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Reproductive life-history traits of the classical biological control agent *Hypena opulenta* (Lepidoptera: Erebidae): Using agent biology to support post release monitoring and establishment

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ABSTRACT

It is important to develop efficient and cost-effective methods for monitoring the establishment and fitness of biological control agents. Understanding how simple and obtainable measurements of insects or their body parts relate to reproductive life-history traits could facilitate assessing the fitness of biological control agent populations in the field. Across many insect taxa, female size represents a principal constraint on potential fecundity. Here, we investigate the relationship between pupal measurements and aspects of potential fecundity in *Hypena opulenta* (Lepidoptera: Erebidae), a recently released biological control agent against *Vincetoxicum rossicum* and *V. nigrum* (Apocynaceae) in Ontario Canada. We dissected adult *H. opulenta* females of different ages to assess their strategy of oogenesis by counting and measuring the number of eggs in their ovarioles and establishing the relationship between pupal measurements and potential fecundity. A second experiment was conducted to determine the relationship between pupal weight and adult longevity. While moths emerged with eggs in their ovarioles, oogenesis continued throughout the adult stage, and mean egg size increased with time after emergence. These observations place the moth closer to being an income breeder on the ovigeny index scale. We observed no significant relationship between pupal weight and total number of eggs; however, pupal weight was positively correlated with adult longevity. These results demonstrate the limited use of general size-fecundity relationships in post-release assessments for insects that are income breeders. However, they also highlight how the understanding of reproductive strategy in *H. opulenta* can provide important information to aid in its establishment and spread at release sites.

1. Introduction

Classical biological control programs have correctly placed a great emphasis on pre-release testing of the host-range of candidate agents, in order to minimize the risk to non-target species (Schaffner et al., 2018). Field efficacy and impact is a second important criterion that is considered prior to release, but it can be challenging to predict field efficacy from laboratory studies (Morin et al., 2009). While there is a significant research investment that is required in order for a biocontrol agent to be approved for release, it is equally important to ensure that the financial investment is made to address the long-recognised need for post-release monitoring of biocontrol agents (Simberloff and Stiling, 1996; McFadyen, 1998; Hinz et al., 2014). Such studies can be logistically difficult, as the rate of establishment differs wildly among agents (Lockwood et al., 2013; Schwarzländer et al., 2018). Moreover, securing funding to track agent establishment and impact, which can take decades, is a challenge. Thus, for any biological control release, it is important to develop efficient and cost-effective methods for monitoring agent establishment, and the fitness of introduced populations. Understanding how easily obtained morphometric measurements of insects relate to their reproductive life-history traits could facilitate assessing the fitness of biological control agent populations in the field.

Under constant environmental conditions, insect fecundity is usually positively correlated with female size (Evans, 1982; Gilbert, 1984; Honěk, 1993). The extent to which female size can be used to predict potential fecundity; however, is highly dependent on the reproductive strategy employed by any given insect. Lepidopterans exhibit a spectrum of reproductive strategies ranging from capital breeding to income breeding (Tammaru and Haukioja, 1996). True 'capital breeders', many of which lack adult mouthparts, reproduce

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entirely using resources sequestered during the larval stage. At the other end of the spectrum, 'income breeders' are heavily dependent on resources consumed during the adult stage for successful reproduction (Dunlap-Pianka et al., 1977). The position of an insect on the continuum of reproductive strategies as described above can be quantified using a single metric, the 'ovigeny index' (OI). The OI is the ratio (expressed as a proportion) of female egg load at emergence to lifetime potential fecundity (Jervis et al., 2001). True capital breeders, therefore, have an OI of one, while income breeders have an OI as low as zero.

Vincetoxicum rossicum (Kleopow) Barbar. (Apocynaceae) and *V. nigrum* (L.) Moench, collectively known as swallow-wort or dog-strangling vine are perennial vines native to southwestern Ukraine and the Iberian Peninsula respectively (Pobedimova, 1952). Both species were introduced to North America in the 1800 s, and quickly became established (Monachino, 1957). Over the past 50 years their abundance has increased dramatically, reaching invasive status in several regions including Ontario, Quebec, and the northeastern United States (DiTommaso et al., 2005). The ecological impacts of invasive swallow-worts are wide ranging. Both species reduce native plant diversity by smothering neighbouring plants (Christensen, 1998). This displacement of native plants has cascading effects on native arthropod assemblages (Ernst and Cappuccino, 2005), and has even been shown to reduce the numbers of breeding birds in grassland habitats (DiTommaso et al., 2005). Rare alvar communities in Ontario are increasingly threatened by encroaching swallow-worts (Lawlor, 2000) and the weed is a potential oviposition sink for monarch butterflies (Casagrande and Dacey, 2007). Conventional weed control methods like mechanical removal or the use of herbicides are expensive, damage non-target plants, and can be both difficult and cost prohibitive to apply at the scale of the invasion and for remote infestations. Biological control represents our best hope of achieving long-term sustainable control of *V. rossicum* and *V. nigrum* in their invasive range.

The defoliating moth, *Hypena opulenta*, (Christoph) (Lepidoptera: Erebidae), was identified as a defoliator of swallow-worts in the Ukraine (Weed and Casagrande, 2010), and a petition for its release in North America was submitted in 2011 (Casagrande et al., 2011) after host-range tests demonstrated it to be specific to European swallow-worts (Weed and Casagrande, 2010; Hazlehurst et al., 2012). *Hypena opulenta* overwinters as a pupa in the soil, emerging as an adult in the spring. Females oviposit largely on the underside of leaves, and the resulting larvae develop through four or five instars before pupating in the soil or in tied up leaf material. Diapause is facultative, so multiple generations are possible in a single year (Hazlehurst et al., 2012). Releases of *H. opulenta* began in Ontario, Canada in fall 2013 (Young and Weed, 2014) and successful overwintering has been confirmed at several sites. The first release location from 2014 is confirmed as established and has spread from the initial release site (Bourchier et al., 2019). Post-release studies are continuing to confirm establishment and spread at additional release sites, and to explore factors that might be affecting *H. opulenta* establishment in the introduced range.

Here, we examine the relationship between pupal measurements and aspects of potential fecundity in *H. opulenta*. Understanding this relationship will benefit the biological control program in two key ways. First, measurements taken from pupae collected at release sites could provide information about the fitness of field populations, and help to prioritize sites where augmentative releases are required. Second, pupal measurements of insects from laboratory colonies could be used to track the need for infusions of new genetic material into rearing colonies, and to ensure that the reproductive potential of released individuals remains high.

We dissected adult *H. opulenta* females at three times post-emergence. Counting the eggs in the ovarioles allowed us to determine the relationship between pupal measurements and potential fecundity, and to determine whether or not oogenesis was conducted exclusively using resources acquired during the larval stage. Additionally, we conducted an experiment to compare adult longevity in males and females across a range of pupal weights. Our overall goal was to develop useful tools to assess the fitness of *H. opulenta* populations in the field.

2. Methods

2.1. Insect rearing

All insects used in this study were from laboratory reared colonies maintained at Agriculture and Agri-Food Canada in Lethbridge, AB, and at the University of Toronto, ON. Both of these laboratory populations originate from insects collected in 2006 and 2012 from Donetsk, Ukraine, by CABI-Switzerland (Weed and Gassmann, 2006). Newly-emerged adults were placed into plastic oviposition cages (55 × 40 × 22 cm) containing cut *V. rossicum* stems. Plant material was replaced every 2–3 days to ensure that the moths had sufficient space to lay their eggs, and that emerging larvae were not overcrowded. Developing larvae were transferred to smaller plastic boxes (35 × 20 × 12 cm) with screened windows for ventilation. Both the larvae and cut *V. rossicum* foliage were placed on chicken wire (1.5 cm mesh) a few centimeters above the base of the box to allow frass to drop down for easier cleaning. The bottom of the box was covered with wet paper towel to increase humidity. Boxes were checked daily for newly formed pupae, which were kept in rearing boxes for an additional 24 h to allow for sufficient hardening of the cuticula. Pupae were then sexed, based on the distance between the anal and genital orifices (Miller et al., 2015), and weighed using a digital balance (Adventurer, Ohaus Corp, Pine Brook, NJ). Measurements of pupal length and width were taken at the widest and the longest extremes of up-facing pupae using a digital microscope (Dino-Lite, digital microscope pro; Software: Dino-Capture 2.0 version 1.5.24). Pupal length was measured excluding the cremaster. Pupae were then placed on moist cotton wool in clear plastic cups (250 ml), and after adult emergence the length and width of empty pupal casings were measured as above. The entire rearing process was conducted at room temperature (23 ± 2 °C) and in a 16:8h (L:D) diel period. All *V. rossicum* foliage originated from a field site in Uxbridge, Ontario, Canada (44.088681, −79.106804).

2.2. Potential fecundity

We measured potential fecundity in *H. opulenta* females, and identified the development of egg production over time by dissecting adult female moths and counting the number of eggs in their ovarioles. A total of 72 unfertilized females were dissected, 24 at each of three time points after their emergence as adults (1-, 3-, and 6-day treatments). To examine the relationship between pupal weight and potential fecundity at emergence, 59 1-day old females (24 from the previous analysis plus 35 additional females) were dissected. Pupal weights were measured 1 day after pupation for all dissected individuals. For the 1-day treatment, individuals were placed in the freezer within 24 h after adult emergence, to be dissected at a later date. For 3- and 6-day treatments, adult moths were kept in 250-ml clear plastic cups and provided with honey water on a strip of cheese cloth hung from the lid. Moths were kept on the lab bench at room temperature with a 16:8h (L:D) diel period. After three or six days, moths were placed in the freezer for later dissection.

Prior to dissection, abdomens of the females were removed using forceps and transferred to a saline solution of 0.9 g of NaCl in 100 ml of distilled water. The ovarioles were then separated from the abdomen using forceps, and the unfertilized mature eggs were counted under a dissecting microscope. During the period prior to freezing, moths laid unfertilized eggs in the cups. These eggs were also recorded to determine the total egg-count. In addition to counting the number of eggs, 10 eggs of 10 females in each time treatment were separated from their ovariole and their diameter was measured under a Dino-lite digital microscope. The 10 eggs selected were the intact eggs closest to the

oviducts. These data were then used to calculate the average egg size per female.

2.3. Adult longevity

To determine adult longevity, 60 weighed pupae were placed individually in 250-ml clear plastic cups with a thin layer of moist cotton wool at the base. Pupae were chosen such that their weights were highly variable: males weighed 0.044–0.095 g, while females weighed 0.052–0.100 g. Pupae were sprayed lightly with water every three days prior to adult emergence. After adult emergence, cotton wool was removed from the cups, and moths were provided with honey water as described above. Moths were kept on the lab bench at room temperature and exposed to a 16:8h (L:D) diel period. High humidity was maintained in the rearing cups by spraying the cheesecloth with water daily. The cheesecloth was replaced every week to prevent mould. Moths were monitored daily, and the date of death was recorded. Of the 60 pupae designated for the study, 22 males and 18 females emerged as adults.

2.4. Pupal weight and size measurements

To examine the relationship between pupal weight and size measurements, larvae were reared until the pupal stage and measurements were taken 24 h after pupation, as described in detail above. Weight, length, and width of live pupae were taken for 115 males and 158 females. After adult eclosion, measurements of pupal casings were taken for 63 males and 75 (76 for width) females. Some live pupae, for which initial measurements were taken, were used in other experiments that are not described here, resulting in the lower number of empty pupal casings measured.

2.5. Statistical analyses

The number of eggs over time was analysed separately for eggs found in ovarioles, eggs laid in the cup, and the total number of eggs (sum of eggs in ovariole + in the cup) using one-way Analyses of Variance (ANOVAs) and posthoc comparisons of means with Tukey adjustments (*aov* function of the STATS package in R, R Core Team, 2018). Data for eggs laid in the cups was log(x + 1)-transformed to meet the assumptions of normality and homoscedasticity of residuals.

The relationship between potential fecundity at emergence and pupal weight was analysed using two separate analyses: first, the 1-day old females (n = 59) were assessed with a Kendall-Theil Sen Siegel nonparametric (median-based) linear regression using the *mblm* function of the MBLM package in R (Komsta, 2019) because the data did not meet the assumptions of normality and homoscedasticity of residuals. Second, to take the time after emergence into account for the fecundity-weight relationship, we used a general linear model with potential fecundity as a function of weight, time, and their interaction (n = 72; 24 females per time treatment) (*lm* function of the STATS package in R, R Core Team, 2018).

A Kruskal-Wallis Test (*kruskal.test* function of the STATS package in R, R Core Team, 2018) and Kruskal-Nemenyi posthoc comparisons of means (*posthoc.kruskal.nemenyi.test* function of the PMCMR package in R, Pohlert, 2014) were used to analyse the effect of time after emergence on egg size because the residuals did not meet the assumptions for a parametric analysis. The relationship between egg size and potential fecundity over time was best described with a logarithmic curve of the form: egg size ~log(number of eggs) fitted with the *lm* function of the STATS package in R (R Core Team, 2018).

Differences in longevity between males and females were analysed using a one-way ANOVA with sex as the explanatory variable (*aov* function, STATS package, R Core Team, 2018). However, for differences in pupal weight between the sexes a Kruskal-Wallis rank sum test was used (*kruskal.test* function, STATS package, R Core Team, 2018) because the residuals were not normally distributed.

Multiple regressions were used (*lm* function of the STATS package in R, R Core Team, 2018) to analyse the effect of pupal weight and sex on longevity (the latter was transformed to ageing rate (1/longevity) to ensure the assumption of normally distributed residuals), the effect of pupal length and sex on pupal weight, and the effect of pupal width and sex on pupal weight. Where significant differences between the sexes were found, simple linear regressions (*lm* function of the STATS package in R, R Core Team, 2018) or, in cases where the residuals did not meet the assumptions of normality and/or homoscedasticity, median-based regressions (mblm function of the MBML package in R, Komsta, 2019) were fitted to the data for males and females separately.

3. Results

3.1. Female fecundity

Female fecundity significantly increased over time for the total number of eggs produced ($F = 32.72$; $df = 2, 69$; $P < 0.001$), the number of eggs in ovarioles ($F = 23.43$; $df = 2, 69$; $P < 0.001$), and the number of eggs laid in cups ($F = 13.23$; $df = 1, 45$; $P = 0.001$). More precisely, the total number of eggs produced increased significantly between day 1 (69.75 ± 6.09) and day 3 (132.67 ± 8.05) after adult emergence. No significant difference was observed; however, between day 3 and day 6 (159.17 ± 9.57; Fig. 1A). Likewise, the number of eggs in the ovarioles increased significantly between day 1 (69.75 ± 6.09) and day 3 (129.92 ± 7.85) after adult emergence, but no significant difference was observed between day 3 and day 6 (145.50 ± 10.30; Fig. 1B). The estimated OI (mean number of eggs at emergence/mean number of eggs six days after emergence) for *H. opulenta* was 0.44. No moths laid any eggs into the cups within the first 24 h post-emergence but the number of eggs laid between 3 (3.04 ± 0.77) and 6 (14.08 ± 3.61) days significantly increased (Fig. 1C).

The relationship between pupal weight and total female egg production at 1 day after emergence was not significant ($F = 0.13$; $df = 1, 57$; $P = 0.717$). Similarly, when time after emergence was considered, by analyzing females dissected at 1, 3, and 6 days after emergence, there was still no relationship between pupal weight and total female egg production (weight × time: $F = 0.79$; $df = 2, 66$; $P = 0.457$).

The average size of unfertilized eggs in the ovarioles increased significantly over time after emergence ($\chi^2 = 16.03$; $df = 2$; $P < 0.001$). Within the first three days post-emergence, the mean egg diameter significantly increased from 0.54 to 0.63 mm; however, no significant increase was measured thereafter (Fig. 2). The relationship between egg size and the number of eggs produced by females over time after emergence was best described by a logarithmic curve ($F = 49.14$; $df = 1, 28$; $P < 0.0001$; Fig. 3). The increase in egg size with the number of eggs was most apparent 1–3 days after adult emergence. The relationship between egg size and number was less severe in older females (3 and 6 days old), although not lost completely.

3.2. Adult longevity

Females lived significantly longer (13.33 ± 1.21 days) than males (9.95 ± 1.12 days; $F = 4.17$; $df = 1, 38$; $P = 0.048$) and had the tendency to be heavier (84.8 ± 3.3 mg) than males, although the difference of the latter was not significant (77.2 ± 2.9; $\chi^2 = 3.37$; $df = 1$; $P = 0.066$). There was a significant positive relationship between longevity and pupal weight ($F = 5.40$; $df = 1, 36$; $P = 0.026$; Fig. 4), but no significant differences between the sexes were found ($F = 3.43$; $df = 1, 36$; $P = 0.072$). The interaction of sex and pupal weight was also not significant ($F = 0.73$; $df = 1, 36$; $P = 0.400$).

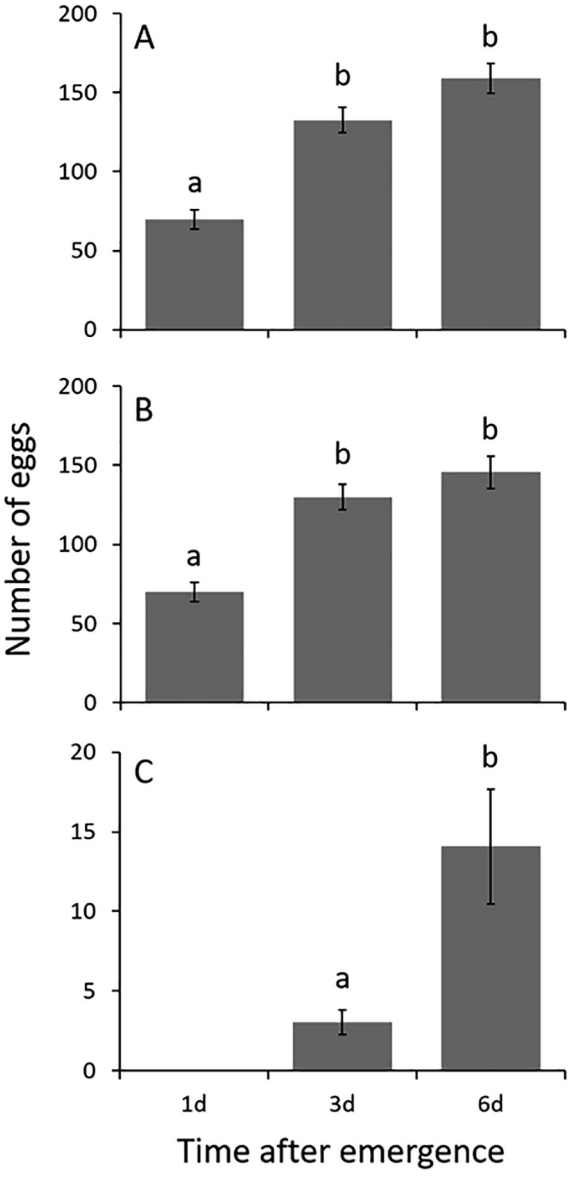

Fig. 1. Mean (± SE) number of *Hypena opulenta* eggs over time (days); (A) total number of eggs, (B) eggs in ovarioles, and (C) eggs laid in cups. A total of 24 females were dissected at each time period.

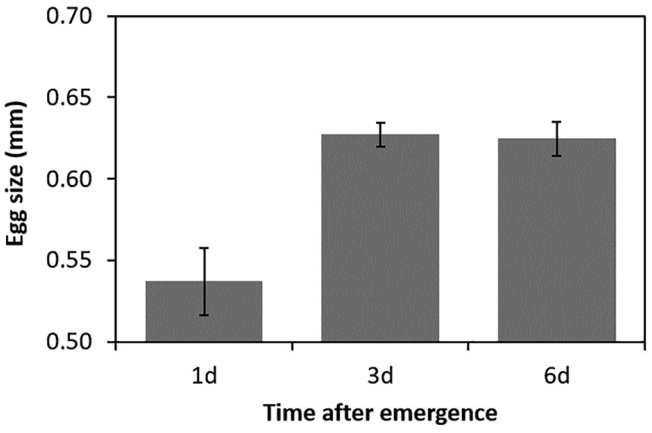

Fig. 2. Mean (± SE) *Hypena opulenta* egg size (diameter) over time (1, 3, and 6 days) after emergence. Egg size was measured for 10 eggs per female, there were 10 females in each time treatment.

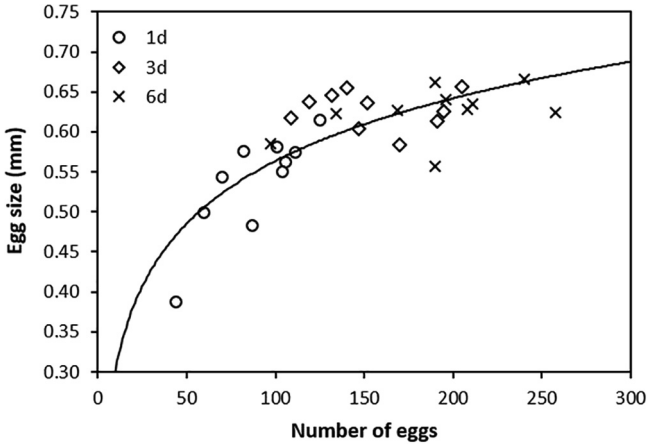

Fig. 3. The relationship between egg size (mean diameter of ten eggs per moth) and the total number of eggs (developed in ovarioles and laid in rearing cups) over time (1, 3, and 6 days) after emergence for laboratory-reared *Hypena opulenta* (n = 30).

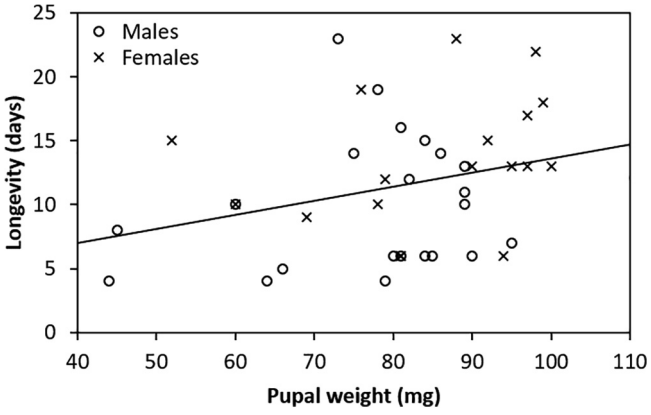

Fig. 4. The relationship between adult longevity and pupal weight for *Hypena opulenta* males (circles, n = 22) and females (x, n = 18). Regression for both sexes combined: y = 0.110x + 2.580, R^2 = 0.083.

3.3. Pupal weight and size measurements

For both live pupae and empty pupal casings, there was a significant positive correlation between pupal weight and length, with females being significantly heavier than males across the measures of pupal lengths (Table 1A; Fig. 5A and C). The simple regressions accounted for 92% and 85% of the variance in the data for live males and females respectively. An R^2-value could not be calculated for the relationship of initial weight and length of empty pupal casings because of the use of a

Table 1
Statistical results for multiple regressions testing the influence of pupal length, sex, and their interaction, as well as pupal width, sex, and their interaction on pupal weight for live *Hypena opulenta* pupae and empty pupal casings.

Variable	Live *H. opulenta* pupae			Empty pupal casings		
	F	df	*P*	*F*	df	*P*
A: Model: weight ~ length*sex						
Length	2014.77	1, 269	< 0.0001	247.59	1, 134	< 0.0001
Sex	85.88	1, 269	< 0.0001	7.65	1, 134	0.0065
Length*Sex	1.98	1, 269	0.1604	0.61	1, 134	0.4354
B: Model: weight ~ width*sex						
Width	1757.40	1, 269	< 0.0001	306.66	1, 133	< 0.0001
Sex	27.21	1, 269	< 0.0001	0.23	1, 133	0.6359
Width*Sex	3.63	1, 269	0.0578	2.19	1, 133	0.1416

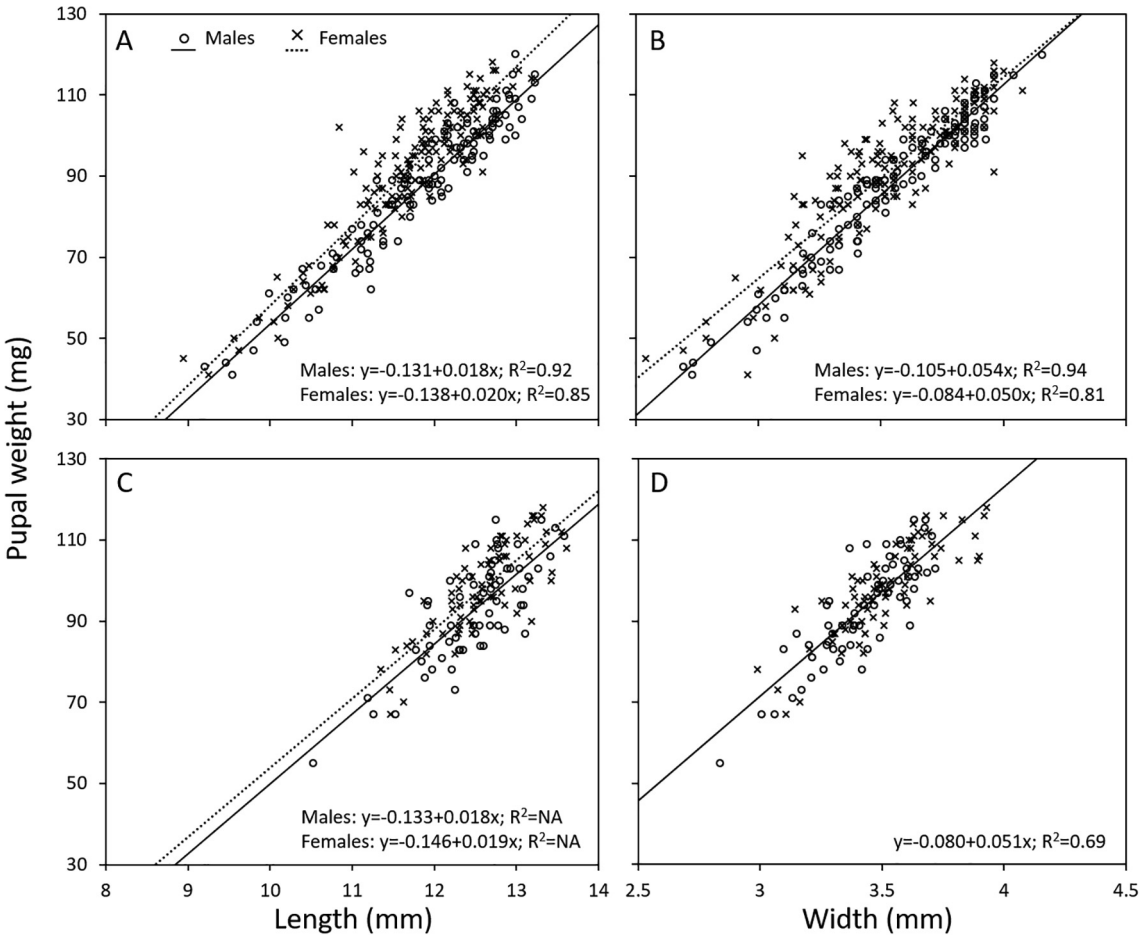

Fig. 5. Pupal weight of *Hypena opulenta* regressed on (A) length and (B) width of live pupae (n = 115 males and 158 females), and (C) length (n = 63 males and 75 females) and (D) width (n = 63 males and 76 females) of empty pupal casings.

non-parametric regression. However, the regression lines comparing each sex's live pupae and empty pupal casings revealed almost identical intercepts and slopes (Fig. 5A & C). There was also a significant positive correlation between pupal weight and width for both live pupae and pupal casings, with females of live pupae being significantly heavier than males over the range of measured widths (Table 1B; Fig. 5B & D). The simple regressions of weight and width for live pupae resulted in very similar R^2-values compared to the regressions of weight and length, for both males (0.94) and females (0.81). The intercept and slope of the regression line for pupal weight before adult emergence and width of empty pupal casings was similar to the line for live females; however, the variance explained by the regression was markedly lower (69%; Fig. 5B & D). In all cases, the interaction terms of pupal length or width with sex were not significant (Table 1).

4. Discussion

The importance of post-release monitoring or assessment of biological control agents and their impact in release sites has been stressed by many authors (Blossey, and Skinner, 2000; Hopper, 2001; Barratt et al., 2006; Morin et al., 2009). In most cases, such assessments are aimed at determining the establishment of an agent or, if establishment was successful, the impact of the agent on target and non-target organisms. Here, we studied reproductive life-history strategies of *H. opulenta* and explored relationships between simple measurements on their immobile pupal stage and individual reproductive life-history traits to see if they might help in assessing the fitness of *H. opulenta* at release sites.

Female *H. opulenta* continued to produce eggs for a period of at least six days post-emergence, and we calculated the ovigeny index (OI) to be 0.44. This estimate is undoubtedly high as we did not measure lifetime potential fecundity. *Hypena opulenta* females have been shown to lay a mean of 410 (± 157) eggs during a mean lifespan of 17 (± 4) days under laboratory conditions (Weed and Casagrande, 2010). Thus, the slowing down of egg production that we observed after six days, with a mean of 159 (± 10) eggs, is most probably due to capacity limits of the female's ovariole. This is also supported by the observation that females started laying eggs into the rearing cups after 3 days, even in the absence of their host plant. Based on Weed and Casagrande's observations of lifetime oviposition (Weed and Casagrande, 2010), a more accurate OI value for *H. opulenta* may be 0.17 (69.75/410), placing this species markedly closer towards a true income breeder.

Estimates of OI can provide insight into various elements of a species life-history. For example, OI in Lepidoptera is negatively correlated with the need for adult food resources (O'Brien et al., 2004), and with the male provision of nuptial gifts (Boggs, 1990). Lepidopterans with an OI close to zero generally live longer as adults (Jervis et al., 2003), and are more often capable of egg resorption and subsequent reallocation of resources (Jervis et al., 2001). Being somewhere between the mid to low range on the OI spectrum, *H. opulenta* can be expected to exhibit life-history traits that are not clearly related to either capital or income breeders.

In our experiments we observed no significant relationship between pupal weight and female egg production in *H. opulenta*. The relationship between pupal size and adult fecundity; however, is far from consistent, even among capital breeders (Fenemore, 1977; Slansky,

1980; Wiklund and Persson, 1983; Karlsson and Wiklund, 1984; Boggs, 1986). In cases where the correlation between pupal weight and egg-count is strong, other factors may limit the usefulness of pupal weight as a predictor of fecundity. In *Pieris rapae* (L.) (Lepidoptera: Pieridae), for example, pupae of similar size, but with different genotypes produced different weight/fecundity relationships (Gilbert, 1986). In the codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), although larger females produce more eggs, a higher proportion of those eggs tend to be infertile (Deseo, 1971). Our results, along with these studies, highlight that pupal weight alone cannot be used as a direct predictor of potential fecundity in *H. opulenta*.

A possible limitation of the present study was that egg counts were conducted in unmated females. In the tobacco budworm, *Heliothis virescens* (Fabricius) (Lepidoptera: Noctuidae), pupal weight is only correlated with fecundity if mating occurs within 48 h of emergence (Proshold et al., 1982). This kind of relationship between mating efficiency and egg production is likely common among species in which males provide females with a nuptial gift. However, the role of *H. opulenta* males in providing resources for reproduction is unknown.

In addition to the number of eggs, average egg size in *H. opulenta* also increased over the three days following adult emergence. Unfortunately, the limited distribution of pupal weights of the dissected individuals for which egg size was measured meant that we were not able to establish a meaningful relationship between pupal weight and egg size. However, we observed a positive logarithmic relationship between total egg count over time after emergence and mean egg size. The increase was strongest within 24 h of emergence, and became weaker with increasing female age. In other lepidopterans, a negative relationship between egg size and egg number has been reported, exhibiting a trade-off between egg size and fecundity (Ehrlich and Ehrlich, 1978; Richards and Myers, 1980; Leather and Burnand, 1987). This relationship is not supported by our findings. However, as a species that tends towards income breeding and that has a preoviposition period of 1.8 ± 0.8 days (Weed & Casagrande, 2010), it is possible that *H. opulenta* females are dependent on allocating resources towards their eggs during the first days after emergence, before oviposition. Thus, not only the number of eggs but also egg size would increase during this period. The 1-day-old females were probably at variable points in the maturation of their eggs because they were sampled at any time within 24 h of emergence. Thus, females that were more mature may have laid sooner and had further developed (and thus larger) eggs in their ovarioles. It is also possible that, in the absence of host-plants on which to oviposit, post-emergence resource allocation was biased towards producing larger eggs rather than producing a greater number of eggs. Indeed, some Lepidoptera have been shown to produce larger eggs when available host plants are of low quality in order to improve the survival chances of the resulting larvae (Leather and Burnand, 1987).

While pupal measurements were not correlated with total egg production in *H. opulenta*, we did observe a positive relationship between pupal weight and adult longevity. Adult longevity was assessed here under laboratory conditions with *ad-libitum* access to honey water. Under field conditions, longevity will be influenced by post-emergence factors such weather and the availability of adult food sources. We currently have little knowledge about the feeding habits of adult *H. opulenta* at release sites, and the influence of adult food source on oogenesis, oviposition behaviour, and adult longevity have not been studied. However, for many lepidopterans, longevity is a more reliable predictor of realized fecundity than pupal weight, and this is particularly true for income breeders (Leather, 1984). This is likely because even moths that live a 'long time' relative to their conspecifics rarely come close to laying their full egg load. For example, in the pine beauty moth, *Panolis flammea* (Denis & Schiffermüller) (Lepidoptera: Noctuidae), maximum estimates of field fecundity are only 28% of their reproductive potential (Leather, 1985). Future work on *H. opulenta* should explore the relationship between adult longevity and the proportion of eggs laid. Any attempt to use longevity for an estimation of realized fecundity must take the above mentioned preoviposition period into account.

The relationship between pupal weight and longevity in *H. opulenta* can be of practical use for post-release assessments of a population's fitness in sites where the moth has become successfully established. However, the relatively low R^2-value for that relationship here implies that only means from a sufficiently high number of measurements (i.e., at least 10 individuals) should be compared between sites or with laboratory-reared individuals. Taking precise measurements of pupal weight in the field is difficult or can add logistical issues because pupae may have to be returned to the laboratory. Therefore, we established the relationship between pupal weight and pupal length or width here, as these measurements can be taken from photos taken at field sites, if a reference scale is also photographed. We show that pupal length or width are good proxies for pupal weight; 81–92% of variation in pupal length or width can be explained by pupal weight. There was also a significant relationship between pupal weight before emergence and length or width of pupal cases after emergence. These relationships can be used when only pupal cases are found in the field and assessment of pupal weight is impossible. Unfortunately, the latter relationship was not as strong as for live pupae and caution must be taken when inferring a population's fitness from empty pupal casings.

The tendency of *H. opulenta* towards an income breeder on the OI scale makes it difficult to directly relate measurements such as pupal weight to its fitness for use in post-release studies. However, the biological control program against *V. rossicum* and *V. nigrum* in North America is still in its early stages, and the results of this study can be used in several key areas. First, the relationship between pupal weight and adult longevity means that there is potential to use pupal measurements in the field to assess the fitness of *H. opulenta* populations at release sites. These assessments can inform the release strategy by prioritizing sites where fecundity is likely to be low or declining. Second, the knowledge that *H. opulenta* continues to produce eggs after adult emergence places a greater importance on the availability of adult food sources. Artificial food sources can and should be provided at release sites, as these may support egg production and improve adult longevity (Leather, 1984). Extending the lifespan of *H. opulenta* adults at release sites could not only increase reproductive fitness, but may also mitigate Allee effects that often affect newly introduced species (Liebhold and Tobin, 2008). Application of this new knowledge about *H. opulenta*'s reproductive biology will aid in the establishment and spread of this crucial biological control agent.

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Declaration of Competing Interest

None.

CRediT authorship contribution statement

M. Lukas Seehausen: Conceptualization, Writing - review & editing, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing - original draft. **Carla Timm:** Conceptualization, Writing - review & editing, Data curation, Investigation, Methodology. **Ian M. Jones:** Conceptualization, Writing - review & editing, Investigation, Methodology, Supervision, Validation, Writing - original draft. **Robert S. Bourchier:** Conceptualization, Writing - review & editing, Funding acquisition, Project administration, Resources, Supervision, Validation. **Sandy M. Smith:**

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