Seasonal Trends in the Condition of Nesting Females of a Solitary Bee: Wing Wear, Lipid Content, and Oocyte Size

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During the nesting season, adult females of the solitary bee *Megachile rotundata* (F.) face considerable physical and energy demands that could include increasing wear and tear on their bodies and decreasing lipid reserves. Consequently, their reproductive performance may be affected not only by extrinsic factors (e.g. weather and floral resource availability), but intrinsic changes in their own bodies. Because of the potential fitness effects of seasonal changes in body condition, our objectives were to determine how wing wear, lipid reserves, and oocyte sizes vary during nesting seasons, beginning when females emerge as adults. As nesting progressed, females in two populations experienced a steady increase in wing wear, which is known to reduce foraging efficiency and increase risk of mortality in other bees. Soon after emergence, females exhibited sharp declines in lipid content which remained low for the remainder of the season. Newly-emerged females ingested pollen, an activity known to be correlated with the initiation egg maturation in this species. Additionally, the early summer drop in lipid stores was correlated with an increase in the size of the oocytes carried. However, by ~6 weeks after emergence, oocytes began to decrease in length and volume, perhaps due to nutrient deficiencies related to loss of stored lipids. Our results suggest management of *M. rotundata* should include rearing bees at temperatures that maximize stored lipid reserves in adults and timing bee release so that significant pollen resources are available for both adults and offspring.

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INTRODUCTION

The nesting season of the leafcutting bee *Megachile rotundata* (F.) (Hymenoptera: Megachilidae) is a period of intense physical activity, each female acting on her own to modify and provision a nest cavity (Pitts-Singer & Cane, 2011). During this time, females experience considerable energy demands and physical stresses from the large number of flights taken to collect pollen, nectar, and nesting materials. Soon after emerging as adults, females also begin to produce relatively large eggs (Richards, 1994), as is the case in other solitary, nest-provisioning Hymenoptera (Iwata, 1955, 1960; K.M. O'Neill, 2001). Thus, we expect females to experience seasonal declines in both physical condition and the lipid reserves they carry over from earlier developmental stages (K.M. O'Neill et al., 2011).

Questions concerning reproductive performance of adult *M. rotundata* are relevant to their value as pollinators of alfalfa grown for seed production (Pitts-Singer & Cane, 2011). Following removal of bee cells from winter storage, *M. rotundata* are reared from prepupae to adults over 3-4 weeks before being released into field nest shelters containing thousands of potential nest sites. Rearing is timed so that cells are placed in fields as the alfalfa starts blooming and as adult females begin to emerge. Unlike honey bees and bumble bees, which produce new batches of foragers throughout the growing season, adults of solitary bees like *M. rotundata* emerge more synchronously. Even if a partial second generation appears later in the summer, it is the adult females from the overwintering generation that are present over the 4-6 weeks when most of the alfalfa flowers are pollinated (Strickler & Freitas, 1999; Bosch & Kemp, 2005). Thus, it is of interest to determine whether the condition of adult females changes seasonally, in ways that could affect the consistency of their performance as pollinators and as parents of the generation of bees that will be released during the following year's growing season.

The effect of management strategies on the survival and development of the immature stages of *M. rotundata* has been studied during the summer nesting season (Pitts-Singer & James, 2008; Pitts-

45   Singer, 2013b), during fall and winter storage of bee cells (Richards, Whitfield & Schaalje, 1987; Pitts-

46   Singer & James, 2005, 2009), and while bees are being reared prior to release into fields (Yocum et al.,

47   2010; K.M. O'Neill et al., 2011). However, few studies have examined factors that could change

48   seasonally and affect the fitness of adult females while they are nesting. First, wings of female solitary

49   bees accumulate wear (Mueller & Wolf-Mueller, 1993; Wuellner, 1999; López-Uribe, Oi & Del Lama,

50   2008) that is known to affect the foraging success and reproductive performance of bees (Cartar, 1992;

51   Higginson & Barnard, 2004; Foster & Cartar, 2010; Rehan & Richards, 2010). Second, lipid stores

52   within fat bodies may decline, potentially influencing egg production, as they do in other insects

53   (Arrese & Soulages, 2010). The importance of lipid stores is suggested by the fact that ~20% of fresh

54   weight of diapausing prepupae of *M. rotundata* consists of lipids. That value is greater than in most

55   other insects, including honey bees (Buckner, Kemp & Bosch, 2004). Although much of the lipid in

56   fat bodies may be metabolized during the pupal-to-adult transition, some females emerge with lipids

57   comprising >25% of their dry weight (K.M. O'Neill et al., 2011). A question that remains, however, is

58   how rapidly these stores are depleted in adults. Third, the decline in lipid stores, whenever it occurs,

59   may also be correlated with a reduction in the size of developing oocytes, which peak in volume about

60   five weeks after emergence (Richards, 1994). Of these three female qualities, only temporal changes in

61   ovary condition have been quantified (Richards, 1994), and questions remain regarding changes in

62   oocyte size.

63   Given the potential fitness effects of seasonal changes in female body condition, the objectives

64   of this study were to determine how wing wear, body lipid reserves, and oocyte sizes vary across

65   nesting seasons, beginning at the time when females appear as adults. Our study was conducted during

66   two field seasons at one site in Montana (MT) and one season at a site in Utah (UT), where we

67   collected bees weekly or bi-weekly following their release into alfalfa fields. We also recorded

68   qualitative assessments of the pollen carried in the guts of females, pollen being the only significant

69   source of protein in adult female diets. We discuss the implications of the results for reproductive

70   strategies of solitary bees and for management of this widely-used pollinator.

71

72   **MATERIALS AND METHODS**

73   **Study sites and collection methods**

74   We collected bees at two sites: 1) ~3 km west of Laurel, MT (45°39' 10.99" N, 108°49'39.36"W)

75   (2011 and 2012) and 2) Lewiston, UT (44°55' 31.73" N, 111°54'00.20"W) (2012). At both sites,

76   cooperating growers released *M. rotundata* into nest shelters within commercial seed alfalfa fields.

77   Each year, our first sample was taken within 1-2 days of the release, by using sweep nets to collect

78   females entering and leaving nest entrances. Most, if not all, females were recently eclosed and in the

79   early stages of nesting activities. We returned to the sites weekly (or bi-weekly for most samples in

80   2011 in MT) to obtain further samples in the same manner. We continued sampling until 1)

81   populations declined and few females remained active or 2) the growers removed the nests from fields

82   (see below). All samples were immediately placed in a cooler with ice and frozen later the same day to

83   reduce the likelihood of females sustaining further wing wear or metabolizing lipids after capture. In

84   2012 in MT, a second set of sweep samples was collected at nest shelters on five dates and used for

85   analysis of ovary condition and relative oocyte size. Females in these samples were immediately

86   placed in Kahle's solution upon return to the lab. We originally planned to sample through the time

87   when numbers of females declined later in the summer, but the grower at the MT site removed bee

88   shelters from the field earlier than usual because of the condition of the alfalfa.

89

90   **Quantification of wing wear**

91   To quantify wing-wear, we used the index of wing wear (WW) developed for bees by Mueller & Wolf-

92   Mueller (1993). For each bee, we used a stereomicroscope to record the number of nicks on the apical

93   edge of each forewing, the relative size of the nicks, and the proportion of intact margin to determine

94   WW as follows: 0 = a completely intact apical margin; 1 = 1-2 nicks on the apical margin; 2 = 3-10

95   nicks on the margin; 3 = some wing margin intact, though heavily serrated, with >10 nicks; 4 =

96   completely serrated with no apical wing margin intact, but with excisions less than half the width of the

97   distal submarginal cell; 5 = wing as described in 4, but with excisions more than half, but less than the

98   entire width of the distal submarginal cell; and 6 = wing as described in 4, but with excisions greater

99   than the width of the distal submarginal cell (Mueller & Wolf-Mueller, 1993). Like Mueller & Wolf-

100   Mueller, we ignored large excisions present on one wing only, because they seemed not to be

101   indicative of gradual wing wear. We evaluated the margins of the left and right forewing of each bee

102   and scored them separately according to the WW classes described above, and then averaged the scores

103   from the forewings to derive a single WW score for each bee. We tested the hypothesis that WW

104   varied with date of collection using Kruskal-Wallis Tests, followed by Dunn's test for multiple

105   comparisons (Zar, 1999; SigmaPlot v. 11.0; Systat Softfware, Inc.).

106

107   **Quantification of body lipid content**

108   We quantified body lipid content using the same bees for which we measured WW, following a

109   method used previously for solitary bees (Richards & Packer, 1994; K.M. O'Neill et al., 2011) and

110   wasps (Strohm, 2000). Bees were removed from the freezer and placed within separate glass vials in a

111   55ºC drying oven. After four days, a subset of 15 bees was removed and their dry masses were

112   individually determined to the nearest 0.01 mg on a Sartorius TE64 balance (Goettingen, Germany).

113   After returning them to the oven, we reweighed the same 15 bees 24 h later to determine if their body

114   masses had stabilized; if not, they were again returned to the oven and the procedure was repeated

115   daily until stable masses were obtained. At that time, all bees were weighed. To extract lipids from

116   dried bees, we added 10 ml of petroleum ether to each vial as soon as the bees were removed from the

117   oven and capped it. After 10 days, we decanted the ether and air-dried the vials containing the bees for

118   one hour under a laboratory hood. The vials were then placed back into the oven until the masses

119   again stabilized (~72 h) before a post-extraction body mass was obtained. We subtracted post-

120   extraction dry mass from the pre-extraction dry mass to estimate of the amount of lipid extracted. The

121   result was then divided by the pre-extraction dry mass to obtain an estimate of the proportion of dry

122   mass comprised of lipids (P_L) for each bee. Each year, all bees collected during the summer were

123   subjected to lipid analysis at the same time to avoid any variation that might be introduced by minor

124   variations in handling technique or conditions in the lab.

125   Using one-way analyses of variance (ANOVA; SigmaPlot v. 11.0, Systat Softfware, Inc.), we

126   tested the hypothesis that the P_L varied with 1) collection date and 2) WW. Before analysis, P_L values

127   were transformed as the arcsine of the square root of P_L (Zar, 1999). Pairwise comparisons were

128   conducted using Student-Newman-Keuls (SNK) tests. We compared proportions of females with P_L

129   values ≥ 0.10 and ≤ 0.05, contrasting the first collection date each year with all other collection dates

130   combined using Fisher Exact Tests.

131

132   **Quantification of ovary condition and oocyte size**

133   The females stored in Kahle's solution were dissected under ethanol so we could measure oocytes using

134   a stereomicroscope equipped with an ocular micrometer. We dissected from 26-32 females collected

135   on each of five dates in 2012 (total $N = 145$). To reduce the likelihood of having second generation

136   females included in the 3 August sample, we dissected only bees with WW ≥ 1 for that date. For each

137   female, we recorded: 1) head width (HW; to the nearest 0.5 mm), as an indicator of overall body size,

138   2) WW as described above, but for the right forewing only (the latter was deemed sufficient because

139   we had determined in the other wing wear analysis that WW values for the two wings on each female

140   differed by ≤ 1 in >97% of females), 3) length (L) and diameter (D, at the midpoint of its long axis) of

141   each of the three longest oocytes (if the oocyte was somewhat flattened by being pressed against the

142   inner wall of the exoskeleton, we recorded the midpoint value between the greatest and smallest

143   width), and 4) whether the largest oocyte was allantoid (i.e., sausage-shaped, or a cylinder with

144   hemispherical ends), as is typical of mature oocytes (Iwata, 1955). For allantoid basal oocytes (i.e.,

145   those next in line for oviposition and the largest oocyte in each female), we estimated their volume (V

146   in mm^3) as $V = (4/3)(\pi r^3) + (\pi r^2)(L-2r)$, where $r = D/2$. Other oocytes approximated a prolate spheroid

147   in shape, so we estimated their volume as $V = (4/3)(\pi)(r^2)(L/2)$.

148   The mean head widths of females dissected did not differ significantly among the five sample

149   dates (one-way ANOVA: $F = 1.61$; $df = 4, 140$; $P = 0.17$). Nevertheless, to test the hypothesis that

150   oocyte size variables changed across the nesting season independent of body size, we standardized

151   oocyte variables across head widths by calculating the ratios of four oocyte size variables to HW. The

152   four variables were the maximum length (L_{basal}) and volume (V_{basal}) of the basal oocyte and the

153   summed maximum lengths (L_{total}) and summed volumes (V_{total}) of the three measured oocytes. We

154   then used Kruskal-Wallis Tests to compare ratios among dates (with Dunn's Test for comparisons

155   among pairs of dates; $\alpha = 0.05$).

156   To test the hypothesis that oocyte variables differed among females with different degrees of

157   wing wear, we began by regressing L_{basal}, L_{total}, V_{basal}, and V_{total} on HW. We used the resulting linear

158   regression equations to determine which females had negative residuals (i.e., smaller than expected

159   oocytes) and positive residuals relative to predicted values from the regressions. We used Mann-

160   Whitney Tests to determine if females with negative residuals had WW values different from those

161   with positive residuals. We also correlated WW with the four oocyte variables using Spearman Rank

162   Correlation tests.

163

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166 **Observations of pollen loads in guts**

167 For each female dissected, we recorded whether their crops and midguts were mostly full of pollen, as

168 opposed to having a few pollen grains. To determine whether most of the pollen was likely from

169 alfalfa plants, we dissected ten randomly chosen females each from the 22 June and 3 August samples;

170 these females were not included in the oocyte analyses. We chose those two days because our previous

171 research showed that females collect mainly alfalfa pollen early in the nesting season, but often add

172 significant amounts of pollen from other plant species later in the nesting season (R.P. O'Neill & K.M.

173 O'Neill, 2010). Thus, we expected any changes that might occur in pollen loads in guts would be

174 detected by examining females from the first and last collection dates of the year. We used a

175 micropipette to remove a sample of pollen from their guts and examined the pollen using 10x and 40x

176 power under a compound microscope. One hundred pollen grains counted along a transect on the slide

177 were scored as being either alfalfa or non-alfalfa pollen based on comparison to reference collections

178 that we made for previous studies (K.M. O'Neill et al. 2004; R.P. O'Neill & K.M. O'Neill, 2010).

179

180 **RESULTS**

181 **Seasonal trends in wing wear**

182 Tattering of the forewings of *M. rotundata* occurs mainly along the apical margins where they are

183 unsupported by bordering wing veins beyond the distal terminus of the costal vein. Wing wear varied

184 significantly over the course of each nesting season at both locales (Fig. 1; Kruskal-Wallis Tests, $P <$

185 0.001 for each of the three sets of samples), but there was considerable variation among females

186 collected after the first sampling dates. After a steady rise in mean WW early in the nesting season, it

187 reached a plateau in both years at the MT site and declined at the UT site. Most bees collected on the

188   first date each year exhibited WW values of ≤0.5, 96% for MT in 2011, 97% for MT in 2012, and 77%

189   for UT in 2012.

190   In the two sets of MT samples, mean WW rose during the first 3-4 weeks following release.

191   During the last three sampling dates combined, WW values were ≥4.0 in 43% of females in 2011 and

192   63% in 2012. For the UT bees, mean WW increased from <0.5 on the first collection date to 3.7 about

193   three weeks following bee release, with 57% of WW values at that time being ≥4.0. However, mean

194   WW then declined to 2.1 on 18 July (JD = 200), with 30% of the values being ≤0.5 and just 12% being

195   ≥4.0; as discussed later, this likely occurred because of an influx of a second generation of bees into the

196   population. The following week (JD = 207), the mean rose to its seasonal peak of 4.5, with all values

197   exceeding 2.5 and 83% being ≥4.0. During the last three dates, the mean again declined to ~1.5-2.5.

198   Wing wear was generally symmetrical between the left and right forewings. Among the 748

199   females examined, scores for the two wings were identical in 61.5% of females and differed by just one

200   in another 35.7% of females. Wing wear scores never differed by more than two in any one female.

201

202   **Seasonal trends in lipid content**

203   The lipid content of females varied significantly over the course of the nesting season in all three sets

204   of samples (Table 1, Fig. 2, left). Body lipids declined after field release, the initial decrease in mean

205   P_L ranging from 30-39% in the three sets of samples. The P_L values then remained relatively constant

206   for the remainder of each summer, with the exception of the brief increase in the 18 July (JD = 200)

207   sample in Utah in 2012 (i.e., the same date on which WW values declined). The proportions of

208   females with P_L ≥0.10 on the first collection date was greater than the proportions for all other dates

209   combined (Table 2). Conversely, the proportion of females on the first collection date with P_L ≤ 0.05

210   was smaller than the proportions on all other dates combined.

211   Given the great variation in female WW values on most dates, it seems clear that date of

212   collection is not an accurate estimate of females' physiological ages (i.e., the cumulative amount of

213   physiological/behavioral activity in their adult lives that is reflected in the degree of wing wear); this is

214   especially the case later in the season when some second generation females emerge. In each sample

215   P_L, varied significantly across WW categories (Kruskal-Wallis Tests, $P < 0.001$ for each site/date

216   combination; Fig. 2, right), with the decline in P_L across WW classes, being more gradual than that

217   observed across dates. Females with WW values ≥ 4.5 had mean P_L values about 35% (MT 2011) to

218   48% (MT and UT 2012) that of females with WW values of zero (Table 1).

219

220   **Seasonal trends in oocyte size**

221   In dissected females, the crop occupied most of the anterior half of the metasoma. The ovaries shared

222   the limited space in the posterior half with other organs. Particularly when the crop was inflated with

223   nectar/pollen, the interior cavity of the metasoma was often so crowded that larger oocytes were

224   pressed against the inner surface of the integument and sometimes had indentations conforming to

225   boundaries between metasomal segments. As oocytes matured and moved towards the position of

226   being basal oocytes, they typically changed from being prolate spheroid-shaped to allantoid. Just 50%

227   of the 30 females dissected from the 22 June sample had allantoid basal oocytes. Over the next three

228   dates combined, 99% ($N = 85$) of females had at least one allantoid oocyte, but the percentage declined

229   to 73% by 3 August ($N = 30$).

230   Our primary objective was to determine if oocyte size variables (L_{basal}, L_{total}, V_{basal}, and V_{total})

231   changed as the nesting season progressed, but analysis is complicated by the correlation of body size

232   with oocyte size (K.M. O'Neill, Delphia & R.P. O'Neill, 2014). To remove the confounding effect of

233   body size, we calculated ratios of each of the four oocyte variables to head width. All four ratios

234   varied significantly across the five sampling dates (Fig. 3; Kruskal-Wallis Tests, $P < 0.001$ for each of

235   the four variables), primarily because of the low ratios for 22 June. However, the L_{total}/HW and

236   V_{basal}/HW ratios rose at the beginning of the nesting season, but then declined significantly by early

237   August.

238   To test the hypothesis that wing wear was correlated with oocyte size (independent of date of

239   collection and head width), we determined which females collected after 22 June had smaller- or

240   larger-than expected values for oocytes-size variables based on the regressions of those variables on

241   HW. Females with positive residuals (larger than expected oocyte sizes) had significantly lower WW

242   values than those with negative residuals for L_{basal} (Mann-Whitney Test, $P < 0.01$), V_{basal} ($P < 0.01$),

243   and V_{total} ($P < 0.001$), but not L_{total} ($P = 0.68$) (Fig. 4). These results are reflected in the overall

244   correlations between the oocyte variables and WW which were significant and negative for L_{basal}

245   (Spearman Correlation, $r = -0.22$, $P < 0.05$), V_{basal} ($r = -0.27$, $P < 0.01$), and V_{total} ($r = -0.27$, $P < 0.01$),

246   but not L_{total} ($r = -0.14$, $P = 0.14$). Thus, although we observed considerable variation among females,

247   and those with high WW scores sometimes produced large oocytes, the more wing-worn females

248   tended to have smaller oocytes than would be predicted from their body sizes.

249

250   **Pollen counts**

251   Of the 2000 pollen grains identified, 100 from each of 10 females from 22 June and 10 from 3 August,

252   all but one was from alfalfa. That single grain appeared to be from a plant of the family Asteraceae.

253   After identifying the 100 grains from each female, we scanned the whole sample on each slide and

254   found no other non-alfalfa pollen.

255

256   **DISCUSSION**

257   **Seasonal trends in wing wear**

258 We documented a steady decline in wing condition over the first 3-4 weeks of each nesting season at

259 both sites, with some females having wings tattered along their entire distal margins by the second or

260 third week following bee release. Many late-season females had deep excisions in their wings. Based

261 on mark-recapture observations of individual bees, wing wear of bumble bees (Foster & Cartar, 2011)

262 and honey bees (Higginson & Barnard, 2004) accelerates with age. The lack of an accelerating, or

263 even a steadily increasing, seasonal trend in wing wear for *M. rotundata* in our data could have several

264 causes. First, unlike the studies of the social bees, we did not score WW on the same bees each week.

265 If *M. rotundata* females with extremely worn wings die late in the season, as occurs in bumble bees

266 (see below), they would be absent from our samples. Second, there may be an inherent non-linear

267 relationship between age and wing wear, in which females progress through the first three (i.e., lowest)

268 WW classes quickly, but then more slowly through the remaining WW classes, perhaps because the

269 open wing margin is now closer to supporting wing veins. Third, the appearance of an overlapping

270 second generation of females with less wing wear late in the season would result in smaller mean WW

271 values if females of both generations are mixed in samples. Based on the reappearance of females with

272 low WW values late in the season, second generation females did not show up in the MT samples until

273 the seventh (2012) or eighth week (2011) following the initial release of the bees. Based both on WW

274 values and the appearance of emergence holes in nests, they appeared at the UT site earlier, perhaps by

275 weeks four and five. The great variability of wing wear in *M. rotundata*, even prior to emergence of a

276 second generation, suggests that it is a weak predictor of mean calendar age, as is the case for the

277 solitary bees *Anthidium manicatum* (L.) (Mueller & Wolf-Mueller, 1993), *Dieunomia triangulifera*

278 (Vachal) (Wuellner, 1999), and *Euglossa* spp. (López-Uribe, Oi & Del Lama, 2008).

279 Rapid accumulation of wing wear in *M. rotundata* is expected from the high level of flight

280 activity of nesting females. Klostermeyer & Gerber (1969) estimated that completion of a single nest

281 cell involved an average of 15 leaf-collecting trips and 17 pollen- and nectar-foraging trips, and later

282   estimated that females typically provision 12-16 cells during their lives (Gerber & Klostermeyer,

283   1972). Szabo & Smith (1972) reported that females visited an average of 7.5 flowers per foraging trip

284   at air temperatures of 17-21ºC, and 17.8 per trip at 21-25ºC. Based on these combined estimates,

285   females would make 384-512 total trips during their adult lives, depending on cell number and

286   temperature, visiting thousands of flowers in the process. This may be an underestimate, because some

287   females are known to provision >25 cells in a lifetime (T. Pitts-Singer, unpublished data). The values

288   for the variables leading to these estimates are certainly time- and site-specific, but they illustrate the

289   amount of time females spend foraging. To this, we can add the unknown number of visits to flowers

290   to collect nectar and pollen for self-maintenance. Although females probably forage near nests when

291   flowers are available during the peak of alfalfa bloom, *M. rotundata* females sometimes forage

292   hundreds of meters from nests (Tepedino, 1983; St. Amand, Skinner & Peaden, 2000). Finally, it is

293   also possible that females sustain wing wear when 1) fighting with nest-intruding females over

294   possession of tunnels, 2) colliding with other bees in crowded commercial bee shelters, 3) interacting

295   with males during attempted matings, and 4) moving about in the confines of nest tunnels, which are

296   often barely wider than their bodies (K.M. O'Neill et al., 2010).

297   Insight into the potential causes and consequences of wing wear in *M. rotundata* is gained from

298   studies of other bees. In worker bumble bees, variation in wing area lost was related not to flight

299   frequency or time spent in flight, but to collisions of wings with vegetation during foraging (Foster &

300   Cartar, 2011). Greater degrees of wing wear increased mortality rates (Cartar, 1992) and influenced

301   the choice of flower patches visited (Foster & Cartar, 2010), but had no effect on pollen load sizes, the

302   duration of foraging trips (Cartar, 1992), or the energetic cost of flight (Hedenström, Ellington & Wolf,

303   2001). Rodd, Plowright & Owen (1980) suggest that the rapid increase in mortality rates of *Bombus*

304   workers after two weeks of adult life is due to accumulated wing damage that hinders their ability to

305   escape predators. In honey bees (*Apis mellifera* L.), workers with greater wing wear tended to accept

306   smaller, lower-quality inflorescences, leading to reduced foraging efficiency (Higginson & Barnard,

307   2004). Similarly, an experimentally-induced reduction in wing area significantly decreases the rate of

308   food delivery and increases mortality of worker honey bees (Dukas & Dukas, 2011). Overall, this brief

309   review suggests that wing wear in *M. rotundata* might also be linked to foraging success, with

310   consequences for the sustainability of nesting performance as the summer progresses. More direct

311   experimental tests of this hypothesis are required, but such tests also need to take into account the

312   possibility that declining performance of wing-worn females late in the season is also related to the

313   smaller body lipid reserves and floral resource availability. In addition, there is also the question of

314   whether the fitness consequences of declining flight performance related to wing wear are similar in

315   social and solitary bees. In eusocial bees, older, physically-worn workers are replaced by younger bees

316   as the season progresses, but there is no such compensating mechanism for solitary bees. One study of

317   a solitary bee *Ceratina calcarata* showed that females with greater wing wear produced smaller

318   clutches and smaller overall masses of adult offspring, and that wing wear appeared to be the primary

319   correlate of declining levels of parental investment across the nesting season (Rehan & Richards,

320   2010).

321

322   **Seasonal trends in lipid content**

323   In a previous study of adults active in the field in 2006 and 2007 at the same MT site in this study,

324   female *M. rotundata* reared under controlled laboratory conditions from early fall to early summer

325   emerged with a mean proportion body lipids of 15-18% (K.M. O'Neill et al., 2011). In the present

326   study, the fact that the initial mean values for P_L of newly-emerged field-collected females were just 7-

327   11% could be related to several differences between the studies. First, yearly differences in floral

328   resource quality and quantity could affect the lipid content of the bees at the time they entered diapause

329   the previous year. Second, there might be differences in stored-lipid metabolism between bees reared

330   under tightly-controlled laboratory conditions (K.M. O'Neill et al., 2011) and those experiencing

331   variable on-farm rearing conditions (present study). In our earlier study, variation in temperature

332   during pre-release rearing explained 13-27% of the variance in the proportion of body lipids of newly-

333   emerged adults, with the optimal rearing temperatures for maintaining maximum lipid reserves being

334   27-29ºC. In addition, brief exposure of bees to 38ºC during the last week of adult rearing in the lab

335   causes significant lipid loss. In the field, bees could regularly experience such spikes in temperature

336   when cells are placed into field shelters at the time of release. In late June or early July, when females

337   complete pupal-adult development, it would not be unusual for shelter temperatures to exceed optimal

338   temperatures for lipid conservation. Finally, bees reared in the laboratory were frozen for lipid analysis

339   within several minutes to several hours of their emergence as adults (K.M. O'Neill et al., 2011). In the

340   present study, however, they were likely to be at least 1-2 days old when collected; some may have

341   been older than that because, even in controlled laboratory conditions, females reared at the same

342   temperature do not all emerge on the same day (K.M. O'Neill et al., 2011). Thus, during the brief

343   interval between emergence and collection, females in the present study may have already converted a

344   considerable portion of their fat-body lipids to proteins destined for egg production (Sihag, 1985,

345   1986). In the halictid bee *Lasioglossum malachurum*, a species that overwinters as adults, depletion of

346   fat reserves during solitary nest-founding is rapid and more lipids are lost during that period than

347   during hibernation (Weissel et al., 2012).

348   Whatever the reason for the initial P_L values, our results indicate that lipid content in adult

349   females declines rapidly after field release and then stabilizes at a lower level later in the season. Some

350   of the temporal variation we observed after the initial decline is likely due to emergence of a second

351   generation that introduced individuals with greater lipid content into later samples. In the UT samples,

352   the significant mid-season increase in lipid content on 18 July (i.e., JD 200), which was about one

353   month after bee release, corresponded to an influx of newly-emerged females with lower WW values.

354 Apparently, as these second-generation females aged, mean P_L values again declined one week later.

355 Examining the trend of P_L as function of wing wear (as a measure of physiological age) rather than

356 date, removed much of the temporal fluctuation probably because it removed the influence of second-

357 generation females on the trend.

358 Because we extracted lipids in bulk, we do not know what proportion of lipids remaining after

359 the initial decline were present in fat bodies, where they could act as nutrient reserves. Lipids extracted

360 from tissues not involved in lipid storage likely create a baseline value to lipid content below which

361 living females cannot decline, even after most fat body lipids have been depleted. Thus, as with wing

362 wear, females whose lipid levels dropped below a critical level may have disappeared from the

363 population. In addition, an unknown amount of lipids in the samples might have been derived from

364 pollen in the guts of females so its contribution deserves further analysis. Alfalfa pollen, which

365 constituted almost all of what we identified in dissected females, contains about 8.5% lipids (Roulston

366 & Cane, 2002). Alfalfa pollen is typically most of what females collect when nesting in commercial

367 alfalfa fields in Montana (K.M. O'Neill et al., 2004; R.P. O'Neill & K.M. O'Neill, 2010). The volume

368 of pollen in the guts of females is unknown, but the estimated lipid content of an average pollen load at

369 a time when alfalfa is the main source of pollen is just 0.06 mm^3 (R.P. O'Neill & K.M. O'Neill, 2010).

370 Based on qualitative assessment of pollen loads within the gut, however, we found no tendency for

371 females to increase pollen consumption as their lipid content dropped. In fact, pollen consumption

372 may have declined because all females collected on 22 June had crops containing large masses of

373 pollen, whereas after that date most had very little pollen in their crops even when their midguts were

374 full of pollen. Richards (1994) reported that 100% of *M. rotundata* females had pollen in their crops,

375 but noted only its presence or absence, so his results are not comparable to ours. We should also re-

376 emphasize that lab-reared females without access to pollen had even higher P_L values (K.M. O'Neill et

377 al., 2011) than the field-collected females in this study that carried pollen in their guts.

378   At present, it seems clear that females used up a considerable proportion of their body lipids

379   soon after emergence, and it is reasonable to assume that this resulted mainly from fat body materials

380   being converted to materials used to make oocytes (Sihag, 1985, 1986; Klowden, 2002; Arrese &

381   Soulages, 2010). A more detailed analysis of the anatomical sources of lipids extracted from females

382   and the types of lipids extracted at different times of the year would be needed to further explore the

383   significance of our results. There is also the question of the significance of the decline in fat body

384   lipids for aspects of female reproductive performance. First, flight metabolism of bees is thought to be

385   fueled by carbohydrates derived from nectar and stored in the crop rather by nutrients mobilized from

386   fat bodies (Suarez et al., 2005). Second, fat body lipids accumulated during larval development are

387   important sources of nutrients for egg production and adult maintenance for insects in general (Pan,

388   Bell &Telfer, 1969; Keely, 1985; Arrese & Soulages, 2010). Bees, however, have to consume pollen

389   to initiate oogenesis (see below), so some of the materials for oocyte production may come from the

390   adult diet. Nevertheless, as in *Lasioglossum malachurum* (Weissel et al., 2012), depletion of lipid

391   levels in *M. rotundata* continues during the early part of the season despite consumption of pollen soon

392   after adult emergence.

393   **Seasonal trends in oocyte size**

394   In solitary nest-provisioning Hymenoptera, eggs are larger relative to body size than they are in related

395   parasitoid and social species, and each female lays relatively few eggs in her lifetime (Iwata, 1955,

396   1960; Iwata & Sakagami, 1966; K.M. O'Neill, 2001; Rozen, 2003). Such large eggs require substantial

397   investment from females. Our data indicate that oocyte size varied with both female size (K.M.

398   O'Neill, Delphia & R.P. O'Neill, 2014) and collection date (present study). These results are consistent

399   with several of Richards' (1994) conclusions concerning seasonal trends in oocyte development in *M.*

400   *rotundata*. First, females in his samples did not fully mature oocytes until the second week after

401   eclosion; one of us (T. Pitts-Singer, unpublished data), nevertheless, has observed females ovipositing

402   just two days after emergence. *Megachile rotundata* females lay eggs only when a cell is fully

403   provisioned, so newly-emerged females can make progress towards constructing and provisioning their

404   first nest cells before they are capable of laying eggs. Second, although we were unable to collect

405   females beyond ~6 weeks past adult emergence, oocyte size showed evidence of a decline by 3 August.

406   The pollen masses seen in guts of recently-released females are consistent with experimental

407   evidence that pollen consumption is a prerequisite for oocyte development. Richards (1994) showed

408   that female *M. rotundata* fed honey, but lacking access to pollen, failed to initiate vitellogenesis. In

409   fact, the broad taxonomic occurrence of a link between pollen consumption by newly-emerged adult

410   female bees and their schedule of egg development suggests that it is an evolutionarily-ancient trait in

411   apiform Apoidea. Adult consumption of pollen is known to be necessary for the initiation of

412   vitellogenesis and oocyte development in bees of the families Apidae (Human et. al., 2007), Halictidae

413   (Bell, 1973; Wuellner, 1999), and Megachilidae (Sihag 1986; Richards, 1994); bees of these three

414   families are estimated to have diverged from one another 90-100 million years ago during the

415   Cretaceous (Brady, Larkin & Danforth, 2009). Sihag (1986) also demonstrated that the inclusion of

416   protein, rather than just carbohydrates, in an artificial diet was required for vitellogenesis in three

417   species of south Asian *Megachile* managed for alfalfa pollination. Females of two of the species,

418   which were multivoltine (Sihag, 1983), exhibited their highest rates of oocyte development when they

419   emerged at the time of year when alfalfa flowers were most abundant (Sihag, 1986). At that time, the

420   average delay between adult female emergence and first oviposition was slightly longer than four days

421   in both species. On a natural diet of alfalfa pollen and nectar, vitellogenesis commenced in the second

422   day following emergence and the first oocytes to develop chorions appeared on day four. In *Megachile*

423   *flavipes* Spinola, vitellogenins that eventually provide proteins for eggs appear in hemolymph on the

424   second day after emergence (Sihag, 1985).

425   Older *M. rotundata* females apparently reach an age when they begin to produce smaller

426   oocytes. Richards (1994) followed a population for ~9 weeks after emergence, and recorded a steady

427   decline in basal oocyte volume after ~5 weeks. Because we could follow bees only until the seventh

428   week after emergence, we observed only the initiation of that decline. The relationship of diminishing

429   oocyte size with advancing physiological age was substantiated by the negative correlations we

430   observed between wing wear and oocyte size. Greater degrees of wing wear are associated with

431   declining clutch size and total brood mass in the bee *Ceratina calcarata* (Rehan & Richards, 2010),

432   and with fewer and smaller mature oocytes in the brood parasitic apoid wasp *Stizoides renicinctus*

433   (K.M. O'Neill & Pearce, 2007). Egg size also declines with maternal age in honey bees (Al-Lawati &

434   Bienefeld, 2009).

435   **Conclusions**

436   Even when nesting in commercial alfalfa fields with high floral densities, *M. rotundata* females face

437   seasonally-dynamic environments. First, higher field temperatures result in greater prevalence of a

438   condition referred to as "pollen ball", that is cells in which the pollen masses are not consumed and in

439   which no bee offspring develop (Pitts-Singer & James, 2008). Second, the prevalences of chalkbrood

440   disease and pteromalid wasp parasitism change seasonally (R.P. O'Neill, 2004). Third, floral resource

441   quality and quantity declines as alfalfa senesces (Strickler & Freitas, 1999; see also Kim & Thorp,

442   2001; Pitts-Singer, 2013a), with the result that pollen types used change seasonally (R.P. O'Neill &

443   K.M. O'Neill, 2010). Lower resource levels for caged females in experimental studies resulted in

444   smaller offspring size and fewer offspring per female (Peterson & Roitberg, 2006). Seasonal declines

445   in investment per offspring have also been documented for the megachilid bee *Osmia lignaria* (Torchio

446   & Tepedino, 1980), though not for the megachilid *Hoplitis anthocopoides* (Schenk) (Strickler, 1982).

447   Fourth, in nest shelters provided for *M. rotundata*, the number of available nest tunnels may decline

448   later in the summer and higher ratios of females to available nest sites have been associated with lower

449   offspring production (Mayer, 1994; but see Pitts-Singer, 2013a).

450   The results of this and other studies (Richards, 1994) make it clear that, besides the

451   environmental factors discussed above, seasonal changes in the condition of the females themselves

452   might also affect the temporal consistency of reproductive performance. Females experience a steady

453   increase in wing wear that could affect their flight efficiency and resource choices. Soon after

454   emergence, they face a rapid drop in lipid stores that can be ameliorated only by feeding on pollen, and

455   any pollen collected for personal consumption cannot be used for nest provisioning. Lastly, relative

456   oocyte size starts to decline within six weeks of emergence, continues through the remainder of the

457   summer (Richards, 1994), and is perhaps related to declining lipid reserves.

458   During management of *M. rotundata* for pollination services (Pitts-Singer & Cane, 2011), not

459   all factors that affect female reproductive success can be controlled (e.g. weather, wing wear).

460   However, certain management practices have the potential to impact adult female reproductive

461   performance in ways related to our findings. First, because females vary considerably in the amount of

462   lipids they carry into adult life and then rapidly deplete these reserves upon emergence, it is important

463   to rear bees at temperatures that enhance conservation of lipid reserves sequestered during larval

464   development (Pitts-Singer & James, 2009; K.M. O'Neill et al., 2011). Second, because females require

465   a source of pollen following emergence, in order to stimulate oogenesis and supplement nutrients no

466   longer supplied by fat body reserves, care must be taken to release females at a time when sufficient

467   bloom is present to support female pollen requirements (as has also been recommended by Richards,

468   1994). The date of commencement of alfalfa bloom is influenced by the vagaries of weather.

469   However, manipulation of pre-release rearing temperatures can be used to slow bee development if

470   bloom is delayed (Yocum et al., 2010), not only to ensure adequate pollen resources for females, but to

471   enhance levels of pollination (Bosch & Kemp, 2005). Nevertheless, although seasonal changes in

472   condition have been documented, the degree to which these changes affect physiological performance

473   and fitness remains to be fully explored.

475 **ACKNOWLEDGEMENTS**

479

480 **ADDITIONAL INFORMATION AND DECLARATIONS**

481 **Funding**

486

487 **Grant Disclosures**

493

494

495 **Competing Interests**

496 The authors declare that they have no competing interests.

497

498 **Author Contributions**

499   •   Kevin M. O'Neill, Casey M. Delphia, and Theresa L. Pitts-Singer conceived, designed, and

500   performed the research, analyzed the data, wrote the paper, prepared figures and tables, and

501   reviewed drafts of the paper.

502

504 **REFERENCES**

505 **Al-Lawati H, Bienefeld K. 2009.** Maternal age effects on embryo mortality and juvenile

506   development of offspring in the honey bee (Hymenoptera: Apidae). *Annals of the*

507   *Entomological Society of America* **102:** 881-888.

508 **Arrese EL, Soulages JL. 2010.** Insect fat body: energy, metabolism, and regulation. *Annual Review*

509   *of Entomology* **55**: 207-225.

510 **Bell WJ. 1973.** Factors controlling initiation of vitellogenesis in a primitively social bee

511   *Lasioglossum zephyrum* (Hymenoptera: Halictidae). *Insectes Sociaux* **20**: 253-260.

512 **Bosch J, Kemp WP. 2005.** Alfalfa leafcutting bee population dynamics, flower availability, and

513   pollination rates in two Oregon alfalfa fields. *Journal of Economic Entomology* **98**: 1077-1086.

514 **Brady SG, Larkin L, Danforth, BN. 2009.** Bees, ants, and stinging wasps (Aculeata) In: Hedges SB,

515   Kumar S, eds. *The Timetree of Life*. New York: Oxford University Press, 264–269.

516 **Buckner JS, Kemp WP, Bosch J. 2004.** Characterization of triacylglycerols from overwintering

517   prepupae of the alfalfa pollinator *Megachile rotundata* (Hymenoptera: Megachilidae). *Archives*

518   *of Insect Biochemistry and Physiology* **57**: 1-14.

519 **Cartar RV. 1992.** Morphological senescence and longevity: an experiment relating wing wear and

520   life span in foraging wild bees. *Journal of Animal Ecology* 61: 225-231.

521 **Dukas R, Dukas L. 2011.** Coping with non-repairable body damage: the effects of wing damage on

522   foraging performance in bees. *Animal Behaviour* **81**: 635-638.

523 **Foster DJ, Cartar RV. 2010.** Wing wear affects wing use and choice of floral density in foraging

524   bumble bees. *Behavioral Ecology* **22**: 52-59.

525 **Foster DJ, Cartar RV. 2011.** What causes wing wear in foraging bumble bees? *Journal of*

526   *Experimental Biology* **214**: 1896-1901.

527 **Gerber HS, Klostermeyer EC. 1972**. Factors affecting the sex ratio and nesting behavior of the

528   alfalfa leafcutting bee. *Washington Experiment Station Technical Bulletin* 73, Pullman:

529   Washington State University.

530 **Hedenström A, Ellington CP, Wolf TJ. 2001.** Wing wear, aerodynamics and flight energetics in

531   bumblebees (*Bombus terrestris*): an experimental study. *Functional Ecology* **15**: 417-422.

532 **Higginson AD, Barnard CJ. 2004.** Accumulating wing damage affects foraging decisions in

533   honeybees (*Apis mellifera* L.). *Ecological Entomology* **29**: 52-59.

534 **Human H, Nicolson SW, Strauss K, Pirk CWW, Dietemann V. 2007.** Influence of pollen quality

535   on ovarian development in honeybee workers (*Apis mellifera scutellata*). *Journal of Insect*

536   *Physiology* **53**: 649-655.

537 **Iwata K. 1955.** The comparative anatomy of the ovary in Hymenoptera. Part I. Aculeata. *Mushi* **29**:

538   17-34.

539 **Iwata K. 1960.** The comparative anatomy of the ovary in Hymenoptera. Supplement on Aculeata

540   with descriptions of ovarian eggs of certain species. *Acta Hymenopterologica* **1**: 91-97.

541 **Iwata K, Sakagami SF. 1966.** Gigantism and dwarfism in bee eggs in relation to modes of life, with

542   notes on the number of ovarioles. *Japanese Journal of Ecology* **16**: 4-16.

543 **Keely LL. 1985.** Physiology and biochemistry of the fat body. In: Kerkut GA, Gilbert LI, eds.

544   *Comprehensive insect physiology, biochemistry and pharmacology* volume 3. Oxford, U.K.:

545   Pergamon Press, 211-248.

546 **Kim J, Thorp RW. 2001.** Maternal investment and size number trade-off in a bee, *Megachile*

547   *apicalis*, in seasonal environments. *Oecologia* **126**: 451-456.

548 **Klostermeyer EC, Gerber HS. 1969.** Nesting behavior of *Megachile rotundata* (Hymenoptera:

549   Megachilidae) monitored with an event recorder. *Annals of the Entomological Society of*

550   *America* **62**: 1321-1325.

551 **Klowden M. 2002.** *Physiological systems in insects*. Academic Press, New York, NY.

552 **López-Uribe MM, Oi CA, Del Lama MA. 2008.** Nectar-foraging behavior of Euglossine bees

553   (Hymenoptera: Apidae) in urban areas. *Apidologie* **39**: 410-418.

554 **Mayer DF. 1994.** Effects of male to female ratio and number of females per nesting tunnel on sex

555   ratio and number of progeny of the alfalfa leafcutter bee *Megachile rotundata* (Hymenoptera:

556   Megachilidae). *Journal of the Entomological Society of British Columbia* **91**: 43-46.

557 **Mueller UG, Wolf-Mueller B. 1993.** A method for estimating the age of bees: age-dependent wing

558   wear and coloration in the wool-carder bee *Anthidium manicatum* (Hymenoptera:

559   Megachilidae). *Journal of Insect Behavior* **6**: 529-537.

560 **O'Neill KM. 2001.** *Solitary wasps: behavior and natural history*. Ithaca NY: Cornell University

561   Press.

562 **O'Neill KM, Pearce AM. 2007.** Ovary structure and oocyte size in relation to female size and age in

563   the brood parasitic wasp *Stizoides renicinctus* (Say) (Hymenoptera: Crabronidae). *Proceedings*

564   *of the Entomological Society of Washington* **109**: 836-840.

565 **O'Neill KM, Delphia CM, O'Neill RP.** 2014. Oocyte size, egg index, and body lipid content in

566   relation to body size in the solitary bee *Megachile rotundata*. *PeerJ* 2:e314; DOI

567   10.7717/peerj.314.

568 **O'Neill KM, O'Neill RP, Blodgett S, Fultz JE. 2004.** Variation in *Megachile rotundata* pollen load

569   composition in relation to flower diversity (Hymenoptera: Megachilidae). *Journal of the*

570   *Kansas Entomological Society* **77**: 619-625.

571 **O'Neill KM, O'Neill RP, Kemp WP, Delphia CM. 2011.** Effect of temperature on post-wintering

572   development and total lipid content of alfalfa leafcutting bees. *Environmental Entomology* **40**:

573   917-930.

574   **O'Neill KM, Pearce AM, O'Neill RP, Miller RS. 2010.** Offspring size and sex ratio variation in a

575   feral population of alfalfa leafcutting bees (Hymenoptera: Megachilidae). *Annals of the*

576   *Entomological Society of America* **103**: 775–784.

577   **O'Neill RP. 2004.** Seasonal and spatial patterns of mortality and sex ratio in the alfalfa leafcutting

578   bee, *Megachile rotundata* (F.). M.S. Thesis, Montana State University.

579   **O'Neill RP, O'Neill KM. 2010.** Pollen load composition and size in the leafcutting bee *Megachile*

580   *rotundata* (Hymenoptera: Megachilidae). *Apidologie* **42**: 223-233.

581   **Pan ML, Bell WJ, Telfer WH. 1969.** Vitellogenic blood protein synthesis by insect fat body.

582   *Science* **165**: 393-394.

583   **Peterson J H, Roitberg BD. 2006.** Impact of resources levels on sex ratio and resource allocation in

584   the solitary bee, *Megachile rotundata*. *Environmental Entomology* **35**: 1404-1410.

585   **Pitts-Singer TL. 2013a.** Intended release and actual retention of alfalfa leafcutting bees

586   (Hymenoptera: Megachilidae) for pollination in commercial alfalfa seed fields. *Journal of*

587   *Economic Entomology* **106**: 576-586.

588   **Pitts-Singer TL. 2013b.** Variation in alfalfa leafcutting bee, *Megachile rotundata,* reproductive

589   success according to location of nests in U.S. commercial domiciles. *Journal of Economic*

590   *Entomology* **106**: 543-551.

591   **Pitts-Singer TL, Cane JH. 2011.** The alfalfa leafcutting bee, *Megachile rotundata*: the world's most

592   intensively managed solitary bee. *Annual Review of Entomology* **56**: 221-237.

593   **Pitts-Singer TL, James RR. 2005.** Emergence success and sex ratio of commercial alfalfa leafcutting

594   bees from the United States and Canada. *Journal of Economic Entomology* **98**: 1785- 1790.

595   **Pitts-Singer TL, James RR. 2008.** Do weather conditions correlate with findings in failed,

596   provision-filled cells of *Megachile rotundata* (Hymenoptera: Megachilidae) in western North

597   America. *Journal of Economic Entomology* **101**: 674-685.

598   **Pitts-Singer TL, James RR. 2009.** Prewinter management affects *Megachile rotundata*

599   (Hymenoptera: Megachilidae) prepupal physiology and adult emergence and survival. *Journal*

600   *of Economic Entomology* **102**: 1407-1416.

601   **Rehan S, Richards MH. 2010.** The influence of maternal quality on brood sex allocation in the small

602   carpenter bee, *Ceratina calcarata*. *Ethology* **116**: 876-887.

603   **Richards KW. 1994.** Ovarian development in the alfalfa leafcutter bee, *Megachile rotundata*.

604   *Journal of Apicultural Research* **33**: 199-203.

605   **Richards MH, Packer. L 1994.** Trophic aspects of caste determination in *Halictus ligatus*, a

606   primitively eusocial sweat bee. *Behavioral Ecology and Sociobiology* **34**: 385-391.

607   **Richards KW, Whitfield GH, Schaalje GB. 1987.** Effects of temperature and duration of winter

608   storage on survival and period of emergence for the alfalfa leafcutter bee (Hymenoptera:

609   Megachilidae). *Journal of the Kansas Entomology Society* **60**: 70-76.

610   **Rodd FH, Plowright RC, Owen RE. 1980.** Mortality rates of adult bumble bee workers

611   (Hymenoptera: Apidae). *Canadian Journal of Zoology* **58**: 1718-1721.

612   **Rozen JG. 2003.** Eggs, ovarioles numbers, and modes of parasitism of cleptoparasitic bees, with

613   emphasis on neotropical species (Hymenoptera: Apoidea). *American Museum Novitates* **3413**:

614   1-36.

615   **Roulston TH, Cane JH. 2002.** Pollen nutritional content and digestibility for animals.

616   *Plant Systematics and Evolution* **222**: 617-643.

617   **Sihag RC. 1983.** Life cycle pattern, seasonal mortality, problem of parasitization and sex ratio pattern

618   in alfalfa pollinating megachilid bees. *Zeitschrift für angewandte Entomologie* **96**: 368-379.

619   **Sihag RC. 1985.** Vitellogenin of alfalfa pollinating bee *Megachile flavipes*. *Current Science* **54**: 92-

620   94.

621   **Sihag RC. 1986.** Reproduction in alfalfa pollinating sub-tropical megachilid bees. 4. Vitellogenesis

622   and oosorption, and factors inducing these processes. *Zoologischer Anzeiger* **216**: 231-249.

623   **St. Amand PC, Skinner DZ, Peaden RN. 2000.** Risk of alfalfa transgene dissemination and scale-

624   dependent effects. *Theoretical and Applied Genetics* **101**: 107-114.

625   **Strickler K. 1982.** Parental investment per offspring by a specialist bee: does it change seasonally?

626   *Evolution* **36**: 1098-1100.

627   **Strickler K, Freitas S. 1999.** Interactions between floral resources and bee (Hymenoptera:

628   Megachilidae) in commercial alfalfa seed fields. *Environmental Entomology* **28**: 178-187.

629   **Strohm E. 2000.** Factors affecting the body size and fat content in a digger wasp. *Oecologia* **123**:

630   184-191.

631   **Suarez RK, Darveau CA, Welch KC, O'Brien DM, Roubik DW, Hochachka PW. 2005.** Energy

632   metabolism in orchid bee flight muscles: carbohydrate fuels all. *Journal of Experimental*

633   *Biology* **208**: 3573-3579.

634   **SzaboTI, Smith MV. 1972.** The influence of light intensity and temperature on the activity of the

635   alfalfa leaf-cutter bee *Megachile rotundata* under field conditions. *Journal of Apicultural*

636   *Research* **11**: 157-165.

637   **Tepedino VJ. 1983.** An open-field test of *Megachile rotundata* as a potential pollinator in hybrid

638   carrot seed fields. *Journal of Apicultural Research* **22**: 64-68.

639   **Torchio P, Tepedino VJ. 1980.** Sex ratio, body size, and seasonality in a solitary bee, *Osmia lignaria*

640   *propinqua* Cresson (Hymenoptera: Megachilidae). *Evolution* **34**: 993-1003.

641   **Weissel N, Mitesser O, Poethke HJ, Strohm E. 2012.** Availability and depletion of fat reserves in

642   halictid foundress queens with a focus on solitary nest founding. *Insectes Sociaux* **59**: 67-74.

643   **Wuellner CT. 1999.** Alternative reproductive strategies of a gregarious ground-nesting bee,

644   *Dieunomia triangulifera* (Hymenoptera: Halictidae). *Journal of Insect Behavior* **12**: 845-863.

645 **Yocum GD, Rhinehart JP, West M, Kemp WP. 2010.** Interrupted incubation and short-term

646   storage of the alfalfa pollinator *Megachile rotundata* (Hymenoptera: Megachilidae): a potential

647   tool for synchronizing bees with bloom. *Journal of Economic Entomology* **103**: 234-241.

648 **Zar JH. 1999.** Biostatistical Analysis (4th edition). Prentice Hall, Englewood Cliffs, NJ.

649

1

Seasonal changes in the index of wing wear (WW).

Light blue circles represent raw data, dark blue circles and solid lines represent the mean values for each date. Means associated with different letters are significantly different at $P < 0.05$ (Dunn's Test). Total sample sizes for each date were $N = 50$ (MT 2011) and $N = 30$ (MT 2012 and UT 2012).
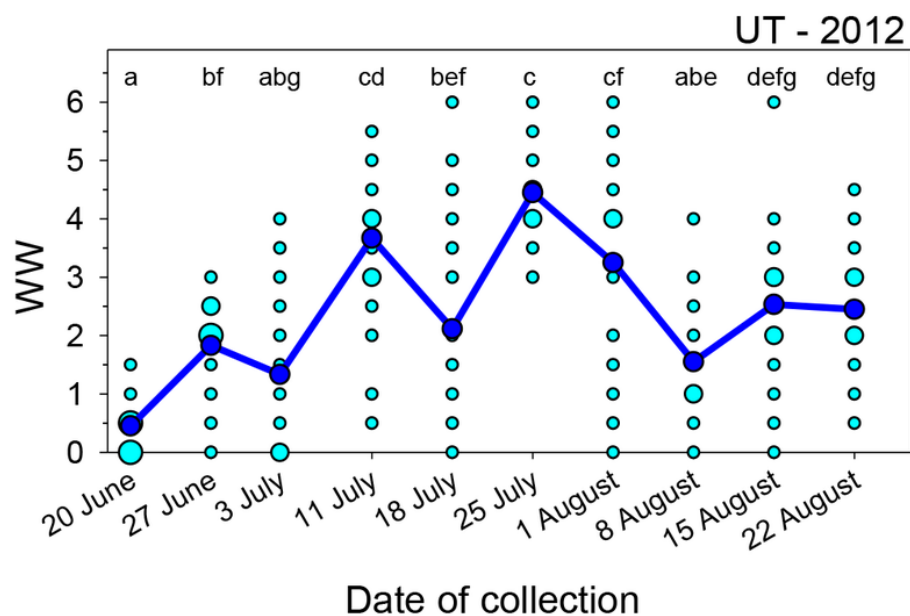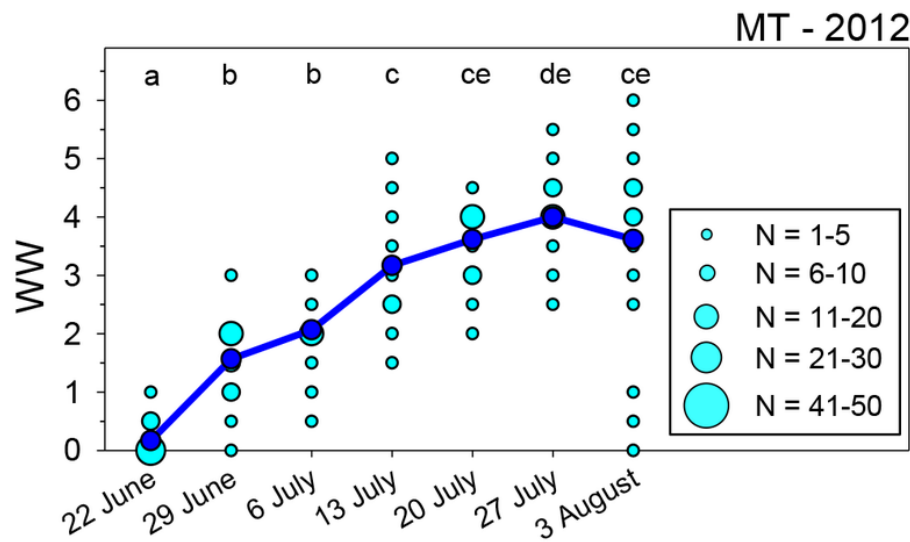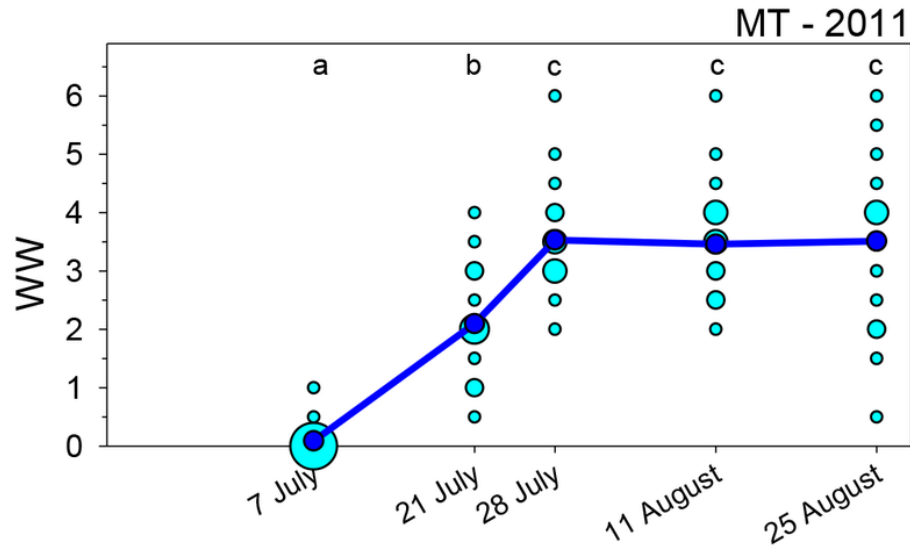
2

Seasonal changes in the relationship of P_L for female *M. rotundata* to date of collection (left; red lines) and WW (right;blue lines).

Means associated with different letters are significantly different at $P < 0.05$ (SNK Test).
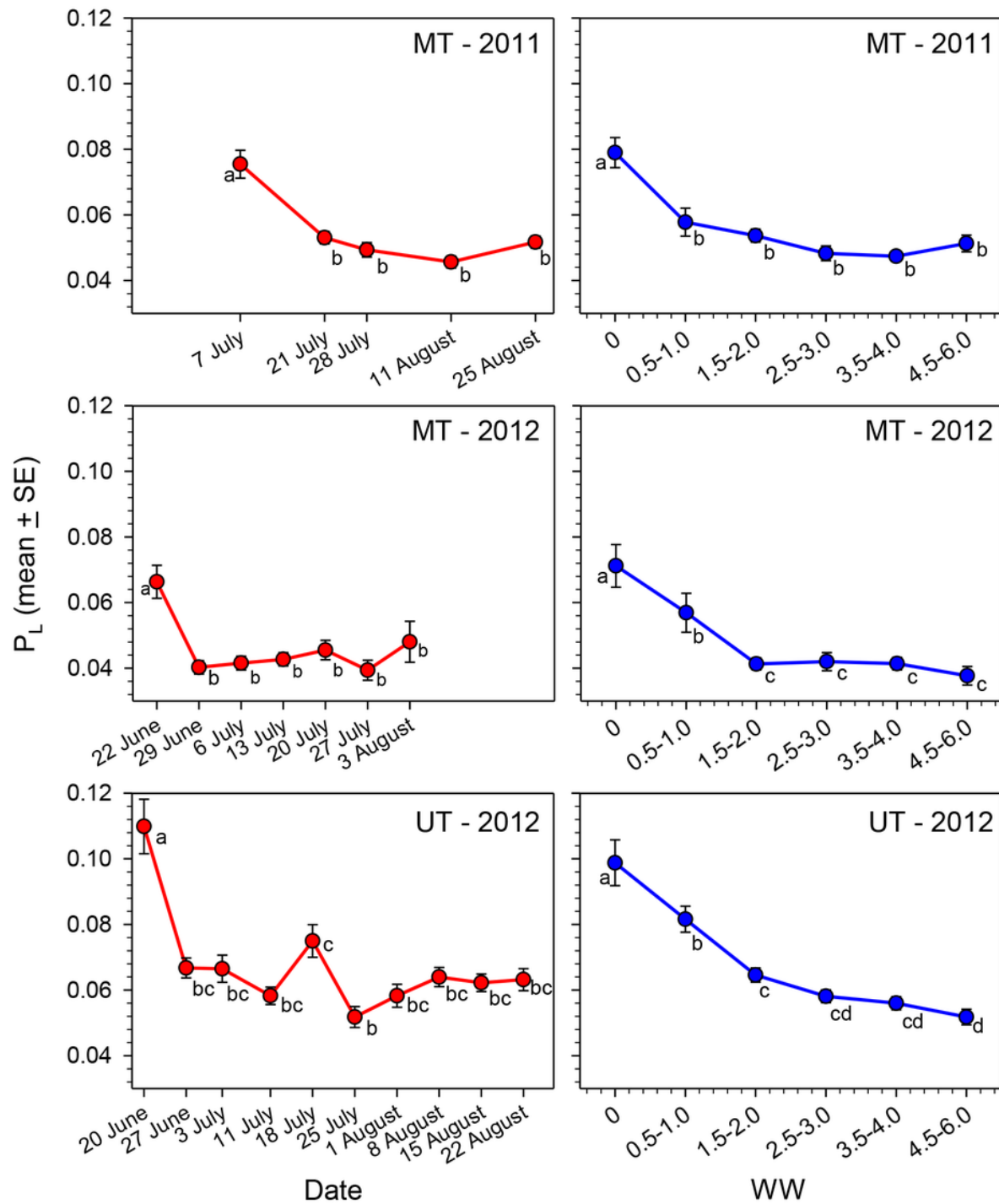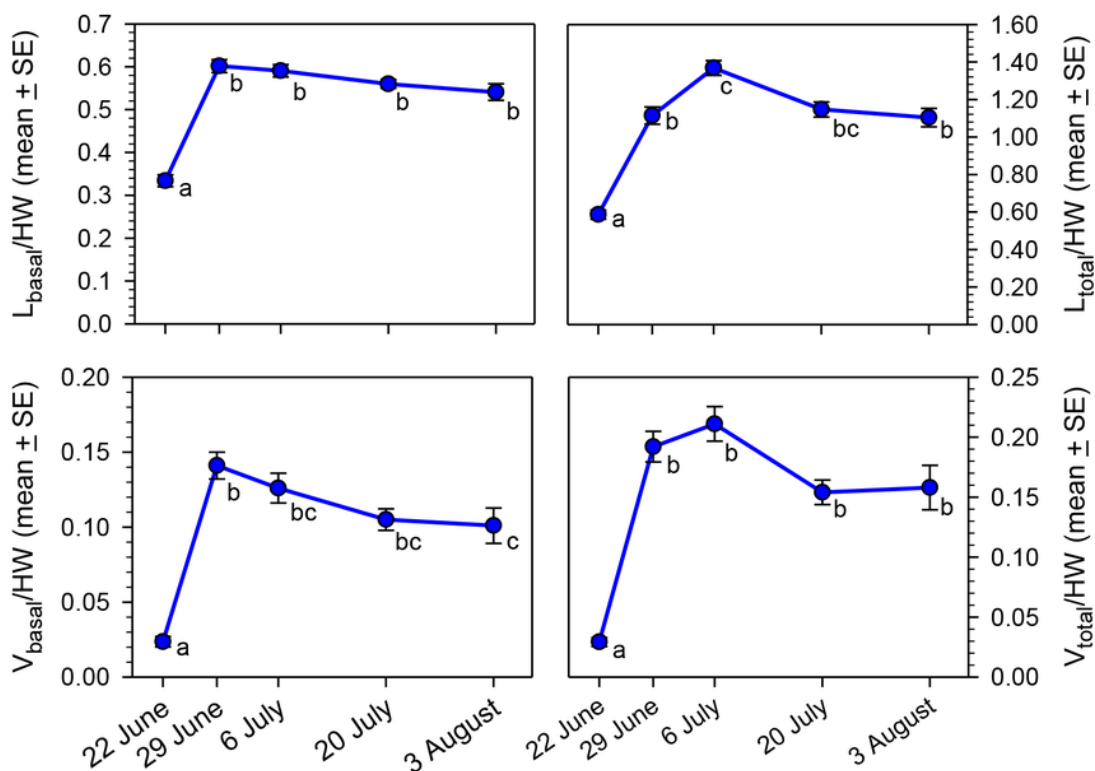
3

Seasonal changes in body size-adjusted oocyte size variables by sampling date for bees collected in MT in 2012.

Means associated with different letters are significantly different at $P < 0.05$ (Dunn's Test). Samples sizes for each date were $N = 30$ for 22 June, $N = 26$ for 29 June, $N = 32$ for 6 July, $N = 27$ for 20 July, and $N = 30$ for 3 August.

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Proportions of females in different wing wear index (WW) categories with either negative or positive residuals for the regressions of L_{basal}, L_{total}, V_{basal}, and V_{total} (all transformed as the square root of the val

Females with negative residuals had smaller than expected oocyte sizes based on the HW-oocyte size linear regression equation. The regression equations for oocyte variable (square-root transformed) on HW were: 1) L_{basal} = 0.329 + (0.341)(HW); F = 81.6, df = 1, 111, P < 0.001, 2) L_{total} = 0.0981 + (0.615)(HW); F = 48.1, df = 1, 111, P < 0.001; 3) V_{basal} = 0.0422 + (0.279)(HW); F = 56.4, df = 1, 111, P < 0.001, and 4) V_{total} = -0.0167 + (0.374)(HW); F = 56.4, df = 1, 111, P < 0.001. Mean ± SE WW values are superimposed above the bars (sample sizes for means range from 52 to 61).
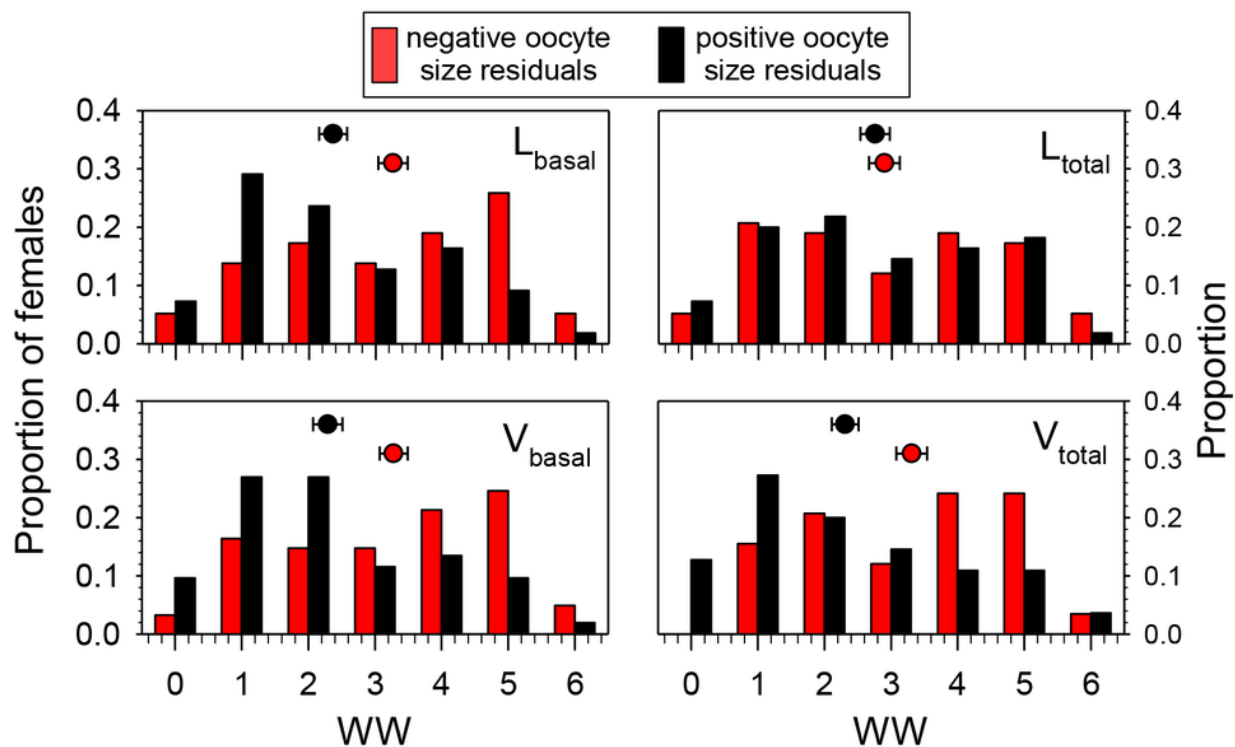
Table 1(on next page)

One-way analyses of variance for the relationship of P_L to date of collection and WW for
M. rotundata females. All analyses were done with arcsine-transformed P_L values.

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					Percent decline in mean P_L from:	
Site	**Year**	*F*	**d.f.**	**Probability**		
					First to second	**First to final**
P_L as function of date*					**collection date**	**collection date**
MT	2011	17.80	4, 245	*P* < 0.001	29.7%	31.5%
	2012	5.58	6, 203	*P* < 0.001	39.2%	27.5%
UT	2012	12.81	9, 290	*P* < 0.001	39.2%	42.5%
					WW = 0 to	**WW = 0 to**
P_L as function of WW*					**WW = 0.5-1.0**	**WW = 4.5-6.0**
MT	2011	17.31	5, 244	*P* < 0.001	26.8%	35.1%
	2012	10.53	5, 204	*P* < 0.001	20.1%	47.5%
UT	2012	24.33	5, 294	*P* < 0.001	17.4%	47.5%

Table 1 One-way analyses of variance for the relationship of P_L to date of collection and WW for *M. rotundata* females.

3   * All analyses were done with arcsine-transformed P_L values.

Table 2(on next page)

Proportions of females with P_L values ≥ 0.10 and ≤ 0.05, comparing the first collection date each year with all other collection dates combined.

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Table 2. **Proportions of females with P$_L$ values ≥0.10 and ≤0.05, comparing the first collection date each year with all other collection dates combined.**

| Site | Year | Proportion females with P$_L$ ≥0.10 | | Fisher Exact Test Probability | Proportion females with P$_L$ ≤0.05 | | Fisher Exact Test Probability |
		First collection date	All other collection dates		First collection date	All other collection dates	
MT	2011	0.260	0.005	*P < 0.001*	0.300	0.525	*P < 0.01*
	2012	0.166	0.011	*P < 0.001*	0.233	0.761	*P < 0.001*
UT	2012	0.566	0.048	*P < 0.001*	0.033	0.265	*P < 0.001*

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