# Taylor's checkerspot (*Euphydryas editha taylori*) Captive Rearing and Reintroduction: South Puget Sound, Washington, 2013-2014


2014 Annual Report to the USFWS Recovery Program, Joint Base Lewis-McChord Fish and Wildlife Program and ACUB Technical Review Committee

**30 November 2014**

Mary Linders, Washington Department of Fish and Wildlife, 600 Capitol Way N. Olympia, WA 98501; 360-902-8135; lindemjl@dfw.wa.gov
Karen Lewis, Oregon Zoo, 4001 SW Canyon Rd., Portland, OR 97221-9704; Karen.Lewis@oregonzoo.org
Lindsey Hamilton, The Evergreen State College, 2700 Evergreen Parkway NW, Olympia, WA 98505; hamiltonl@evergreen.edu

# Table of Contents

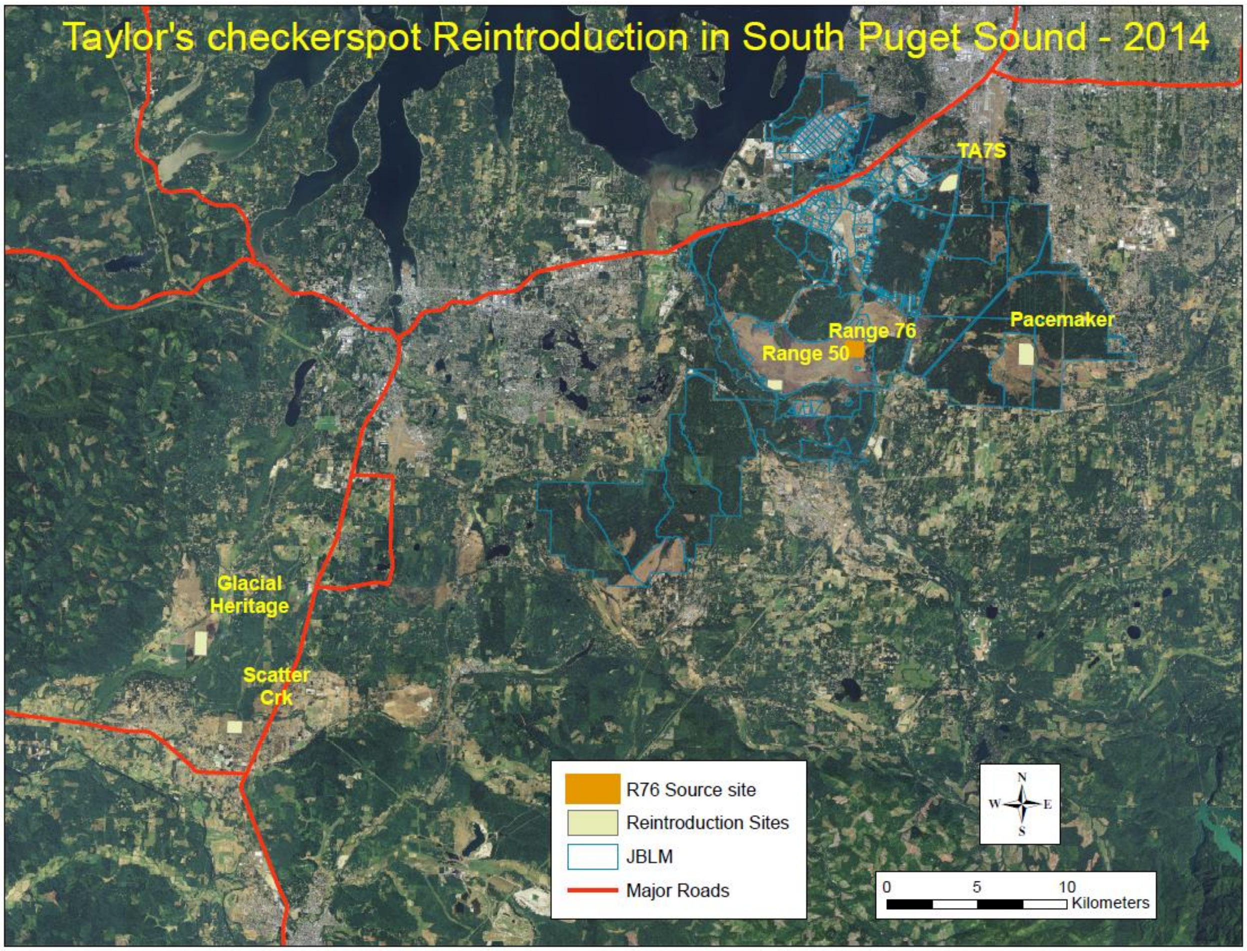


# List of Tables

## List of Figures

## *Project Sites*

Captive rearing locations are: Oregon Zoo, Portland, OR; Mission Creek Corrections Center for Women, Belfair, WA. Release and monitoring sites (see map below) are: Scatter Creek Wildlife Area – South Unit, (SCS; 2007-2013 releases); Range 50, Joint Base Lewis-McChord (JBLM) (R50; 2009-2011 releases); Pacemaker Airstrip, JBLM (PCM; 2012 release); Glacial Heritage Preserve (GHP; 2012-2013 releases); and Range 76, JBLM (R76; reintroduction source population).



## Executive Summary

In 2014, one new site in South Puget Sound was selected for reintroduction of Taylor's checkerspot: Training Area 7S (TA7S) on Joint Base Lewis-McChord. Larval releases continued at Glacial Heritage Preserve (GHP; initiated in 2012) in 2014, and Scatter Creek South (SCS; initiated in 2007) received adults. Range 50 (R50; initiated in 2009) received three sequential releases of Taylor's checkerspot through 2011; monitoring for establishment is ongoing there and at Pacemaker (PCM; initiated in 2012), where no further releases are planned. A total of 8,258 eggs were produced between the Oregon Zoo and Mission Creek captive rearing facilities, with 54.2 percent (4,475) of eggs produced by known lineage captive-mated females and 45.8 percent (3,783) of eggs produced by wild females. A total of 1,522 postdiapause larvae were released at TA7S on 7 March 2014 (670 in Plot A, 778 in Plot B, 25 each in Control Plots 1 and 3, and 24 in Control Plot 2). Also on 7 March 2014, 1,086 larvae were released at Glacial Heritage Preserve (871 in Plot H, 165 in Plot 165, and 25 each in Control plots 1 and 2). In addition, a total of 56 adult Taylor's checkerspots were released from the Mission Creek facility once their roles in mating trials and oviposition were complete. We used distance sampling to quantify daily population density, daily population size, and to illustrate the distribution of adults at the release sites and three other sites, PCM, R50 and R76 (source site). A combined total of 4,901 checkerspots were counted across six sites (R76, R50, SCS, PCM, GHP and TA7S) during distance sampling surveys in 2014, a 60 percent increase compared to the 2013 count. Long-term monitoring and population goals developed in fall 2012 were used to assess progress at R50. Based on those criteria, that site far exceeded the target of 250 adults on a single day in both 2012 and 2013, based solely on natural reproduction. The peak single day abundance estimate in 2013 was 1,166 adults (range 891-1,527). The peak raw count for 2014 (406) suggests abundance estimates will exceed the target in 2014 as well. Adults were distributed across the majority of the 22-ha monitoring area in 2013, and while their distribution in 2014 was somewhat reduced, they still occupied the site from north to south and east to west. Baseline standards will need to be met for five consecutive years based solely on reproduction in the field to meet the actual threshold established for success. This report summarizes captive-rearing work conducted in May 2013-May 2014, and reintroduction work from July 2013-July 2014.

## Project Goals and Objectives

The goal of this project is to establish new Taylor's checkerspot populations in Washington's South Puget Sound to reduce the likelihood of local extinction, and eventually move toward species recovery. To accomplish this, WDFW and its partners intend to establish at least three new populations at three sites in the next decade. This project employs a strategy of captive rearing and reintroduction which has been funded jointly by JBLM's ACUB and Fish and Wildlife programs, and the USFWS Recovery Initiatives Program, with in-kind support from JBLM, the Oregon Zoo, The Washington State Department of Transportation Habitat Enhancement fund, the Washington Department of Corrections, and ACUB cooperators. This report mainly summarizes activities from the 2013-2014 field season, although some longer term summaries are included. In 2014 we proposed to 1) release a total of about 4,000 postdiapause larvae at a minimum of two reintroduction sites, 2) conduct follow-up surveys during postdiapause larval, and adult life stages at reintroduction sites, 3) conduct prediapause larval surveys at one reintroduction site, and 4) conduct surveys of adult butterflies at three additional reintroduction sites, using a combination of USFWS ACUB, and JBLM funds. To insure clarity and cohesion, this report covers all activities involved in captive propagation and reintroduction of Taylor's checkerspots in 2013-2014, regardless of funding source.

This project has five main objectives, which also form the structural framework of this report. Some objectives include a suite of smaller tasks and objectives, which are emphasized by additional headings in the text. The objectives are:

I. Select areas for release of Taylor's checkerspot larvae in South Puget Sound, Washington

II. Produce larvae for release via captive propagation and collection of wild stock

III. Release captive and associated wild stock

IV. Monitor success of the reintroductions

V. Long-term monitoring and population goals

# I. Select areas for the release of Taylor's checkerspot larvae in South Puget Sound, Washington

## *Methods*

A suite of historic and potential sites within the known range of Taylor's checkerspot in South Puget Sound were initially scored in 2006 (Linders 2006). The objective of Taylor's checkerspot habitat restoration in South Puget Sound has been to return degraded grasslands to a forb-rich condition that can be readily characterized as containing dense and diverse host and nectar plants with a low, open vegetation structure (Fimbel and Dunn 2013). Priority host and nectar species have been identified (Linders and Lewis 2013) and preliminary restoration targets described (Fimbel and Dunn 2013) which are designed to insure access to food plants (host and nectar), basking and roosting sites, and oviposition locations. Habitat conditions on potential reintroduction sites are reviewed annually by project partners with final site selection occurring on an as-needed basis. In addition, in 2013 the ACUB Butterfly Habitat Enhancement project initiated a new sampling method as a cooperative project with JBLM, to increase the scientific basis of habitat enhancement planning and determination of site readiness for Taylor's checkerspot reintroductions (see Kronland and Dunwiddie 2014b). These data are being used to: 1) set quantifiable restoration targets, 2) measure progress toward achieving them, and 3) determine site readiness for reintroduction.

Release areas were selected and enhanced in the same manner as in previous years (Linders and Lewis 2013). A site may be deemed suitable to begin receiving larvae when at least 1,200 square meters of habitat dominated by host plants (enough for two release plots) and at least 20,000 square meters (about 5 acres) of supporting (matrix) habitat (fewer host resources but otherwise as defined above) have been prepared (Kronland and Dunwiddie 2014b). These figures originate from field observation at Range 76, where postdiapause larvae have often been observed at a density of 1 larva per square meter and an approximation of the spread of adults in the first year following release. In addition, two 10 x 10-m Survival method test plots were established at GHP and three at TA7S; these were used to test a new approach to post-release sampling. This amount of habitat is expected to be added annually to reintroduction sites to achieve a total of 25 acres of matrix habitat with a total of 5 acres of enhanced checkerspot resources (Kronland and Dunwiddie 2014a) prior to the $5^{th}$ year of release. Ideally all of this restoration would be complete prior to initiating reintroduction, but restoration has proved challenging as a result of recent burn bans and shortages of plug and seed resources. Fortunately, a great deal of effort has gone toward overcoming these obstacles, and the pace of restoration efforts is improving, with a goal of initiating reintroductions on fully restored sites by 2017.

### *Results and Discussion*

Five sites have been selected for reintroduction to date from the larger list of potential sites in the South Puget lowlands (Linders 2006, Fimbel and Dunn 2013). Three sites, SCS (since 2007), R50 (since 2009) and GHP (since 2012) have received multiple sequential releases of Taylor's checkerspot (Appendix A) consistent with the scheduled approach (Appendix A, Linders 2013); one site (PCM) received a single release (2012) and releases were initiated at one new site in 2014 (TA7S). Two main release areas were established at each site. These measured a total of 715 square meters at GHP and 2,492 square meters at TA7S (Table 1); in addition two control plots at GHP measured at total of 40 square meters and three control plots at TA7S measured 60 square m. TA7S exceeded the minimum requirement (1200 square meters) necessary to begin reintroductions, whereas GHP fell short of its annual goal.

Table 1. Size of individual release plots and total area available for release of Taylor's checkerspot larvae at Training Area 7S and Glacial Heritage, South Puget Sound, Washington, Spring 2014.

| **Site/plot** | **Hectares** | **Acres** |
|---|---|---|
| Training Area 7S | | |
| Plot A | 0.094 | 0.232 |
| Plot B | 0.155 | 0.383 |
| Glacial Heritage Preserve | | |
| Plot G | 0.016 | 0.039 |
| Plot H | 0.056 | 0.137 |

## II. Produce larvae for release via captive propagation and collection of wild stock

Our objective with respect to captive propagation is to produce the target numbers of eggs, larvae and adults for reintroductions proposed (Linders 2013). Captive propagation methods were developed at the Oregon Zoo (Barclay et al. 2009) and adapted to our second rearing facility at Mission Creek Corrections Center for Women in Belfair, Washington, under the supervision of the Sustainability in Prisons Project at The Evergreen State College.

### Continue captive propagation at the Oregon Zoo and Mission Creek to achieve target numbers of eggs, larvae and adults for reintroductions

### *Methods*

### *Collection of wild checkerspots*

To increase the number of founders contributing to reintroduction and reduce the potential influence of captive propagation on stock used for reintroduction, we proposed to collect wild females from the source population at R76 each year. A population size of at least 1000 adults at Range 76 is sufficient to supply the minimum of 20 females needed for oviposition in captivity (not to exceed 2 percent of the local population) for oviposition. In fact, single day population estimates often exceed 1000 adults (Linders 2010, 2011b, 2012, 2013). Should something happen to the wild population, 10 females per rearing facility annually is sufficient to sustain a captive population based on guidelines provided by the Population Management Center (Schad 2008). Wild females are collected with the aim that they will supply two thirds of the 10,000 eggs needed (5,000 per facility), with the remaining eggs (n = 3330) supplied by captive-mated females. A total of 24 females were collected from R76 on 8 May 2013 and distributed to the rearing facilities (12 to each). In addition, 8 wild males were collected the same day and provided to the Oregon Zoo to be mated with a subset of second diapause adults. Collection methods and transport procedures followed those reported previously (Linders and Lewis 2013).

### *Captive mating*

A total of 291 offspring from 12 maternal lines originating as eggs in 2012 (12FL), were available for inclusion in the 2013 captive breeding colony (Table 2). In addition, 12 second diapause (11FL) adults from 5 maternal lines were available to breed (Table 2). Each year, a few larvae also proceed to the adult stage without entering diapause. In 2013, this led to 2 mature larvae, 2 pupae and 2 adult butterflies from at least 5 matrilines at the Oregon Zoo. These were transferred to Mission Creek where two males and one female eclosed and were used for breeding (Hamilton et al. 2014).

Pairings for the 12FL cohort were determined using the daisy chain approach (Fig. 1) previously described (Lewis et al. 2013). Our objective was to obtain two successful copulations from each mating dyad. Pairings for the 11FL cohort were more opportunistic due to the strong sex bias, and several females were paired with wild males. Mating methods were consistent with those reported previously (Barclay et al. 2009). Males were allowed to copulate only once to increase the genetic contribution from each line, although one 11FL male was allowed to copulate a second time. All adults fit for release at the completion of the mating period are released to a selected field site (see Adult release).

Table 2. Number of 12FL (2012 cohort) male and female Taylor's checkerspots by matriline included in captive mating trials at the Oregon Zoo, Portland, Oregon and Mission Creek Corrections Center, Belfair, Washington, Spring 2013 (from Hamilton et al. 2014, Lewis et al. 2014, in prep.).

| Matriline | # of Females | # of Males |
|---|---|---|
| **Oregon Zoo** | | |
| 11FL03 | 4 | 1 |
| 11FL05 | 3 | 1 |
| 11FL21 | 0 | 1 |
| 11FL26 | 1 | 0 |
| 11FL27 | 1 | 0 |
| **Total** | **9** | **3** |
| 12FL03 | 14 | 9 |
| 12FL07 | 6 | 4 |
| 12FL10 | 19 | 8 |
| 12FL13 | 10 | 18 |
| 12FL20 | 12 | 13 |
| 12FL23 | 3 | 4 |
| **Total** | **64** | **56** |
| **Mission Creek** | | |
| 12FL02 | 11 | 14 |
| 12FL04 | 12 | 16 |
| 12FL09 | 14 | 8 |
| 12FL12 | 15 | 15 |
| 12FL18 | 17 | 12 |
| 12FL22 | 12 | 13 |
| **Total** | **81** | **78** |

### *Captive rearing, egg to adult*

Taylor's checkerspots readily oviposit in captivity. Our goal was to collect about 150 eggs from each gravid wild and captive female to meet our target of 10,000 eggs in 2013 (Linders 2013). Oviposition and rearing methods followed Barclay et al. (2009). Larval numbers are assessed at 3rd instar when they are hardy enough to be manipulated individually; at this point an official "hatch" count is obtained. Both pre- and postdiapause larvae are reared exclusively on freshly-cut *Plantago lanceolata* leaves rather than on native host plants because it is 1) easy to grow and handle in the lab, 2) it is less prone to mold and desiccation, and 3) results in high survival (Linders 2007). Oviposition host plant choice in adult females is a genetically derived trait that is not affected by the host plant on which they fed as larvae (Singer 2004).

Once larvae entered diapause no additional handling occurred. Larvae are checked periodically throughout the diapause period following procedures outlined in Barclay et al. (2009). We refer to the time period from diapause initiation until the time the weather cools and larvae are moved outdoors as "warm" diapause. Subsequently, larvae were moved outdoors for the remainder of the "cold" diapause period. Mission Creek began using its diapause shed to house larvae in fall 2013, which proved to be a suitable location.

Larvae were removed from diapause on 18 (Mission Creek) and 22 (Oregon Zoo) February 2014 following increased activity levels, sunny weather and emergence of wild progeny. Upon removal from diapause, larvae were placed in a high humidity environment and cared for according to established procedures (Barclay et al. 2009). Once they began to eat, a subset of larvae from wild females captured in 2013 was retained at each facility for inclusion in the 2013 breeding colony; the remainder, including all offspring of captive-mated females, was released. Eleven multi-diapause larvae from Mission Creek were also retained and transferred to the Oregon Zoo for breeding. Multi-diapause larvae are the offspring of wild females; these are retained and paired with other multi-diapause adults whenever possible because this trait is likely important to population persistence and genetic mixing.

Postdiapause larvae destined for release are reared indoors for only a few weeks to maximize development time in the wild and reduce the likelihood they will re-enter diapause. An unknown ailment at the Oregon Zoo rendered this colony unfit for release (see Results-Captive rearing egg to adult), and most larvae were humanely euthanized upon removal from diapause (Lewis et al. 2014, in prep.). A subset of larvae from 12 female lines were retained and reared to the adult stage for meconium (first excrete after eclosion) sampling, at which time all remaining checkerspot were also euthanized regardless of life stage. Postdiapause larvae at the Oregon Zoo were reared under warmer-than-normal conditions to speed their development time and allow ample time to sterilize the lab prior to bringing in a new cohort from the wild. Pupae were handled as in the past (Barclay et al. 2009), however adults were held inside yogurt cups after then eclosed to facilitate collection of the meconium (Lewis et al. 2014, *in prep*.). Rearing of larvae, pupae and adults at Mission Creek followed standard protocols (Barclay et al. 2009).

### *Rearing conditions*

During all stages of larval growth at the Oregon Zoo, full spectrum fluorescent lights were maintained on a 12-hour light/12-hour dark cycle from 0700-1900 using timers. Indoors, overhead lights were turned on during the day to increase light intensity. Conditions during mating were generally the same as for adult males, but mating tents were also placed next to a window or outside on a sunny day. Oviposition chambers were placed outside on sunny days. At Mission Creek, no supplemental light or heat was provided as the glass greenhouse provided ample amounts. Target conditions for temperature, relative humidity, and supplemental heat and light are summarized by life stage in Table 3. Temperature and relative humidity during all rearing stages were measured as min/max data at the same time each day (1100-1300 h).

Table 3. Target conditions for temperature and relative humidity, summarized by life stage for Taylor's checkerspot in captivity at the Oregon Zoo, Portland, Oregon and Mission Creek Corrections Center, Belfair, Washington, 2013-2014. Supplemental heat and light was provided only at the Oregon Zoo.

| Life stage | Temp (°F) | RH (%) | Supplemental Heat/Light |
|---|---|---|---|
| Males | ambient | ambient | 160-watt mercury vapor lamps 30 min @ 1000 & 1400 h |
| Oviposition | ambient | ambient | 160-watt mercury vapor lamps 30 min @ 1000 & 1400 h |
| Egg/prediapause | 65-68 | 50 | |
| Warm diapause | ambient | ambient | |
| Cold diapause | ambient | ambient | |
| Postdiapause | <65 | ≥55 | 50-watt tungsten lamps at 1000-1400 h |
| Pupation | >65 | >50 | |

## *Results and Discussion*

### *Captive mating*

The Oregon Zoo conducted 48 breeding introductions resulting in 15 successful copulations (31.3 percent) between 4 and 11 May 2013, a significant decline in efficiency from 2012 (Lewis et al. 2013). Successful copulations are those that result in eggs that hatch. Copulations occurred among all six recommended breeding dyads (Fig. 1 and Table 4). The average duration for successful copulations at the Oregon Zoo was 96 minutes (SD=35). Twenty-seven females did not copulate successfully. Mission Creek conducted 94 pairings that resulted in 9 successful copulations (9.6 percent) between 29 April and 10 May 2013; copulations occurred among all six recommended dyads (Fig. 1 and Table 5). Similar to results at the Oregon Zoo, this was a significant decline in efficiency from 2012 (Lewis et al. 2013).

### *Captive rearing egg to adult*

Captive-mated females at the Oregon Zoo laid 2,614 eggs in 2013, and 1,861 eggs were laid by captive-mated females at Mission Creek (Table 4). At the Oregon Zoo, 11 of 12 wild females (Table 5) laid eggs, as did 10 of 12 wild females at Mission Creek. Females with eggs that failed to hatch are not included in these totals. A total of 8,258 eggs were produced between the two facilities with 54.2 percent (4,475) of eggs produced by known lineage captive-mated females and 45.8 percent (3,783) of eggs produced by wild females. These results fell short of our target production of 10,000 eggs for 2013 (both facilities combined). At the Oregon Zoo, wild females produced 48.3 percent of all eggs, and captive-mated females contributed 51.7 percent. At Mission Creek, wild females laid 41.9 percent of the eggs produced and captive-mated females produced 58.1 percent (Tables 5 and 6). Both facilities fell failed to meet the target for two-thirds of eggs contributed from wild females.

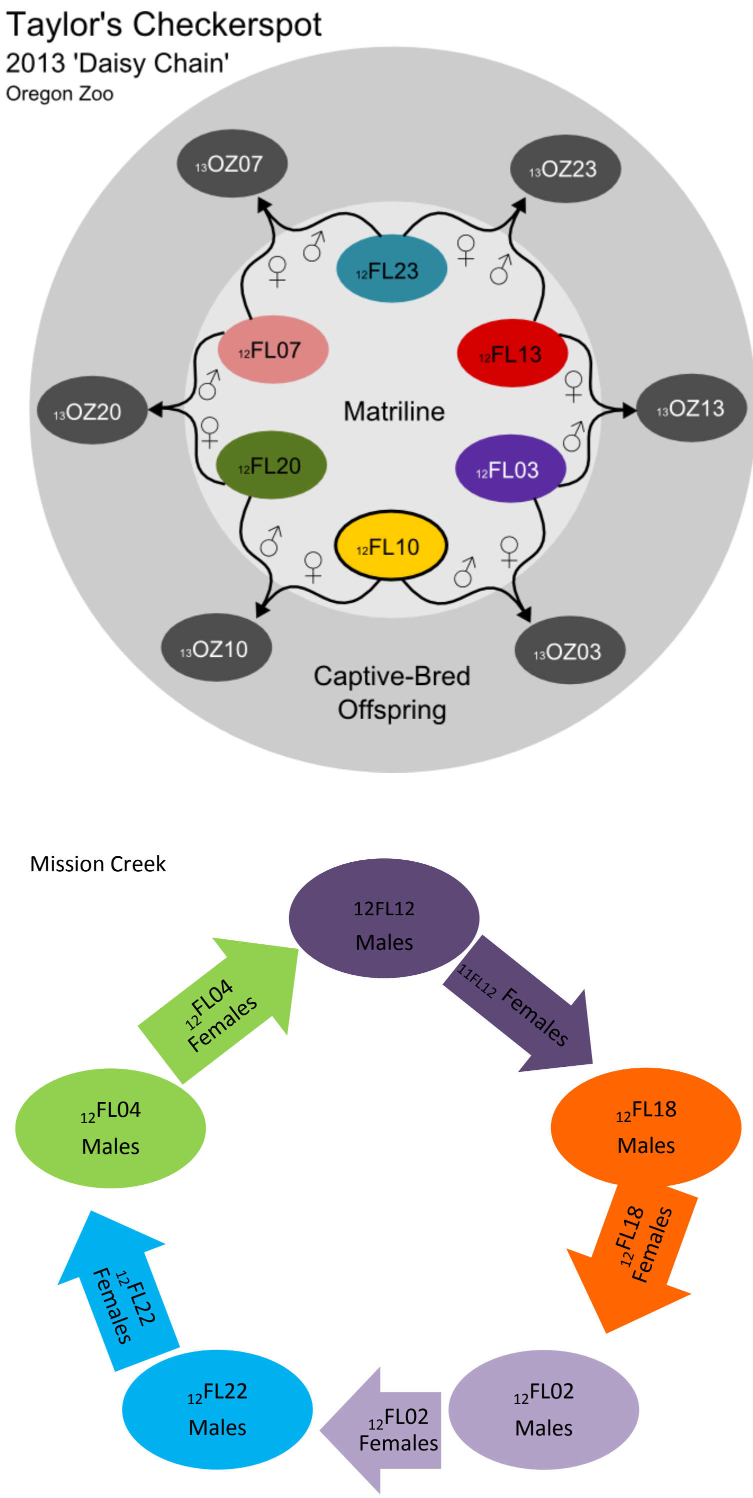



Figure 1. "Daisy chain" pairing strategy used to manage breeding introductions in 2013 at the Oregon Zoo, (top) Portland, Oregon, and Mission Creek Corrections Center (bottom), Belfair, WA, within the captive colony co-managed by these institutions (Images from Lewis et al. 2014, *in prep*., Hamilton et al. 2014, *in prep*.).

Table 4. Number of eggs laid and survival to diapause by individual for captive-mated Taylor's checkerspot females at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, 2013. Number hatched is taken at 3rd instar. Data from Hamilton et al. (2014) and Lewis et al. (2014).

| Female ID | # eggs | # hatched | # to diapause |
|---|---|---|---|
| **Oregon Zoo** | | | |
| 11FL03-04 | 247 | 37 | 28 |
| 11FL03-05 | 89 | 14 | 12 |
| 11FL05-04 | 34 | 23 | 11 |
| 11FL26-01 | 62 | 30 | 18 |
| 11FL27-01 | 215 | 184 | 118 |
| 12FL03-17 | 181 | 57 | 35 |
| 12FL03-19 | 165 | 43 | 42 |
| 12FL07-03 | 154 | 31 | 30 |
| 12FL07-08 | 96 | 45 | 34 |
| 12FL10-21 | 184 | 4 | 4 |
| 12FL13-18 | 199 | 102 | 95 |
| 12FL13-28 | 162 | 154 | 151 |
| 12FL20-14 | 207 | 37 | 36 |
| 12FL23-07 | 280 | 192 | 152 |
| 12FL23-08 | 339 | 100 | 41 |
| **Total** | **2614** | **1053** | **807** |
| **Ave (SD)** | **174.3 (35.5)** | **70.2 (81.7)** | **53.8 (49.9)** |
| **Mission Creek** | | | |
| 12FL02-11 | 260 | 227 | 223 |
| 12FL04-13 | 179 | 51 | 51 |
| 12FL12-27 | 140 | 62 | 62 |
| 12FL18-15 | 111 | 125 | 123 |
| 12FL18-27 | 160 | 149 | 150 |
| 12FL18-28 | 260 | 200 | 199 |
| 12FL22-09 | 245 | 184 | 183 |
| 12FL22-10 | 191 | 154 | 153 |
| 12FL22-20 | 315 | 320 | 314 |
| **Total** | **1861** | **1472** | **1458** |
| **Ave (SD)** | **206.7 (66.8)** | **163.6(82.9)** | **162(81.2)** |

Eggs from captive-mated females at the Oregon Zoo began hatching on 13 May and finished 6 June 2013; those from wild females began hatching on 21 May and completed hatching on 5 June 2013. Eggs from captive-mated females at Mission Creek began hatching on 15 May 2013 and finished 7 June 2013, whereas those from wild females hatched between 24 May and 4 June 2013. In addition, of 82 of 125 eggs laid by 13FL54-01 (female that bypassed diapause), hatched in on 6 August 2013 and entered diapause by 29 August 2013. Larvae from wild females at the Oregon Zoo had an 83.4 percent hatch rate (Table 5), which was considerably higher than offspring of captive-mated females (40.3 percent, Table 4), which was unusually low (M. Linders, *unpub. data*). The hatch rate for offspring of wild

Table 5. Number of eggs laid and survival to diapause by individual for Taylor's checkerspot females collected from the Range 76 source population and reared at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, 2013. Number hatched is taken at 3rd instar. Data from Hamilton et al. (2014) and Lewis et al. (2014).

| Female ID | # eggs | # hatched | # into diapause |
|---|---|---|---|
| **Oregon Zoo** | | | |
| P13FL50 | 368 | 351 | 315 |
| P13FL51 | 192 | 53 | 52 |
| P13FL52 | 425 | 391 | 366 |
| P13FL53 | 126 | 77 | 75 |
| P13FL54 | 111 | 105 | 103 |
| P13FL55 | 176 | 128 | 107 |
| P13FL56 | 221 | 199 | 188 |
| P13FL58 | 295 | 264 | 68 |
| P13FL59 | 113 | 70 | 53 |
| P13FL60 | 214 | 209 | 153 |
| P13FL61 | 199 | 187 | 161 |
| **Sum:** | **2440** | **2034** | **1641** |
| **Ave (SD)** | **221.8(102.4)** | **184.9(113.6)** | **149.2(105.3)** |
| **Mission Creek** | | | |
| P13FL01 | 175 | 176 | 177 |
| P13FL02 | 345 | 332 | 329 |
| P13FL03 | 285 | 292 | 290 |
| P13FL04 | 231 | 211 | 211 |
| P13FL07 | 98 | 131 | 130 |
| P13FL08 | 33 | 33 | 33 |
| P13FL09 | 50 | 46 | 45 |
| P13FL10 | 51 | 62 | 62 |
| P13FL11 | 45 | 47 | 47 |
| P13FL12 | 30 | 3 | 3 |
| **Total** | **1343** | **1333** | **1327** |
| **Ave (SD)** | **134.3(116.7)** | **133.3(115.3)** | **132.7(114.6)** |

females at Mission Creek (99.3 percent, Table 5) was also high relative to captive-mated females (79.1 percent, Table 4).

The greatest losses are typically observed from egg to hatching (defined as 3$^{rd}$ instar), with most remaining life stages producing survival rates above 90 percent (Linders 2010, 2011b, 2012, Linders and Lewis 2013). For this reason, high post-hatch mortality at the Oregon Zoo, which was particularly notable in some lineages, caused Zoo staff to become concerned about the health of their larvae. Larvae first showed signs of ill health (e.g., lethargy, vomiting and diarrhea, flaccidity, and molting issues) on 16 June 2013. Containers housing symptomatic larvae were isolated; larvae in these containers appeared smaller than the other groups, and did not successfully enter diapause. Samples for both groups were sent for pathology screening to the Insect Pathology Lab at Mississippi State University; no pathogens were identified. As remaining larvae had entered diapause, no further action was taken at that time.

The first larvae at the Oregon Zoo entered diapause on 21 June and the last on 12 August 2013. At Mission Creek, the first larvae entered diapause on 28 June and the last of the 2013 cohort on 7 July 2013. In addition, 82 larvae from female 13FL54-01 and two larvae transferred from the Oregon Zoo entered diapause by 29 August 2013. In all, 5,317 diapausing larvae (2,349 captive and 2,968 wild) from the 2013 cohort (Tables 4 and 5) were moved outdoors on either 4 September (Oregon Zoo) or 22 September 2013 (Mission Creek). In addition, 40 multi-diapause larvae (29 at Oregon Zoo and 11 at Mission Creek) and 82 larvae from female 13FL54-01 were also placed in cold diapause at their respective facilities.

Larvae at Mission Creek were removed from diapause on 22 February 2014; those at the Oregon Zoo were removed on 18 February 2014. Larvae at the Zoo were again experiencing higher than normal mortality rates, which now also included maternal lines that had exhibited little prediapause mortality. Samples were again sent to the lab in Mississippi for testing, but no evidence of pathogens could be found. While frustrating, it can be difficult to screen for the multitude of potential pathogens that might be present. In addition, suboptimal environmental conditions during early development can have lasting effects on larval health, and the Zoo had concerns about a period of low humidity that occurred from 17-20 June 2013, just prior to the observation of initial symptoms. Due to concerns about the health of these larvae and the potential risk of spreading an unknown pathogen onto a reintroduction site where it could persist for years, a collective decision was made by WDFW, USFWS and the Oregon Zoo that no larvae would be released from the Oregon Zoo in 2014.

Postdiapause larvae at Mission Creek were divided into two groups depending on their destination. A total of 2,599 postdiapause larvae (1,455 captive, 1,051 wild, 82 from female 13FL54-01 and 11 QC larvae) were sent for field release in 2014. In addition, 300 larvae were retained for captive mating (Table 6). Because no larvae were released from the Oregon Zoo, we fell 35 percent short of our target

Table 6. Postdiapause larval numbers and outcomes by matriline for 13FL cohort of Taylor's checkerspots retained for captive-mating at Mission Creek Corrections Center for Women, Belfair, WA, Spring 2014. Improperly eclosed adults (IE) are a subset of adults. Discrepancies between number of pupae and number of adults signify pupae that never developed to eclosion.

| Matriline | # larvae | 2nd diapause | # pupae | # adults | IE | Adults released |
|---|---|---|---|---|---|---|
| 13FL01 | 30 | 18 | 12 | 12 | - | 5 |
| 13FL02 | 30 | 15 | 10 | 10 | 2 | - |
| 13FL03 | 30 | 3 | 25 | 25 | 1 | 12 |
| 13FL04 | 30 | 8 | 21 | 21 | 3 | 13 |
| 13FL07 | 29* | 6 | 22 | 21 | 1 | 13 |
| 13FL08 | 31* | 6 | 23 | 23 | 3 | 9 |
| 13FL09 | 30 | 9 | 17 | 17 | 2 | 1 |
| 13FL10 | 30 | 10 | 19 | 19 | - | - |
| 13FL11 | 30 | 2 | 25 | 25 | 1 | 7 |
| **Subtotal** | **300** | **77** | **174** | **173** | **13** | **60** |

* One larva placed in wrong bin.

of 4000 postdiapause larvae (both rearing facilities). The Oregon Zoo did retain 374 postdiapause larvae to rear to the adult stage (Table 7) for meconium testing, because some pathogens can only be detected in that manner. All checkerspots regardless of their life stage were humanely euthanized at the conclusion of this process.

Table 7. Postdiapause larval outcomes by matriline for larvae retained for meconium testing at the Oregon Zoo, Portland, OR in 2014; see text for details.

| Matriline | # retained | 2nd diapause | # pupae | # adults |
|---|---|---|---|---|
| 11/12FL-OZ | 11 | 6 | 5 | 5 |
| 12FL-MC | 14 | 1 | 13 | 12 |
| 13FL50 | 50 | 0 | 44 | 42 |
| 13FL52 | 30 | 17 | 12 | 12 |
| 13FL53 | 29 | 15 | 13 | 12 |
| 13FL54 | 30 | 5 | 21 | 20 |
| 13FL55 | 44 | 13 | 26 | 24 |
| 13FL58 | 29 | 7 | 18 | 17 |
| 13OZ03 | 34 | 14 | 18 | 15 |
| 13OZ13 | 30 | 12 | 17 | 17 |
| 13OZ23 | 50 | 20 | 26 | 25 |
| 11-13OZ27 | 23 | 9 | 13 | 12 |
| **Total** | **374** | **119** | **226** | **213** |

Larvae at Mission Creek pupated between 6 and 23 April 2014, with 25.7 percent returning to diapause. Adult checkerspots eclosed between 30 April and 21 May 2014; 7.5 percent eclosed improperly (Table 6). Temperature, humidity, sunlight and airflow at the time of eclosion influence the unfolding and hardening of wings in adults both in captivity and in the wild, and is typically unrelated to genetics. Of 173 adults at Mission Creek, three could not be identified to sex, leaving 170 suitable for breeding in 2014. The first pupa was found at the Oregon Zoo on 22 March and the last on 3 April 2014. Adults at the Oregon Zoo eclosed between 1 and 14 April 2014; the number of improper eclosions was not reported, but numbers by life stage are reported in Table 7.

### *Rearing conditions*

Temperature (°F) and relative humidity (%) readings recorded at the Oregon Zoo and Mission Creek in 2013-2014 are presented in Table 8. Overall, conditions at Mission Creek were cooler and somewhat drier than at the Oregon Zoo, with Mission Creek notably cooler at night and generally warmer during the day. For 2015, it would be helpful to review the existing data and set new target conditions based on existing data for average minimum and maximum temperatures and humidity. These are likely to be more useful than the simple averages identified in Table 3.

## Assess the efficacy of the captive propagation program and identify opportunities for improvement

### *Methods*

### *Survival of captive animals*

We use stage-specific survival rates to track success within and between years and identify areas for improvement. We calculated Kaplan-Meier (1958) survival rates for all captive stock between the

Table 8. Average temperature and average minimum, average maximum and (range) of temperature (°F) and relative humidity (%RH) during Taylor's checkerspot rearing by life stage, including time frame and location at the Oregon Zoo, Portland, OR, and Mission Creek Corrections Center for Women, Belfair, WA, 2013-2014. Min/max data read daily at 1100 – 1300 h.

| Life Stage | Date range | Location[1] | Ave temp (°F; range) | Avg. min temp (°F; range) | Avg. max temp (°F; range) | Avg min RH (%; range) | Avg. max RH (%; range) |
|---|---|---|---|---|---|---|---|
| **Oregon Zoo** | | | | | | | |
| **Adult Males** | 28 Apr-18 May 2013 | Mezz. | 68 (58 - 80) | 64 (58 - 71) | 74 (66 - 80) | 53 (41 - 69) | 79 (53 - 95) |
| **Oviposition** | 4 May- 6 June 2013 | Mezz. | 71 (54 - 112) | 64 (54 - 73) | 89 (68 - 112) | 39 (18 - 58) | 75 (50 - 93) |
| **Egg & prediapause** | 4 May- 12 Aug 2013 | Mezz. | 72 (54 - 85) | 68 (54 - 76) | 77 (64 - 85) | 56 (41 - 71) | 74 (60 - 88) |
| **Warm diapause** | 13 May-4 Sep 2013 | Mezz. | 73 (54 - 85) | 68 (54 - 76) | 78 (64 - 85) | 56 (41 - 71) | 73 (57 - 84) |
| **Cold diapause** | 4 Sep 2013- 18 Feb 2014 | Larva Land | 46 (18 - 85) | 42 (18 - 70) | 51 (22 - 85) | 82 (36 - 100) | 91 (50 - 100) |
| **Wake up** | 18 Feb-14 Mar 2014 | Mezz. | 57 (49 - 80) | 52 (49 - 58) | 62 (57 - 80) | 69 (54 - 79) | 86 (68 - 92) |
| **Postdiapause[2]** | 14 Mar-3 Apr 2014 | Lab | 71 (66 - 84) | 66 (58 - 67) | 77 (74 - 85) | 56 (40 -75) | 84 (66 - 91) |
| **Pupation** | 22 Mar-14 Apr 2014 | Lab | 71 (65 - 85) | 67 (65 - 68) | 77 (74 - 85) | 56 (44 -75) | 85 (66 -92) |
| **Mission Creek** | | | | | | | |
| **Adult Males** | 22 Apr-10 May 2013 | Main | 60 | 42 (31-49) | 82 (65-101) | 43 (27-56) | 51 (37-70) |
| **Oviposition** | 1 May–14 Jun 2013 | Main | 62 | 48 (31-58) | 79 (57-101) | 46 (27-62) | 54 (37-68) |
| **Egg & prediapause** | 1 May–7 Jul 2013 | Main | 65 | 51 (31-64) | 82 (57-105) | 47 (27-62) | 54 (37-68) |
| **Warm diapause** | 7 Jul-22 Sep 2013 | Main, low | 70 | 56 (46-65) | 88 (63-104) | 45 (28-64) | 53 (37-70) |
| **Cold diapause** | 22 Sep-22 Feb 2014 | Small | 45 | 38 (14-56) | 58 (35-83) | 75 (38-99) | 81 (66-99) |
| **Postdiapause** | 22 Feb-23 Apr 2014 | Main | 57 | 49 (35-57) | 68 (48-79) | 59 (37-81) | 71 (52-86) |
| **Pupation** | 6 Apr–24 Apr 2014 | Main | 61 | 52 (48-62) | 72 (65-79) | 56 (41-66) | 68 (54-81) |

[1] Location descriptions: Mezzanine: a large, open, unfinished indoor area with minimal climate controls adjoining the main butterfly lab. Larva Land: outdoor overwintering area located under an overhang of the Animal Management building. Lab: temperature and humidity controlled room with a single southeast-facing window; animals are housed on multi-shelved, rolling racks. Main: 16 x 10-ft room in glass greenhouse; low refers to lower shelves of tables. Small: 8 x 10-ft room at in glass greenhouse.

[2] Larvae retained for meconium testing were reared under warmer conditions than usual; see text for details.

following stages: egg, hatching (2nd instar), diapause, postdiapause, pupa, and adult; we also calculated the rate at which postdiapause larvae returned to diapause, averting development to the adult stage.

### *Adult measurements*

Pupae and adults from each female line were measured and weighed using standardized procedures (Barclay et al. 2009) for comparison with measures obtained from wild adults. This is in keeping with best management practices (Crone et al. 2007) and allows us to determine whether adults produced in captivity are undersized relative to their wild counterparts, which could result in reduced mobility in the field. Left side ventral hind wing area was calculated using ImageJ ver. 1.46r (Rasband 2012). Wing measurements will be compared between facilities and with those from wild adults using an ANOVA spreadsheet constructed by Shannon Knapp (WDFW biometrician) in Excel 2007. Left side ventral wing photos were also collected for wild females brought into Mission Creek in 2013. Weights for wild females were not collected.

### *Results and Discussion*

### *Survival of captive animals*

Most mortality typically occurs prior to 3rd instar, which is also the stage at which survival varies the most from year to year (Linders, *unpub. data*). Both eggs and early instar larvae are difficult and time-consuming to count. Because we expect survival to vary during this stage both in captivity and in the wild (Kuussaari et al. 2004), a simplified approach is being applied, whereby we get a reasonable estimate of the number of eggs, but avoid counting larvae until they reach third instar. During all remaining life stages survival is typically in the 90th percentiles at both facilities (Linders 2012, Linders and Lewis 2013). This was again the case at Mission Creek in 2013-2014 (Table 9). As has already been discussed, mortality rates at the Oregon Zoo were higher than normal, a trend that continued throughout the remainder of the rearing period. While it was a difficult decision to destroy these animals at the Oregon Zoo, the high survival exhibited by both rearing institutions on a consistent basis leaves us optimistic that our contributions to on-the-ground recovery outweigh the cost of a bad year.

### *Adult measurements*

Data for the ANOVA have been obtained from the Oregon Zoo for 2013, but have not yet been received from Mission Creek. Analyses comparing adult and pupal size between institutions and the wild will be run once all data are in hand.

## III. Release captive and associated wild stock

### *Postdiapause larval release 2014*

Postdiapause larvae are the preferred stage for release because they are robust and nearly mature. Larvae were brought to the field packed in labeled deli containers containing freshly-cut leaves of *Plantago*; containers were packed in coolers without ice or heat. A total of 1,522 postdiapause larvae were released at TA7S on 7 March 2014 (670 in Plot A, 778 in Plot B, 25 each in Survival Plots 1 and 3, and 24 in Survival Plot 2). Also on 7 March 2014, a total of 1,086 larvae were released at Glacial Heritage Preserve (871 in Plot H, 165 in Plot G, and 25 each in Survival plots 1 and 2). Larvae at both locations were released in groups of 2-5 on large and/or dense host plants/patches (*Plantago lanceolata, Castilleja hispida* or *Castilleja levisecta*) within restored prairie. Weather on release day was excellent, with temperatures ranging from 10.0-16.0 °C, average wind speed ranging from 1.5-4.0 mph, and scattered to broken clouds with distinct to soft shadows. At least 9 people assisted with releases, working from about 1000-1700 to complete the task.

Table 9. Number of individuals and Kaplan-Meier (1958) survival by life stage for captive Taylor's checkerspot butterflies at the Oregon Zoo (OZ), Portland, Oregon, and Mission Creek Corrections Center for Women (MC), Belfair, Washington, 2013-2014. Only females with eggs that hatched are included; number of hatched larvae recorded at 3rd instar. Labels refer to the rearing institution combined with source as either Wild: Joint Base Lewis-McChord (FL) or Captive-mated origin: OZ or MC, for offspring in the 2013 (13) cohorts.

| Life stage | OZ Wild 13FL | | OZ Captive 13OZ | | MC Wild 13FL | | MC Captive 13MC | | All 2013 stock | |
|---|---|---|---|---|---|---|---|---|---|---|
| | # | % | # | % | # | % | # | % | # | % |
| **Eggs** | 2440 | - | 2614 | - | 1343 | - | 1861 | - | 8258 | - |
| **Eggs to hatch** | 2034 | 83.4 | 1053 | 40.3 | 1333 | 99.3 | 1472 | 79.1 | 5892 | 71.3 |
| **Hatch to warm diapause** | 1641 | 80.7 | 807 | 76.6 | 1327 | 99.5 | 1458 | 99.0 | 5233 | 88.8 |
| **Warm to cold diapause** | 1188 | 72.4 | 593 | 73.5 | 1324 | 99.8 | 1458 | 100.0 | 4563 | 87.2 |
| **Diapause to wake-up** | 1176 | 99.0 | 589 | 98.9 | 1324 | 100.0 | 1453 | 99.9 | 4542 | 99.5 |
| **Captive colony larvae** | 212 | 18.0 | 137 | 23.4 | 300 | 22.7 | | | 649 | 14.3 |
| **Return to diapause** | 57 | 26.9 | 55 | 40.1 | 77 | 25.7 | | | 189 | 29.1 |
| **Pupae** | 134 | 63.2 | 74 | 54.0 | 174 | 58.0 | | | 382 | 83.0 |
| **Adults** | 127 | 94.8 | 69 | 93.2 | 173 | 99.4 | | | 369 | 96.6 |

### *Adult release 2014*

A total of 56 adult Taylor's checkerspots from Mission Creek were released from captivity once their roles in mating trials and oviposition were complete; seven of the 63 adults from Mission Creek died prior to release. Adults were released at SCS (Appendix A) on 30 May 2014. As in previous years, adults were transported in net enclosures and released directly into the environment by allowing them to fly from the cage or placing them on a nectar plant. Weather at the time of release was 19.0 °C, wind speed averaged 3.6 mph, and skies were clear with distinct shadows.

## IV. Monitor success of the reintroduction

Documenting presence and relative abundance through various life stages provides near-term measures of survival and improves the likelihood that factors affecting success will be detected. Population targets and population monitoring goals are used to evaluate long term success in population establishment (see below) and demonstrate progress toward species recovery.

### Document postdiapause larval presence and abundance in release areas

Past releases have shown that larvae and adults exhibited normal behaviors immediately following release (e.g., feeding, basking, mating, and ovipositing). We have also relocated animals in release areas in the days, months and years following release even when no successive releases have occurred, indicating that checkerspots are surviving.

### Methods

To confirm site occupancy, quantify larval abundance, and identify issues that may be cause for concern, we conducted surveys for postdiapause larvae in active release areas on two occasions in the weeks following release. Our objective in estimating abundance was to assess survival relative to the number of larvae released and evaluate relative success between reintroduction sites and years. All search methods and sampling protocols were the same as those used previously (Linders 2011a). Sampling plots (2 m wide x 5 m long) were laid out along transects identified in ArcGIS by placing the first transect 10 m from the southern border and continuing with that spacing. The first sampling plot was randomly placed 2-6 m from the transect start with a 2-m space between each successive 2 x 5-m plot. Each 2 x 5-m plot was also divided into two 2 x 2-m plots separated by 1 m to increase sample size. The aim was to sample 10 percent of the release areas.

Survival method test plot surveys evolved over the sampling period. Our main objective in surveying these plots was to determine whether we could directly measure the survival of released larvae. Initially, Survival plots (4 x 5-m each) were searched thoroughly by a single observer (Table 10; 11-13 March). During the second survey all plots were surveyed by both observers in succession (17, 20 March). Finally, on the last round of surveys, both observers searched plots in succession and mapped the location of each animal based on a series of flags. To calculate survival, the locations of mapped larvae were compared between surveyors to determine which larvae were recorded by both observers and which were unique individuals.

### Results and Discussion

Two complete surveys of the main release areas were conducted at GHP on 12-13 and 20 March 2014. Three complete surveys of the main release areas were conducted at TA7S on 11-12, 17-18 and 24 March 2014. Post-release survey results are still being summarized so detailed results are not presented. Typically more than one day was required to survey an entire site due to the short duration of suitable weather in March. All surveys met protocol conditions. Some days were quite warm, with temperatures reaching 20.0 °C. Larvae were readily observed in most plots at both sites until they entered the pupal stage in late March, although numbers in Plot A at TA7S dropped markedly during the survey period. Flocks of robins, starlings and individual bluebirds were observed foraging in the area, particularly in Plot A. Only bluebirds were photographed collecting larvae (Fig. 2). Final counts of surviving larvae found in Control plots at reintroduction sites are shown in Table 10. Survival was considerably higher at GHP than at TA7S. This is assumed to be the result of the depredation observed at TA7S, as habitat quality there appeared higher based on food plant abundance.

## Evaluate reintroduction success based on adult presence, relative abundance and distribution

### Methods

### Adult presence and relative abundance

We used line transect sampling to quantify daily density, daily population size, and to illustrate the distribution of adults within the sampling area annually. Five reintroduction sites (SCS, R50, PCM, GHP and TA7S) plus one extant site (R76) were surveyed for adult checkerspots during the 2014 flight season. Field sampling methods followed Linders and Olson (2014), except that adults were recorded by 25-m segments rather than 50-m segments. Distance sampling was conducted up to 3 times per week during

Table 10. Summary of Taylor's checkerspot larval counts following release in survival method test plots at two reintroduction sites in South Puget Sound, Washington, Spring 2014. TA7S = Training Area 7 South; GHP = Glacial Heritage Preserve.

| | GHP | | TA7S | |
|---|---|---|---|---|
| | **LTR** | **MJL** | **LTR** | **MJL** |
| **11-Mar-14** | | | | **6** |
| C3 | | | | 6 |
| **12-Mar-14** | | **14** | **10** | |
| C1 | | 6 | 2 | |
| C2 | | 8 | 1 | |
| C3 | | | 7 | |
| **13-Mar-14** | **17** | | | **12** |
| C1 | 14 | | | 6 |
| C2 | 3 | | | 6 |
| **17-Mar-14** | | | **12** | **13** |
| C1 | | | 3 | 3 |
| C2 | | | 7 | 8 |
| C3 | | | 2 | 2 |
| **20-Mar-14** | **20** | **16** | | |
| C1 | 9 | 6 | | |
| C2 | 11 | 10 | | |
| **23-Mar-14** | **25** | **26** | | |
| C1 | 10 | 12 | | |
| C2 | 15 | 14 | | |
| **24-Mar-14** | | | **14** | **10** |
| C1 | | | 2 | 2 |
| C2 | | | 6 | 6 |
| C3 | | | 6 | 2 |
| **Numbers of surviving larvae based on mapped locations relative to numbers released** | | | | |
| **Plot ID** | **# released** | **GHP** | **TA7S** | **Survival (%)** |
| C1 | 25 | 16 | | 64 |
| C2 | 25 | 15 | | 60 |
| C1 | 25 | | 3 | 12 |
| C2 | 24 | | 7 | 29 |
| C3 | 25 | | 6[1] | 24[1] |

[1] One additional larva was observed just outside the plot edge; if counted, survival is 28 percent.

the flight season. Survey transects at reintroduction sites included all release plots and a buffer of sufficient size (up to 200 m) to capture the anticipated adult use area, except at R50, where access to some areas is restricted. Transects at R76 covered the majority of the occupied area to which we have access. Transect length and spacing by site and year is shown in Table 11. The closer transect spacing at reintroduction sites insures that a sufficient number of butterflies are detected to calculate abundance estimates. All surveyors received pre-season training and distance estimation skills were tested weekly throughout the flight season.

We defined release success as the production of 10-30 adults per 1000 postdiapause larvae released based on the results of Harrison's (1989) work, but beyond the first year of release it is impossible to quantify how many adults originate from newly released larvae vs. those originating from reproduction in the wild. Instead, the presence and relative abundance of adults is an indication of year-to-year reintroduction success. Similarly, increases in the distribution of adults across the sampling area from year-to-year are an indication that the reintroduction is likely succeeding. We currently lack the methods to reliably estimate annual population size, which would be useful for making more direct comparisons between sites and years. In addition, an annual abundance estimate is needed for population modeling to determine which life stages are the biggest drivers of population size. On the other hand, because the viability of a demographic unit may ultimately hinge on its ability to survive population lows brought on by environmental extremes of drought and deluge (Ehrlich and Murphy 1987), annual population size is not the only important measure of long-term success.

Figure 2. Western bluebird depredating Taylor's checkerspot larvae at Training Area 7 South (TA7S) on Joint Base Lewis-McChord, Washington, Spring 2014. Photograph by R. Gilbert.

<u>Data analyses.</u> Analyses were conducted using Program Distance, Version 6.0 (Thomas et al. 2010) with density estimates computed for each survey date because population numbers can change daily. Detection functions were fitted using both the Conventional Distance Sampling (CDS) and the Multiple Covariate Distance Sampling (MDCS) engines. Summary statistics, including observation frequency tables calculated by observer and date, and sometimes by transect line, were calculated first. We also generated tables of encounter rates (observations per unit line length surveyed) by date and observer. For the MCDS analyses, we also computed univariate statistics and plots of distance data for potential covariates, such as observer, butterfly behavior, survey protocol, and weather, as recommended by Marques et al (2007). The best detection functions were chosen using a combination of default settings and user specified settings to select which of the many available models best fit the data, including Akaike's Information Criterion (AIC) and goodness-of-fit tests. More information on observer differences and detection functions used for 2009-2011 distance sampling analyses can be found in

Linders (2010, 2011b, 2012); data for 2013 are reported here. Data and density estimates are presented for three reintroduction sites (SCS, R50, and GHP) plus R76 from 2013.

After determining the detection function(s) to use, density estimates were computed by date. Variance estimates of density were calculated using a relatively new method (Fewster et al. 2009) that takes advantage of the sequential (evenly spaced) layout of transects to reduce variance estimates over those, assuming that transects are placed randomly. Of the two methods of this type available in Program Distance, we chose to use method O2, which is generated by creating overlapping strata among adjacent transects and has been shown to increase precision with little change in bias (Fewster et al. 2009). Variances generated from the O2 method were used to estimate 95% Confidence Intervals. Density estimates were computed by survey date because of the expectation that population numbers change on nearly a daily basis due to eclosion and mortality of individuals.

### *Adult distribution*

To illustrate distribution of adults within the survey grids at each site, all 2014 Taylor's checkerspot observations collected during distance sampling, regardless of date, were spatially joined to a GIS polyline layer representing transects and sections, then shaped into category classes and symbolized using a standard color ramp. Category classes were scaled so the midpoint of each successive bin increased by a factor of two. Observations were overlaid on 2011 National Agriculture Imagery Program (NAIP) color aerial photos with 1-m resolution.

In addition to the standard transects at Range 76, 4 transects were added to the north end of the monitoring area at Range 76 to map distribution in that area. Standard distance sampling methods were employed on two dates, 30 April and 13 May, 2014. These transects were also spaced 50 meters apart and employed 50-meter segments.

Reconnaissance surveys were also conducted along the east edge of the Artillery Impact Area and in Training Area 6 in 2014 to search for evidence of dispersal and colonization, and to identify potential and suitable habitat. Surveys were scheduled at all occupied sites, but the 2014 flight season was too brief to permit visits to all sites.

## *Results and Discussion*

### *Adult abundance 2013*

Scatter Creek South. Eight surveys were conducted between 22 April and 2 June by four observers at SCS in 2013; no butterflies were recorded on 22 April or after 2 June (Linders and Lewis 2013). In total, 194 butterflies were counted, with a peak daily count of 64 on 1 May 2013 (Table 12). Total line length surveyed was 50.4 km. As in 2012, the survey area was reduced to focus on the area where adults were most likely to be observed (see Linders and Lewis 2013), with a single complete survey (9,100 m) conducted on 14 May for distribution. Two of the four observers had similar levels of effort with the other two being higher and lower than those. Allocation of effort per day was fairly evenly distributed, although the number of observers per day varied from 2 to 4. The composition of observers differed by day with no consistent pattern that would likely cause observer differences to be confounded with other potential effects on detectability. The number of butterflies observed per survey date was too small to estimate detection functions by date, so three models were fit using CDS and compared to MCDS models with a global detection function and date/ and or observer covariates. There was very little difference in AIC between models in which observer effects were accounted for in detectability. The best model was the MCDS model with a Global DF and observer covariate, and this was used to generate density estimates. Data were truncated at 8m to eliminate a few outliers and improve the fit of detection functions near the transect line. Density estimates with 95% Confidence Intervals are presented by date in Table 12 along with daily abundance estimates for the entire survey area.

Range 50. Nine surveys were conducted between 21 April and 1 June by four observers in 2013 at R50; no butterflies were observed on the first survey date, and only one adult was observed on the last (Linders and Lewis 2013). In all, 963 adults were counted, with a peak single day count of 373 on 3 May (Table 12). All transects were surveyed on all dates, but data were lost from some transects on May 6, and total effort on that date was reduced to 6800m for a total line length surveyed of 86.0 km. These transects were dispersed throughout the survey area and their loss is not expected to affect the extrapolation of density estimates based on the remaining transects to the entire area; any comparisons of raw numbers of observations will be affected. There were usually 3 surveyors on any given date but occasionally 2 or 4, with the composition of observers varying by day. Similar to SCS, effort varied by

Table 11. Number and length (m) of distance sampling transects and segments by site for extant (R76) and reintroduced populations of Taylor's checkerspot in the Puget Trough, Washington, 2010-2014.

| Site | # transects | Transect spacing | # segments | Segment spacing (m) | Transect length (m) | Total line length (m) | Survey area (ha) |
|---|---|---|---|---|---|---|---|
| 2010 | | | | | | | |
| SCS | 11 | 25 | 11 | 50 | 550 | 6050 | 15.13 |
| R50 | 13 | 25 | 8 | 50 | 400 | 5200 | 13.00 |
| R76 | 12 | 50 | 14 | 50 | 700 | 8400 | 42.00 |
| 2011 | | | | | | | |
| SCS | 14 | 25 | 12 | 50 | 600 | 8400 | 21.00 |
| R50 | 16 | 25 | 11 | 50 | 550[1] | 8600 | 21.50 |
| R76 | 12 | 50 | 14 | 50 | 700 | 8400 | 42.00 |
| 2012 | | | | | | | |
| SCS | 14 | 25 | 12 | 50 | 600 | 8400 | 21.00 |
| R50 | 14 | 25 | 13 | 50 | 650[2] | 8900 | 22.25 |
| PCM | 14 | 25 | 8 | 50 | 400 | 5600 | 14.00 |
| GHP | 12 | 25 | 8 | 50 | 400 | 4800 | 12.00 |
| R76 | 12 | 50 | 14 | 50 | 700 | 8400 | 42.00 |
| 2013 | | | | | | | |
| SCS[3] | 14 | 25 | 7-12 | 50 | 350-600 | 5900 | 14.75 |
| R50 | 16 | 25 | 13 | 50 | 650[2] | 9900 | 24.75 |
| PCM | 14 | 25 | 8 | 50 | 400 | 5600 | 14.00 |
| GHP | 12 | 25 | 8 | 50 | 400 | 4800 | 12.00 |
| R76 | 12 | 50 | 14 | 50 | 700 | 8400 | 42.00 |
| 2014 | | | | | | | |
| SCS | 14 | 25 | 14-24 | 25 | 350-600 | 5900 | 14.75 |
| R50 | 16 | 25 | 18-26 | 25 | 450-650[2] | 9900 | 24.75 |
| PCM | 14 | 25 | 16 | 25 | 400 | 5600 | 14.00 |
| GHP | 15 | 25 | 18 | 25 | 450 | 6750 | 16.88 |
| R76 | 12 | 50 | 28 | 25 | 700 | 8400 | 42.00 |
| TA7S | 14 | 25 | 8-13 | 25 | 200-325 | | |

[1] Two short transects measure 450 m each.
[2] Four short transects measure 450, 500, 550 and 550 m, respectively; ; two transects were excluded from distance analyses.
[3] Survey area was reduced in 2013 to focus on areas with highest sighting likelihood.
[4] Four short transects measure 450, 500, 550 and 600 m, respectively.

observer. In general, sample sizes were sufficient to attempt all date and observer models. A series of CDS and MCDS models were analyzed, which looked at global and date-specific models with observers and/or dates as covariates. Data were truncated at 8m to reduce unnecessary adjustment terms and satisfy criteria associated with MCDS models. The best model was the MCDS model with date-specific detection functions and an observer covariate. This model was used to generate density estimates for most survey dates, but it did not produce reasonable estimates for 25 May or 1 June (due to small sample sizes), so density estimates for these dates were obtained from the MCDS model with a global detection function and observer covariate. Both of the models had a half-normal key function with cosine adjustment terms, with σ scaling. Density estimates with 95% Confidence Intervals are presented by date in Table 12 along with daily abundance estimates for the entire survey area.
Pacemaker. Five complete and one partial survey were completed at PCM in 2013 for a total survey effort of 31.6 km. No checkerspots were observed during distance sampling surveys; however four opportunistic sightings were reported (see Linders and Lewis 2013, Appendix C, Fig. 3).

Glacial Heritage Preserve. Eight surveys were conducted at GHP between 23 April and 2 June (Linders and Lewis 2013) by four observers in 2013; no butterflies were observed 23 April or 2 June. A total of 231 butterflies were observed with a peak count of 75 on 7 May (Table 12) during a total survey effort of 38.4 km. Three observers had similar amount of effort and one had much less. Effort by all observers was distributed throughout the season and varied by day so that no systematic pattern was evident. The number of butterflies observed per survey date was sufficient to estimate detection functions by by date or by observer with the CDS models, but not both simultaneously. This was done in addition to the global detection model. Surprisingly, the CDS model with the detection function by date was slightly better than the global model, and fit far better than the observer detection function model. This was supported by the MCDS model results. Of the latter, a Hazard rate model (with no adjustment terms) with a global detection function and date covariate fit best and resulted in reasonable density estimates. Density estimates with 95% Confidence Intervals are presented by date in Table 12 along with daily abundance estimates for the entire survey area.

Range 76. Seven complete surveys were conducted between 22 April and 1 Jun (Linders and Lewis 2013) by four observers at R76 in 2013. No checkerspots were observed on three survey dates (22, 23 April and 1 June). On most days effort was evenly distributed among observers with at least 3 observers per day for a total effort of 58.8 km surveyed. In total 1,676 adult checkerspots were counted, with the greatest number of checkerspots (865) observed on 6 May. All dates with observations had sufficient sample sizes to run date-specific detection function models; CDS models for 28 Apr and 19 May 19 had no covariates. For 28 Apr the detection function was hazard rate with 1 cosine adjustment. For 19 May, the detection function was a half-normal with no adjustments. For May 6 and May 10, the best models were MCDS with an observer covariate and σ scaling. For May 6 the detection function was a half-normal with 2 cosine adjustments and for May 10 it was a hazard-rate with no adjustments. Density estimates with 95% Confidence Intervals are presented by date in Table 14 along with daily abundance estimates for the entire survey area.

### Adult abundance 2014

Eight to 10 complete distance sampling surveys were conducted at each site in 2014; no partial surveys were conducted, although a few extra transect lines were added to TA7S during the survey season (Table 13). Effort varied by site, with the greatest effort expended at R50 (86.0 km), R76 (58.8 km) and SCS (50.4 km). The size of the survey area at SCS was reduced again as in 2013, to minimize the influence of areas with excessively low densities on population estimates. Two complete surveys of the 2014 grid was conducted on 11 and 20 May 2014 for distributional purposes, but no butterflies were observed outside of the standard 2012 survey area (Appendix C, Fig. 1).

A combined total of 4,121 checkerspots were counted across six sites (R76, R50, SCS, PCM, GHP and TA7S) during distance sampling surveys in 2014 (Table 13). About 1,057 more checkerspots were counted in the standard monitoring areas in 2014 than in 2013 (Linders and Lewis 2013), although in the absence of analyzed data we can't say whether this represents an increase in population size(s). In addition, a total of 780 checkerspots were counted on the two days when 4 additional transects were surveyed at the north end of the Range 76monitoring area. These data will not be included in daily abundance estimates. All told the 4,901 checkerspots comprised 4,629 groups. Butterflies were in groups of 1-5 with 4,402 single butterflies observed; 194 groups of two; 25 groups of three; 4 groups

Table 12. Raw counts, density estimates and adult abundance estimates including 95% Confidence Intervals for Taylor's checkerspot survey areas at Scatter Creek South (SCS), Range 50 (R50), Pacemaker (PCM), Glacial Heritage (GHP) and Range 76 (R76) in South Puget Sound, Washington, Spring 2013. See text for details regarding derivation of density estimates.

| | | Density | | | Abundance | | |
|---|---|---|---|---|---|---|---|
| **Date** | **Count** | **#/ha** | **Lower CI** | **Upper CI** | **#/survey area** | **Lower CI** | **Upper CI** |
| **SCS** | | | | | | | |
| 26-Apr | 21 | 3.80 | 1.67 | 8.64 | 56 | 25 | 127 |
| 01-May | 64 | 10.25 | 5.89 | 17.84 | 151 | 87 | 263 |
| 07-May | 55 | 9.30 | 5.68 | 15.22 | 137 | 84 | 224 |
| 11-May | 33 | 5.50 | 3.6 | 8.42 | 81 | 53 | 124 |
| 14-May | 16 | 2.66 | 1.41 | 5.01 | 39 | 21 | 74 |
| 20-May | 5 | 0.76 | 0.16 | 3.58 | 11 | 2 | 53 |
| **R50** | | | | | | | |
| 28-Apr | 52 | 6.80 | 4.16 | 11.10 | 168 | 103 | 275 |
| 3-May | 373 | 47.12 | 36.00 | 61.68 | 1166 | 891 | 1527 |
| 6-May | 208 | 37.27 | 21.29 | 65.25 | 922 | 527 | 1615 |
| 10-May | 228 | 27.48 | 19.09 | 39.57 | 680 | 472 | 979 |
| 14-May | 83 | 8.65 | 5.77 | 12.97 | 214 | 143 | 321 |
| 19-May | 10 | 1.26 | 0.56 | 2.86 | 31 | 14 | 71 |
| 25-May | 8 | 1.12 | 0.49 | 2.60 | 28 | 12 | 64 |
| 01-Jun | 1 | 0.14 | 0.02 | 0.86 | 3 | 0 | 21 |
| **GHP** | | | | | | | |
| 26-Apr | 21 | 5.68 | 2.44 | 13.22 | 68 | 29 | 159 |
| 01-May | 57 | 16.37 | 7.59 | 35.29 | 196 | 91 | 423 |
| 07-May | 75 | 20.71 | 14.05 | 30.53 | 249 | 169 | 366 |
| 11-May | 60 | 18.04 | 12.37 | 26.30 | 216 | 148 | 316 |
| 17-May | 13 | 3.34 | 1.77 | 6.29 | 40 | 21 | 75 |
| 20-May | 5 | 1.82 | 0.67 | 4.96 | 22 | 8 | 60 |
| **R76** | | | | | | | |
| 28-Apr | 74 | 23.85 | 8.38 | 64.07 | 1002 | 352 | 2691 |
| 06-May | 865 | 129.70 | 99.28 | 169.45 | 5447 | 4170 | 7117 |
| 10-May | 698 | 84.06 | 70.66 | 100.00 | 3531 | 2968 | 4200 |
| 19-May | 39 | 8.58 | 5.42 | 13.60 | 360 | 228 | 571 |

four; and 4 groups of five. Distance estimates ranged from 0.0 to 30.0 m, with an average detection distance of 3.67 m across all sites, which is slightly longer than in 2012 and 2013 (Linders, 2012, Linders and Lewis 2013). Site-based averages ranged from 3.20 (R50) to 4.06 (GHP) meters. Site order by average distance was: R50, SCS, R76, TA7S, and GHP, which roughly corresponds with relative vegetation height and density. Adult counts at Glacial Heritage were lower than expected based on postdiapause larval surveys (Table 10) and the abundance of prediapause larvae observed in Spring 2013 (Linders and Lewis 2013). Numbers at SCS were higher than expected, given no larvae were released in 2014. This is encouraging and a sign that a small resident population continues to persist.

Table 13. Number of Taylor's checkerspots counted by site and date during distance sampling surveys at extant (R76= Range 76) and reintroduction sites (SCS = Scatter Creek South; R50 = Range 50, PCM = Pacemaker, GHP = Glacial Heritage Preserve, and TA7S = Training Area 7S) in South Puget Sound, Washington, Spring 2014. R76: standard survey area is 12 transects; four northern transects (count for T. 13-16 in Notes) added for distribution.

| Date | GHP | PCM | R50 | R76 | SCS | TA7S | Comments |
|---|---|---|---|---|---|---|---|
| 10-Apr | 0 | | | | | | |
| 13-Apr | | 0 | 0 | 0 | | 0 | |
| 14-Apr | 1 | | | | 0 | | |
| 18-Apr | 3 | 0 | | | 0 | 0 | TA7S: one line added |
| 21-Apr | | | | 44 | | 0 | |
| 25-Apr | | 0 | | | 12 | 14 | |
| 26-Apr | 11 | | | | | | |
| 28-Apr | | | 331 | 232 | | | |
| 29-Apr | | | | | | 52 | TA7S: two transects added |
| 30-Apr | | 0 | | 672 | 60 | | R76: 422 on T. 13-16 |
| 1-May | 48 | | | | | | |
| 2-May | | | | 500 | | | |
| 3-May | | | 16 | | | | R50: Non-protocol survey |
| 6-May | | | 146 | | | 50 | |
| 7-May | | 0 | | 754 | | | |
| 11-May | | | | | 30 | | |
| 12-May | 32 | | 201 | | | | |
| 13-May | | 0 | | 561 | | 23 | R76: 358 on T. 13-16 |
| 14-May | | | | | 26 | | |
| 15-May | 10 | | | | 16 | 7 | |
| 16-May | | | 29 | 144 | | | |
| 20-May | 0 | 0 | | | 3 | 2 | |
| 21-May | | | 14 | | | | |
| 24-May | | 0 | | 2 | | 0 | |
| 27-May | | | 0 | | 0 | | SCS: 56 adults released 30 May 2014 |
| 2-Jun | | | | 0 | | | |
| **Total** | **105** | | **812** | **2909** | **147** | **148** | **Number of observations** |

Nectaring observations were recorded opportunistically during distance sampling surveys for Taylor's checkerspot. Thirteen different nectar species were recorded across five sites in 2014 (Table 14). The number of nectar observations in 2014 was nearly twice that of 2013. As in previous years, three plant species, *Balsamorhiza deltoidea*, *Lomatium triternatum* and *Saxifrage integrifolia,* accounted for the majority (91.2 percent) of all nectaring observations in 2014.

Table 14. Number and plant species on which Taylor's checkerspots nectared during distance sampling surveys at four reintroduction sites (PCM = Pacemaker, R50 = Range 50, SCS = Scatter Creek South, TA7S = Training Area 7 South) and one extant site (R76 = Range 76) in South Puget Sound, Washington, 2011-2014. ACMI – *Achillea millefolium*; ARMA – *Armeria maritima*; BADE - *Balsamorhiza deltoidea*; CAHI – *Castilleja hispida; CAQI -Camassia quamash*; CEAR – *Cerastium arvense* ; COPA – *Collinsia parviflora*; CYSC – *Cytisus scoparius;* ERLA - *Eriophylum lanatum*; FRVI - *Fragaria virginiana*; HYRA – *Hypochaeris radicata*; LECA – *Lepidium campestre*; LOSP – *Lomatium* species; LOTR- *Lomatium triternatum*; LOUT-*Lomatium utriculatum*; LULE – *Lupinus lepidus*; PLCO- *Plectritis congesta*; POGR - *Potentilla gracilis*; RAOC - *Ranunculus occidentalis*; SAIN - *Saxifraga integrifolia*; ZIVE – *Zigadenus venenosus*.

| Site | ACMI | ARMA | BADE | CAHI | CAQU | CEAR | COPA | CYSC | ERLA | FRVI | HYRA | LECA | LOSP | LOTR | LOUT | LULE | PLCO | POGR | RAOC | SAIN | ZIVE | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **2011** | | | | | | | | | | | | | | | | | | | | | | |
| R50 | 0 | 0 | 113 | | | | | | | | | | | | | | | | | 6 | | 119 |
| R76 | 3 | 14 | 618 | | 3 | | | | 12 | 46 | 1 | 1 | | 621 | 5 | 3 | | 3 | 4 | 37 | | 1371 |
| SCS | 0 | 2 | 2 | | 1 | | | | | | | | | 1 | | | 4 | | | | | 10 |
| **Subtotal** | **3** | **16** | **733** | **0** | **4** | **0** | **0** | **0** | **12** | **46** | **1** | **1** | **0** | **622** | **5** | **3** | **4** | **3** | **4** | **43** | **0** | **1500** |
| **2012** | | | | | | | | | | | | | | | | | | | | | | |
| R50 | | | 86 | 1 | 4 | 1 | | | | 3 | | | 13 | 4 | | | | | | 30 | | 142 |
| R76 | | 6 | 465 | | 3 | | | 1 | | 8 | | | 161 | 305 | 21 | | | | 8 | 113 | | 1091 |
| SCS | 1 | 2 | 6 | | | | | | | | | | | | | | 1 | | | | | 10 |
| PCM | | | 1 | | | | | | | | | | 2 | 2 | | | | | 1 | | | 6 |
| **Subtotal** | **1** | **8** | **558** | **1** | **7** | **1** | **0** | **1** | | **11** | **0** | **0** | **176** | **311** | **21** | **0** | **1** | **0** | **9** | **143** | **0** | **1249** |
| **2013** | | | | | | | | | | | | | | | | | | | | | | |
| R50 | | | 67 | | | | | | | | 2 | | | 6 | | | | | 1 | 17 | | 93 |
| R76 | | 1 | 219 | 1 | 1 | | | | | 2 | | | | 48 | 6 | | | | 1 | 3 | | 282 |
| SCS | | 1 | | | | | | | | | | | | | | | | | | | | 1 |
| GHP | | | 2 | 1 | 5 | | | | | | | | | | | | | | | | | 8 |
| **Subtotal** | **0** | **2** | **286** | **1** | **1** | **0** | **0** | **0** | **0** | **2** | **2** | **0** | **0** | **54** | **6** | **0** | **0** | **0** | **2** | **20** | **0** | **376** |
| **2014** | | | | | | | | | | | | | | | | | | | | | | |
| R50 | | | 35 | | | | | | | | | | 1 | 2 | | | | | 1 | 8 | | 47 |
| R76 | | 6 | 484 | | 12 | | | | | 8 | 1 | | 14 | 35 | 1 | | | | | 35 | 4 | 600 |
| SCS | | 1 | 6 | | | | | | | | | | | | | | | | 1 | | | 8 |
| GHP | | | | | 2 | | | | | | | | | | | | | | | | | 2 |
| TA7S | | 2 | 6 | | | 1 | 2 | | | | | | 1 | | 1 | | | | | | | 13 |
| **Subtotal** | **0** | **9** | **531** | **0** | **14** | **1** | **2** | **0** | **0** | **8** | **1** | **0** | **16** | **37** | **2** | **0** | **0** | **0** | **2** | **43** | **4** | **670** |
| **Total** | **4** | **35** | **2108** | **2** | **26** | **2** | **2** | **1** | **12** | **67** | **4** | **1** | **192** | **1024** | **34** | **3** | **5** | **3** | **17** | **249** | **4** | **3795** |
| **Percent** | **0.1** | **0.9** | **55.5** | **0.1** | **0.7** | **0.1** | **0.0** | **0.0** | **0.3** | **1.8** | **0.1** | **0.0** | **5.1** | **27.0** | **0.9** | **0.1** | **0.1** | **0.1** | **0.4** | **6.6** | **0.0** | |

Mardon skipper butterfly sightings were also recorded opportunistically during surveys for Taylor's checkerspot at three sites visited in 2014 where they occur. In total 41 mardon skippers were observed (Table 15). While this is nearly double the number detected in 2013 (Linders and Lewis 2013), these numbers are still disturbingly low, and are inadequate for generating the abundance estimates we had hoped for. All were observed as singles except for one group of 2.

Table 15. Number of mardon skipper butterflies observed by date and site during distance sampling surveys targeting Taylor's checkerspot butterflies in Spring 2014 at three sites in South Puget Sound, Washington. Only the first "0" survey date is included in the table. R50 = Range 50; R76 = Range 76; SCS = Scatter Creek South.

| Date | R50 | R76 | SCS |
|---|---|---|---|
| 6-May | 0 | 0 | |
| 7-May | | | 0 |
| 03-May | 1 | | |
| 07-May | | 1 | |
| 11-May | | | 1 |
| 12-May | 8 | | |
| 13-May | | 2 | |
| 15-May | | | 1 |
| 16-May | 14 | 6 | |
| 20-May | | | 3 |
| 21-May | 4 | | |
| Total | 27 | 9 | 5 |

### *Adult distribution*

Adult checkerspots were distributed across the majority of the sampling areas at R50 and R76 (Appendix C, Fig. 2 and 5, respectively). The distribution of adults at TA7S (Appendix C, Fig. 6) tracked the areas of release fairly closely, whereas distributions at SCS and GHP, were more widespread. Adults were distributed across 26.3 percent (62 of 236) of the segments surveyed at SCS (Appendix C, Fig. 1), with the greatest concentration of adults in the west-central portion of the grid, where many of the recent releases occurred. There was also expanded use of the southern portion of the survey area, where recent habitat restoration was focused. Adults occupied 45.2 percent (179 of 396 segments) of the R50 survey area over the course of the flight season (Appendix C, Fig. 2). Although no sightings of Taylor's checkerspots were observed on distance sampling surveys at PCM in 2014, three observations were noted on the site (Appendix C, Fig. 3), indicating it remains occupied. Adults were distributed across 19.3 percent (52 of 270) of the segments surveyed at GHP (Appendix C, Fig. 4). This is the greatest extent observed at this site since reintroductions began. At R76, the only extant site in South Puget Sound, 93.8 percent (315 of 336 segments) of the survey area was occupied in 2014 (Appendix C, Fig. 5), which is not different from survey results in 2014. Surveys conducted north of the main grid at R76, however, indicated the Taylor's checkerspots occupied this area in high numbers (Appendix C, Fig. 5), although this area has not been surveyed in a standardized fashion. At TA7S, a new reintroduction site in 2014, adults occupied 24.8 percent (34 of 137 segments) of the survey area, with sightings observed along several edges. Scattered sightings outside of the standard monitoring areas were also collated and included on site maps except on the Artillery Impact Area at JBLM (Appendix C, Fig. 7), where sightings were numerous and widespread. Scattered sightings and associated checkerspot resources are mapped for Training Area 6 (Appendix C, Fig. 8) along the east edge of the AIA (Appendix C, Fig. 9).

## Evaluate short-term success of releases based on reproduction

### *Methods*

The first step toward establishing a naturally-occurring population is documenting local reproduction of released animals. We used 5 x 2-m belt transects placed randomly along transects at 5-10 m intervals to search for larval clusters in and around release plots on one newly established site (TA7S). PCM was not re-surveyed due to the small number of adults observed there (Appendix C, Fig. 3) and because funding is sufficient to conduct surveys on only one site per year. Sampling methods followed those described in (Linders and Lewis 2013). Based on the results of similar surveys at R50 (Linders 2010) and the size of the area searched, we consider location of at least 10 larval clusters, where the majority of larvae and clusters persist to at least 3$^{rd}$ instar, a sign of successful reproduction.

### *Results and Discussion*

Eight 5 x 2-m plots were searched at TA7S on 24-25 June 2014. Only one fourth instar larva was observed, although forage sign was evident in many locations. Consistently warm and dry weather conditions in Spring 2014 appears to have advanced larval development ahead of the drying vegetation, which was already widely evident by this date (Fig. 3). A reconnaissance survey for prediapause larvae at Range 76 revealed similar patterns of low larval numbers, with nearly all remaining larvae in 4$^{th}$ instar and feeding damage widespread. Presumably the larvae observed at both TA7S and R76 were those that were not yet ready to enter diapause.

Figure 3. Prediapause larval feeding damage on desiccating *Castilleja hispida* from reproduction of reintroduced Taylor's checkerspots at Training Area 7S in South Puget Sound, Washington, 24 June 2014.

## Long-term monitoring and population goals

### *Methods*

Abundance and distribution data from R76 are critical to understanding annual variations in population size and the shape and phenology of population curves (Weiss and Weiss 1998), which in turn helped us set appropriate population targets, evaluate population growth potential, and set long-term monitoring goals. Monitoring will be used to measure progress toward population establishment 1) during the five-year release period, and 2) after the five-year release period is complete. For the purpose of this project, an established population is defined as follows:

> *A reintroduced population of Taylor's checkerspots will be considered established when a minimum of 250 adult butterflies (single day abundance estimate) are widely distributed across a monitoring area >20 ha (50 ac) in size and they occupy the site solely through natural reproduction each year for five consecutive years.*

The monitoring area at R76 covers about 42 ha (100 ac) or twice that of most reintroduction management units. The minimum single day abundance estimate is based on the peak single day abundance estimate at R76 in 2009 (Appendix C). In that year it was difficult to find 2 or 3 butterflies together on any given day, yet the population was able to recover from this point giving us confidence that other populations could as well. A five-year monitoring window is a widely used standard for establishing occupancy by butterflies. The monitoring scheme and distance sampling protocol already developed (Linders and Olson 2012) will be used as the basis for monitoring reintroduction sites. Reintroduction sites will be evaluated against this standard annually and across years.

### *Results and Discussion*

Based on these criteria, R50 exceeded the target of 250 adults on a single day for the second consecutive year, based solely on reproduction, with a peak single day abundance estimate of 1,166 adults (range 891-1,527) in 2013. The peak raw count for 2014 (Table 13) suggests abundance estimates exceeded the target in 2014 as well. Adults were distributed across the majority of the 22-ha monitoring area in 2013 (Linders and Lewis 2013). Distribution was somewhat more restricted in 2014 (Appendix C, Fig. 2), although it still stretched from edge to edge in both directions. Although checkerspot were rarely observed along the southern edge where food resources are lacking, they were observed at the northern edge of the survey area, where resources extend beyond the survey grid.

## Literature Cited


Barclay, E., M. Arnold, M. Andersen, and D. Shepherdson. 2009. Husbandry Manual: Taylor's checkerspot (*Euphydryas editha taylori*). September 2009, 1st edition. Oregon Zoo, Portland, Oregon.

Crone, E. D. Pickering, and C. Schultz. 2007. Can captive rearing promote recovery of endangered butterflies? An assessment in the face of uncertainty. Biological Conservation 139:103-112.

Ehrlich, P. R., and D. D. Murphy. 1987. Conservation lessons from long-term studies of checkerspot butterflies. Conservation Biology 1:122-131.

Fewster, R.M., S.T. Buckland, K.P. Burnham, D.L. Borchers, P.E. Jupp, J.L. Laake, and L. Thomas. 2009. Estimating the encounter rate variance in distance sampling. Biometrics 65: 225-236.

Fimbel, C. and P. Dunn. 2013. Unoccupied Butterfly Habitat Enhancement: A Proposal to ACUB Cooperators. The Center for Natural Lands Management, Olympia, WA.

Harrison, S. 1989. Long-distance dispersal and colonization in the bay checkerspot butterfly, *Euphydryas editha bayensis*. Ecology 70: 1236-1243.

Hamilton, L., E. Louie, M. Dittamore, M. Pruett, and M. Linders. 2014. 2014 Mission Creek captive rearing report: Taylor's checkerspot butterfly (*Euphydryas editha taylori*). Report to the Washington Department of Fish and Wildlife, *in prep.*

Kaplan, E. L. and P. Meier. 1958. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 53:457-481.

Kronland, B. and P. Dunwiddie. 2014a. Butterfly Habitat Enhancement. A Proposal to ACUB Cooperators, 13 February 2014. 9 pp. + appendices.

Kronland, B. and P. Dunwiddie. 2014b. Butterfly Habitat Enhancement. Annual Progress Report to ACUB Cooperators, August 30, 2014. 10 pp. + appendices.

Kuussaari, M., S. Van Nouhuys, J. Hellman, and M. Singer. 2004. Larval biology of checkerspots. Pp. 138-160 in Ehrlich, P. R., and I. Hanski, eds. On the wings of checkerspots: a model system for population biology. Oxford University Press New York City, New York.

Lewis, K., M. J. Andersen, J. Low, and D. Shepherdson. 2013. 2013 Oregon Zoo captive rearing report: Taylor's checkerspot butterfly (*Euphydryas editha taylori*). Final report to the Washington Department of Fish and Wildlife, Olympia, WA.

Lewis, K., J. Low, M. J. Andersen, K. McEuen, and D. Shepherdson. 2014. 2014 Oregon Zoo Captive rearing report: Taylor's checkerspot butterfly (*Euphydryas editha taylori*). Final report to the Washington Department of Fish and Wildlife, Olympia, WA, *in prep.*

Linders, M. J. 2006. Captive rearing and translocation methods development for Taylor's checkerspot (*Euphydryas editha taylori*), South Puget Sound, Washington. Washington Department of Fish and Wildlife, Wildlife Program, Olympia. 26 pp + appendices.

Linders, M. J. 2007. Development of captive rearing and translocation methods for Taylor's checkerspot (*Euphydryas editha taylori*) in south Puget Sound, Washington: 2006-2007 Annual Report. Washington Department of Fish and Wildlife, Wildlife Program, Olympia. 29 pp.

Linders, M. J. 2010. 2010 ACUB Project progress report: Development of captive rearing and translocation methods for Taylor's checkerspot in south Puget Sound: 2009-2010 and an update for 2010-2011. Report to the ACUB Technical Review Committee. 29 September 2010. 15 pp.

Linders, M. J. 2011a. Work plan for Taylor's checkerspot captive rearing and translocation in 2011. Report to ACUB technical review committee. 30 January 2011. 10 pp.

Linders, M. J. 2011b. 2011 ACUB Project progress report: Captive rearing and translocation of Taylor's checkerspot in south Puget Sound: 2010-2011. Report to the ACUB Technical Review Committee. 20 September 2011. 22 pp.

Linders, M. J. 2012. 2012 ACUB Annual Progress Report: Captive rearing and translocation of Taylor's checkerspot in south Puget Sound: 2011-2012. Report to the ACUB Technical Review Committee. 4 October 2012. 29 pp. + appendices.

Linders, M. J. 2013. Taylor's checkerspot (*Euphydryas editha taylori*) Captive Rearing and Reintroduction: A Proposal to ACUB Cooperators. The Washington Department of Fish and Wildlife, Olympia, WA.

Linders, M. J., and K. Lewis. 2013. Captive rearing and translocation of Taylor's checkerspot butterfly (*Euphydryas editha taylori*) in south Puget Sound, Washington, 2012-2013. 2013 Annual Report to the US Fish and Wildlife Service, Joint Base Lewis-McChord Fish and Wildlife Program and JBLM-ACUB Technical Review Committee, 17 December 2013. 40 pp. + appendices.

Linders, M. J. and G. S. Olson. 2014. Taylor's checkerspot monitoring South Puget Sound, Washington, Jan 2014. Unpublished methodology of the Washington Department of Fish and Wildlife, Olympia, Washington.

Marques, T.A., L. Thomas, S.G. Fancy, and S.T. Buckland. 2007. Improving estimates of bird density using multiple covariate distance sampling. The Auk 124(4):1229–1243.

Rasband, W. S. 2012. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA, imagej.nih.gov/ij/, 1997–2012.

Schad, K., editor. 2008. Amphibian Population Management Guidelines. Amphibian Ark Amphibian Population Management Workshop; 2007 December 10-11; San Diego, CA, USA. Amphibian Ark, www.amphibianark.org; 2008. 31 p.

Singer, M. C. 2004. Measurements, correlates, and importance of oviposition preference in the life of checkerspots. Pp 112-137 in On the Wings of Checkerspots: a Model System for Population Biology. P. R. Ehrlich and I. Hanski, editors. Oxford University Press, New York. 371 pp.

Thomas, L. S.T. Buckland, E.A. Rexstad, J.L. Laake, S. Strindberg, S.L.Hedley, J.R.B. Bishop, T.A. Marques, and K.P. Burnham. 2010. Distance software: design and analysis of distance sampling surveys for estimating population size. Journal of Applied Ecology 47: 5-14.

Weiss, S.B. and A. D. Weiss. 1998. Landscape-level phenology of a threatened butterfly: a GIS-based modeling approach. Ecosystems 1:299-309.

## Future Plans and Recommendations

This is the ninth year of a multi-year recovery project. Translocation data from 2007-2013 have been compiled and preliminary results for many cross-year analyses are complete. Analyses on 2014 distance sampling data are scheduled for completion in winter 2014-2015. Plans for 2015 include continuing releases at Glacial Heritage Preserve (Thurston County) and Training Area 7S (JBLM). Given the robust response of the R50 population, no further reintroductions are recommended there at this time, however population monitoring will continue there and at all remaining reintroduction sites to track the establishment and dynamics of these populations over time. Funds for the 2014-2015 field season and rearing year have been secured; those for 2015-2016 will be requested from USFWS, ACUB and JBLM. Goals are to continue relatively large releases, while maintaining a number of release sites and microsites to offset the influence of climatic perturbations on translocation success. Additional sources of funding and new conservation partnerships are being explored.

In 2014 we initiated searches in areas surrounding the current occupied sites to document potential colonization. The main part of the flight season was limited to a two-week period in 2014, which limited our ability to conduct expansive searches. We plan to continue this work in 2015 to follow up on recent sighting information and areas identified as having potential habitat. Documenting new breeding populations is especially important to recovery and will improve our understanding of checkerspot demography and ultimately reduce the expense of captive propagation and reintroduction as a path to increasing the size and number of populations.

## Questions for Further Research

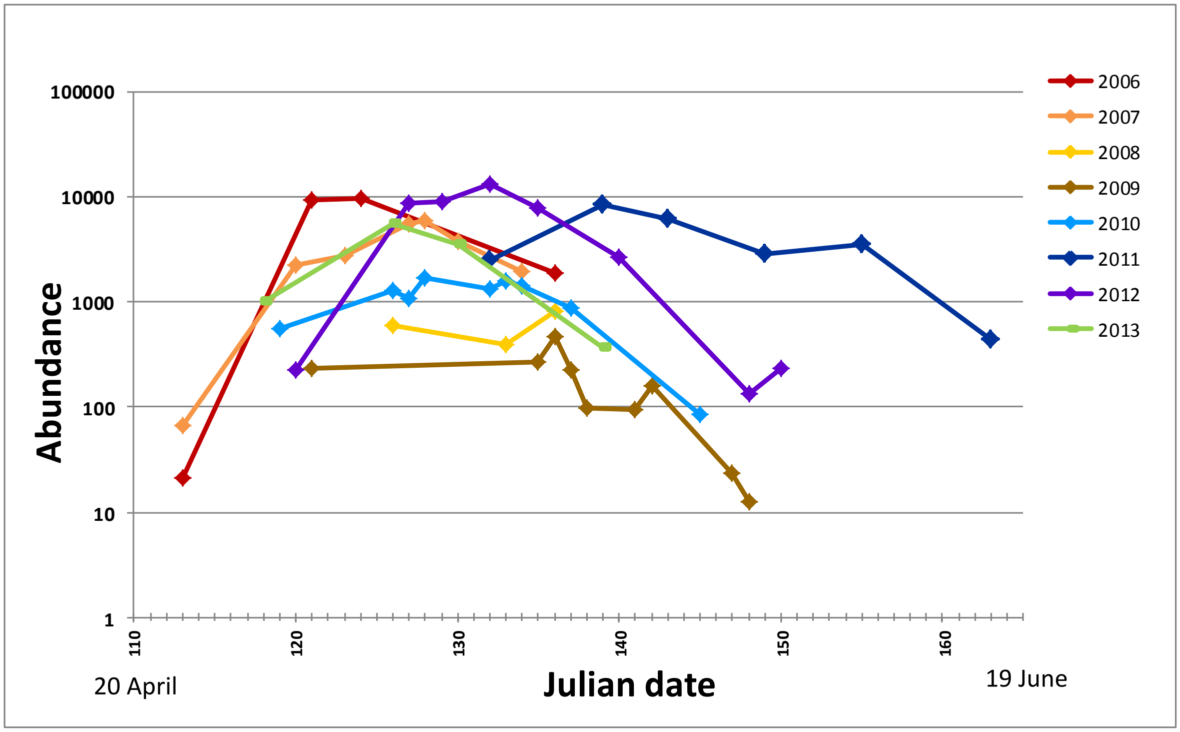
1) What is the total population size of adult butterflies in any given year?

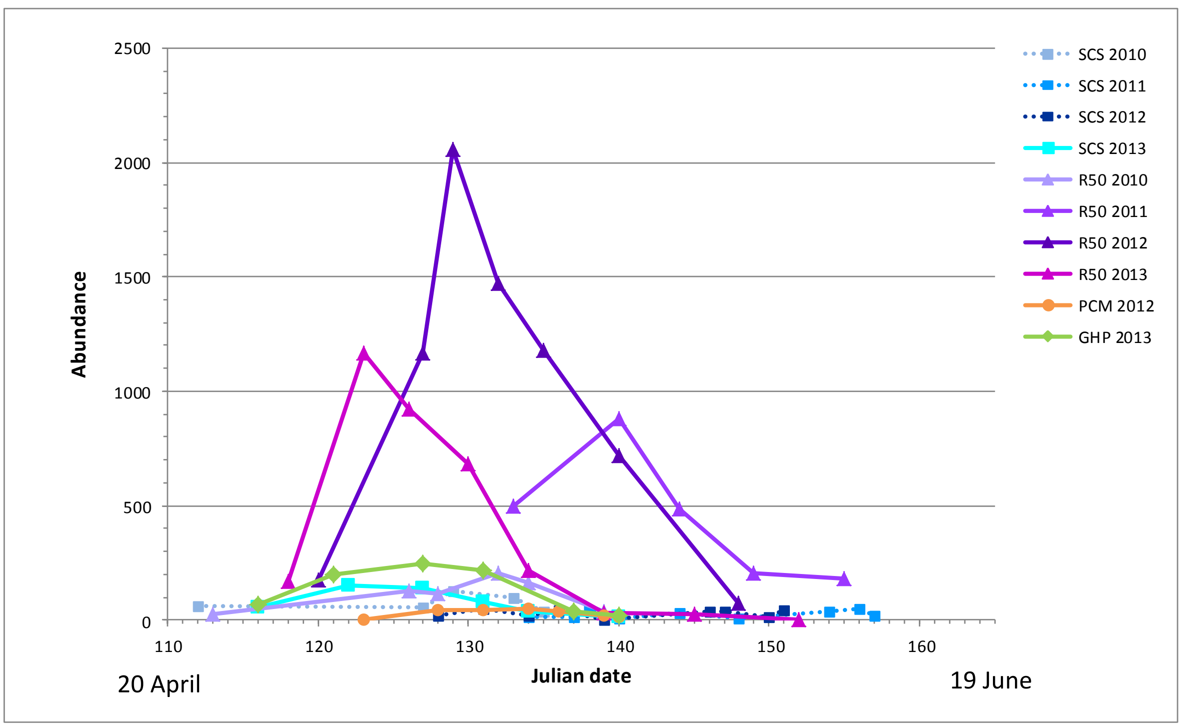
This question remains problematic for certain types of analyses because daily counts of butterflies, or abundance estimates based on such counts, reflect only a portion of the total population during any given flight season. Subsequent counts include some individuals that were part of previous counts, plus new individuals, such that numbers reflect emergence and death rates that vary through the flight season. Without estimates of these rates, estimating a total population size is difficult, but may be accomplished based on the pattern of the counts and some assumptions. Various methods have been used to address this problem, but these are often very sensitive to the validity of the assumptions. Recent methods applying Bayesian modeling techniques to estimate salmon escapement appear to have application to the butterfly abundance estimation problem, and these are worth investigating as at least a starting point to developing a methodology for Taylor's checkerspot.

*Appendix A.* Approximate number of Taylor's checkerspot butterflies released or proposed () for release by life stage (Pre = prediapause larvae; Ad = adult, Post = postdiapause larvae) and site (SCS = Scatter Creek South; R50 = Range 50; GHP = Glacial Heritage Preserve; PCM = Pacemaker Airstrip; TA7S = Training Area 7 South; SCN= Scatter Creek North; TNQ = Tenalquot Prairie; WPR – West Rocky Prairie; T15 = Training Area 15; BHP= Bald Hill Preserve).

| | 2006 | | | 2007 | 2008 | 2009 | | 2010 | | 2011 | | | 2012 | | 2013 | | 2014 | | (2015) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Post | Egg | Pre | Post | Post | Post | Pre | Post | Ad | Post | Ad | Pre | Post | Ad | Post | Ad | Post | Ad | Post | Ad |
| SCS | | 639 | | 199 | 340 | 747 | 2487 | 891 | 202 | 1109 | 167 | 1036 | | 133 | 3250 | | | 56 | | 100 |
| R50 | | | | | | | 2956 | 1145 | | 1141 | | | | | | | | | | |
| GHP | 30 | | | | | | | | | | | | 975 | | 3372 | 107 | 1522 | | 2000 | |
| PCM | | | 307 | | | 741 | | | | | | | 1565 | | | | | | | |
| TA7S | | | 301 | | | | | | | | | | | | | | 1086 | | 2000 | |
| SCN | | | | | | 759 | | | | | | | | | | | | | | |
| TNQ | | | | | | | | | | | | | | | | | | | | |
| WRP | | | | | | | | | | | | | | | | | | | | |
| T15 | | | | | | | | | | | | | | | | | | | | |
| BHP | | | | | | | | | | | | | | | | | | | | |

*Appendix B.* Estimated daily abundance of Taylor's checkerspots using distance estimation and timing of the flight season at Range 76 (R76, source site) in 2006-2013 (top) and at four reintroduction sites: Scatter Creek South (SCS) and Range 50 (R50) in 2010-2013, Pacemaker (PCM) in 2012, and Glacial Heritage preserve (GHP) in 2013 (bottom). The R76 survey area was 42.0 ha (2006-2013); note log scale on the y-axis. R50 survey areas were 16.8 ha (2010), 22.6 ha (2011), 22.3 (2012) and 24.8 (2013); SCS was 19.8 (2010), 22.5 (2011, 2012) and 24.8 (2013); PCM was 12.0 ha (2012); and GHP was 12.0 ha (2013).



***Appendix C.*** Distribution of adult Taylor's checkerspots observed during distance sampling surveys at five reintroduction sites (SCS, R50, PCM, GHP and TA7S) and one extant site (R76) in South Puget Sound, Washington, 2014.

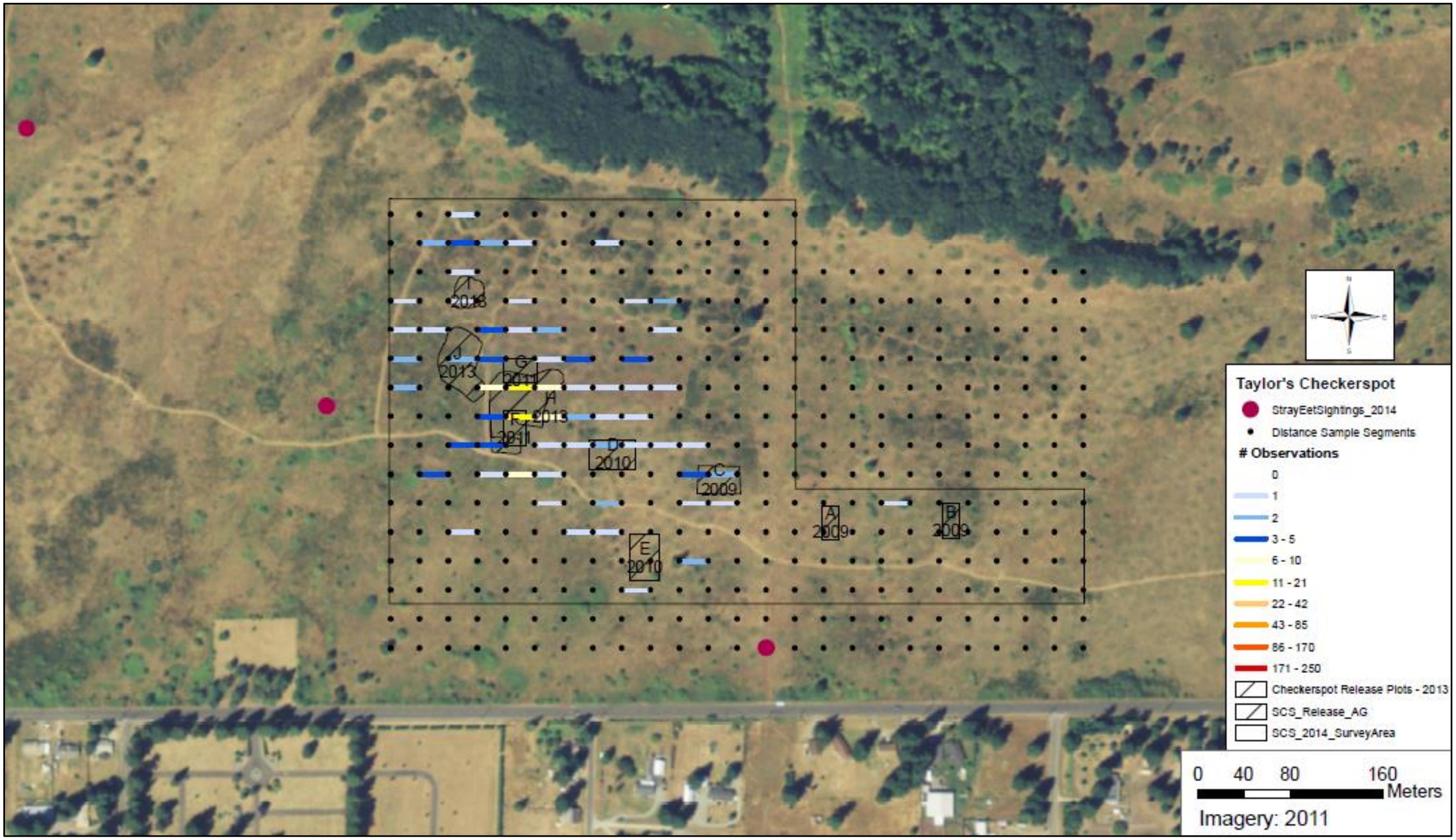



Figure 1. Distribution of adult Taylor's checkerspots observed during distance sampling surveys at Scatter Creek South, combined across all survey dates in Spring 2014, South Puget Sound, Washington.

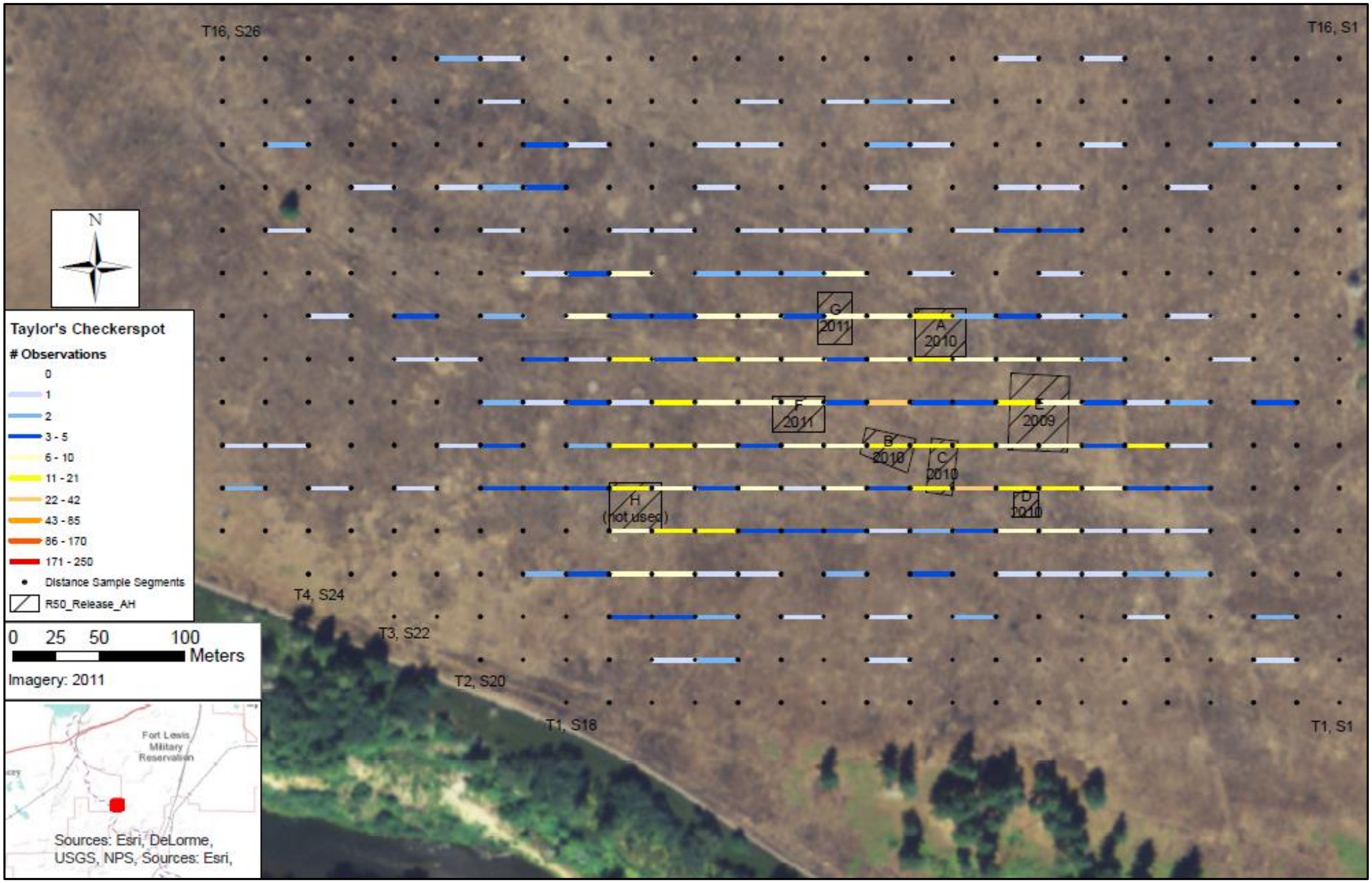



Figure 2. Distribution of adult Taylor's checkerspots observed during distance sampling surveys at Range 50, combined across all survey dates in Spring 2014, South Puget Sound, Washington.

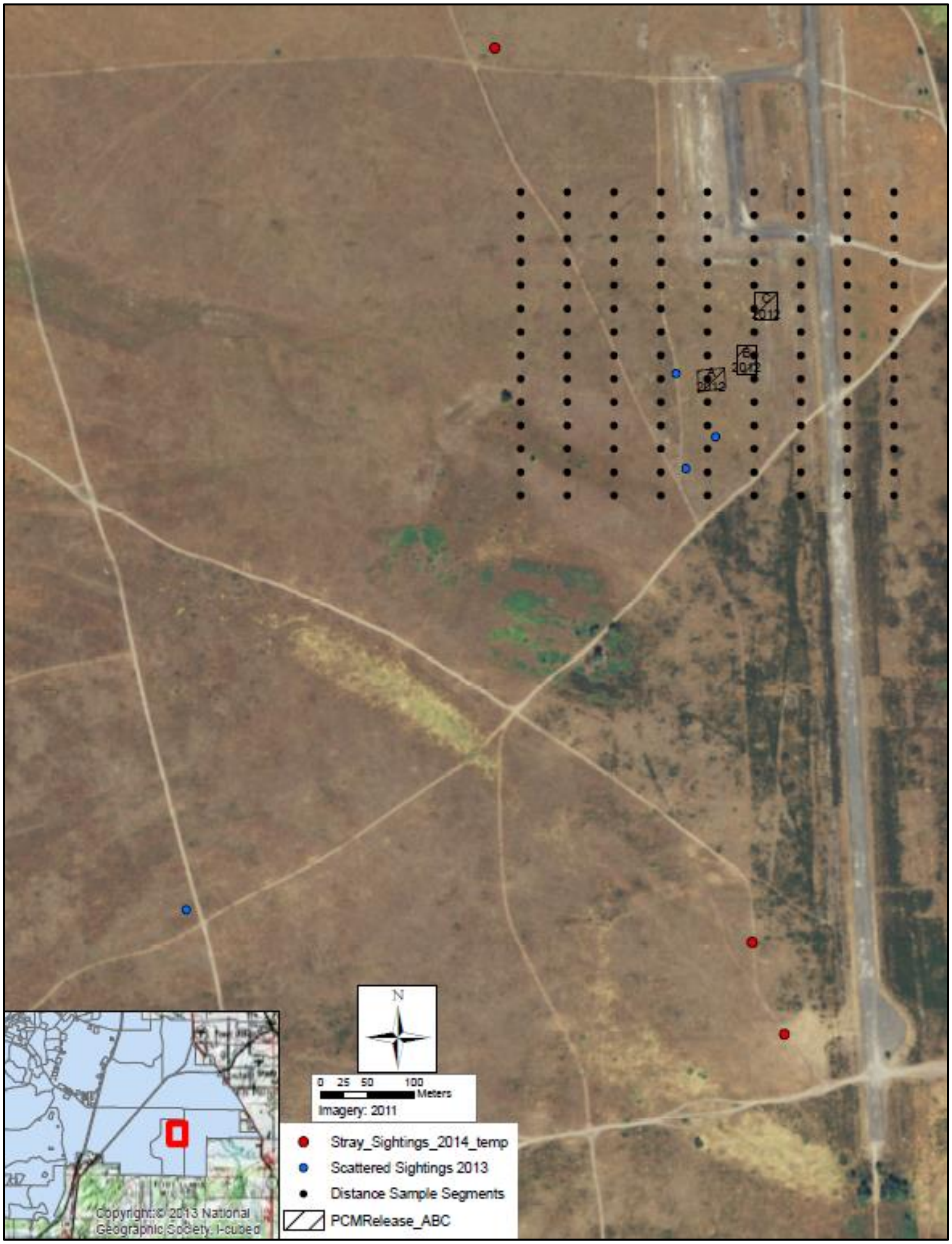



Figure 3. Distribution of adult Taylor's checkerspots observed at Pacemaker in Spring 2014, South Puget Sound, Washington. No checkerspots were observed during distance sampling surveys.

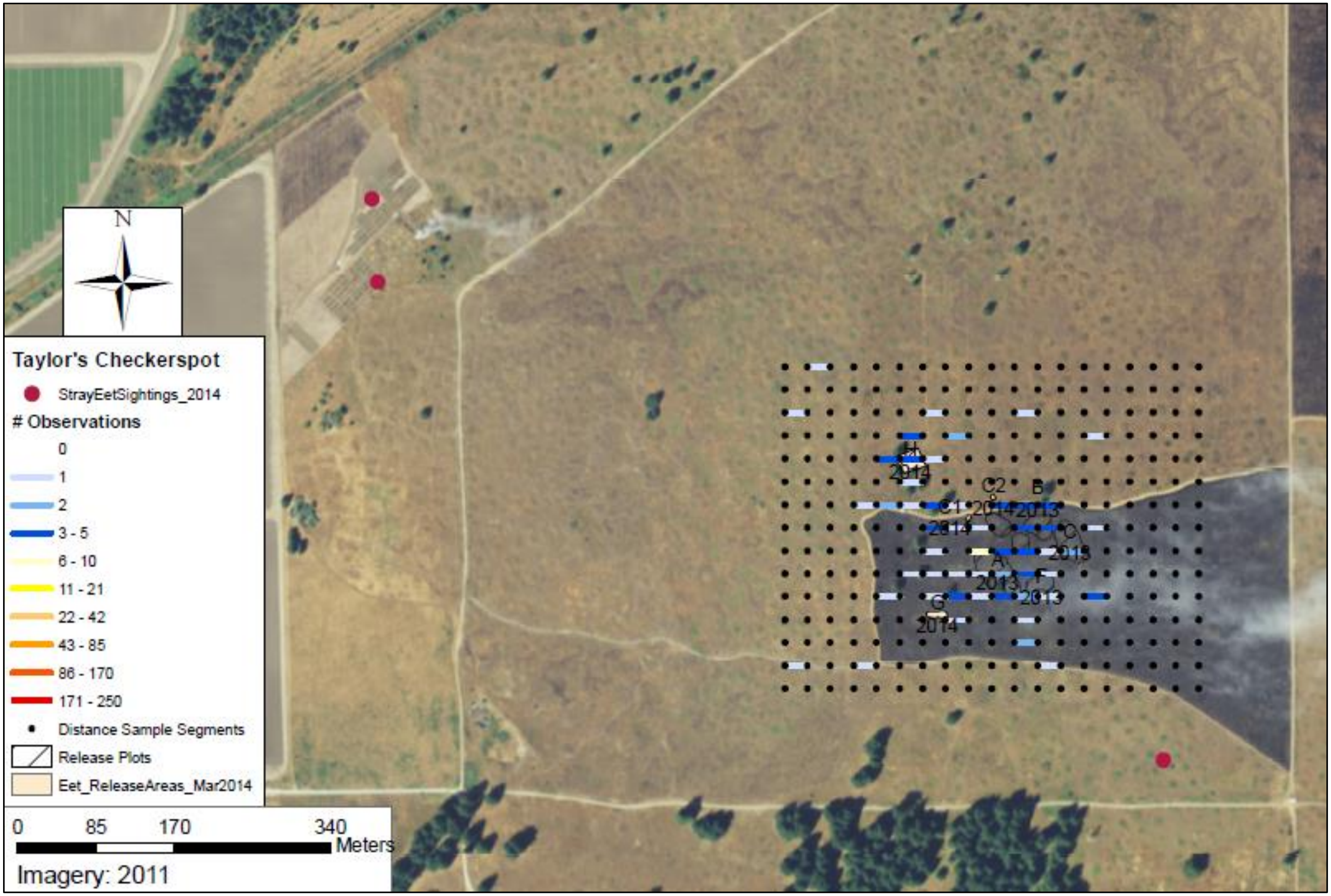



Figure 4. Distribution of adult Taylor's checkerspots observed during distance sampling surveys at Glacial Heritage Preserve, combined across all survey dates in Spring 2014, South Puget Sound, Washington.

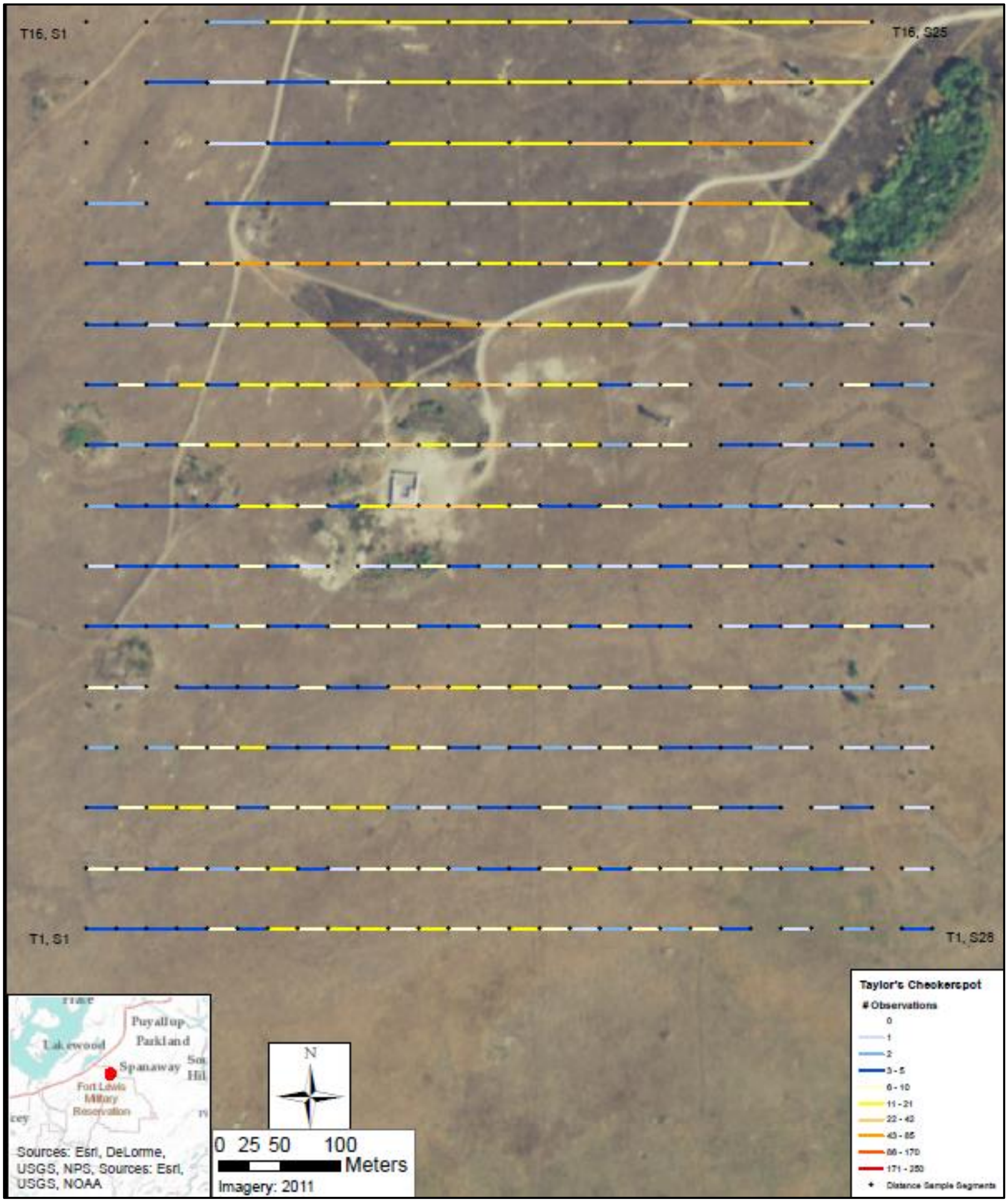



Figure 5. Distribution of all adult Taylor's checkerspots observed during distance sampling surveys at Range 76, combined across all survey dates in Spring 2014, South Puget Sound, Washington. Four transect at north end were included for distribution in 2014.

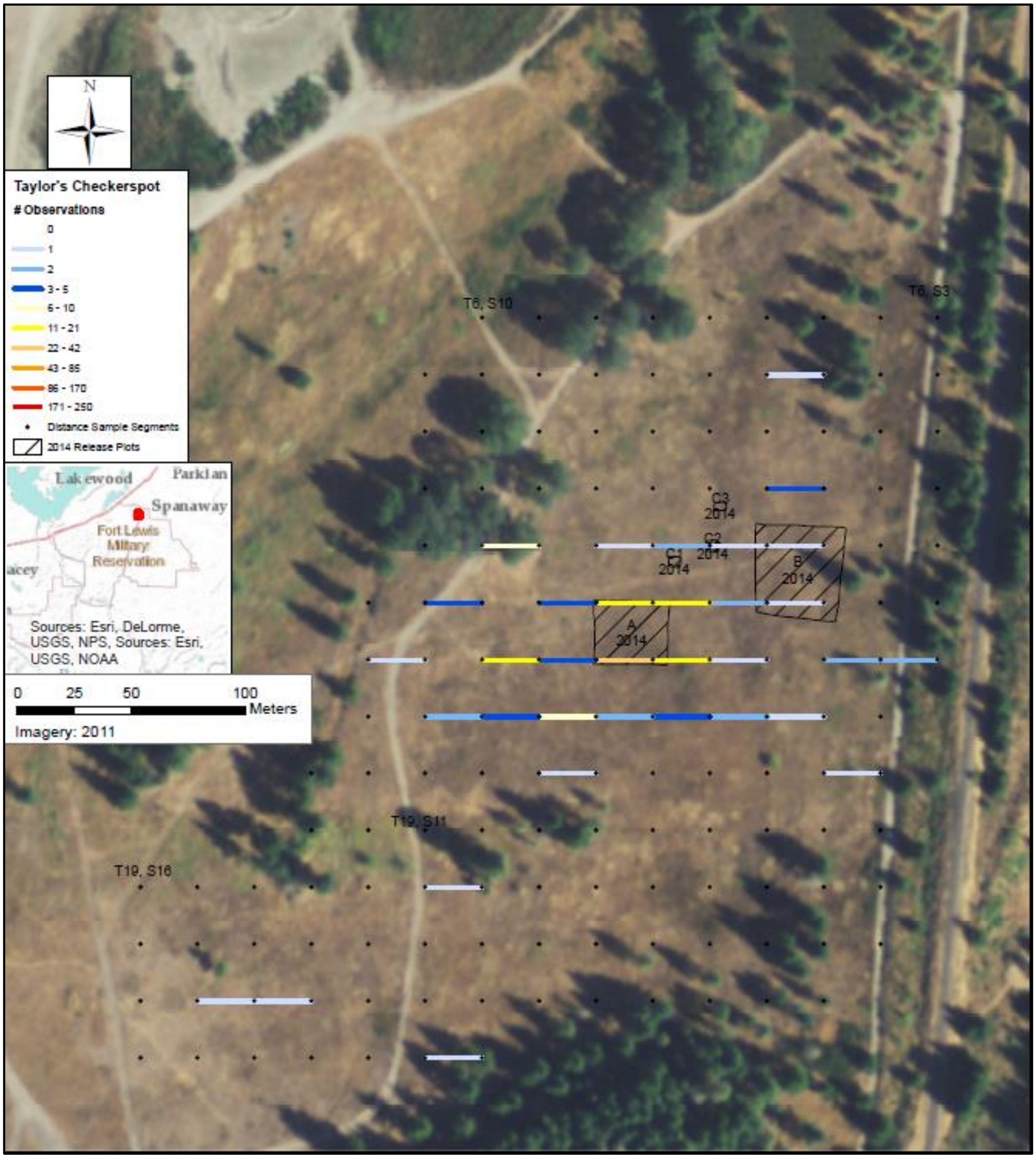



Figure 6. Distribution of adult Taylor's checkerspots observed during distance sampling surveys at Training Area 7S, combined across all survey dates in Spring 2014, South Puget Sound, Washington.

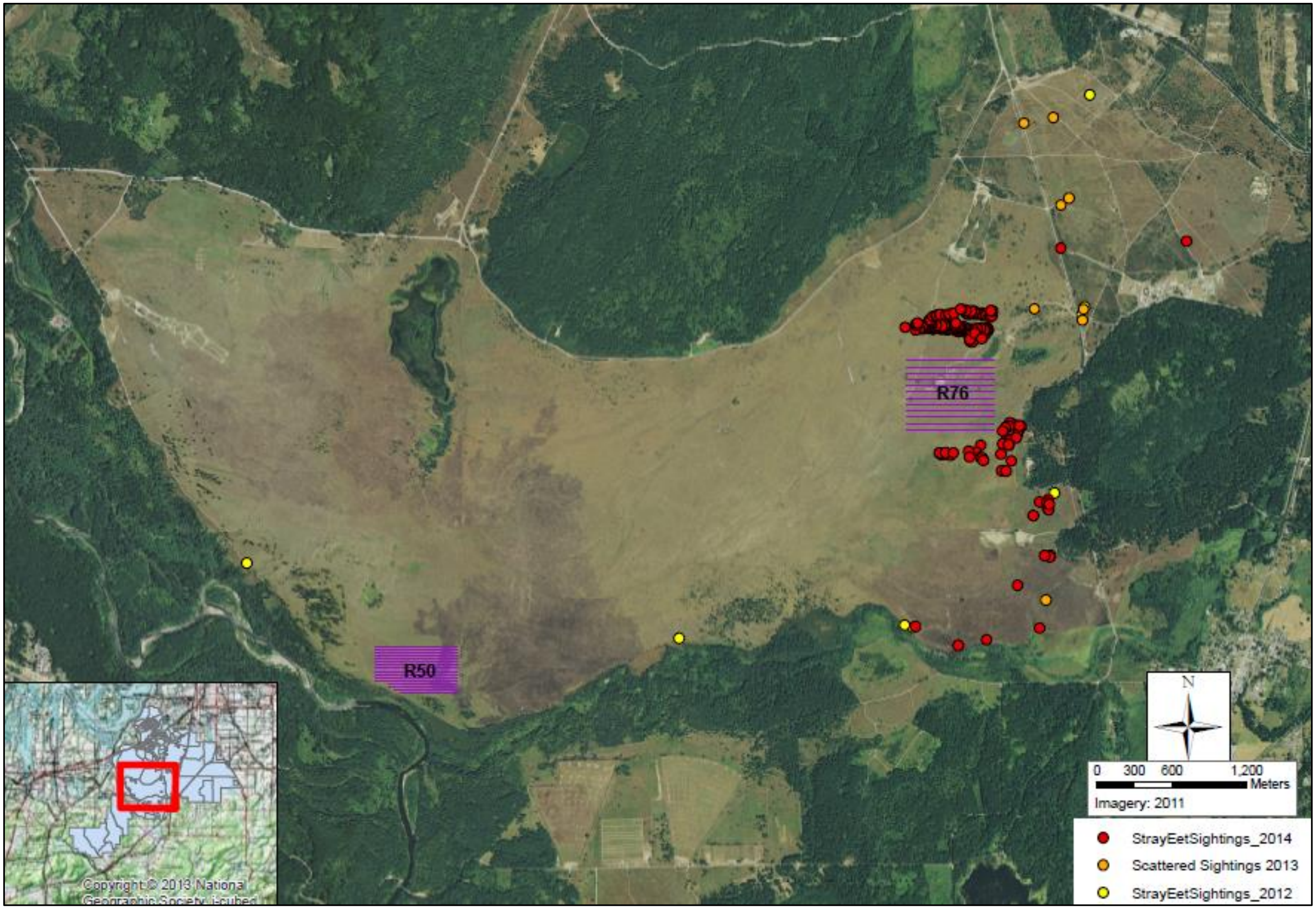



Figure 7. Scattered sightings of adult Taylor's checkerspots observed on the Artillery Impact Area on Joint Base Lewis-McChord relative to standard monitoring areas at R76 (extant site) and R50 (reintroduction site), 2012-2014, South Puget Sound, Washington. Sightings east and west of R50 represent new locations since monitoring began in 2003.

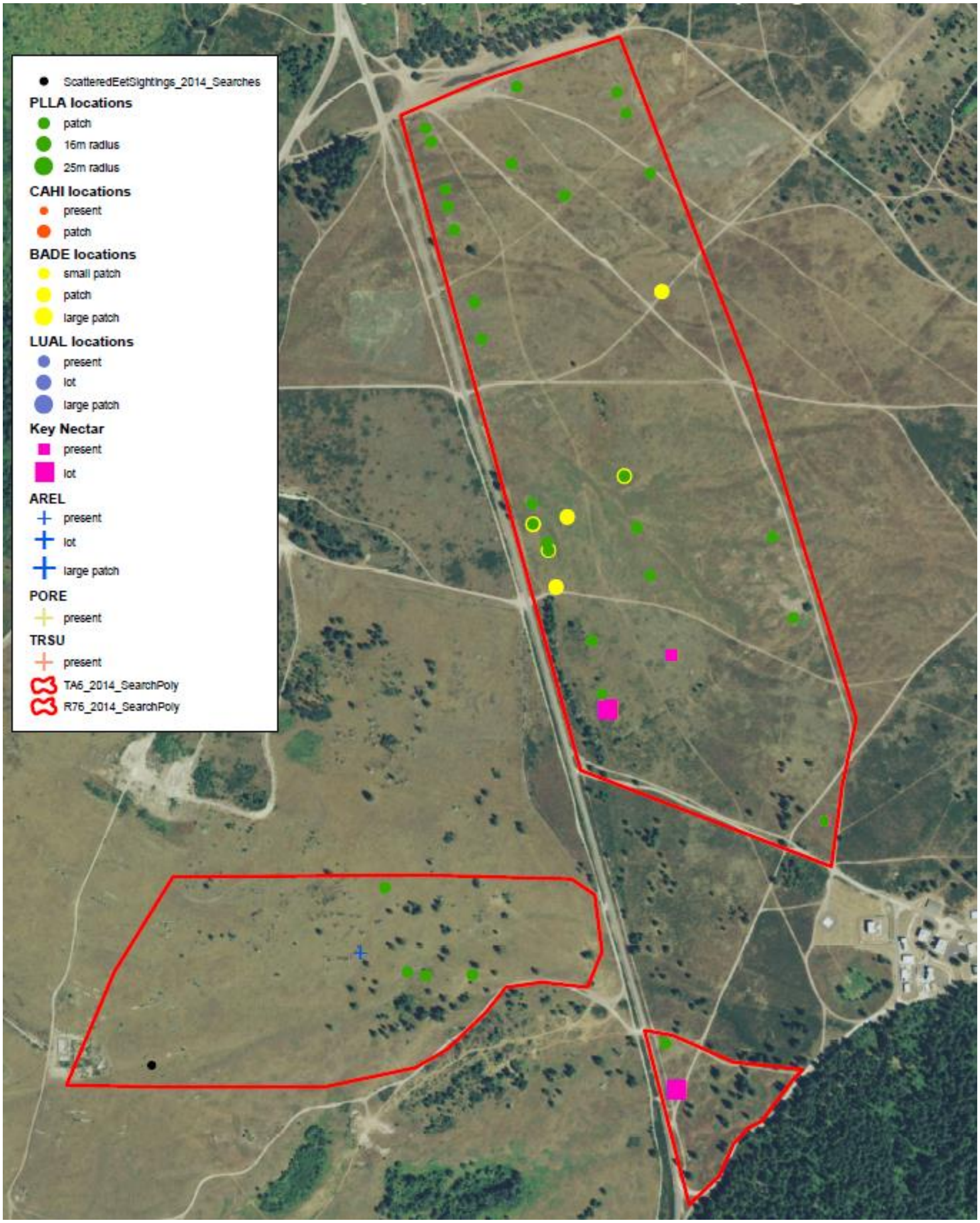



Figure 8. Scattered sightings and associated resources for Taylor's checkerspot mapped during reconnaissance surveys in Training Area 6 at the north edge of the Artillery Impact Area on Joint Base Lewis-McChord, 2014.

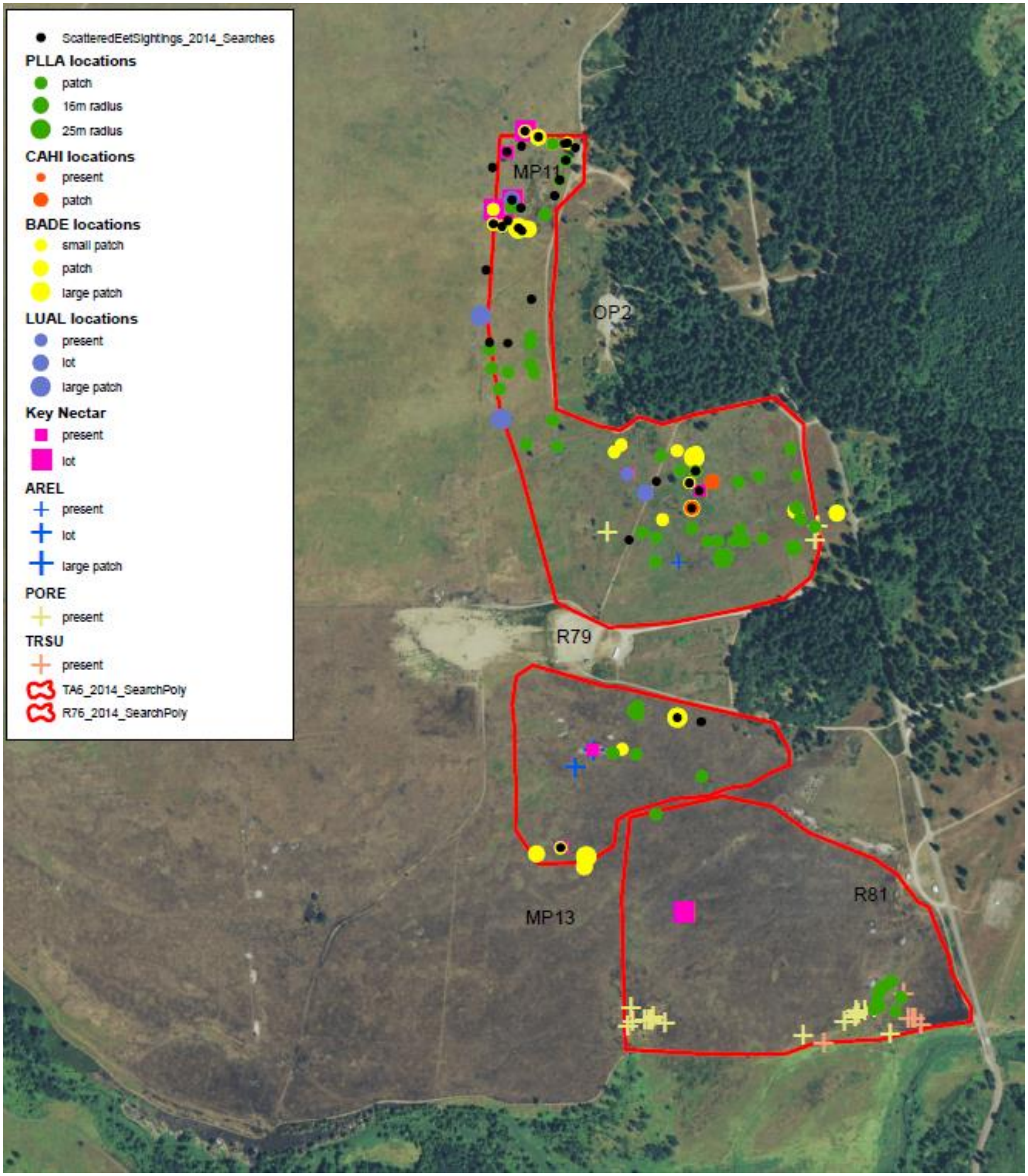



Figure 9. Scattered sightings and associated resources for Taylor's checkerspot mapped during reconnaissance surveys along the east edge of the Artillery Impact Area at Joint Base Lewis-McChord, 2014.