

Appendices

Sarah Amundrud (2020) “Brown marmorated stinkbug age structure and reproductive development in Kelowna, British Columbia”

Appendix A. Study sites and sampling methods

I sampled BMSBs weekly or biweekly between May and November 2019 at four sites in Kelowna (Fig. A1), using the beating sampling technique. At each site except Bordon, where only one tree was sampled, I selected 5 potential host plants. For each plant, I performed three sets of beating samples (one sample consisted of three sharp taps with a stick), using a 1m x 1m sheet to catch falling BMSBs. I recorded the life stage of each individual [1st instar, 2nd instar, 3rd instar, 4th instar, 5th instar, and adult (male or female)], and collected female adults for later determination of reproductive developmental stage. I also collected any other females I saw in the field. I euthanized and stored female adults in a freezer at the BC Ministry of Agriculture in Kelowna until further processing. After beating, I performed a timed visual search of 3 min on the undersides of leaves at each sampled plant for eggs. However, I never found any egg masses.

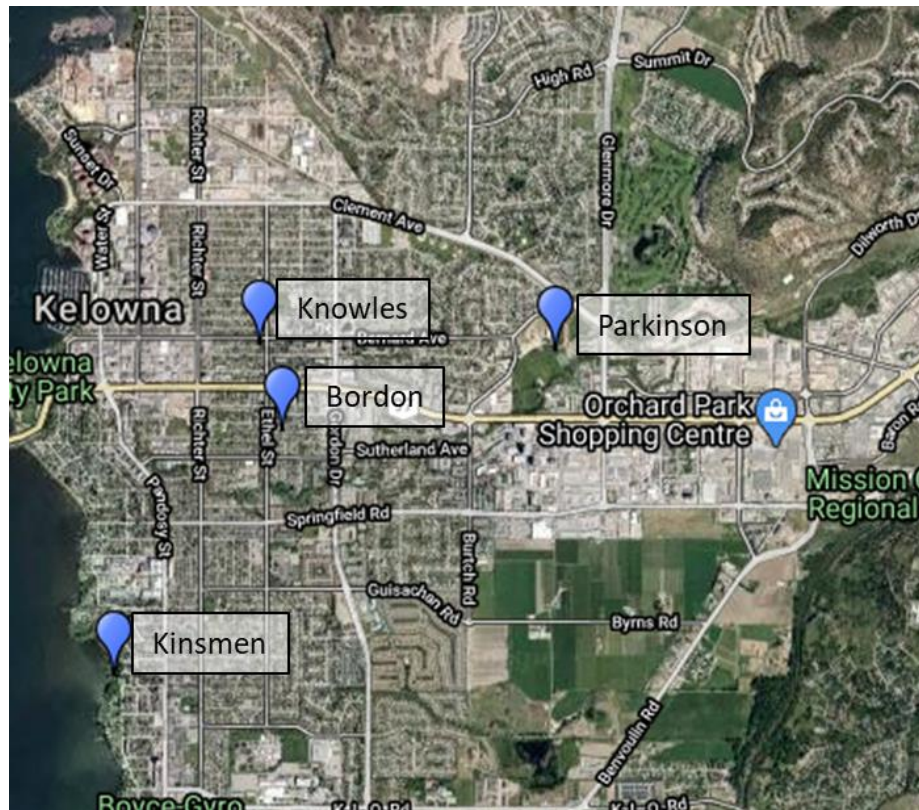


Figure A1. Map showing BMSB sampling sites in Kelowna in 2019.

Appendix B. Age structure of BMSB nymphs

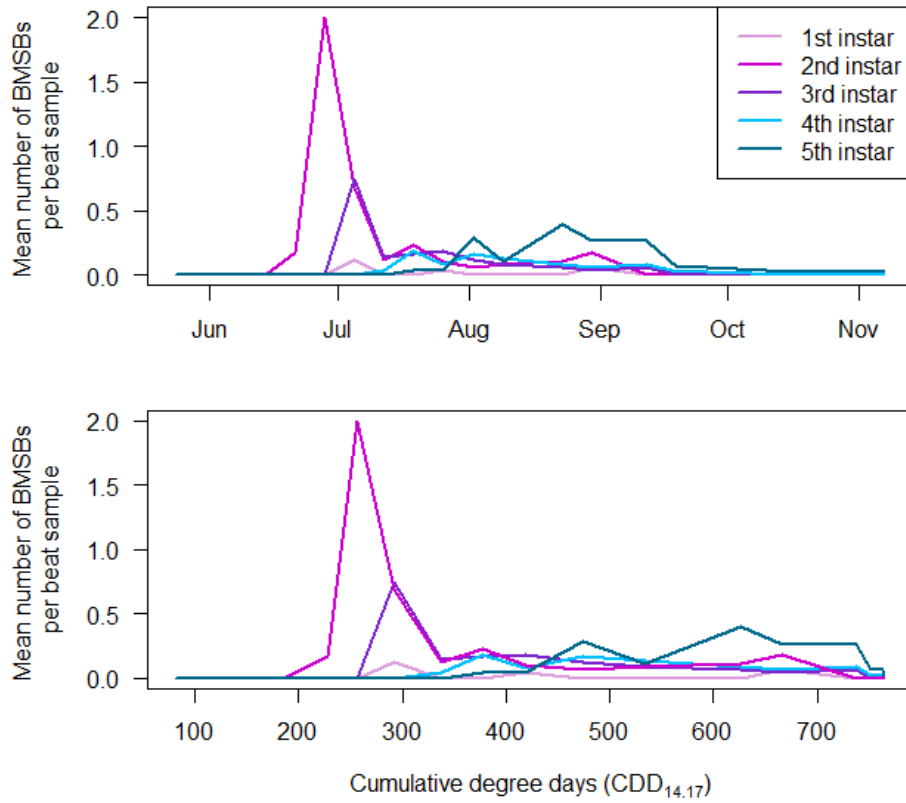


Figure B1. BMSB nymph densities in 2019 in Kelowna (all sites combined) over time (**top**) and cumulative degree days (**bottom**).

Appendix C. Dissections of female BMSBs to determine reproductive development

I prepared female BMSBs for dissections while still frozen. First, I removed the limbs with tweezers (Fig. C1a). I then pinned the specimen (dorsal side down) on a piece of Styrofoam (Fig. C1b), and used a scalpel to first cut along both sides from the 2nd abdominal segment to the posterior end, and then cut above the 2nd abdominal segment (Figs C1c and C2) to loosen the abdominal area of the exoskeleton (Fig C1d). I then transferred the insect to a Petri dish, submerged it in isotonic Ringer's solution (Fig. C1e), and placed it under a dissecting microscope. Using forceps, I separated the abdominal section from the rest of the insect, and carefully teased out the organs to expose the reproductive system (Fig. C1f).

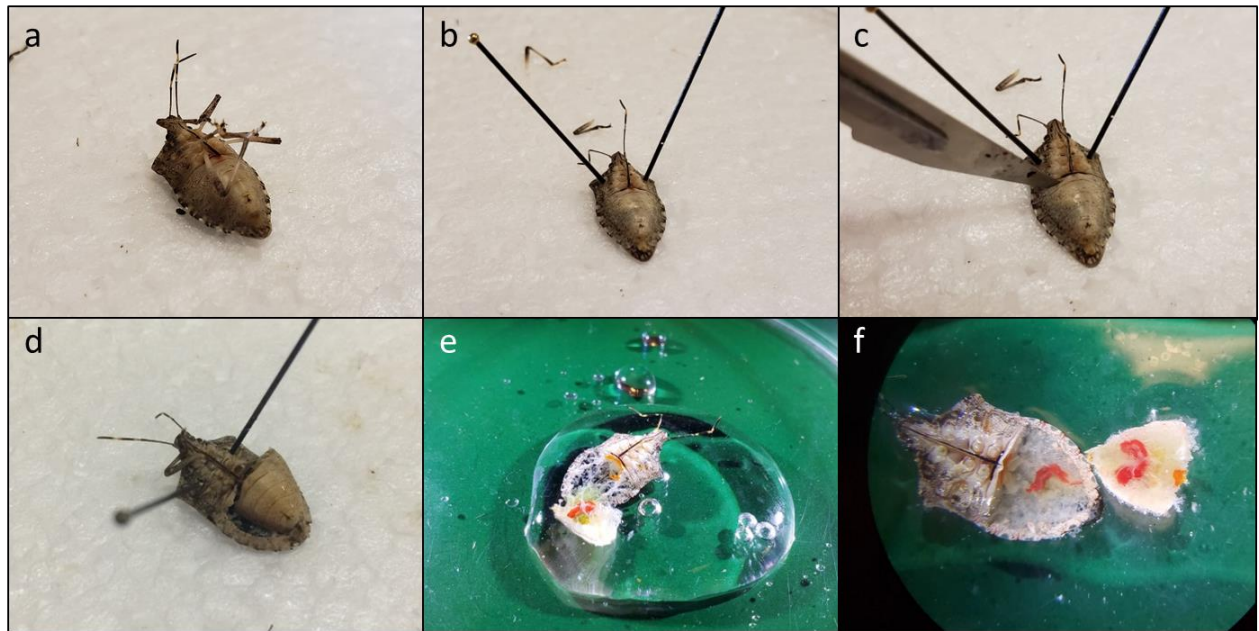


Figure C1. Steps to prepare female BMSBs for ovarian dissection.

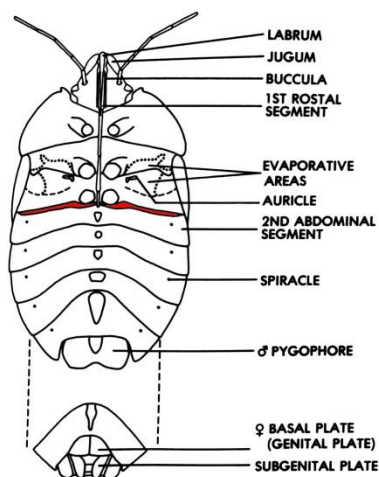


Figure C2. General ventral view of a stink bug.

Source: McPherson, J.E. 1941. The Pentatomoidea (Hemiptera) of northeastern North America with emphasis on the fauna of Illinois.

Appendix D. Ovarian dissections for determination of reproductive and mated ranks

I ranked female BMSBs into five categories of reproductive status and three categories of mated status according to Nielsen et al. (2017). Females in reproductive rank 1 had undeveloped ovaries and thin translucent oviducts, with no or one immature oocyte per ovariole (Fig. D1a). Females in reproductive rank 2 had thicker oviducts and more than one immature oocyte per ovariole, but no mature oocytes (determine by testing whether the oocyte was hardened using forceps; Fig. D1b). Females in reproductive ranks 1 and 2 were previtellogenic, or immature. Females in reproductive rank 3 contained at least one mature oocyte per ovariole (Fig. D1c), and females in reproductive rank 4 contained mature oocytes and at least one oocyte in the lateral oviducts (Fig. D1d). Females in reproductive ranks 3 and 4 were vitellogenic, or reproductively mature. Females in rank 5 were postvitellogenic, and had follicular relics that were dark yellow to black. Females with mated rank 0 were unmated, and had spermatheca that were translucent and not distended (Fig. D1a,b,g). Females with mated rank 2 were mated, and had enlarged and opaque spermatheca (Fig. D1c,d,f). Females that had possibly mated were assigned mated rank 1. These females had slightly opaque or enlarged spermatheca.

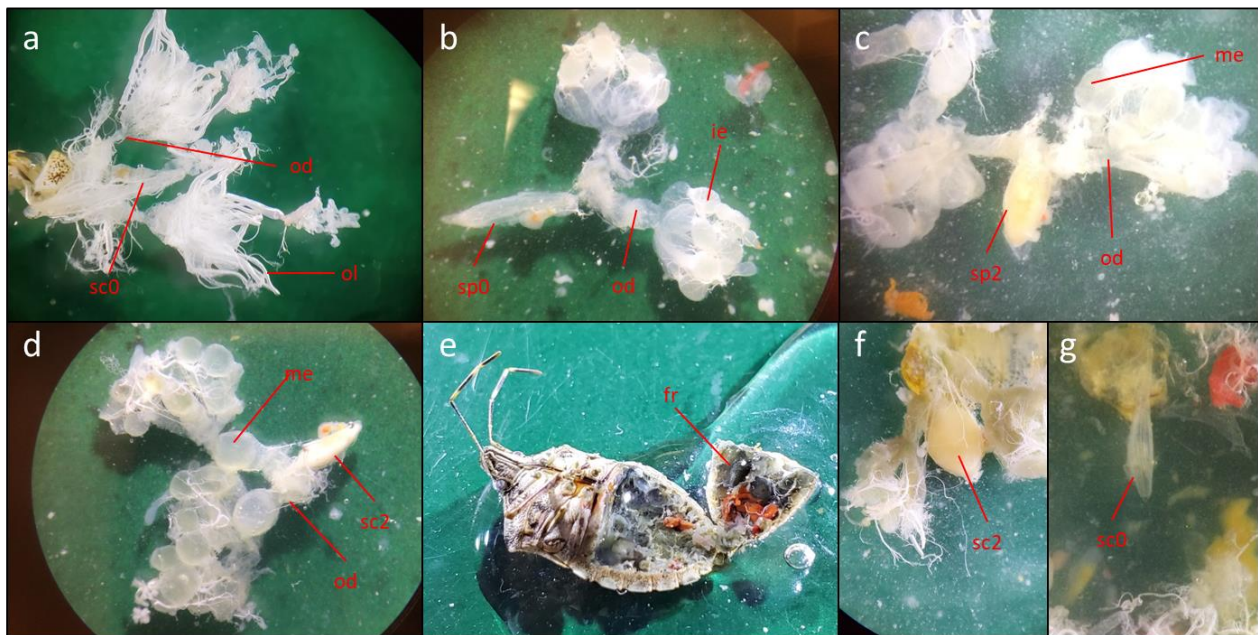


Figure D1: Reproductive ranks and mated ranks of female BMSBs adapted from Nielsen et al. (2017).

Reproductive ranks are rank 1 = previtellogenic (no or one immature oocytes, **a**); rank 2 = previtellogenic (more than one immature oocyte, **b**); rank 3 = vitellogenic (at least one mature oocyte, **c**); rank 4 = vitellogenic (at least one oocyte in the lateral oviducts, **d**); and rank 5 = post reproductive (**e**). Mated ranks are rank 0 = unmated (thin and transparent spermatheca, **a,b,g**); rank 1 = possibly mated (thicker and more opaque spermatheca); and rank 2 = mated (thick and opaque spermatheca, **c,d,f**). Labels in red: sp0: spermatheca, unmated; sp2: spermatheca, mated; od: oviduct; ol: ovariole; ie: immature egg; me: mature egg, fr: follicular relics.