Brown marmorated stinkbug age structure and reproductive development in Kelowna, British Columbia

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The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Heteroptera, Pentatomidae; Fig. 1), is a serious invasive pest that is expanding rapidly throughout the world [1]. BMSB feeds on more than 100 plant species including a variety of fruit and vegetable crops, as well as ornamental and non-crop plants [2]. Native to eastern Asia [3], BMSB first appeared in North America in 1998, and has since spread rapidly throughout the continent, including Canada (British Columbia, Quebec, and Ontario) [4]. It was first detected in the Okanagan Valley (including Kelowna), British Columbia, in 2016, where it is now feared to impact the local economy by affecting important agricultural industries such as apple, cherry and grape.

Figure 1. BMSB adult (right) and 5th instar nymphs (middle and left) on 21 September 2019 at Kinsmen Park, Kelowna.

Throughout their range, BMSBs spend the winter in diapause as non-feeding adults. After overwintering, BMSBs become reproductively mature and females lay eggs. BMSB nymphs transition through five developmental instars before the final molt to adult (Fig. 2), and the new generation of adults can either go into diapause or become reproductively mature and produce a new generation. In parts of its native range, BMSB produces four to six generations per year [5], while one (e.g., Ontario [4] and Switzerland [6]) or two (e.g., Italy [2] and southern United States [7,8]) generations per year are more typical in its non-native range. Whether or not BMSB has multiple generations in the same year at a given location likely depends on the local temperature as well as on photoperiod [2].

Figure 2. From left to right: BMSB eggs and first instars, 2nd instar, 3rd instar, 4th instar, 5th instar, adult male, and adult female. Source: https://www.stopbmsb.org/stink-bug-basics/life-stages/

In order to manage the BMSB outbreak in Kelowna, it is important to understand the timing and duration of the oviposition period of BMSB. Prior observations (2017-2018) show that early instar BMSB nymphs are still present in the fall, suggesting that either: (i) the oviposition period of females emerging from overwintering extends for several months; or alternatively (ii) at least some first-generation adults (i.e., those developing to adults in the summer) are maturing eggs and not entering into reproductive diapause (i.e., there is a partial second generation). However, the timing of oviposition is difficult to determine directly because BMSB eggs are hard to find. To determine the number of BMSB generations in Kelowna, I (1) surveyed BMSB and quantified the change of the age structure of the population throughout one year (2019), and (2) assessed the reproductive development of field-caught adult females. Initially, my supervisors and I also planned on conducting this study at another site in British Columbia, Chilliwack, where BMSB is common. However, my collaborators only collected BMSB until mid-July. As such, I also (3) assessed the reproductive development of female BMSB in Chilliwack, but only in the early summer.

Growing degree-days (DDs) and biofix are often used to quantify temperature and photoperiod for BMSB development [9]. DDs are calculated from the daily accumulated heat units received within a physiologically meaningful developmental temperature range. For BMSB, the developmental minimum and maximum threshold temperatures are 14.17°C and 35.76°C, respectively, and development is halted beyond this range [10]. Biofix is used in degree day modeling and defines the time at which development is initiated. For BMSB, the biofix that initiates reproductive development is a 12.7 hour photoperiod [11], after which reproductive development is primarily driven by temperature. To calculate DDs for BMSBs in Kelowna in 2019, I downloaded hourly temperature data for Kelowna from Environment Canada (https://kelowna.weatherstats.ca/about.html). I calculated DDs with the sine-wave method [12] and a lower threshold set to 14.17 °C [10,11]. I found that BMSBs in Kelowna accumulated a total of 765 $DD_{14.17}$ in 2019, and the majority of these DDs were accumulated between early May and late September, making this the prime window for BMSB development (Fig. 3). Because the biofix coincided with the start of DD accumulation (less than two degree days accumulated before March 28, the date of the biofix where photoperiod = 12.7 h; Fig. 3), I did not correct degree days in the analysis of BMSB phenology using the biofix.

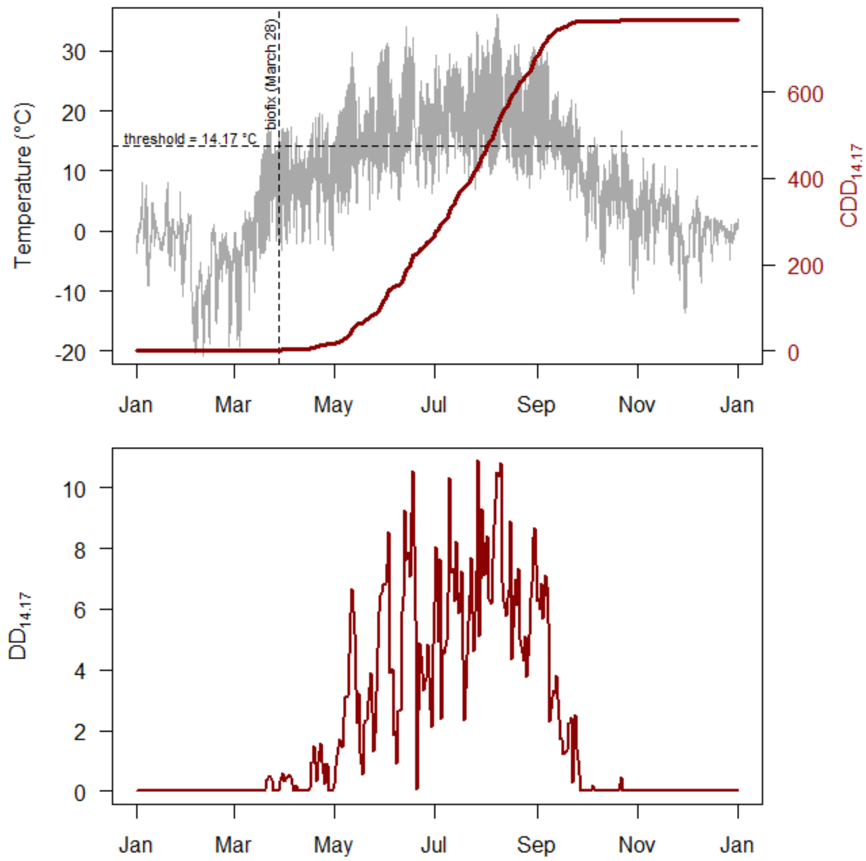

Figure 3. Top: Daily hourly temperature in Kelowna in 2019 (gray), and cumulative degree days ($CDD_{14.17}$) for BMSB (red). Horizontal dashed line shows threshold to calculate degree days for BMSB (14.17°C). Vertical dashed line shows time of biofix for BMSB (12.7 h photoperiod: March 28). Red line shows cumulative degree days ($CDD_{14.17}$). **Bottom:** Degree days ($DD_{14.17}$) per day.

1. Population survey of BMSB in Kelowna

I used the beating sampling technique to survey BMSBs weekly or biweekly between May and November 2019 at four sites in Kelowna (see Appendix A for sampling sites and detailed methods). I recorded the first overwintering adults in mid-June (at ~ 180 accumulated $DD_{14.17}$) with the beating technique (Fig. 4), although I observed a few adults as early as May (however, population densities were not high enough to be captured by the beating method). Adult BMSBs were relatively scarce until late August (~ 650 accumulated $DD_{14.17}$), at which point the adult population more than quadrupled (Figs 4 and 5). This sudden increase in adults was due to the emergence of a new adult generation (evidence supporting a new generation will be discussed below). While the sex ratio of BMSB adults was roughly 1:1 until mid-August, males tended to be more common than females after the adult population peaked (Fig. 5). This could be because males may develop quicker than females, have higher survival rates, or the sex ratio of eggs is biased towards males.

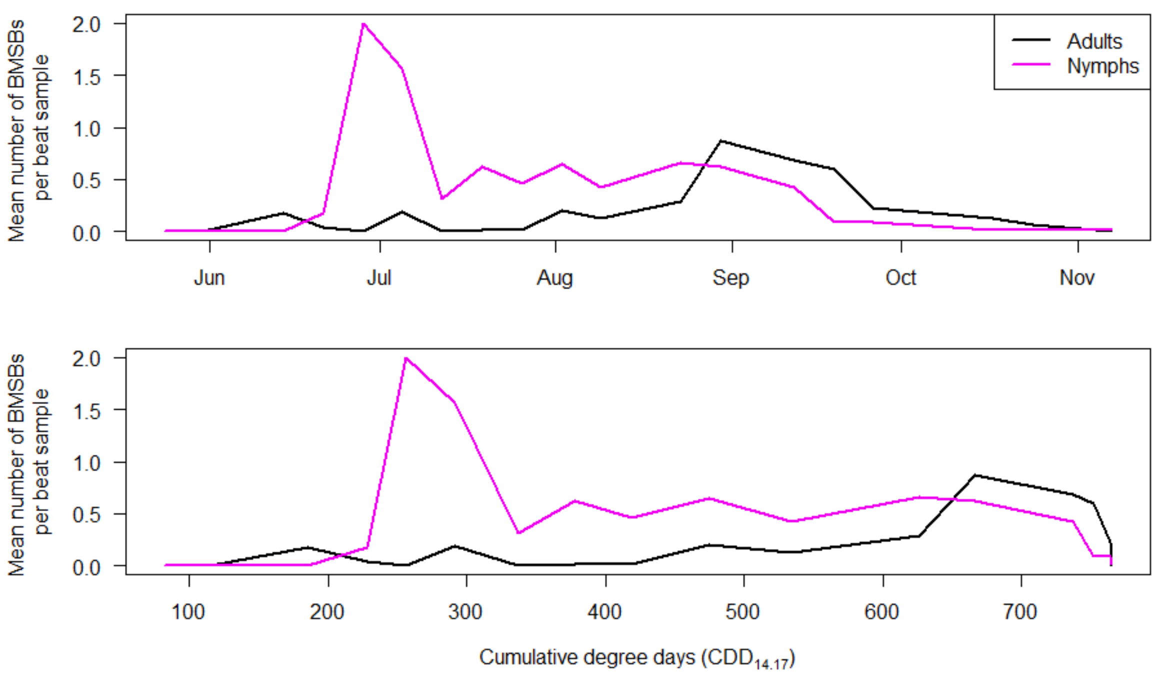

Figure 4. Average number of BMSBs per beat sample over time (**top**) and by cumulative degree days ($CDD_{14.17}$; **bottom**). Adults (black) are males and females combined; nymphs (magenta) are all instars (1-5) combined.

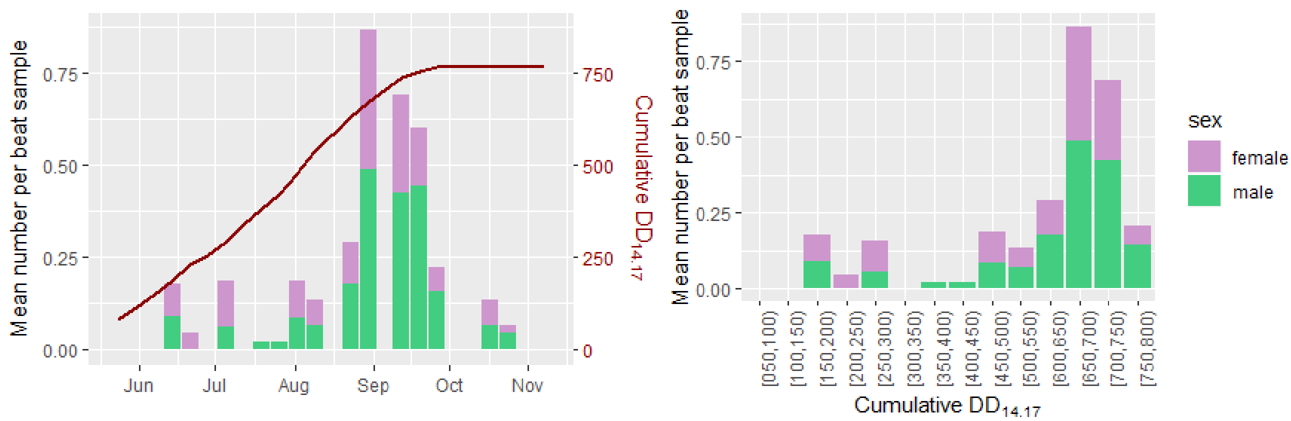

Figure 5. Average number of BMSB adults over time (**left**) and by cumulative degree days ($CDD_{14.17}$; **right**). After mid-August (~ 600 $CDD_{14.17}$), the sex ratio was slightly biased towards males.

I recorded the first BMSB nymphs in late June (~ 220 accumulated $DD_{14.17}$). Nymph populations quickly peaked in late June (at ~ 260 accumulated $DD_{14.17}$), and then stayed relatively constant until populations decreased to almost zero in mid-September (~ 750 accumulated $DD_{14.17}$; Fig. 6). The BMSB nymph population displayed a clear succession in developmental stage throughout the season (Fig. 6). 1^{st} instar nymphs were not readily sampled, as they tended to stay with the egg masses until they developed into 2^{nd} instars, but 2^{nd} to 5^{th} instar nymphs followed a clear pattern. The 2^{nd} and 3^{rd} instars displayed distinct peaks early in the summer [2^{nd} instar nymphs peaked in late June (~ 250 accumulated $DD_{14.17}$), followed by a peak in 3^{rd} instar nymphs about two weeks later (~ 300 accumulated $DD_{14.17}$)]. 2^{nd} instar nymphs were recorded from mid-June until early September, consistent with the findings of other studies that the oviposition period of BMSB can last for several months [2], as non-diapausing females continue to reproduce throughout their lifespan [6,10]. 4^{th} instar nymphs started to appear in mid-July (~ 350 accumulated $DD_{14.17}$), and 5^{th} instar nymphs were first observed in early August (~ 480 accumulated $DD_{14.17}$). The populations of these later instars didn't exhibit the district peaks of the earlier instars, but were relatively constant until mid-September (~ 750 accumulated $DD_{14.17}$; Fig. 6). The adult population experienced a steep increase about one month (~ 200 $DD_{14.17}$) after 5^{th} instar nymphs first appeared, suggesting that a new generation of adults emerged in late August (~ 650 accumulated $DD_{14.17}$). Adult populations were high for about a month, and then decreased rapidly in mid to late September when temperatures fell below the threshold of 14.17 °C (Figs. 3 and 6).

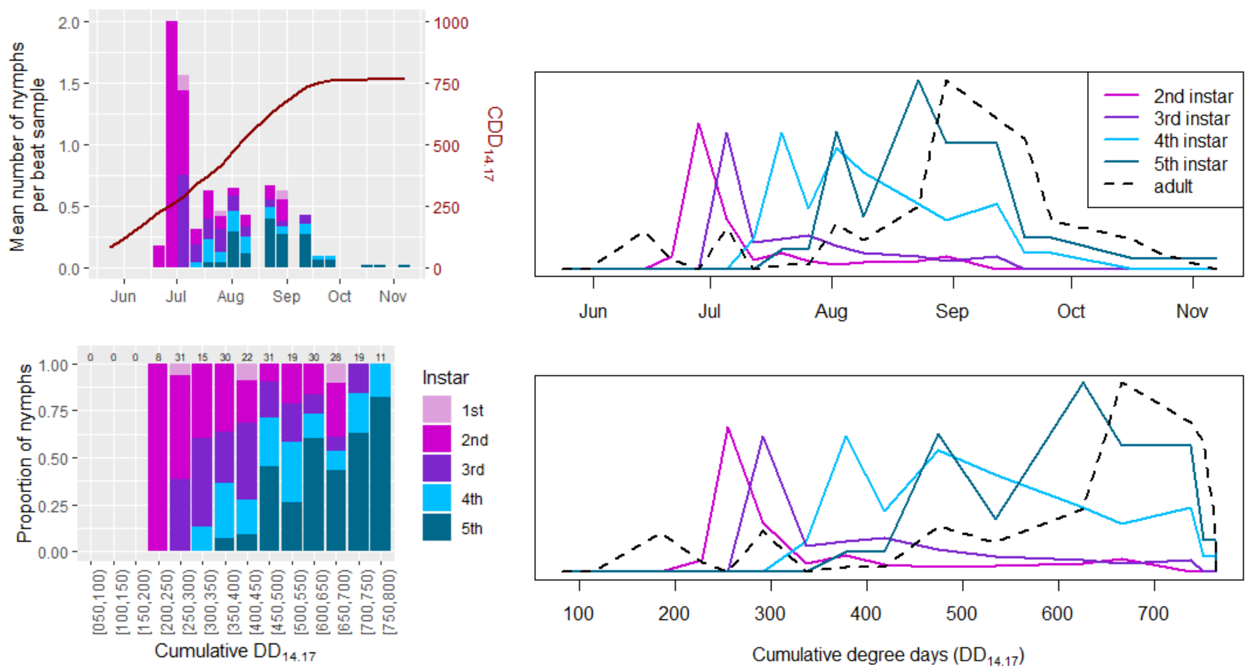

Figure 6. Age structure of BMSB nymphs. **Left side** shows total number of the various instars over time (**top**) and the proportion of the various instars by cumulative degree days ($CDD_{14.17}$; **bottom**). Numbers on top of each bar are total BMSB nymphs in each CDD interval. **Right side**: Scaling the abundances (y-axis) of the various instars illustrates a clear progression of early to late instar nymphs throughout the year. The data with the unscaled y-axis is shown in Appendix B.

2. Reproductive development of female BMSBs in Kelowna

The reproductive development of BMSB varies by region, owing to differences in photoperiod and temperature [11]. To assess the reproductive development of female BMSBs in Kelowna, I dissected field-caught adult females under a dissecting microscope and classified their reproductive and mating ranks according to Nielson *et al*. (2017). Detailed dissection and ranking methods (with pictures) are in Appendix C and D, respectively. The reproductive stage of female BMSBs strongly depended on season. Individuals caught before August were exclusively in reproductive ranks 3 and 4 (vitellogenic, or mature) and mated ranks 3 (mated) or 2 (possibly mated), suggesting that the adult population in Kelowna until early August consisted of only overwintering adults (Fig. 7). Together with the observation that early instar nymphs occurred throughout early summer, as well as findings from other studies that suggest that female BMSBs continue to reproduce throughout their lifetime which can last for several months post diapause termination [6,10], these results suggest that female BMSBs in Kelowna reproduced until well into the summer. Only in the first two weeks of August did I observe an overlap of reproductively mature and immature adults (Fig. 8). In mid-August, the adult population transitioned entirely to immature adults (i.e., the new generating), indicated by females that were in reproductive ranks 1 and 2 (previtellogenic, or immature) and mated ranks 0 (unmated) and 1 (possibly mated) (Fig. 7). In Switzerland and Italy, adult female BMSBs that emerge under short photoperiodic conditions (< 15 hours light per day) go straight into diapause instead of becoming reproductively mature [2]. In Kelowna, the photoperiod dropped to < 15 hours light per day on August 5, around the same time the first new adults emerged. As such, the overall pattern in reproductive development of female BMSB in Kelowna is consistent with a single generation per year under currently climatic conditions. It is possible, however, that warmer spring conditions could lead to earlier ovipositions and faster developmental rates (such as happens in Italy [2]), so I cannot exclude the possibility of a second or partial second generation during warmer years based on the single year of data in this study. Indeed, my model suggests that after ~ 700-800 $DD_{14.17}$, some adults may reach reproductive maturity again (Fig 8). However, in 2019, we did not accumulate more than 765 DDs in Kelowna.

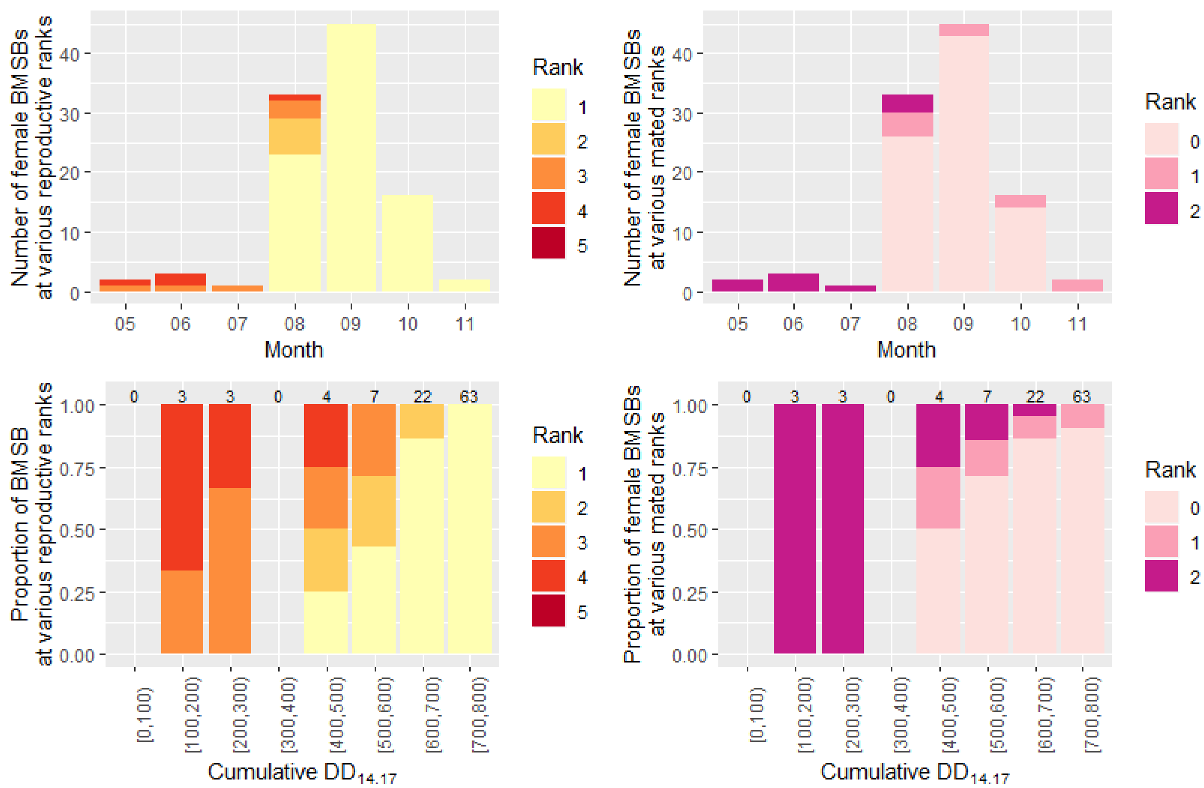

Figure 7. Reproductive (**left**) and mated (**right**) ranks of adult female BMSBs captured between May and November 2019 in Kelowna over time (**top**) and by cumulative degree days with a lower threshold set to 14.17 °C (**bottom**). Reproductive ranks are rank 1 = previtellogenic (no or one immature oocytes); rank 2 = previtellogenic (more than one immature oocyte); rank 3 = vitellogenic (at least one mature oocyte); rank 4 = vitellogenic (at least one oocyte in the lateral oviducts); and rank 5 = post reproductive (Appendix D). Mated ranks are rank 0 = unmated (thin and transparent spermatheca); rank 1 = possibly mated (thicker and more opaque spermatheca); and rank 2 = mated (thick and opaque spermatheca) (Appendix D). Numbers on top of each bar on bottom graphs show total number of female BMSBs in each DD interval.

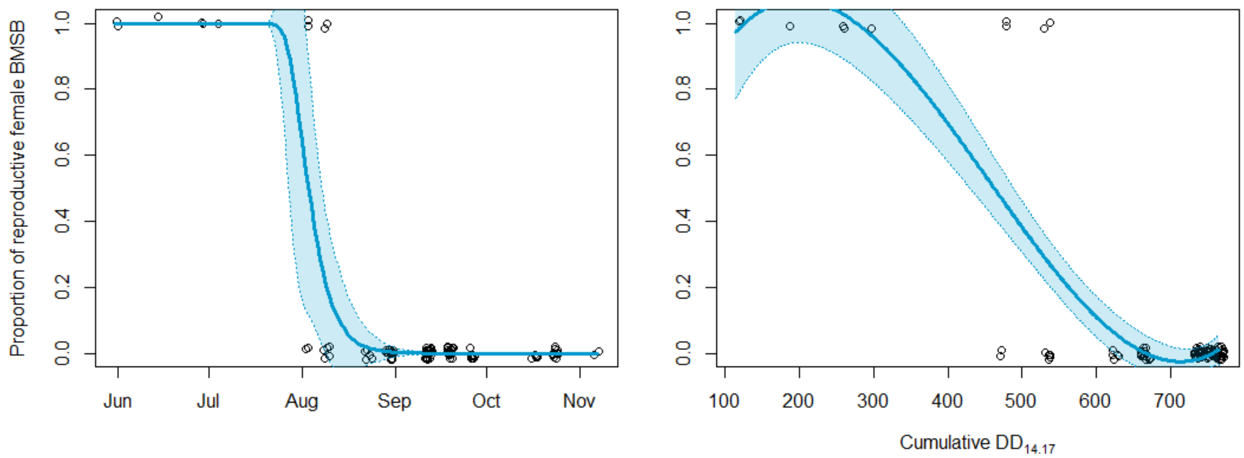

Figure 8. The proportion of reproductively active (vitellogenic, rank 3 and rank 4; Appendix D) adult female *Halyomorpha halys* captured in Kelowna in 2019 over the year (**left**) and by cumulative degree days ($DD_{14.17}$; **right**). When modeling the proportion of reproductively mature female BMSBs over the year, a logistic regression (with link = "cloglog" due to many zeros) was the best suited model (left figure). There was an abrupt change from mature to immature females with only ~ 2 weeks overlap between the stages ($X^2_{101} = 14.6$, $p < 0.001$, pseudo-$R^2 = 77.7$). This model predicts that the female populations transitioned from primarily overwintering (mature) adults to primarily diapausing (immature) adults on August 1 (± 3 days 95% C.I.). When predicting the reproductive maturity of females from cumulative degree days, a third order polynomial model provided the best fit. This model predicts a shift from primarily mature to primarily immature females at about 480 DDs ($F_{3,98} = 86.7$, $p < 0.001$, adjusted $R^2 = 0.72$). Shaded areas represent 95 % confidence intervals.

3. Reproductive development of female BMSBs in Chilliwack

In Kelowna, I obtained the first specimen for dissection in early June, and I was only able to obtain specimens at very low sample sizes until the start of August, at which point the new generation of adults emerged. Thus, I was not able to estimate when overwintering adults reached reproductive maturity, as the first adults in my samples were already mature (Figs 7 and 8). By contrast, the specimens from Chilliwack were collected much earlier in the season (starting in early May). My colleagues from Chilliwack were able to achieve relatively high sample sizes because they collected BMSBs from traps rather than the beating method I employed in Kelowna. Unfortunately, my collaborators in Chilliwack stopped collecting BMSBs after mid-July, making it impossible to say when the new generation of adults emerged (I also do not have any data on population surveys from Chilliwack). Still, the findings from Chilliwack suggest that, on average, BMSB reach maturity at the end of June (the point in time at which > 50% of adults were mature; Fig. 9). However, there appears to be a very large range of when adults reach maturity, indicated by the large overlap in mature and immature females (Fig. 9).

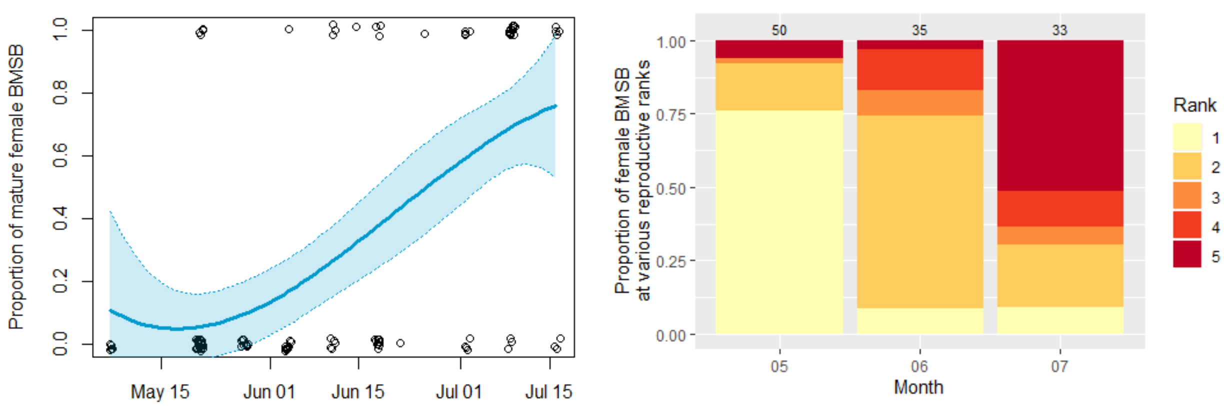

Figure 9. The reproductive ranks of adult female BMSBs trapped in 2019 around Chilliwack, BC, from early May to mid-July. **Left**: the proportion of mature (vitellogenic: rank 3 and rank 4; and post reproductive: rank 5) females. **Right**: the proportion of females in each reproductive rank. Reproductive ranks are rank 1 = previtellogenic (no or one immature oocytes); rank 2 = previtellogenic (more than one immature oocyte); rank 3 = vitellogenic (at least one mature oocyte); rank 4 = vitellogenic (at least one oocyte in the lateral oviducts); and rank 5 = post reproductive (Appendix D). Numbers on top of each bar show total number of female BMSBs trapped each month.

Overall conclusions and future directions

In summary, these findings suggest that BMSB in Kelowna only produced one new generation in 2019. This conclusion is supported by the analysis on population age structure, which showed a clear progression of early nymphs to adults throughout the year, as well as the observation of a clear transition of overwintering adults to a new generation of adults (based on the examination of the reproductive development of females) in early August. Because the new generation of adults emerged relatively late in the summer (at or below the critical photoperiod of 15 h to initiate reproductive development), the new generation of adults likely went straight into diapause rather than becoming reproductively mature. Because of the short sampling period in Chilliwack, I was not able to assess whether or not BMSB in Chilliwack produce more than one generation per year, but there are still some interesting insights this data provided. In Chilliwack, the first mature adults were observed about a month after the first immature adults. This observation agrees with other studies [4] that demonstrate that overwintering females are immature early in the year when they emerge from diapause. Interestingly, unlike in Kelowna, where female BMSB populations were strongly structured in terms of their reproductive stage, there was a strong overlap of immature and mature adults throughout the early summer in Chilliwack (Fig. 9), suggesting that overwintering females reach maturity more gradually. The lack of immature females in Kelowna early in the year could be an artefact of the very low sample size of female adults in Kelowna. Alternatively, the greater overlap of mature and immature females in Chilliwack could have resulted from the possibility that sampling occurred at more sites that were further apart and thus had different climatic conditions leading to different rates of development. Unlike in Kelowna, BMSB in Chilliwack were collected in traps, which were able to achieve a much higher sampling size that the beat sampling technique I employed. It is possible that immature females are less likely to be sampled by the beat sampling technique than by traps, either because they are not as active (e.g., they are not in search of oviposition sites), or because they are better at holding on to leaves and avoid being beaten off branches than mature adults because of their smaller weight. Another study that also used traps to collect BMSBs showed that the BMSB population in Niagara, Canada, also consists of some immature females early in the year [4]. As such, it would be informative to repeat this study this year, but sample earlier and to a greater extend, as well as assess the reproductive development of females that are collected in traps.

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