$$

The optimal temperature of 25°C for development found in this study aligns with other Delphacid species, including *Peregrinus maidis* (Ashmead) (Hemiptera: Delphacidae), whose optimal development temperatures are between 21.1°C and 26.7°C (Tsai & Wilson, 1986) and *Nilaparvata lugens* (Stål) (Hemiptera: Delphacidae) whose optimal development temperatures are 25–30°C (Krishnaiah et al., 2005). In *Megamelus scutellaris*

Berg (Hemiptera: Delphacidae), which is native to the same region as *L. pistiae*, the optimal temperature for instar development was also 25°C (May & Coetzee, 2013). Similar to *L. pistiae*, *M. scutellaris* egg development time also decreases with increased temperature, but at the highest tested temperature (30°C) 1st instars do not survive (May & Coetzee, 2013).

The limited survival of *L. pistiae* females at the highest temperature tested, 30°C, in conjunction with the longer lifespan at 15°C is a pattern seen in other Delphacid species. *Peregrinus maidis* can survive 96.6 ± 15.6 days and 107.6 ± 6.2 days for males and females, respectively, at 15.6°C but only 10-20% as long at 32.2°C (Tsai & Wilson, 1986). *Nilaparvata lugens* also has a longer adult lifespan at cooler temperatures, 19.6 ± 0.7 days at 15°C and 30.0 ± 1.1 days at 20°C, but only one half to one third as long at higher temperatures. The trend towards increased reproductive rate at higher temperatures with sharp declines as the maximum threshold is reached also occurs in *N. lugens* (Krishnaiah et al., 2005).

Evaluating insect responses to constant temperature may not be predictive of the continuum of *L. pistiae* responses to environmental conditions in Florida but it does provide broad guidance and prediction of the natural history of this species, namely, that the shortest development time, the highest nymph survival rate, and the most nymphs produced per female per week should occur at 25°C. These experiments also predict that adults could persist up to a month or more through colder conditions, but require at least temperatures of 17°C for eggs to develop. Any mass production regimen for this species should include a higher temperature setting for egg development along with lower temperatures for nymph development and adult reproduction. This information can also be used to inform other biocontrol activities, including climate matching of potential release locations, modelling of establishment and dispersal, and integration into existing pest management programmes.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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