1 Biological Control

2 **Reproductive and developmental biology of the emerald ash borer parasitoid *Spathius***
3 ***galinae* (Hymenoptera: Braconidae) as affected by temperature**

4 Timothy J. Watt,^{1,2*} Jian J. Duan,² Douglas W. Tallamy,¹ Judy Hough-Goldstein,¹ Thomas W.
5 Ilvento,³ Xin Yue,³ and Hui Ren³

6 ¹Department of Entomology and Wildlife Ecology, University of Delaware, Newark, DE 19713

7 ² USDA ARS, Beneficial Insects Introduction Research Unit, Newark, DE 19713

8 ³Department of Applied Economics and Statistics, University of Delaware, Newark, DE 19713

9 *Corresponding author: tjwatt@udel.edu

10
11 Phone: (231) 632-3081

12 Fax: (302) 737-6780

13 Corresponding address: 77 University Avenue

14 New Castle, DE 19720

15
16 Keywords: *Agrilus planipennis*, mass-rearing, life tables, biological control, fecundity, survival

Abstract

Emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is an invasive pest of serious concern in North America. To complement ongoing biological control efforts, *Spathius galinae* Belokobylskij and Strazenac (Hymenoptera: Braconidae), a recently-described specialist parasitoid of emerald ash borer larvae, is being released for the first time in the United States. To better understand its reproductive and developmental biology, and to improve outcomes of mass-rearing, we investigated the effects of constant ambient temperature (15, 20, 25, 30, and 35°C) on immature development time, sex ratio, pre-oviposition period, fecundity and survivorship. Results show that between 20 and 30°C immature development time was inversely associated with temperature, with adults emerging $\approx$ 38, 32, and 25 d after oviposition at 20, 25, and 30°C, respectively. At 15°C larvae developed to 5th instars and spun cocoons before development was arrested, while at 35°C parasitoid eggs desiccated and did not hatch. Sex ratio of adult progeny that were exposed to different temperatures, from eggs, was not affected. When newly ($\leq$ 24 h-old) emerged female parasitoids were provided larval hosts throughout their lifetimes, adults parasitized at all temperatures except 35°C, although parasitism rate was highest (35%), as was lifetime fecundity (43 progeny/female), when females lived at 25°C. Survivorship was inversely associated with temperature, with ovipositing females living for >60 d when maintained at 15°C, and females held at 35°C living for <10 d. Life table analyses showed *S. galinae* had the highest net reproductive rate ($R_0=27.8$) and intrinsic rate of increase ($r_m=0.29$) when female parasitoids were maintained at 25°C for their lifetimes, suggesting this is the optimal temperature to rear *S. galinae*.

40

41 **1. Introduction**

42 Emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) is one of the
43 most ecologically and economically devastating insect pests to ever invade North America
44 (Gandhi and Herms, 2010; Herms and McCullough, 2014; Kovacs et al., 2010). Since its initial
45 detection in southeast Michigan in 2002, the wood-borer has spread rapidly throughout the
46 Midwest and eastern United States, including southeastern Canada, facilitated primarily by
47 movements of infested timber wood or nursery stock. In 2003 a classical biological control
48 program for emerald ash borer was initiated in the United States that has centered on the
49 introduction of three natural enemy parasitoids from a region of the pest's indigenous range in
50 China (Duan et al., 2014; Liu et al., 2003). To complement these efforts, *Spathius galinae*
51 Belokobylskij and Strazenac (Hymenoptera: Braconidae), a specialist parasitoid of emerald ash
52 borer larvae (Duan et al., 2015), is being released for the first time in the United States (USDA
53 APHIS, 2015). *S. galinae* was first described in 2009 and originates from the Vladivostok region
54 of the Russian Far East (Belokobylskij et al., 2012). Climate matching (Duan et al., 2012)
55 indicates it is well-suited for introduction in northern regions of the United States where ash is
56 abundant and where the Chinese congener *Spathius agrili* Yang (Hymenoptera: Braconidae) has
57 had little success (Hooie et al., 2015). Compared to *Tetrastichus planipennisi* Yang
58 (Hymenoptera: Eulophidae), currently the most successful Chinese parasitoid (Duan et al.,
59 2013a), which has a short (≤ 2.5 mm) ovipositor and is capable of attacking larvae only in small
60 ash trees, *S. galinae* has an ovipositor up to twice as long, allowing it to attack hosts in large,
61 reproductive-age trees (Abell et al., 2012).

Key to any classical biological control program is understanding how climatic factors affect the ability of an introduced organism to establish and proliferate (Hoelmer and Kirk, 2005). For example, a parasitoid's invasion success may reasonably be predicted if it is known how temperature impacts estimates of population growth (Pilkington et al., 2014). Insects are ectothermic; that is, their physiological and metabolic functions, including growth and development of immatures and egg production of adults, are controlled directly by temperature (Patton, 1963; Taylor, 1981). Of all the climatic variables, temperature is perhaps the most influential on insect distribution, reproduction, development, and survival (Baffoe et al., 2012; Bale et al., 2002; Ju et al., 2011; Infante 2000). Thus, identifying which temperatures favor rapid population growth is important not only for optimizing release strategies (Pilkington and Hoddle, 2006a), but this knowledge is critical for mass-rearing programs that aim to produce the most quality (fittest) biological control agents in the shortest time frames (Qiu et al., 2012).

A commonly employed tool for studying the effects of temperature on reproductive and developmental parameters of insects used in biological control is a fertility life table (Force and Messenger, 1964; Pratissoli and Parra, 2000; Staubli Dreyer et al., 1997). Fertility life tables summarize data from individuals (e.g. fecundity and survivorship) to describe how key demographic parameters, such as net reproductive rate (R_0), generation time (T_c), intrinsic rate of increase (r_m), and population doubling time (T_d) are affected by changes in temperature. A major shortcoming of life table studies, however, is that data collected on individuals are compiled to make single estimates for life table terms (from which demographic parameters are calculated). For these studies achieving replication is costly and time and labor-intensive, but without replication estimating variance and making statistical comparisons is impossible. To overcome

this, re-sampling techniques such as jackknifing or bootstrapping may be applied to life table data to create pseudo-replicates and obtain estimates of variance (Meyer et al., 1986).

S. galinae is a gregarious idiobiont ectoparasitoid of late (2nd-4th) instar emerald ash borer larvae with typically multiple (2-15) progeny developing on a host (Duan et al., 2014; USDA APHIS, 2015). Because this parasitoid is not native to the United States, much of what is currently known of its life history results from laboratory studies conducted in quarantine. Recently, Duan et al. (2014) investigated the developmental and reproductive biology of *S. galinae* under normal laboratory rearing conditions ($25 \pm 1^{\circ}\text{C}$, $\approx 65\%$ RH, 16: 8 h L: D photoperiod). To date, however, no studies have addressed the role of temperature. The purpose of our study was to describe, in the laboratory, (i) the effects of temperature on immature development time (egg to adult emergence) and progeny sex ratio, (ii) the effects of temperature on adult pre-oviposition period (time between emergence and first egg-laying), survivorship and fecundity, and then (iii) use these data to construct fertility life tables to calculate key demographic parameters. Our results will be used to optimize a mass-rearing protocol for *S. galinae*, and may assist timing of field releases and predictions about its establishment North America.

2. Materials and Methods

2.1 Emerald ash borer larvae

Larvae used in experiments were 3rd-4th instars reared in evergreen (tropical) ash (*Fraxinus uhdei* [Wenzig] Lingelsh) sticks (9 cm length x 1-1.5 cm diameter) or logs (12 cm length x 3-4 cm diameter) using methods established by Duan et al. (2011; 2013b). To produce larvae in sticks (1 larva/stick) and logs (3-4 larva/log), emerald ash borer eggs freshly (≤ 7 d-old)

oviposited on unbleached coffee filter paper (HomeLife, Eden Prairie, MN) were placed individually or in groups (2-3 eggs) against the surface of the ash wood and secured with Parafilm (Bemis Co., Inc., Neenah, WI) wrap for hatching. One egg was used to infest sticks targeting only one larva and five eggs were used for sticks targeting 3-4 larvae, accounting for some early larval mortality. Sticks and logs infested with eggs were maintained on distilled water-saturated floral foam bricks (Smithers-Oasis Company, Kent, OH) and maintained in AR-360 environmental growth chambers (Percival Scientific, Perry, IA) set at $30 \pm 1^{\circ}\text{C}$ ($\approx 65\%$ RH, 16:8 h L:D photoperiod) for ≈ 4.5 wk when larvae were 3rd-4th instars. Prior to exposure to parasitoids, sticks were checked for emerald ash borer larvae by shaving back a thin strip of bark to expose frass from developing larvae; sticks without larvae (or if larvae were dead or unhealthy) were discarded. Sticks and logs being exposed to parasitoids were wrapped at the base with wet paper towel, stood vertical on small petri dishes (Falcon[®], Becton Dickinson, Franklin Lakes, NJ), and wrapped together at the base with Parafilm and prepared for experimental use.

2.2 Parasitoids

S. galinae adults were F₁₀₋₁₂ progeny of a founder colony collected near Vladivostok, Russia in fall 2010 (Duan et al., 2012). Upon emergence and prior to experimental use, parasitoids were maintained in ventilated acrylic cylinders using a $\approx 1:2$ m:f ratio and provided streaks of clover honey for a food source.

2.3 Experimental temperatures

Developmental and reproductive parameters were studied at each of 5 temperature treatments. For each treatment, individual growth chambers were programmed to constant 15,

20, 25, 30, or $35 \pm 1^\circ\text{C}$ ($\approx 65\%$ RH, 16: 8 h L: D photoperiod). A HOBO data logger (Onset Computer Co., Bourne, MA) was used in each chamber to monitor temperature and RH.

2.4 Effect of temperature on development time and sex ratio

Groups (15-20) of ash sticks, each containing one host larva, were exposed to gravid female parasitoids at 25°C for 24 h using a high (6:1) parasitoid: host ratio. Female parasitoids were naïve (i.e. no prior host exposure) and 2-3 weeks of age. Previous research shows these conditions are best to achieve high parasitism under normal rearing conditions (Duan et al., 2014; Watt et al., 2015). An 18.5 x 13 x 10 cm polystyrene crisper box (Tri-State Plastics, Dixon, KY) was used as the exposure arena. Immediately after exposure, sticks were randomly assigned to emergence containers (ventilated, clear plastic 1 L SOLO cups with mesh-screened lids; SOLO Cup Co., Urbana, IL), which were previously assigned to one of the experimental temperatures. A sheet of moist paper towel was added to emergence containers and re-wetted as needed to provide humidity. This procedure was repeated (replicated) until a total of 10 *S. galinae* broods produced emerged progeny at each temperature. Containers were checked daily for parasitoid emergence. Emerged progeny and the sex of each individual were tallied for each date of emergence. No parasitoids emerged from either 15 or 35°C so these temperatures were excluded from the data analysis.

2.5 Effect of temperature on reproduction and survivorship of “ovipositing” females

For each experimental temperature, 15 pairs (replicates) of newly (≤ 24 h) emerged female and male parasitoids were isolated in individual rearing cages (≈ 16 cm length x 6 cm dia. polycarbonate tubing fitted with plastic caps, US Plastics) and provided emerald ash borer larvae constantly throughout their lifetimes (until female mortality). Clover honey was *ad libitum*

150 streaked onto the screens of cages to provide a food (energy) source. Because *S. galinae* often
151 live for several weeks under normal laboratory conditions (Duan et al., 2014), larval host logs
152 were changed twice per week (Tuesday and Friday). On these days, mortality (if any) was
153 recorded. Survivorship curves were constructed for each temperature based on median survival
154 (longevity) of each of 15 females.

155 Parasitoid-exposed logs were placed in emergence containers (as above) and incubated at
156 $26 \pm 1^\circ\text{C}$ for ≈ 2 wk. Logs were then dissected and larvae scored for parasitism. Parasitism was
157 easily observed by the presence of silken cocoons spun by the 5th instar *S. galinae* larvae. For
158 each log (exposure period), we counted the number of parasitized larvae as well as the number of
159 cocoons on each parasitized host.

160 *2.6 Effect of temperature on survivorship of “host-deprived” females*

161 In addition to the “ovipositing” females, we tracked the survival of 15 “host-deprived”
162 females that were held in cages with males, as described above, and provided honey but never
163 offered host larvae. Cages were checked twice weekly and the time of natural mortality was
164 recorded. Survivorship curves were constructed for each temperature based on median survival
165 (longevity) of each of 15 females. Survivorship of “host-deprived” and “ovipositing” females
166 was also compared for each temperature.

167 *2.7 Calculation of S. galinae demographic parameters*

168 To assist calculations of demographic parameters for each temperature, weekly fecundity
169 and survivorship data from each of the 15 “ovipositing” females dying naturally were used to
170 construct $l_x m_x$ life tables, where m_x is age-specific female fecundity (number of daughters born to
171 females age x) and l_x is the probability of surviving to age x . To estimate m_x , the proportion of *S.*

galinae progeny that are female (Watt and Duan, 2014) was multiplied by the number of parasitoid cocoons produced during each week of life.

Demographic parameters (R_0 , r_m , λ , T_d) were calculated for all temperatures using equations by Southwood and Henderson (2000). Net reproductive rate ($R_0 = \sum l_x m_x$) expresses the per generation growth rate of a population ($R_0 > 1$, population increasing; $R_0 < 1$, population declining; $R_0 = 1$, population at equilibrium). Generation time (T_c) is the average time separating births of one generation to births of the next, and was estimated directly for each temperature by adding the immature development time (egg to adult emergence) to the female pre-oviposition period observed at each temperature. Intrinsic rate of increase (r_m) is the exponential growth potential for a population with a stable-age distribution living under defined environmental conditions (Birch 1948) and was calculated by iterating the value of r_m in the equation $1 = \sum l_x m_x e^{r_m}$ until the equation was solved. Finite rate of increase ($\lambda = e^r$) is the constant factor by which a population multiplies per generation. Population doubling time ($T_d = \ln(2)/r_m$) is the amount of time required for a population growing at a given r_m to double in size.

To obtain estimates of means and variance for demographic parameters (R_0 , r_m , λ , T_d) we adopted the bootstrap pseudo-replication approach of Pilkington and Hoddle (2006b). Estimates for generation time T_c (immature development time + pre-oviposition period) were obtained directly, so this parameter remained constant (i.e. no pseudo-replication). Demographic parameters were first calculated using associated $l_x m_x$ life tables from the set of data containing all 15 females. Subsequently, each of 15 females was removed and replaced in the dataset and demographic parameters re-calculated following removals (total 16 sample estimates). These values were placed in a table with identifying rows. We then used MS Excel to randomly choose 1,000 samples of size 16 with replacement to generate 1,000 pseudo-replicates used to calculate

standard errors and confidence intervals. To reduce bias we used the adjustment recommended by Meyer et al. (1986):

$$R_{0,adj} = 2 * R_{0,all} - R_{0,bootstrapped}$$

2.8 Data analysis

Prior to analyses, data were checked to fit the assumptions of normality and homogeneity for parametric tests, and appropriate transformations were made when necessary. For development data, effects of temperature on immature development time and sex ratio were analyzed using one-way analysis of variance (ANOVA), followed by Tukey-Kramer's HSD test to compare mean values (Tukey, 1949). For data on adult reproduction, female pre-oviposition period was estimated as the number of days elapsed from adult emergence to first parasitism. Parasitism rate was estimated by dividing the number of parasitized hosts by the number of available hosts during each exposure period. Lifetime fecundity of ovipositing females was estimated as the total number of progeny (number of cocoons) produced from parasitized hosts throughout the life span. Survivorship of both "ovipositing" and "host-deprived" females were estimated by nonparametric Kaplan-Meier survival analysis (Kaplan and Meier, 1958). Analyses were carried out in JMP Pro 11.2.0 (SAS Institute 2013).

3. Results

3.1 Effect of temperature on development time and sex ratio

At 15°C parasitoid larvae developed to 5th instars (cocoons) but did not develop beyond this (i.e. no adult emergence). At 35°C parasitoid eggs desiccated and did not hatch. Between 20, 25, and 30°C development time from egg to adult emergence was significantly different ($F=53.4$,

df=2, 47, $P<0.0001$; $\alpha=0.05$), with adult emergence occurring around day 25, 32, and 38, respectively (Table 1). Temperature did not affect sex ratio of adult progeny ($F=2.4$, $df=2$, 36, $P=0.1072$; $\alpha=0.05$; Table 1).

3.2 Effect of temperature on reproduction and survivorship of “ovipositing” females

Parasitism occurred at all temperatures except 35°C, however parasitism rate was significantly higher (35%) at 25°C ($F=18.6$, $df=4$, 67, $P<0.0001$; Figure 1). At 15°C pre-oviposition period was 16 d, which was significantly longer than for the other temperatures ($F=13.7$, $df=3$, 54, $P<0.0001$; Table 1). Of the temperatures producing parasitism (15, 20, 25, 30°C), lifetime fecundity was highest at 25°C (43 progeny/female). Temperature significantly affected female survivorship (Log-rank test, $\chi^2=107.8$, $df=4$, $P<0.0001$), with median survival being 11.5 wk (95% CI: 6 — 12 wk), 8 wk (95% CI: 7 — 9 wk), 7 wk (95% CI: 5 — 7 wk) 6.5 wk (95% CI: 5 — 8 wk) and 1 wk (95% CI: 0.5 — 2 wk) at 15, 20, 25, 30, and 35°C respectively (Figure 2).

3.3 Effect of temperature on survivorship of “host-deprived” females

For female parasitoids held without hosts, temperature significantly affected survival (Log-rank test, $\chi^2=116.1$, $df=4$, $P<0.0001$), with median survivorship being 15 wk (95% CI: 9 — 16 wk), 10.5 wk (95% CI: 4 — 13 wk), 9.5 wk (95% CI: 6 — 11 wk), 9.5 wk (95% CI: 3 — 11 wk), and 1 wk (95% CI: 0.5 — 2 wk) at 15, 20, 25, 30, and 35°C, respectively (Figure 2). Compared to “ovipositing” (non-deprived) females, host deprived females lived significantly longer than ovipositing females at 15°C (Log-rank test, $\chi^2=6.99$, $df=1$, $P=0.0082$), 20°C (Log-rank test, $\chi^2=4.87$, $df=1$, $P=0.0273$), 25°C (Log-rank test, $\chi^2=10.01$, $df=1$, $P=0.0016$), and 30°C

(Log-rank test, $\chi^2 = 5.18$, $df=1$, $P=0.0228$), but not 35°C (Log-rank test, $\chi^2 = 0.38$, $df=1$, $P=0.5351$).

3.4 Calculation of *S. galinae* demographic parameters

Demographic parameters were compared for experimental temperatures producing parasitism (15, 20, 25, 30°C). Mean net reproductive rate (R_0) ($F=37038.4$, $df=3$, 3996, $P<0.001$), intrinsic rate of increase (r_m) ($F=40983.6$, $df=3$, 3996, $P<0.001$), finite rate of increase (λ) ($F=37632.2$, $df=3$, 3996, $P<0.001$), and population doubling time (T_d) ($F=27121.2$, $df=3$, 3996, $P<0.001$) were all significantly different between temperatures. R_0 (27.78), r_m (0.29), and λ (1.34) were highest at 25°C, while T_d was lowest (2.77) at this temperature (Figure 3). Generation time (T_c) was longest (45.1 d) at 20°C and least (30.3 d) at 30°C (Table 1). Because progeny arrested development at 15°C T_c could not be calculated for this temperature.

4. Discussion

Knowledge of what temperatures favor or limit parasitoid population growth is important in the context of a biological control program not only to estimate proliferation potential but also for mass-rearing, which aims to produce the most biological control agents in the shortest time frames. Indeed, biological control success may largely be affected by costs of mass-rearing biological control agents (Irvin and Hoddle, 2006). Previously, Watt and Duan (2014) and Watt et al. (2015) showed that *S. galinae* may be reared most optimally using 4th instar emerald ash borer larvae exposed in medium-density host-parasitoid groups. Here, we show that the optimum temperature for rearing *S. galinae* is around 25°C ($\approx 65\%$ RH, 16:8 h L: D photoperiod). At this temperature net reproductive rate (R_0) and intrinsic rate of increase (r_m) are highest (indicating

fastest population growth), and population doubling time (T_d) is least. Therefore, we recommend that this temperature be used to rear *S. galinae* most efficiently.

Currently, the northernmost range of emerald ash borer in the United States is Houghton, MI (47.1°N latitude) where annual mid-summer (June-Aug.) temperature is around 19°C, and its southernmost range is Fulton County, GA (33.7°N latitude), where mid-summer temperature is ≈30°C (Emerald Ash Borer Info, 2016; US Climate Data, 2016). The results of our study indicate that populations of *S. galinae* should be able to establish and grow within this range. A primary reason for introducing *S. galinae* is that it may be more cold-hardy than its Chinese congener *S. agrili*, which lacks consistent recovery data in the northern United States but has promise in more southern regions (Hooie et al., 2015). Although climate matching indicates *S. galinae* is better suited than *S. agrili* to establish in the northern regions, its ability to overwinter in the wild will be critical if sustainable populations are to establish and proliferate in northern regions. It is hoped that future release strategies will be refined such that both parasitoids will prove useful for controlling this invasive pest in different regions of the country.

Our analyses show that *S. galinae* exhibits fastest population growth when temperature is between 20—25°C. Excluding 35°C (where values were zero) *S. galinae* net reproductive rate (R_0) was lowest at 30°C, and intrinsic rate of increase (r_m) was only slightly higher at 30°C than at 15°C, for which r_m was least. This is evidence that temperatures around 30°C and above might slow its population growth, and thus affect its ability to control emerald ash borer in the field. Along the US latitudinal gradient, Cincinnati, OH, is the northernmost city in the Midwest and eastern regions (latitude of 39.1°N) of the United States with annual mid-summer temperatures above 30°C. In these south-central regions reduced rates of parasitism, survival, or fecundity may result when temperatures reach above 30°C, consequently resulting in restricted biological

control. However there are a number of factors that can affect insect performance in the field, including using microclimates to avoid extreme heat (Gibbs et al., 2003). The ability of this parasitoid to adapt to local climates will be critical to its success as a biological control agent, and this should be a priority for future research.

In the experiment where female parasitoids were exposed to hosts for 24 h under normal rearing temperature (25°C), and their offspring (eggs) subsequently moved to different temperatures, immature development time was inversely associated with temperature from 20, 25, and 30°C. However at 35°C parasitoid eggs oviposited on host larvae became desiccated and did not hatch. At 15°C, parasitoid larvae developed to 5th instars and spun cocoons, but did not emerge after three months at this constant temperature. Interestingly, when these cocoons were moved to a higher temperature (25°C) after the three month period, adults began to emerge after <1 wk. By comparison when the larvae of egg-laying females, which were living at 15°C, were maintained at 25°C from eggs most all of these larvae emerged within the normal development period (around 29 d; Duan et al., 2014). Thus, we suspect that 15°C may induce diapause in *S. galinae* larvae, and that diapause is non-maternal in origin (i.e. it is controlled by the thermal experience in the immature stage rather than by the experience of the mother), although this was not confirmed by our study. Additional studies are being conducted to determine the environmental conditions (e.g. threshold temperature and photoperiod) required to induce and break up diapause in *S. galinae*, which will be valuable knowledge for its mass-rearing.

Female parasitoids at all temperatures required a pre-oviposition period, and the temperature-dependent fecundity curves were typical for a synovigenic parasitoid (Calvo et al., 2013). In contrast to pro-ovigenic parasitoid species, where females emerge with their complete complement of eggs, synovigenic parasitoids emerge with immature eggs and use nutrition

acquired as larvae or adults to mature eggs over their lifetimes (Rivero et al., 2001). Because for synovigenic parasitoids ovigenesis is a function of metabolism (Rivero and Casas, 1999), reproductive parameters such as fecundity and pre-oviposition period are directly affected by temperature. Generally, higher temperatures shorten pre-oviposition period, although very high temperatures can induce stress responses that delay or halt ovigenesis and thereby extend this period (Roux et al., 2010). This appears to be true for *S. galinae*, which had its longest pre-oviposition period (16 d) at the lowest temperature tested (15°C), while having a pre-oviposition period of only about 5 d at 25 and 30°C. Although the difference was not statistically significant, pre-oviposition period was slightly longer at 30°C than at 25°C, and females living at 35°C lived for only a short time (< 10 d) and never laid eggs, lending further support that higher temperatures adversely affect population growth.

For all temperatures except 35°C, host-deprived females lived much longer than females offered hosts throughout their life spans (i.e. ovipositing females). This is in part because ovipositing females expend energy parasitizing hosts (via foraging and oviposition) and females that are deprived of hosts will not expel this same energy. In synovigenic parasitoids, females may resorb their eggs after extended periods of host deprivation, which helps conserve energy until hosts become available (Jervis et al., 2005). Therefore it is not surprising that females never offered hosts survived longer (at all temperatures except 35°C) than females using energy to parasitize hosts. Survivorship curves for *S. galinae* were typical type I at all temperatures except 35°C, where it was an extreme type II. Survivorship curves display how risk of mortality relates to time; with type I survivorship mortality risk is lower early in life and increases with time, whereas with a type II curve mortality risk is constant (Jervis et al., 2005).

326 Together, our findings demonstrate that ambient temperature significantly affects the
327 reproductive biology and development of *S. galinae*, which consequently lead to changes in life
328 history and demographic parameters. These findings should be incorporated into current mass-
329 rearing programs for production of this biocontrol agent by using the optimal temperature (25°C)
330 and avoiding the use of 30°C or above. In addition, our results indicate that *S. galinae* can be
331 effective in attacking emerald ash borer larvae in later fall or early spring when ambient
332 temperature is around 15°C. Thus, field releases of *S. galinae* in most emerald ash borer-infested
333 regions of the United States may be conducted early spring (e.g. April – May) or late fall (e.g.
334 September-October).

335 **Acknowledgments**

336 We thank Kristi Larson, Jackie Hoban, Sue Barth (University of Delaware), Jonathan
337 Schmude and Cherie Keenan (United States Department of Agriculture-Agricultural Research
338 Service) for assistance maintaining insect cultures. We are especially grateful to Dick Bean,
339 Kimberly Rice, Charles Pickett, and Steve Bell (Maryland Department of Agriculture) for
340 providing most of the ash logs used during this study. We also thank Mark Hoddle, Leigh
341 Pilkington, and Michael Lewis (University of California-Riverside) for providing the framework
342 for the bootstrap analysis. Finally, we thank Doug Luster (United States Department of
343 Agriculture-Agricultural Research Service) for reviewing the manuscript. This research was
344 supported through funding from United States Department of Agriculture-Animal and Plant
345 Health Inspection Service grant #58-1926-2-282.

References

Abell, K.J., J.J Duan, L. Bauer, J.P Lelito and R.G. van Dreisch. 2012. The effect of bark thickness on host partitioning between *Tetrastichus planipennisi* (Hymen: Eulophidae) and *Atanycolus* spp. (Hymen: Braconidae), two parasitoids of emerald ash borer (Coleop: Buprestidae). *Biol. Control.* 63: 320-325.

Baffoe, K.O., P. Dalin, G. Nordlander, and J.A. Stenberg. 2012. Importance of temperature for the biological control efficiency of the parasitoid *Perilitus brevicollis* (Hymenoptera: Braconidae) on *Salix*. *BioControl.* 57: 611-618. doi: 10.1007/s10526-012-9443-5.

Bale, J.S., et al. 2002. Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Glob. Change Biol.* 8: 1–16.

Belokobylskij, S.A., G.I. Yurchenko, J.S. Strazanac, n.A. Zaldívar-Rivero', and V. Mastro. 2012. A new emerald ash borer (Coleoptera: Buprestidae) parasitoid species of *Spathius* Nees (Hymenoptera: Braconidae: Doryctinae) From the Russian Far East and South Korea. *Ann Entomol. Soc. Am.* 105: 165-178.

Birch, L.C. 1948. The intrinsic rate of natural increase in an insect population. *J. Anim. Ecol.* 17: 15-26.

Calvo, F.J., J.D. Soriano, K. Bolckmans, and J.E. Belda. 2013. Host instar suitability and life history parameters under different temperature regimes of *Necremnus artynes* on *Tuta absoluta*. *Biocontrol Science and Technology.* 23(7): 803-815. doi: 10.1080/09583157.2013.795930

367 **Duan, J.J., T. Watt, and C.B. Oppel. 2011.** An alternative host plant-based method for
368 laboratory rearing of emerald ash borer to produce larval parasitoids for biological control. Pp.
369 107 – 108. *In* Parra G., D. Lance, V. Mastro, R. Reardon and C. Benedict (compiled):
370 Proceeding of 2011 Emerald Ash Borer National Research and Technology Development
371 Meeting October 12 & 13, 2011 Wooster, Ohio. USDA Technology Transfer FHTET-2011-06,
372 Raleigh, North Carolina.

373 **Duan, J.J., G. Yurchenko, and R. Fuester. 2012.** Occurrence of emerald ash borer
374 (Coleoptera: Buprestidae) and biotic factors affecting its immature stages in the Russian Far
375 East. *Environ. Entomol.* 41(2): 245-254.

376 **Duan, J.J., L.S. Bauer, K.J. Abell, J.P. Lelito, and R. van Driesche. 2013a.**
377 Establishment and abundance of *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in
378 Michigan: potential for success in classical biocontrol of the invasive emerald ash borer. *J. Econ.*
379 *Entomol.* 106: 1145-1154.

380 **Duan, J.J., T. Watt, P. Taylor, K. Larson, and J.P. Lelito. 2013b.** Effects of ambient
381 temperature on egg and larval development of the invasive emerald ash borer (Coleoptera:
382 Buprestidae): implications for laboratory rearing. *J. Econ. Entomol.* 106(5): 2101-2108.

383 **Duan, J.J., T.J. Watt, and K. Larson. 2014.** Biology, life history and laboratory rearing
384 of *Spathius galinae* (Hymenoptera: Braconidae), a larval parasitoid of the invasive emerald ash
385 borer (Coleoptera: Buprestidae). *J. Econ. Entomol.* 107(3): 939-946.

386 **Duan, J.J., J.R. Gould, and R.W. Fuester. 2015.** Evaluation of the host specificity of
387 *Spathius galinae* (Hymenoptera: Braconidae), a larval parasitoid of the emerald ash borer
388 (Coleoptera: Buprestidae) in Northeast Asia. *Biol. Control.* 89: 91-97.

389 **Emerald Ash Borer Information. 2016.** Emerald Ash Borer.
390 [<http://www.emeraldashborer.info/index.cfm>].

391 **Force, D.C., and P.S. Messenger. 1964.** Fecundity, reproductive rates, and the innate
392 capacity for increase of three parasites of *Therioaphis maculata* (Buckton). Ecology. 45(4): 706-
393 715.

394 **Gandhi, K.J.K., and D.A. Herms. 2010.** North American arthropods at risk due to
395 widespread *Fraxinus* mortality caused by Alien Emerald ash borer. Biol. Invasions. 12: 1893-
396 1846.

397 **Gibbs, A.G., M.C. Perkins, and T.A. Markow. 2003.** No place to hide: microclimates of
398 Sonoran Desert *Drosophila*. J. Therm. Biol. 28: 353-362.

399 **Herms, D.A. and D.G. McCullough. 2014.** Emerald ash borer invasion of North America:
400 history, ecology, impacts, and management. Annu. Rev. Entomol. 59: 13-30.

401 **Hoelmer, K.A., and A.A. Kirk. 2005.** Selecting arthropod biological control agents
402 against arthropod pests: can the science be improved to decrease the risk of releasing ineffective
403 agents? Biol. Control. 34: 255–264.

404 **Hooie, N.A., G.J. Wiggins, P.L. Lambdin, J.F. Grant, S.D. Powell, and J.P. Lelito.**
405 **2015.** Native parasitoids and recovery of *Spathius agrili* from areas of release against emerald
406 ash borer in eastern Tennessee, USA. Biocontrol Science and Technology. 25(3): 345-351. DOI:
407 10.1080/09583157.2014.971712

408 **Irvin, N.A., and M.S. Hoddle. 2006.** The effect of intraspecific competition on progeny
409 sex ratio in *Gonatocerus* spp. for *Homalodisca coagulata* egg masses: economic implications for
410 mass rearing and biological control. *Biol Control*. 39: 162-170.

411 **Infante, F. 2000.** Development and population growth rates of *Prorops nastua* (Hym.,
412 Bethyilidae) at constant temperatures. *J. Appl. Ent.* 124: 343-348.

413 **Jervis, M.A., M.J.W. Copeland, and J.A. Harvey. 2005.** The life cycle. p. 73-165. *In*
414 Jervis, M.A (ed.). *Insects as Natural Enemies: a Practical Perspective*. Springer. Netherlands.

415 **Ju, R.T., F. Wang, and B. Li. 2011.** Effects of temperature on the development and
416 population growth of the sycamore lace bug, *Corythucha ciliata*. *J. Insect Sci.* 11:16 available
417 online: insectscience.org/11.16.

418 **Kaplan, E. L., and P. Meier. 1958.** Nonparametric estimation from incomplete
419 observations. *J. Amer. Statist. Assn.* 53(282): 457–481.

420 **Kovacs, K.F., R.G. Haight, D.G. McCullough, R.J. Mercader, N.W. Siegert, and A.M.**
421 **Liebhold. 2010.** Cost of potential emerald ash borer damage in U.S. communities, 2009-2019.
422 *Ecol. Econ.* 69: 569-578.

423 **Liu, H., L.S. Bauer, R. Gao, T. Zhao, T.R. Petrice, and R.A. Haack. 2003.** Exploratory
424 survey for the emerald ash borer *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural
425 enemies in China. *The Great Lakes Entomologist*. 36 (3-4): 191-204.

426 **Meyer, J.S., C.G. Ingersoll, L.L. McDonald, and M.S. Boyce. 1986.** Estimating
427 uncertainty in population growth rates: jackknife vs. bootstrap techniques. *Ecology*. 67(5): 1156-
428 1166.

429 **Patton, R.L. 1963.** Introductory Insect Physiology. W.B. Saunders Co. Philadelphia, USA.

430 **Pilkington, L.J., and M.S. Hoddle. 2006a.** Use of life table statistics and degree-day
431 values to predict the invasion success of *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae), an
432 egg parasitoid of *Homalodisca coagulate* (Hemiptera: Cicadellidae), in California. Biol. Control
433 37: 276-283.

434 **Pilkington, L.J., and M.S. Hoddle. 2006b.** Reproductive and developmental biology of
435 *Gonatocerus ashmeadi* (Hymenoptera: Mymaridae), and egg parasitoid of *Homalodisca*
436 *coagulate* (Hemiptera: Cicadellidae). Biol. Control. 37: 266-275.

437 **Pilkington, L.J., M. Lewis, D. Jeske, and M.S. Hoddle. 2014.** Calculation and thematic
438 mapping of demographic parameters for *Homalodisca vitripennis* (Hemiptera: Cicadellidae) in
439 California. Ann. Entomol. Soc. Am. 107(2): 423-434. doi: <http://dx.doi.org/10.1603/AN13144>.

440 **Pratissoli, D., and J.R.P. Parra. 2000.** Fertility life table of *Trichogramma pretiosum*
441 (Hym., Trichogrammidae) in eggs of *Tuta absoluta* and *Phthorimaea operculella* (Lep.,
442 Gelechiidae) at different temperatures. J. Appl. Ent. 124: 339-342.

443 **Qiu, B. Z-S. Zhou, S-P. Luo, and Z-F. Xu. 2012.** Effect of temperature on development,
444 survival, and fecundity of *Microplitis manila* (Hymenoptera: Braconidae). Environ. Entomol.
445 41(3): 657-664.

446 **Rivero, A., D. Giron, and J. Casas. 2001.** Lifetime allocation of juvenile and adult
447 nutritional resources to egg production in a holometabolous insect. Proc. R. Soc. Lond. 268:
448 1231-1237.

449 **Rivero, A., and J. Casas. 1999.** Incorporating physiology into parasitoid behavioral
450 ecology: the allocation of nutritional resources. *Res. Popul. Ecol.* 41: 39-45.

451 **Roux, O., C. Le Lann, J.J.M. van Alphen, and J. van Baaren. 2010.** How does heat
452 shock affect the life history traits of adults and progeny of the aphid parasitoid *Aphidius avenae*
453 (Hymenoptera: Aphidiidae)? *B. Entomol. Res.* 100: 543-549.

454 **SAS Institute. 2013.** JMP® Pro Version 11.2.0. SAS Institute Inc., Cary, NC.

455 **Southwood, T.R.E., and P.A. Henderson. 2000.** Ecological methods, 3rd ed. Blackwell
456 Science, Oxford, UK.

457 **Staubli Dreyer, B., P. Neuenschwander, B. Bouyjou, J. Baumgartner., and S. Dorn.**
458 **1997.** The influence of temperature on the life table of *hyperaspis notata*. *Entomol. Exp. Appl.*
459 84: 85–92.

460 **Taylor, F. 1981.** Ecology and evolution of physiological time in insects. *Am. Nat.* 117(1):
461 1-23.

462 **Tukey, J. 1949.** Comparing Individual Means in the Analysis of Variance. *Biometrics.*
463 5(2): 99-114.

464 **U.S. Climate Data. 2016.** [www.usclimatedata.com]

465 **USDA APHIS. 2015.** Field release of the parasitoid *Spathius galinae* for the biological
466 control of the emerald ash borer (*Agrilus planipennis*) in the contiguous United States, an
467 environmental assessment. Pp. 1-33.

468 **Watt, T.J., and J.J. Duan. 2014.** Influence of host age on critical fitness parameters of
469 *Spathius galinae* (Hymenoptera: Braconidae), a new parasitoid of the emerald ash borer
470 (Coleoptera: Buprestidae). J. Econ. Entomol. 107(4): 1320-1329. doi:
471 <http://dx.doi.org/10.1603/EC14081>

472 **Watt, T.J., J.J. Duan, D.W. Tallamy, and J. Hough-Goldstein. 2015.** Effect of
473 parasitoid: host ratio and group size on fitness of *Spathius galinae* (Hymenoptera: Braconidae):
474 implications for mass-rearing. J. Econ. Entomol. DOI: 10.1093/jee/tov062

475

Figure Legends

Figure 1. Mean ($\pm$ SE) parasitism rate of late instar emerald ash borer larvae by *Spathius galinae* as influenced by temperature. Bars with different letters are significantly different ($\alpha=0.05$).

Figure 2. Weekly survivorship and fertility of *Spathius galinae* as influenced by ambient temperature. Solid lines with black circles are the mean number of progeny (male and female) produced by a single ovipositing female for each week of life. Solid lines with white circles are the life expectancy of ovipositing females; solid lines with grey triangles are the life expectancy of host-deprived females.

Figure 3. Demographic parameters, and fitted quadratic lines, for the emerald ash borer parasitoid *Spathius galinae* as influenced by constant ambient temperature. R_0 (top left) is net reproductive rate; r_m (top right) is intrinsic rate of increase; λ (bottom left) is finite rate of increase; and T_d (bottom right) is the time required for a population growing at a given r_m to double in size.

Graphical Abstract. Demographic parameters, and fitted quadratic lines, for the emerald ash borer parasitoid *Spathius galinae*, as influenced by constant ambient temperature. R_0 (top left) is net reproductive rate; r_m (top right) is intrinsic rate of increase; λ (bottom left) is finite rate of increase; and T_d (bottom right) is the time required for a population growing at a given r_m to double in size.

Table 1

Table 1. *Spathius galinae* immature development time, emerged progeny sex ratio, female pre-oviposition period, fecundity and generation length as influenced by constant ambient temperature.

	Temperature (°C) ^a				
	15	20	25	30	35
Development time (egg to adult) ^b	-*	38.0 ± 0.9a	31.5 ± 1.0b	24.8 ± 0.9c	-**
Sex ratio (f:m)	-	1.5 ± 0.2a	1.9 ± 0.1a	1.9 ± 0.1a	-
Female pre-oviposition period ^b	16.4 ± 1.41a	7.13 ± 1.40b	5.33 ± 1.41b	5.54 ± 1.51b	-
Lifetime fecundity	26.3 ± 4.7a	37.1 ± 4.7a	42.6 ± 4.7a	24.3 ± 4.7a	0.0 ± 0.0b
Generation length (<i>T_c</i>) ^b	-	45.13	36.8	30.3	-

* All larvae entered diapause as cocoons.
** Eggs desiccated prior to hatching.
^a Mean (± SE) values in rows with different letters are significantly different ($\alpha=0.05$).
^b Values in d.

Fig.1

Fig. 2

Fig. 3