

Oxygen Consumption During the Life Cycles of the Prepupa-Wintering Bee *Megachile rotundata* and the Adult-Wintering Bee *Osmia lignaria* (Hymenoptera: Megachilidae)

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ABSTRACT We studied the oxygen consumption of two megachilid bees (Hymenoptera: Megachilidae), *Megachile rotundata* (F.) and *Osmia lignaria* Say, at selected, biologically relevant intervals throughout their respective life cycles. The U-shaped oxygen consumption curve and the static weights of wintering (nonfeeding) prepupae that we observed during the life cycle of *M. rotundata* support previous arguments for a winter diapause similar to that observed in other Hymenoptera. For *O. lignaria*, which overwinters as an adult, we found stepwise increases in oxygen consumption and continuous weight loss throughout the wintering period. However, our observations on adult *O. lignaria* wintering requirements are consistent with the previously published results for overwintering *M. rotundata* prepupae and reveal sharply increasing survival rates when wintered for a minimum of 3 mo. We interpret the greatly reduced survival in both *M. rotundata* and *O. lignaria*, as an indication that a critical biological process, diapause, is disrupted among individuals wintered for <3 mo. In the continued development of these two species as commercial scale pollinators on an ever-increasing list of target crops, any similarities or contrasts observed between the “summer bee,” *M. rotundata*, and the “spring bee,” *O. lignaria*, although of interest from a biological perspective, will probably have important implications in the continued development of sustainable population management protocols.

KEY WORDS diapause, overwintering, crop pollination, respirometry, Akaike Information Criterion

PREPUPAL OVERWINTERING IS a widespread life history trait among the Hymenoptera (Danks 1987, Gauld and Bolton 1988). Typically, these species reach the prepupal stage by mid- or late summer and quickly enter diapause, as evidenced by lowered metabolic rates detected in those few species that have been studied (Wallace and Sullivan 1963, Villacorta et al. 1972, Bauman et al. 1978). These species remain in the prepupal stage through autumn and winter and resume development in the spring or early summer. Similarly, most bees in the family Megachilidae, including those in the primitive subfamily Lithurginae, also overwinter as prepupae and are normally active as adults during the summer (Westrich 1989, Bosch et al. 2001). However, species of the subgenus *Osmia* (*Osmia*) are active in early spring and overwinter only in the adult stage. Overwintered prepupae of these species do not develop into viable adults (Bosch 1994, Bosch and Kemp 2000). Both wintering in the adult stage and spring

activity seem to be derived conditions within the Megachilidae (Bosch et al. 2001).

In this study, we measured oxygen consumption levels and weight loss throughout the life cycles of two megachilid species, the summer-flying, prepupa-wintering *Megachile rotundata* (F.) and the spring-flying, adult-wintering *Osmia lignaria* Say. Because both species are managed as crop pollinators (Richards 1984, Bosch and Kemp 2001), their life cycles and wintering requirements (in terms of adequate temperature regimes) are relatively well known (Johansen and Eves 1973; Taséi and Masure 1978; Richards et al. 1987; Bosch et al. 2000; Bosch and Kemp 2000, 2003; Kemp and Bosch 2000, 2001). However, given the generally acknowledged physiological differences associated with wintering as a prepupa versus adult, we anticipate that the hypothesis of identical life cycle oxygen consumption and weight loss in these two megachilid bees will probably be rejected. Thus, our objectives are 1) to compare the respiration rates of *M. rotundata* and *O. lignaria* throughout their life cycles, and 2) to relate respiration levels to winter temperature requirements for both species. We expect our results to contribute to the establishment of a physiological background for improved rearing regimes for these two increasingly valuable crop pollinators.

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Megachile rotundata is an adventive, gregarious, cavity-nesting, leafcutting bee that has been extensively cultured as a superior pollinator of alfalfa, *Medicago sativa* L., following the first North American records of its presence (Krombein 1948, Stephen and Torchio 1961). At present, *M. rotundata* is the pollinator of choice for alfalfa seed production on >70,000 ha in western North America and is the most widely used commercially managed pollinator, after the honey bee, *Apis mellifera* L.

At most North American latitudes, *M. rotundata* emerge and mate during June and July. Nesting continues for 4–6 wk, and nests consist of a linear series of cells constructed from cut-leaf pieces. Each cell is provisioned with a mass of pollen and nectar, on top of which an egg is deposited. Completed nests are sealed with cut-leaf plugs.

By midsummer, fifth instars of *M. rotundata* complete the consumption of their pollen-nectar provision, defecate, and spin a cocoon with silk-like strands (Stephen and Torchio 1961). In this stage (prepupa), empirical evidence indicates that most individuals in a population pass the winter in diapause (Stephen and Osgood 1965, Klostermeyer 1982). However, apparently due to a combination of maternal effects and high temperatures, a variable portion of the population may avert diapause, complete development, and emerge as adults during the current year (Klostermeyer 1982; Parker and Tepedino 1982; Kemp and Bosch 2000, 2001). Under natural conditions, overwintered *M. rotundata* complete their development through the adult stage and emerge as ambient temperatures increase during the following spring and early summer. Under artificial commercial rearing conditions, prepupae that have received a 7–8-mo wintering period (Richards et al. 1987) are incubated to ensure emergence and nesting coincident with the characteristic flush of bloom in alfalfa seed fields in June or early July (Stephen 1981, Rank and Goerzen 1982, Richards 1984).

Osmia lignaria is a gregarious, cavity-nesting, univoltine, mason bee that is native to North America (Rust 1974). Because of its willingness to accept artificial nesting materials (Torchio 1982) and its superior pollination performance, it has been cultured increasingly as a pollinator of orchard crops (Torchio 1976, 1979, 1985; Bosch and Kemp 1999, 2001, 2002; Bosch et al. 2000).

Osmia lignaria emerges early in the year, March–May, mates, and females begin nesting in natural or artificial cavities. Nesting continues for 4–6 wk, and nests consist of a linear series of cells delimited by mud partitions. Each cell is provisioned with a mass of pollen and nectar, on top of which an egg is deposited, and completed nests are sealed with mud plugs. By late spring, fifth instars of *O. lignaria* complete the consumption of their pollen-nectar provision, defecate, and spin a cocoon with silk-like strands (Torchio 1989). In this stage (prepupa), larvae undergo a month-long “rest” period (Torchio 1989) and subsequently pupate and molt to the adult stage by late summer. Adults remain inside the cocoon, apparently

enter a second quiescent period, and require a cold period of ≈ 6 mo to overwinter successfully and emerge the following spring as vigorous pollinators (Bosch and Kemp 2000, 2001, 2003; Bosch et al. 2000).

Materials and Methods

Populations and Experimental Conditions. Bees used in this study were selected from populations of *M. rotundata* and *O. lignaria* managed at the USDA-ARS Bee Biology & Systematics Laboratory in Logan, UT. We followed methods described previously for the selection and assignment of individuals of *M. rotundata* (Kemp and Bosch 2000, 2001) and *O. lignaria* (Bosch and Kemp 2000) to this study. In 1999, provisions with eggs of each species were transferred to artificial clay wells (Torchio and Bosch 1992) and were labeled with nest number and cell position within the nest and covered with glass slidecovers. Clay wells with eggs and provisions were placed in clear plastic boxes containing two additional clay wells filled with water to provide adequate humidity throughout development and were maintained at room temperature ($\approx 22^\circ\text{C}$) throughout the summer developmental period.

After cocoon completion in both species, cocoons were placed individually in clear gel capsules and transferred to sticky boards (20 by 25-cm boards with double-sided adhesive tape), which were X-rayed weekly (Stephen and Undurraga 1976) to monitor developmental status through to adult emergence the following year. X-ray plates were used to record the dates when bees pupated and subsequently molted to adults.

During October 1999, *M. rotundata* prepupae were acclimatized for 2 wk at a constant 14°C before being transferred to a constant 4°C walk-in cooler for wintering. In early June 2000, after ≈ 220 d of wintering, *M. rotundata* prepupae were transferred to a constant 30°C for development through pupae and emerged adults. During September 1999, *O. lignaria* adults were acclimatized for 2 wk at a constant 14°C temperature before being transferred to a constant 4°C walk-in cooler for wintering. In early April 2000, after ≈ 185 d of wintering, *O. lignaria* adults were transferred to a constant 22°C for emergence.

Oxygen Consumption and Weight Measurement. At selected intervals (15 for each sex of *M. rotundata*, 18 for each sex of *O. lignaria*) throughout the life cycles of both bee species, we used constant volume respirometry to measure consumed oxygen, an indicator of overall metabolic activity. We used a Sable Systems FC-1 oxygen analyzer operating in differential mode with a 100 ml/min flow rate (<http://www.sablesys.com/pushmo2.html>). This allowed accuracy of measurement that exceeded 0.001% in detecting oxygen departures from an undepleted airstream that had been scrubbed of carbon dioxide and water vapor with a Drierite-Ascarite column. For each selected stage in both species, we measured the oxygen consumed by each of seven individual bees of both sexes simultaneously for 2 h at room temperature

(22°C) with lights on, and data were collected via the Sable Systems data acquisition program DATACAN (<http://www.sablesys.com/datacan.html>) following manufacturer's protocols. Upon completion of a respirometry session, individual bees were weighed. For comparison purposes, oxygen consumption levels were adjusted for the weight of each individual and expressed as milliliters of oxygen consumed per gram · hour by using the following transformation (CONVOL refers to the Sable Systems software package used with constant volume respirometry for computing oxygen depletion from an undepleted airstream):

$$\begin{aligned} \text{O}_2 \text{ Consumed (ml/g} \cdot \text{h)} \\ &= (\text{CONVOL output [ml/h]} / \\ &\quad \text{individual weight [g]}) \quad [1] \end{aligned}$$

Because we did not use the same individuals throughout the study, there was the potential for sampling variability in the mean weights of each group selected for oxygen consumption. Thus, to generate a characteristic and consistent weight loss curve over time, we maintained separate lots of both species and sexes for repeated measurements at intervals similar to those selected for assessing respiratory activity. This is of interest because both species spend 9 to 10 mo in a nonfeeding condition. For developmental stages associated with cocoons, weights are reported as individuals removed from the cocoon.

Data Analysis and Model Evaluation. In this study, we used a model-building approach based on information-theoretic model selection indices (Sakamoto et al. 1986). The approach, used previously by Kemp and Dennis (1991) for describing a grasshopper phenology data set, involves fitting all relevant models and choosing the best model according to an index of model quality. The index, known as the Akaike Information Criterion (AIC) after Akaike (1973), is essentially a goodness-of-fit measure that is penalized by the number of parameters estimated in the model. The penalty is scaled so that the difference in the AIC values from two models constitutes an asymptotically unbiased and statistically consistent estimate of the difference in the "distances" of the two models from the true underlying model that generated the data. Burnham and Anderson (1998) have given an excellent recent account of this increasingly popular statistical methodology. In this study, we were interested in describing the oxygen consumption levels of *M. rotundata* and *O. lignaria* over time with the fewest parameters necessary.

With sampling occasions for each bee species and sex that contain oxygen consumption levels spanning their respective life cycles (15 for each sex of *M. rotundata*, 18 for each sex of *O. lignaria*), parameters could be estimated according to several hypotheses. First, data could be pooled, within species, over sex and interval to produce a single set of parameters to describe general trends in oxygen consumption. Alternatively, we could iteratively solve for the model

with the fewest parameters, but with those that explain the most variation in the data.

Our basic model for the observations was that of the familiar one-way analysis of variance (ANOVA) (Neter et al. 1996). The observation y_{ij} ($j = 1, 2, \dots, \eta$): at sampling occasion i was assumed to have a normal distribution with mean μ_i and variance σ^2 . In this study, there were so many sampling occasions, up to 18 for *O. lignaria*, that the conventional pairwise hypothesis testing was not likely to identify the best submodel among all the possible structures of μ_i values.

We were interested in whether a period of relative constancy among the μ_i values could be identified and interpreted consistent with our current knowledge of the life cycles of both species. We regarded the relevant submodels to be all of the models in which any equal means are adjacent to each other. For instance, with four sampling occasions and four possible mean parameters $\mu_1, \mu_2, \mu_3,$ and μ_4 , the relevant submodels are as follows:

- 1 parameter $\mu_1 = \mu_2 = \mu_3 = \mu_4$
- 2 parameters $\mu_1 = \mu_2 = \mu_3$
 $\mu_2 = \mu_3 = \mu_4$
 $\mu_1 = \mu_2$ and $\mu_3 = \mu_4$
- 3 parameters $\mu_1 = \mu_2$
 $\mu_2 = \mu_3$
 $\mu_3 = \mu_4$

For each model, the AIC was calculated as follows:

$$\begin{aligned} \text{AIC} = &-2(\text{maximized log likelihood}) \\ &+ 2(\text{number of estimated parameters}) \quad [2] \end{aligned}$$

The number of estimated parameters included the variance parameter σ^2 . The maximized log likelihood can be shown to be equal to $-(\eta/2) [(2\pi) + 1 + \ln(\eta) + \ln(\text{SSE})]$, where SSE is the sum of squared errors for the fitted model. In the SSE, each estimated mean was calculated as the sample mean of the observations from all the sampling occasions that shared a common mean parameter. The model with the minimum AIC value was selected as the "best" model. The AIC is asymptotically equivalent to selecting the model with the smallest prediction error as established with cross-validation (Stone 1977).

Results

Megachile rotundata. The life cycle of *M. rotundata*, under the conditions imposed during this study, essentially consists of three distinct periods: summer development (egg to prepupa in a completed cocoon), autumn–winter diapause (prepupa in a cocoon), and springtime development (incubation of the prepupa through to the emergence of the adult). AIC results suggest that the life cycle oxygen consumption data for *M. rotundata* could be best described with six (Fig. 1A) and five (Fig. 1B) parameter models for females and males, respectively.

In late summer, respiration levels begin declining as fifth instars of *M. rotundata*, having consumed their

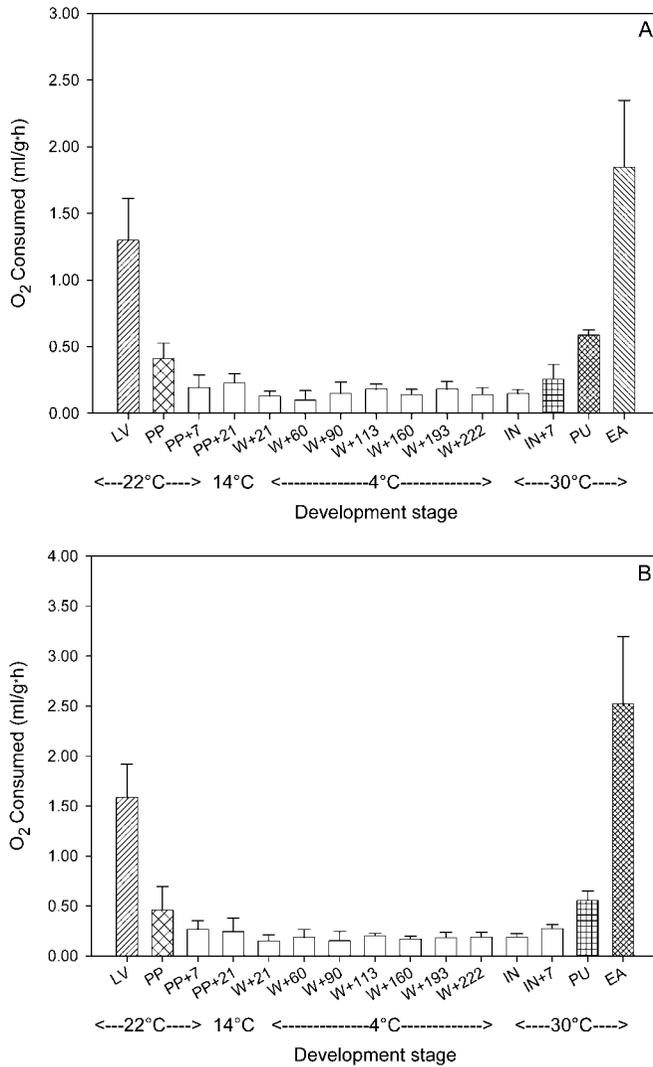


Fig. 1. Oxygen consumption (mean + 95% CI) of *M. rotundata* females (A) and males (B) at selected biologically relevant stages, under artificial management conditions. Development stage LV, cocoon-spinning fifth instar; PP, prepupa; PP+7, prepupa + 7 d; PP+21, prepupa + 21 d; W+21, 21-d-wintered prepupa; W+60, 60-d-wintered prepupa; W+90, 90-d-wintered prepupa; W+113, 113-d-wintered prepupa; W+160, 160-d-wintered prepupa; W+193, 193-d-wintered prepupa; W+222, 222-d-wintered prepupa; IN, start of incubation; IN+7, 7-d-incubated prepupa; PU, pupa; EA, emerged adult. Like-patterned bars indicate no significant difference between means for the model with minimized AIC (females AIC = 1381.51; males AIC = 1398.29).

provision, complete cocoon spinning (Fig. 1). After cocoon completion, individuals enter a "torpid" prepupal stage (Trostle and Torchio 1994), the stage in which they will overwinter. Oxygen consumption of prepupae 7 d after the completed cocoon stage (Fig. 1, PP+7) averages <20% that of earlier actively feeding or spinning forms and is not significantly different from oxygen consumption levels of prepupae (artificially) acclimatizing at 14°C 2 wk later (Fig. 1, PP+21). In fact, there is remarkable similarity in oxygen consumption levels as assayed from the prepupa + 7 d through 7 mo of wintering at 4°C (Fig. 1, PP+7, W+222). After adequate wintering and under artifi-

cial incubation at 29–30°C, oxygen consumption increases (Fig. 1) as *M. rotundata* prepupae transit through 7 d of incubation, molt to pupae by day 23–25, and emerge as adults by day 33–36 (Kemp and Bosch 2001).

Individual weights for both sexes of *M. rotundata* decline after larvae complete provision consumption in late summer, remain very stable during prewintering and wintering, and decline rapidly with incubation and adult emergence (Fig. 2).

Osmia lignaria. The life cycle of *O. lignaria* consists of three well-defined periods: summer development (egg to adult), prewintering (adult at warm temper-

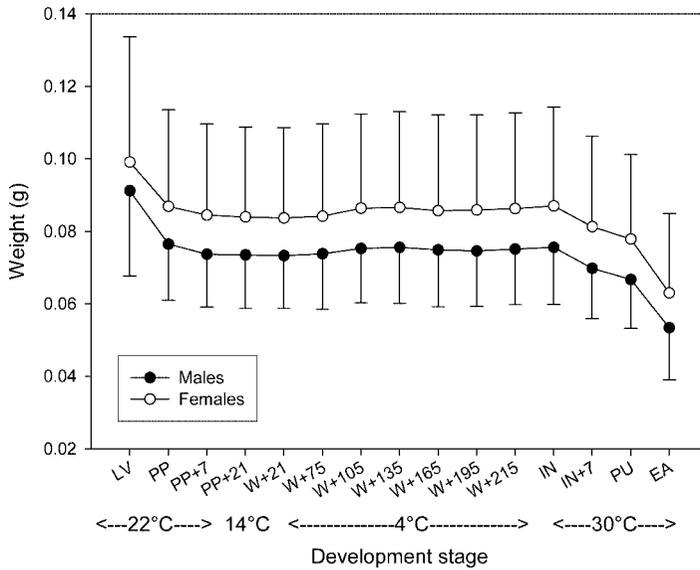


Fig. 2. Weight loss (mean + 95% CI) of *M. rotundata* at selected biologically relevant stages, under artificial management conditions. Development stage LV, cocoon-spinning fifth instar; PP, prepupa; PP+7, prepupa + 7 d; PP+21, prepupa + 21 d; W+21, 21-d-wintered prepupa; W+75, 75-d-wintered prepupa; W+105, 105-d-wintered prepupa; W+135, 135-d-wintered prepupa; W+165, 165-d-wintered prepupa; W+195, 195-d-wintered prepupa; W+215, 215-d-wintered prepupa; IN, start of incubation; IN+7, 7-d-incubated prepupa; PU, pupa; EA, emerged adult.

atures), and wintering (adult at low temperatures), followed by a brief springtime emergence period. AIC results suggest that the life cycle oxygen consumption data for *O. lignaria* could be best described with six parameter models for both females and males (Fig. 3).

As with *M. rotundata*, but in early instead of late summer, respiration levels decline as fifth instars of *O. lignaria*, having consumed their provision, complete cocoon spinning (Fig. 3, PP). At prepupa + 7 d, mean oxygen consumption ranges between only 0.04–0.09 ml/g · h, for females and males (Fig. 3, PP+7), and prepupae look distinctly sack-like and C-shaped; prepupae will later stretch and become more slender as they approach pupation. However, in contrast with diapausing *M. rotundata*, the oxygen consumption of *O. lignaria* increases steadily as individuals continue development during the next 2 mo (at 22°C; Bosch and Kemp 2000), and AIC results suggest that at pupa + 21 d, a transition in oxygen consumption occurs from that characteristic of summer development to that of the prewintering and early wintering period (Fig. 3, PU+21). At constant 22°C, the pupa–adult molt occurs at approximately pupa + 32 d for males and pupa + 33 d for females (Bosch and Kemp 2000).

The oxygen consumption in both sexes of adult *O. lignaria* seems relatively stable from pupa + 21 d (11–12 d before the pupa–adult molt) through the first 45 d of wintering at 4°C (Fig. 3, PU+21, W+45). During early wintering at 4°C, however, the adults of both sexes were sensitive to manipulation, and we found it difficult to remove individuals from their cocoons (after respirometry) and obtain accurate weights without first chilling them on ice for a few

minutes. After 45 d at 4°C, AIC results indicate a stepwise increase in *O. lignaria* oxygen consumption through 185 d of wintering (Fig. 3, W+45, W+185) in both sexes, with males exhibiting apparently delayed but steeper increases, consistent with our expectations for this protandrous species. With the exception of wintering + 75 d and wintering + 105 d (Fig. 3, W+75, W+105), adults required chilling, as noted above, for us to be able to remove individuals from their cocoons and obtain accurate weights after respirometry.

Similar to *M. rotundata* (Fig. 2), the individual weights for both sexes of *O. lignaria* decline slightly after larvae complete provision consumption in early summer (Fig. 4). However, *O. lignaria* weight loss (beyond the prepupa) seems more continuous during prewintering, wintering, and through to spring emergence (Fig. 4), compared with that of *M. rotundata* (Fig. 2).

Discussion

Tauber et al. (1986) describe a generalized seasonal diapause cycle consisting of five periods, each of which “is characterized by species-specific sets of responses.” During the prediapause (1) and diapause induction (2) periods, token stimuli elicit a suite of species-specific responses that result in the expression of generally accepted symptoms of diapause syndrome (Tauber et al. 1986). The subsequent diapause maintenance period (3) begins with diapause syndrome, continues with the maintenance of diapause resulting from species-specific responses elicited by one or more stimuli, and ends either spontaneously or with

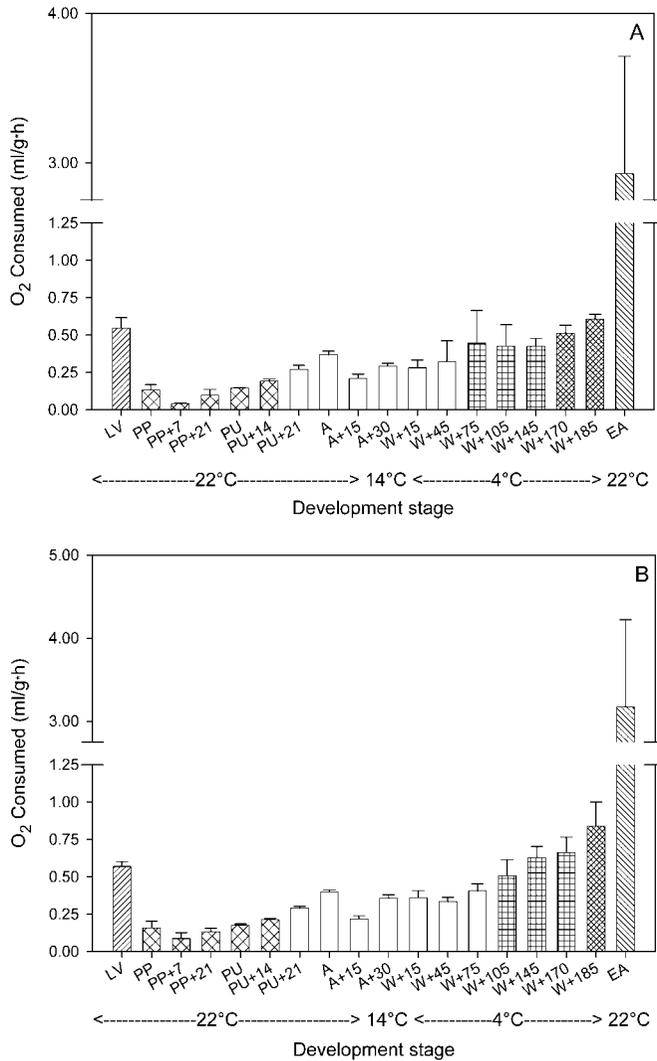


Fig. 3. Oxygen consumption (mean + 95% CI) of *O. lignaria* females (A) and males (B) at selected biologically relevant stages, under artificial management conditions. Development stage LV, cocoon-spinning fifth instar; PP, prepupa; PP+7, prepupa + 7 d; PP+21, prepupa + 21 d; PU, pupa; PU+14, pupa + 14 d; PU+21, pupa + 21 d; A, adult; A + 15, adult + 15 d; A + 30, adult + 30 d; W+15, 15-d-wintered adult; W+45, 45-d-wintered adult; W+75, 75-d-wintered adult; W+105, 105-d-wintered adult; W+145, 145-d-wintered adult; W+170, 170-d-wintered adult; W+185, 185-d-wintered adult; EA, emerged adult. Like-patterned bars indicate no significant difference between means for the model with minimized AIC (females AIC = 1712.94; males AIC = 1711.64).

token stimuli that initiate diapause termination. At this point, immediate environmental conditions, as opposed to token stimuli, regulate growth and development as insects enter the postdiapause transitional period (4) and continue on to the final nondiapause period (5).

Oxygen consumption has been used extensively in the past as a reliable indicator of active versus diapause states in insects (see Table 5 in Danks 1987), and with few exceptions, it follows a U-shaped curve during the course of diapause (Wigglesworth 1972, Tauber et al. 1986). A useful aspect of oxygen consumption as an indicator is the fact that during diapause its rate is

generally low and relatively independent of temperature (reproductive diapause in some adult insects is an exception to this). Thus, the detection of a departure from temperature-sensitive to temperature-insensitive oxygen consumption frequently indicates a transition from active to diapause state, and the reverse can indicate that diapause development is complete and that the insects of interest have reached either the postdiapause transitional period or beyond.

Megachile rotundata. Oxygen consumption of *M. rotundata* prepupae exhibits the frequently encountered U-shaped life cycle pattern described in similar studies on a variety of insect species (Tauber et

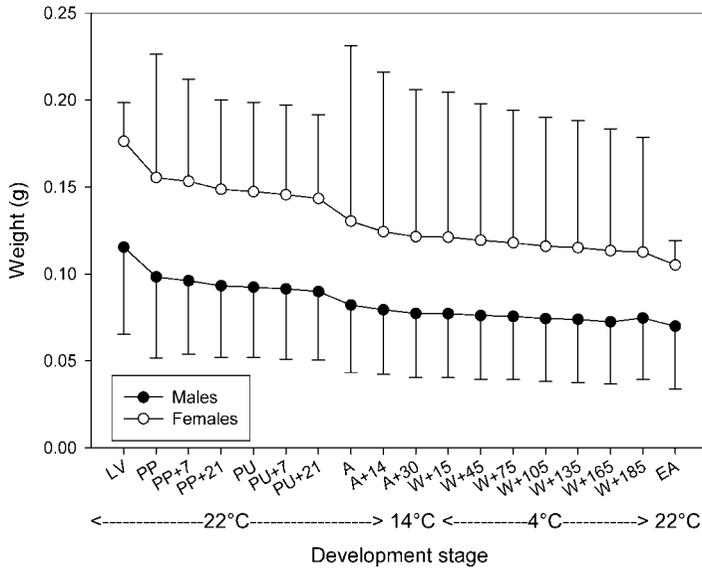


Fig. 4. Weight loss (mean + 95% CI) of *O. lignaria* at selected biologically relevant stages, under artificial management conditions. Development stage LV, cocoon-spinning fifth instar; PP, prepupa; PP+7, prepupa + 7 d; PP+21, prepupa + 21 d; PU, pupa; PU+7, pupa + 7 d; PU+21, pupa + 21 d; A, adult; A + 14, adult + 14 d; A + 30, adult + 30 d; W+15, 15-d-wintered adult; W+45, 45-d-wintered adult; W+75, 75-d-wintered adult; W+105, 105-d-wintered adult; W+145, 145-d-wintered adult; W+170, 170-d-wintered adult; W+185, 185-d-wintered adult; EA, emerged adult.

al.1986, Danks 1987). The precipitous drop in respiration levels at 22°C as nonfeeding larvae complete cocoon spinning (Fig. 1, PP), and the lack of significant differences in oxygen consumption as prepupae transit from completed cocoon + 7 d (Fig. 1, PP+7) through, the 2-wk acclimatization at 14°C (Fig. 1, PP+21), and on through 7 mo of wintering at 4°C (Fig. 1, IN), signal the transition from the active pre-diapause, fifth-instar, spinning-cocoon period, through diapause induction to the diapause maintenance period. Oxygen consumption at prepupa + 7 d (Fig. 1, PP+7) averaged ≈16% that of actively spinning fifth instars (Fig. 1, LV) and 11% that of active, postdiapause, emerged adults (Fig. 1, EA), well within the range of values previously reported for other insects (see Table 5 in Danks 1987). The general pattern of oxygen consumption that we observed throughout the life cycle of *M. rotundata* is very similar to that observed in two other hymenopterans (*Cephus cinctus* Norton [Cephalidae] and *Trypoxylon politum* Say [Sphecoidea]), both of which exhibit a prepupal diapause (Villacorta et al. 1972, Bauman et al. 1978).

It is important to note, however, that the lack of apparent increases in oxygen consumption during the latter portion of the wintering period of *M. rotundata* does not necessarily signal that the maintenance of diapause ends with the onset of incubation temperatures of 30°C (Fig. 1). In fact, only in a few winter-diapausing, temperate-zone insect species have diapause-terminating stimuli been identified (Tauber et al. 1986). In most situations, insects simply seem to cease to respond to diapause-maintaining factors sometime during the winter (they enter the postdia-

pause transitional period), frequently well before the onset of increasing temperatures that regulate physiological processes in general, ATP demand, and thus oxygen consumption (Clarke 1991, 1993). These findings are consistent with the general observation (Tauber et al. 1986, Danks 1987, Denlinger and Tanaka 1999) that under natural conditions, most diapausing temperate-zone insect species are in a true diapause condition only during autumn and early winter, and if such individuals are exposed to warm conditions after the winter solstice they will reinitiate development. However, *M. rotundata* incubated in January will not resume development right away.

In the case of overwintering *M. rotundata*, our results together with existing empirical evidence suggest that storage at appropriate temperatures allows individual prepupae to transit the diapause maintenance period during the first 3 to 4 mo of wintering, but an additional several months are required for a high proportion of the population to complete the postdiapause transitional period. First, the U-shaped oxygen consumption curve (Fig. 1) and the static weights of wintering (nonfeeding) prepupae (Fig. 2) that we observed for *M. rotundata* support previous arguments for a winter diapause similar to that observed in other Hymenoptera (Villacorta et al. 1972, Bauman et al. 1978). Second, the results of Richards et al. (1987), which show that the percentage of survival of *M. rotundata* prepupae reaches maximum levels only after 3 to 4 mo of wintering, strongly suggest that a critical biological process, diapause, is apparently disrupted among individuals wintered for <3 mo. And finally, beyond 3 to 4 mo of wintering required to

complete diapause, Richards et al. (1987) found a steady decline in the variation associated with the length of time required for individual *M. rotundata* to emerge after incubation at constant 30°C, to a low at 8 to 12 mo of wintering, suggesting that from approximately month 4 through 8 of wintering, prepupae (considered as a population) are in postdiapause transition. Thus, it seems that a population wintered at appropriate temperatures for a minimum of 8 mo contains a very high percentage of prepupae that have developed beyond diapause and postdiapause transition and are now in a nondiapause state.

Osmia lignaria. Overall, oxygen consumption (Fig. 3) and the more or less continuous wintering weight loss pattern (Fig. 4) of the "spring bee" *O. lignaria*, which overwinters as an adult, contrast with results for *M. rotundata* and previous studies on *C. cinctus* and *T. politum* (Villacorta et al. 1972, Bauman et al. 1978). The initial declines in oxygen consumption at prepupa + 7 d (Fig. 3, PP+7), however, average $\approx 11\%$ that of actively spinning fifth instars (Fig. 3, LV) and 2% that of emerged adults (Fig. 3, EA), similar to observed reductions in *M. rotundata* prepupae entering diapause and those reported for other insects (see Table 5 in Danks 1987). Although *O. lignaria*, like *M. rotundata* (Fig. 1), exhibits a precipitous drop in respiration levels at 22°C, as nonfeeding larvae complete cocoon spinning and continue development through to prepupa + 7 d (Fig. 1 and 3, PP+7), oxygen consumption in *O. lignaria* increases steadily through to the adult molt inside the cocoon (Fig. 3, A). AIC results suggest that a transition in oxygen consumption occurs from that of summer development (Fig. 3, PP to PU+14) to that characteristic of pre- and early wintering (Fig. 3, PU+21 to W+45).

The declines in oxygen consumption and the minimal weight losses observed at prepupa + 7 d in *O. lignaria* suggest that prepupal summer dormancy in *O. lignaria* may be diapause mediated and further suggest the intriguing possibility that this period of summer diapause may have originated from an evolutionary flexible hibernal diapause (Clarke 1991, 1993; Chown and Gaston 1999); minimal weight losses during the summer prepupal stage have also been documented in another adult-wintering *Osmia* (Bosch and Vicens 2002). Under this hypothesis, which has yet to be tested, the prepupa + 7 d stage from *O. lignaria* populations originating from northern Utah may actually exhibit an abbreviated diapause, the duration of which is reduced in length when compared with populations from southerly latitudes or lower elevations with greater summertime heat loads (Clarke 1991, 1993; Chown and Gaston 1999).

Also worthy of further investigation is our observation that at ≈ 2 wk after the adult molt, both female and male *O. lignaria* exhibit a short period of depressed oxygen consumption (Fig. 3, A+15). In both sexes of adult *O. lignaria*, and in contrast to the patterns observed in *M. rotundata* prepupae, oxygen consumption then seems to increase as individuals transit the 2-wk acclimatization at 14°C, and on through to early wintering at 4°C. The importance of temperature

cues at this developmental transition period should be rigorously tested.

Our results and observations suggest that *O. lignaria* adults make the transition from the diapause maintenance period and enter the postdiapause transitional period at ≈ 3 mo of wintering. This argument is supported first by AIC results, which detect that oxygen consumption increases to a new plateau no longer characteristic of pre- and early wintering levels at 75 and 105 d of wintering for *O. lignaria* females (Fig. 3A, W+75) and males (Fig. 3B, W+105), respectively. Second, our observations on adult *O. lignaria* minimum wintering requirements (Bosch and Kemp 2003), which are consistent with those of Richards et al. (1987) for overwintering *M. rotundata* prepupae discussed above, reveal sharply increasing survival rates for adults wintered for a minimum of 3 mo.

Observed declines in both the average time to emerge as well as the variation in emergence times in *O. lignaria* adults wintered from 3 to 6 mo (Bosch and Kemp 2003) further suggest that a population wintered at appropriate temperatures for a minimum of 6 mo contains a very high percentage of adults that have developed beyond diapause and postdiapause transition and are now in a nondiapause state. Although this determination is consistent with the general argument for a true diapause state only during autumn and early winter among diapausing temperate zone insects (Tauber et al. 1986, Danks 1987, Denlinger and Tanaka 1999), further work is needed to improve our understanding of the developmental biology of overwintering *O. lignaria* adults. At present, however, sufficient evidence exists to support a "classically defined" diapause syndrome in *M. rotundata* prepupae and *O. lignaria* adults that is spontaneously completed at ≈ 3 mo of wintering.

Although our observations were conducted at only one temperature, the stepwise increases in oxygen consumption that we observed in both sexes of *O. lignaria*, between 75 and 105 d of wintering at 4°C and beyond, seem to be consistent with the hypothesis of cascading changes in thermal responses proposed by Sawyer et al. (1993) to explain diapause development in the egg of gypsy moth, *Lymantria dispar* (L.). Under the cascading thermal response hypothesis, no clear demarcation is assumed between diapause and postdiapause development. Instead, initial developmental responses having low thresholds and optimal temperatures gradually give way to responses having higher thresholds and optima (Sawyer et al. 1993). Testing this hypothesis in either of the two megachilid bees that we are studying, however, will likely require molecular techniques in addition to constant volume respirometry and developmental rate observations at multiple temperatures.

Management Implications. Intensive agriculture, as practiced in much of North America, frequently presents large areas of a target crop requiring insect pollination. The need for sufficient numbers of pollinators, ready at the appropriate time, to pollinate such areas has resulted in the development of artificial management protocols for *M. rotundata* (Richards

1984) and *O. lignaria* (Bosch and Kemp 2001). Our results demonstrate large differences in the seasonal metabolic changes between these two species that have important implications to the continued improvement of artificial prewintering and wintering management regimes in the orchard pollinator *O. lignaria*. The apparent seasonal metabolic changes of adult *O. lignaria* observed in this study provide a physiological explanation to the observations of Bosch et al. (2000) who recorded apparent fat body depletion and increased wintering mortality in *O. lignaria* exposed to elevated prewintering temperatures in California. Therefore, we suggest that because *O. lignaria* exhibits proportionally greater oxygen consumption levels during the prewintering and wintering periods, it is important to minimize the exposure of *O. lignaria* to elevated temperatures once they have reached the teneral adult stage (Fig. 3; Bosch and Kemp 2001).

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