Taylor's checkerspot (*Euphydryas editha taylori*) Captive Rearing and Reintroduction: South Puget Sound, Washington, 2015-2016

2016 Annual Report to:
US Fish and Wildlife Service (Cooperative Agreement #F15AC00515),
Joint Base Lewis-McChord (Contract #W911S8-16-2-0006), and the
ACUB Technical Review Committee (Subaward #WA-S-2013-015-3: WDFW3)

15 October 2016

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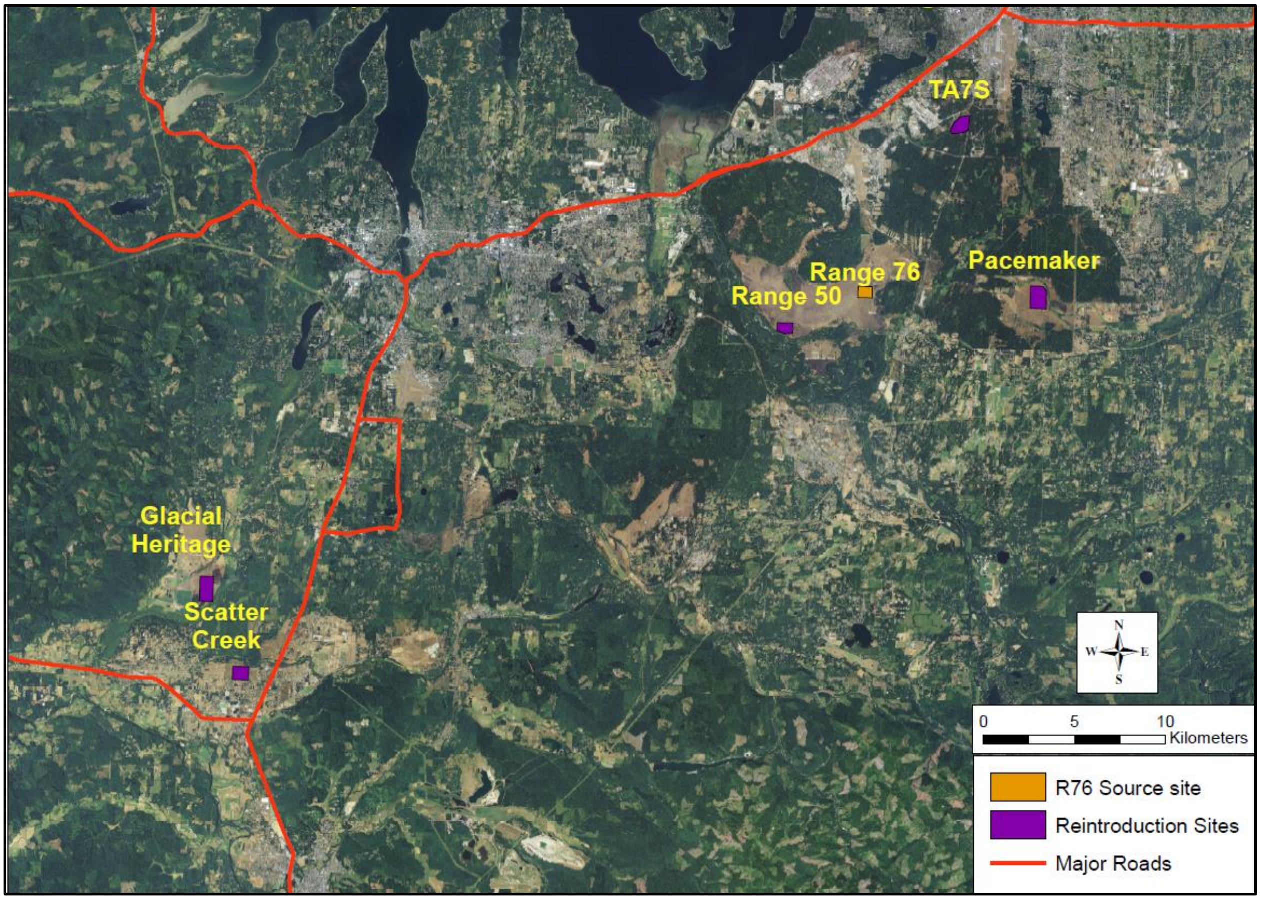
Project Sites

Captive rearing facilities are located at the Oregon Zoo, Portland, OR, and Mission Creek Corrections Center for Women in Belfair, WA. Reintroduction and monitoring sites included Scatter Creek Wildlife Area – South Unit, (SCS; 2007-14, 2016 releases); Range 50, Joint Base Lewis-McChord (JBLM) (R50; 2009-2011 releases); Pacemaker Airstrip, JBLM (PCM; 2012 release); Glacial Heritage Preserve (GHP; 2012-2016 releases); Training Area 7 South (TA7S; 2014-2016 releases) and Range 76, JBLM (R76; population source for reintroductions).



Executive Summary

This report summarizes captive-rearing work conducted in May 2015-May 2016, and reintroduction work from July 2015-July 2016. The captive rearing section borrows heavily from annual reports produced by the Oregon Zoo (Lewis et al. 2016) and Mission Creek (Dorman et al. 2016) programs. Postdiapause larval Taylor's checkerspots were released at two existing reintroduction sites in 2016: Scatter Creek South (SCS; initiated in 2007) and Glacial Heritage Preserve (GHP; initiated in 2012). In addition, adults and prediapause larvae were released at Training Area 7 South (TA7S; initiated 2014). Monitoring for establishment continued at Scatter Creek South and Range 50 (R50; initiated in 2009). Staff from Joint Base Lewis-McChord (JBLM) took over monitoring at Pacemaker (PCM; initiated in 2012), which had no sightings in 2015. A total of 10,317 eggs were produced at the two captive rearing facilities between 20 April and 21 May 2015, with 43.1 percent (4,451) of eggs coming from captive-mated females and 56.9 percent (5,866) produced by wild females collected from the Range 76 (R76) source site on JBLM. Wild females at the Oregon Zoo produced 57.9 percent of eggs there, with 42.1 percent produced by captive females. Conversely, at Mission Creek, wild females produced 44.3 percent of the eggs, with captive-mated females contributing the remaining 55.7 percent. A total of 1,658 postdiapause larvae were released at SCS on 23 Feb 2016. On 25 Feb 2016, a total of 2,029 larvae were released at Glacial Heritage Preserve. Of the 3,687 larvae released, 2,377 were offspring of wild females, 1,210 were offspring of captive-mated females. Eighty-two adult Taylor's checkerspots (50 females and 32 males) were obtained from the Oregon Zoo on 2 May 2016 and released at TA7S; 2 died in transit with 80 released. On 12 May 2016, 131 adults (103 females and 28 males) were released at the same site. In addition, 20 wild females from R76 that were brought to the University of Washington for oviposition were released at TA7S once egg targets were met; 10 females were released on both 5 and 9 May 2016 for a total of 231 adults released. We used distance sampling to quantify daily population density, daily population size, and to illustrate the distribution of adults at four reintroduction sites (R50, SCS, GHP and TA7S) and at R76 (source site). A combined total of 7,402 checkerspots were counted during distance surveys in 2016, with the greatest number observed at R50. Peak counts occurred on 20-21 April (Table 15), but encounter rates varied among sites, with twice as many butterflies observed at R50 (0.126 checkerspots/m) compared to SCS (0.069/m). Numbers were substantially lower at both R76 (0.024/m for transects 1-16; 0.014/m for transects 1-12) and GHP (0.013/m) compared to both R50 and SCS. ***Very low numbers and the apparent dispersal of the R76 population make it unlikely we can collect the requisite adults needed for captive rearing and reintroduction in 2017. Reasons for these trends are unclear but habitat condition and/or military training impacts cannot be ruled out as primary causes, since we do not see similar trends at other sites. Either way, decline or loss of the R76 population could endanger both reintroduction and recovery efforts underway in the Puget lowlands.*** Long-term monitoring and population goals developed in fall 2012 were used to assess progress at R50, which exceeded the target of 250 adults on a single day, based solely on reproduction, for the fourth consecutive year in 2015, with a peak single day abundance estimate of 1,730 adults on both 18 and 27 April. The peak raw count for 2016 (1,246; Table 15) was double that in 2015, and should exceed the single day target for the fifth consecutive year. Adults were again distributed across the majority of the 22-ha monitoring area in 2016, and increased their spread relative to 2014 and 2015. These measures render this site an officially established population based on the project definition herein. Using the same definition, analyzed distance data show that SCS also exceeded the target of 250 adults in a single day abundance estimate in 2015 with a single day estimate of 336 (range 246-459), which was based solely on reproduction. While the release of larvae in spring 2016 will restart the five-year assessment period beginning in 2017, the robust counts recorded there in 2016 suggest that site is well on its way to establishment.

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 by 2022. This project employs a strategy of captive rearing and reintroduction. This report mainly summarizes activities from the 2015-2016 field season, although some longer term summaries are included. Objectives for 2016 were to:

1) Identify areas for release of larvae in collaboration with land managers,
2) Maintain two captive propagation facilities and produce at least 3,000 postdiapause Taylor's checkerspot larvae for release,
3) Conduct postdiapause larval releases at two reintroduction sites (Glacial Heritage and Training Area 7S),
4) Monitor the success of the reintroduction as follows:
    a. Evaluate release success using postdiapause larval and adult surveys at reintroduction sites (Glacial Heritage and Training Area 7S),
    b. Evaluate reintroduction success based on presence, distribution and relative abundance of adults at three past reintroduction sites (Scatter Creek South. Range 50, and Pacemaker)
    c. Evaluate short-term reintroduction success on one site based on reproduction, and
    d. Document actual or potential colonization sites in the vicinity of reintroduction sites.

This set of objectives was accomplished with joint funding provided 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 Department of Corrections, and ACUB cooperators. To insure clarity and cohesion, this report covers all activities involved in captive propagation and reintroduction of Taylor's checkerspots in 2015-2016, regardless of funding source.

This report is organized around the five project objectives that follow. Some objectives include several smaller tasks and objectives, which are emphasized by additional headings in the text.

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

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

III. Release captive and associated wild stock

IV. Monitor success of the reintroductions

V. Conduct long-term monitoring and evaluate progress towards meeting population goals

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

Methods

A suite of historical 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 is to return degraded grasslands to a forb-rich condition with dense and diverse host and nectar plants in a low, open vegetation structure (Fimbel and Dunn 2013). Priority host and nectar species have been identified (Linders 2014) and restoration targets described (Waters et al. 2015) which are designed to insure access to food plants (host and nectar), basking and roosting sites, and oviposition locations. Habitat conditions on occupied and potential reintroduction sites are assessed using the Rapid Habitat Assessment (RHA) protocol, developed in 2013 as part of the ACUB Taylor's checkerspot Habitat Enhancement project and conducted in cooperation with JBLM (see Linders 2014, Waters et al. 2015, and Waters et al. 2016). This protocol is being used to collect standardized baseline data for comparison against a set of Habitat Assessment Criteria (Linders 2015a). Data are being used to: 1) set quantifiable restoration targets, 2) measure progress toward achieving them, and 3) determine site readiness for reintroduction. Project partners also visit a subset of sites annually to discuss habitat conditions, project progress and outstanding needs.

Final site selection for reintroduction occurs on an as-needed basis with input from all stakeholders including ACUB cooperators, USFWS and JBLM personnel. A site may be deemed suitable to receive larvae when at least 1,500 square meters (0.37 ac) of habitat (or 1 sq m per larva) with abundant host plants including at least 20,000 square meters (about 5 acres) of Reintroduction Ready habitat have been prepared (Linders 2015a). These figures originate from field observation at Range 76, where 1) postdiapause larvae have often been observed at a density of 1 larva per square meter and 2) an approximation of the spread of adults in the first year following release. Land managers are expected to restore at least two adjacent 5-ac blocks of Reintroduction Ready habitat prior to the initial release of Taylor's checkerspot larvae, adding at least one 5-ac block annually for the next 3 years for a total of 25 acres of Reintroduction Ready habitat as well as 25 ac of additional habitat to support expansion and establishment of the population (Linders 2015a). Ideally all restoration would be complete prior to initiating reintroduction, but burn bans, seed shortages and other difficult-to-predict events has made this challenging. Some issues are within our ability to address, but others are weather related and beyond the control of our conservation collective. Releases were scheduled to occur at GHP (5^{th} year) and TA7S (3^{rd} year) in 2016, consistent with our strategy of five consecutive years of release (Linders et al. 2015).

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). Four sites, SCS (2007-2014, 2016), R50 (2009-2011) GHP (2012-2016), and TA7S (2014-2016), have received multiple sequential Taylor's checkerspot releases consistent with the scheduled approach (Appendix A); one site (PCM) received a single release (2012), after which releases were restricted. Three release areas were established at each active reintroduction site (SCS and GHP); total area measures are listed in Table 1. While SCS was slightly below the desired area, it still provided more than 1 square meter per released larva (see Postdiapause release and Appendix A). Release plots at GHP exceeded the minimum habitat requirement (1500 square meters). Two of these release areas were used in previous reintroductions, but were considered unoccupied at the time of release.

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

Our objective with respect to captive propagation is to produce the target numbers of eggs, larvae and adults for reintroduction as proposed (Linders 2015b). 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.

Table 1. Size of release plots and total release area for Taylor's checkerspot larvae at two reintroduction sites, Scatter Creek South (SCS) and Glacial Heritage Preserve (GHP), South Puget Sound, Washington, spring 2016.

Site/plot	Sq Meters	Hectares	Acres
SCS			
Plot K	495	0.049	0.122
Plot L	753	0.075	0.186
Plot M	238	0.023	0.059
Site total	**1486**	**0.149**	**0.367**
GHP			
Plot B	315	0.031	0.077
Plot C	560	0.056	0.138
Plot J	795	0.080	0.196
Site total	**1670**	**0.167**	**1.411**

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

Collection of wild checkerspots

Methods

To increase the number of founders contributing to reintroduction and reduce the potential influence of captive propagation on stock used for reintroduction, we collect wild females from the source population at R76 each year (Fig. 1). 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). In fact, single day population estimates often exceed 1000 adults (Linders 2010, 2011b, 2012, Linders and Lewis 2013, Linders et al. 2014, 2015). Should something happen to the wild

Figure 1. Wild females are collected from the source site at Range 76 on Joint Base Lewis-McChord in spring 2016, South Puget Sound, Washington. At the rearing facilities they are placed on oviposition plants. Their offspring become the next generation of larvae available for captive mating or release at reintroduction sites.

population, 10 females per rearing facility annually is considered 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 (5,000 per facility) needed, with the remaining third (n = 3330) supplied by captive-mated females. Wild females are cared for according to established procedures (Barclay et al. 2009, Lewis et al. 2013).

Results and Discussion

A total of 39 females (19 to Oregon Zoo and 20 to Mission Creek) and one misidentified male were collected from R76, 20 each on 26 April and 6 May 2015. These were split between rearing facilities and delivered the same day following methods reported previously (Linders and Lewis 2013).

Captive mating

Methods

A total of 189 adults from 8 maternal lines originating as eggs in 2014 (14FL), were available for inclusion in the captive breeding colony at the Oregon Zoo in 2015. Similarly, 158 adults from 6 maternal lines were included in the breeding colony at the Mission Creek facility (Table 2). Following eclosion, adults were processed and housed according to established procedures (Barclay et al. 2009, Lewis et al. 2013). As in years past, we employed a daisy chain strategy for pairing males and females from different lines (Schad 2008; Lewis et al. 2013). Daisy chains were created using a female driven, late binding strategy recommended in 2014 (Lewis et al., 2015a). A total of 3 daisy chains were created between the two facilities, one at Mission Creek and two at the Zoo (e.g., Fig. 2, Table 3). The first matriline entered the chain when 40% of postdiapause larvae pupated. The remaining matrilines were added to the chain as females from those lines eclosed, insuring males would be available for mating when females are fresh.

Table 2. Number of male and female adult Taylor's checkerspots by matriline available for captive rearing at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington (from Lewis et al. 2016, Dorman et al. 2016), and number provided for release in South Puget Sound, Washington, spring 2015.

Matriline	Females	Males	Released Females	Released Males
Oregon Zoo				
14FL22	13	7	9	6
14FL23	12	13	8	5
14FL25	9	15	6	7
14FL28	17	11	12	8
14FL73	13	15	9	10
14FL77	11	7	6	6
14FL80	17	12	12	3
14FL81	13	4	9	1
OZ Total	**105**	**84**	**71**	**46**
Mission Creek				
14FL21	8	11	5	9
14FL24	11	9	9	5
14FL26	14	19	9	10
14FL30	20	12	16	11
14FL31	14	15	9	13
14FL34	16	9	10	7
MC Total	**83**	**75**	**58**	**55**

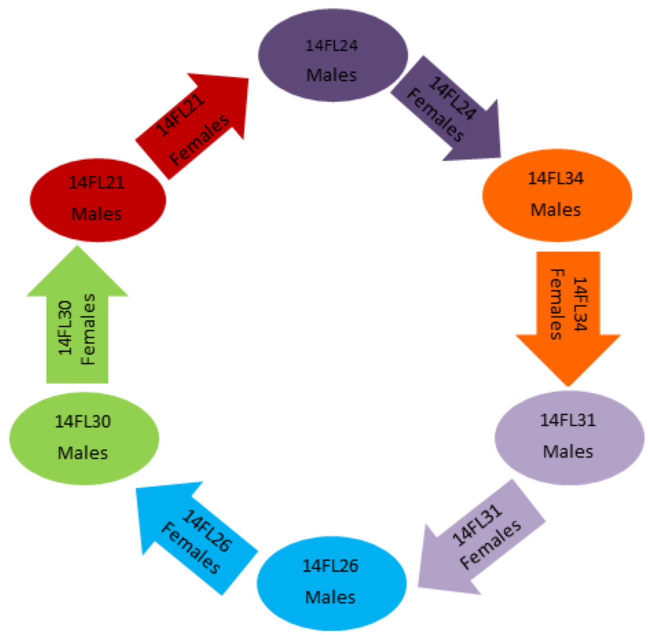


Figure 2. “Daisy chain” pairing strategy used to manage Taylor’s checkerspot breeding introductions in 2015 at Mission Creek Corrections Center for Women, Belfair, Washington.

Breeding introductions for the 14FL cohort were conducted according to established protocols (Barclay et al. 2009) with several modified procedures (Lewis et al. 2013, Hamilton et al. 2014). Our objective was to obtain four copulations from each mating dyad, twice our usual effort, in order to support research conducted by Nate Haan at the University of Washington-Seattle, on prediapause larval survival relative to three host plant species. Males were allowed to copulate only once to increase the genetic contribution from each line. Following copulation, females were moved to oviposition chambers (Fig. 3). All adults that were physically fit at the completion of the mating period were released (see Adult release).

Results and Discussion

Between 16 April and 7 May 2015, the Oregon Zoo conducted 57 planned breeding introductions, resulting in 30 copulations (Table 3), which was nearly twice the rate reported in 2014. Nineteen females did not copulate despite seven of those having multiple opportunities. All but two breeding introductions included more than one male, and two introductions resulted in copulation despite having only two males available. Providing supplemental heat in advance of introductions is believed to have improved male performance.

At the Mission Creek facility, the first copulation occurred on 16 April and the last on 4 May 2015. A total of 30 copulations resulted from 188 breeding introductions (Table 3); 51 females did not copulate despite having multiple opportunities. Copulation rates reported by Mission Creek in the past (Aubrey 2012, Hamilton et al. 2013, 2014, 2015) were calculated using a different definition for “introduction”, and cannot be compared to the current method. Copulations occurred among five of the six recommended breeding dyads, and after numerous failed attempts to get the 14FL31 females to copulate (Fig. 2), five remaining females and 17 males were placed into one large tent. Four of the females copulated and produced eggs, but no eggs hatched (see next section); it is uncertain whether

Table 3. Summary by dyad of Taylor's checkerspot captive mating efforts, including matriline of origin (IDs), and numbers of individual females, copulations, rate (proportion), and number of productive females (i.e., those that laid eggs that hatched), conducted at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington (from Lewis et al. 2016, Dorman et al. 2016), spring 2015.

Female ID	Male ID	Introductions	Females	Copulations	Copulation rate	Productive females
Oregon Zoo						
14FL22	14FL25	7	7	4	0.57	1
14FL23	14FL22	7	6	3	0.43	2
14FL25	14FL28	8	6	3	0.38	3
14FL28	14FL23	10	8	4	0.40	2
14FL73	14FL81	8	6	3	0.38	2
14FL77	14FL73	7	6	5	0.71	3
14FL80	14FL77	6	6	4	0.67	3
14FL81	14FL80	4	4	4	1.00	3
OZ Total		**57**	**49**	**30**		**19**
Mission Creek						
14FL21	14FL24	20	13	5	0.16	3
14FL24	14FL34	23	12	4	0.20	1
14FL26	14FL30	59	14	4	0.18	1
14FL30	14FL21	33	13	5	0.23	5
14FL31	14FL26	22	15	6	0.07	0
14FL34	14FL31	31	14	6	0.26	3
MC Total		188	**81**	**30**		**13**

this is the result of poor copulation or a failure of fertile eggs to develop. Alternatively, there may be unknown genetic issues affecting the likelihood that certain dyads will pair and produce fertile offspring.

Overall, the rearing facilities met their objective to obtain four copulations per dyad in 11 of 14 cases, and exceeded that in 5 cases (Table 3). Unfortunately, many of these females did not produce eggs that hatched. One of our objectives in conducting captive breeding is to develop and maintain the skills required in the event that the source population becomes compromised or is lost. Many lessons have been learned as a result of these efforts, but the process remains challenging and somewhat unpredictable. We have previously reported on the effects of sunlight on both the likelihood that adults will mate as well as egg development rates (Linders 2010). Julia Low, the Lead Butterfly Keeper at The Oregon Zoo, reports that they often have multiple copulations on some days, but little or no success on many others. However environmental conditions are just one set of factors affecting reproductive success. Genetics also influence mate choice and reproductive success. In the absence of detailed genetic data on the R76 population, we assume all females we collect are equally (un)related, and collect from dispersed locations across the site to reduce the likelihood of collecting siblings. This stop-gap measure was not intended to become a long-term approach. The R76 population dropped to very low numbers in 2009 and seemingly again in 2016, with a significant reduction and shift in distribution in recent years (Linders et al. 2015, see also Adult distribution below). We know nothing about this isolated population prior to its discovery in 2003, but there is a reasonable likelihood that the R76 population has undergone significant selection pressure as a result of habitat loss, low population size, and isolation, factors which are known to reduce reproductive fitness (Reed and Frankham 2003, Saarinen 2009). Given the critical importance of the R76 population to recovery efforts in the Puget lowlands, its precarious status and ongoing threats at this site, we highly recommend that a detailed genetic assessment of this population and associated reintroduced populations be conducted as soon as possible to avoid and minimize adverse effects on the success and future viability of the captive propagation and reintroduction program.

Captive rearing, oviposition to adult

Methods

Taylor's checkerspots readily oviposit in captivity. Our objective was to collect about 150 eggs from each gravid wild and captive female to meet our captive rearing target of 10,000 eggs in 2015 (Linders 2015b). We also wanted to produce an additional 54 clusters of about 20 eggs each to support the UW-Seattle prediapause research. 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; this is the point when 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). Female's choice of oviposition host plant species 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 were checked periodically throughout the diapause period following procedures outlined in Barclay et al. (2009). Keepers at the Oregon Zoo also noted the number of larvae that were active in each cup during each diapause check (Lewis et al. 2016). To gain more information about larvae that are active during diapause, a fresh *Plantago* leaf was added to each of two diapause cups beginning on 4 December 2015 and replaced once each week until wake-up; none of the leaves were consumed by larvae. We refer to the time period from diapause initiation until larvae are moved outdoors as "warm" diapause; the remainder of the diapause period we term "cold" diapause as this state is maintained through most of the winter

Figure. 3. Outdoor oviposition set-up employed at the Oregon Zoo, Portland, Oregon in spring 2015.

months. Upon removal from diapause, larvae were placed in a high humidity environment and cared for according to established procedures (Barclay et al. 2009; Lewis et al. 2012; Lewis et al. 2013). Once they began to eat, a subset of larvae from wild females captured in 2015 was retained at each facility for inclusion in the 2015 breeding colony. The remaining larvae, including all offspring of captive-mated females, were released. Prior to release, postdiapause larvae are reared indoors for a few weeks to reduce the likelihood they will re-enter diapause, however because of the warm conditions in winter 2016 all Zoo larvae destined for release were processed and reared in an outdoor shelter out of concern they were too far ahead of their wild counterparts.

Results and Discussion

Sixteen of 19 wild females laid eggs that hatched at the Oregon Zoo in 2015, as did 17 of 20 wild females at Mission Creek (Table 4). In contrast, 19 of 30 captive-mated females laid eggs that hatched at the Oregon Zoo, with that number dropping to 13 of 30 captive females producing viable eggs at Mission Creek (Table 5). Only females with eggs that hatched (i.e., productive) are included in the tables. A total of 10,317 eggs were produced at the two facilities between 20 April and 21 May 2015, with 43.1 percent (4,451) of eggs produced by captive-mated females (Table 5) and 56.9 percent (5,866) produced by wild females (Tables 4). Wild females at the Zoo produced 57.9 percent of eggs, with 42.1 percent produced by captive females. Conversely, at Mission Creek, wild females produced 44.3 percent of the eggs, with captive-mated females contributing the remaining 55.7 percent (Tables 4 and 5).

Eggs from wild females at the Oregon Zoo began hatching on 8 May and finished hatching on 3 June 2015. At Mission Creek they were slightly later, with wild eggs hatching between 15 May and 7 June 2014. Eggs from captive females were similar, hatching between 3 and 28 May at the Oregon Zoo, and from 7-29 May 2015 at Mission Creek.

Larvae from wild females at the Oregon Zoo had a lower hatch rate than those at Mission Creek (Table 4). Hatch rates for eggs from captive females at both facilities (Table 5) were lower than wild females, with the lowest rate observed at Mission Creek (Table 5). The greatest losses are typically observed from egg to hatching, and can be highly variable from year to year (Linders 2010, 2011b, 2012, Linders and Lewis 2013, Linders et al. 2014, 2015). Hatch rates for 2015 are on the lower end of those we've observed, but are not as low as those observed in 2010.

On 19 June 2015, late prediapause larvae from the second group of wild females were still feeding at the Zoo. Larvae suddenly became sick, with symptoms that included vomiting and diarrhea; some mortality also occurred (Table 4). All rearing bins were cleaned, fresh food was given and larvae were sent to Mississippi State University for disease testing; tests were negative. The sudden onset and nature of the symptoms led keepers to suspect the food. Food removed from the bins, food from the Zoo grounds and from the nearby Arboretum (a back-up source) was sent to Synergistic Pesticide Lab in Portland for toxicity testing. Samples were screened for hundreds of chemicals. All tests came back negative except the food taken from the bins with sick larvae, which tested positive for Chlorothalonil at a level of 0.02 ppm. Dr. John Stark, Professor of Ecotoxicology at Puyallup Research and Extension Center and an entomologist working with the USFWS on butterfly toxicology in California, indicated this seemingly low level of toxin was sufficient to be of concern. Chlorothalonil is "an organic compound mainly used as a broad-spectrum, non-systemic fungicide with other uses as a wood protectant, pesticide, acaricide, and to control mold, mildew, bacteria, and algae" (Wikipedia: https://en.wikipedia.org/wiki/Chlorothalonil). It is known to be highly toxic to fish and marine invertebrates, although it is listed as "relatively non-toxic" to honey bees. Chlorothalonil is a commonly-used household chemical that is widely available on the market and is thought to break down quickly in the environment. Interestingly, larvae that became ill in 2013 had also consumed food from the Arboretum (Linders et al. 2014). However, Hoyt Arboretum

Table 4. Number of eggs laid and persistence to diapause for offspring of wild-caught female Taylor's checkerspots collected at the Range 76 source site and reared at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2015 (from Lewis et al. (2016) and Dorman et al. (2016). Number hatched is taken at 3rd instar but includes any mortality to that point.

Female ID	# eggs	# hatched	Hatch rate	# into diapause
Oregon Zoo				
P15FL01	277	178	0.64	178
P15FL02	128	98	0.77	93
P15FL04	11	3	0.27	3
P15FL05	299	241	0.81	227
P15FL06	272	161	0.59	158
P15FL07	326	245	0.75	207
P15FL08	350	210	0.60	190
P15FL09	262	244	0.93	229
P15FL10	311	171	0.55	168
P15FL13	388	277	0.71	147
P15FL14	105	49	0.47	35
P15FL15	29	28	0.97	4
P15FL16	128	59	0.46	14
P15FL18	141	52	0.37	4
P15FL19	51	52	1.02	1
P15FL20	16	2	0.13	0
OZ Total	**3094**	**2070**		**1658**
Ave (SD)	**193.3 (130.1)**	**129.4 (96.3)**	**0.60 (0.24)** [1]	**103.6 (92.3)**
Mission Creek				
P15FL51	217	118	0.54	114
P15FL52	291	268	0.92	266
P15FL53	218	211	0.97	204
P15FL54	244	149	0.61	147
P15FL55	294	229	0.78	226
P15FL56	125	177	1.42	176
P15FL57	182	125	0.69	126
P15FL58	173	151	0.87	150
P15FL59	156	165	1.06	162
P15FL61	81	77	0.95	73
P15FL63	30	31	1.03	31
P15FL64	71	70	0.99	70
P15FL65	100	17	0.17	17
P15FL66	121	65	0.54	61
P15FL67	158	113	0.72	108
P15FL68	72	63	0.88	62
P15FL69	239	196	0.82	193
MC Total	**2772**	**2225**		**2186**
Ave(SD)	**163.0 (79.3)**	**130.9 (71.6)**	**0.75 (0.22)**[1]	**128.6 (70.9)**

[1] Omitted cases with hatch success >1.0 hatchling per egg laid.

Table 5. Females ID, copulation date and duration (minutes), number of eggs laid and persistence to diapause for offspring of captive-mated Taylor's checkerspot females at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, Spring 2015 (from Dorman et al. (2016) and Lewis et al. (2016). Number hatched is taken at 3rd instar but includes any mortality to that point.

Female ID	Date	Duration (min)	# eggs	# hatched	Hatch rate	# to diapause
Oregon Zoo						
14FL22-07	20-Apr	56	201	29	0.14	27
14FL23-19	25-Apr	115	39	4	0.10	3
14FL23-20	21-Apr	65	200	63	0.32	62
14FL25-06	21-Apr	138	133	95	0.71	93
14FL25-12	17-Apr	100	64	39	0.61	40
14FL25-15	21-Apr	55	126	98	0.78	94
14FL28-13	19-Apr	75	221	193	0.87	187
14FL28-16	20-Apr	42	77	8	0.10	8
14FL73-15	7-May	75	60	26	0.43	25
14FL73-23	30-Apr	130	28	14	0.50	14
14FL77-09	27-Apr	50	143	8	0.06	8
14FL77-11	27-Apr	70	2	2	1.00	2
14FL77-16	1-May	85	222	102	0.46	96
14FL80-15	27-Apr	80	149	69	0.46	66
14FL80-22	27-Apr	125	104	49	0.47	49
14FL80-24	27-Apr	85	136	102	0.75	96
14FL81-02	17-Apr	70	49	1	0.02	1
14FL81-04	20-Apr	58	122	5	0.04	5
14FL81-07	25-Apr	150	170	26	0.15	24
QC[1]						16
OZ Total			**2246**	**933**		**916**
Ave (SD)[2]		**85.5 (32.0)**	**118.2 (66.9)**	**49.1 (50.6)**	**0.42 (0.31)**	**47.4 (48.7)**
Mission Creek						
14FL21-02	18-Apr	37	133	3	0.02	3
14FL21-05	17-Apr	48	261	248	0.95	241
14FL21-24	2-May	56	133	133	1.00	59
14FL24-31	22-Apr	-	124	18	0.15	18
14FL26-31	21-Apr	-	237	21	0.09	7
14FL30-01	16-Apr	65	215	1	0.00	1
14FL30-04	18-Apr	40	197	45	0.23	42
14FL30-11	17-Apr	46	96	4	0.04	4
14FL30-14	19-Apr	-	314	53	0.17	53
14FL30-32	19-Apr	77	30	11	0.37	11
14FL34-06	21-Apr	26	201	126	0.63	126
14FL34-13	24-Apr	63	205	68	0.33	68
14FL34-18	24-Apr	45	59	4	0.07	4
QC[1]						26
MC Total			**2205**	**735**		**663**
Ave (SD)[2]		**50.3 (15.1)**	**169.6 (82.0)**	**56.5 (73.0)**	**0.31 (0.34)**	**49.0 (68.3)**

[1] Quality Control (QC) larvae became separated from their cohort and original ID.
[2] QC larvae excluded from average and standard deviation.

had no record of Chlorothalonil use in the last 10 years. Testing larvae for chemical exposure has been challenging because most pre-existing toxicity screens are for plants, and there are thousands of potential compounds to test for. So identification of Chlorothalonil as a potential toxin was ground-breaking news. In order to implicate it as the cause of the illness, however, the larvae needed to contain critical levels of the toxin. In addition, we just learned that Chlorothalonil produces one metabolite, but that metabolite is 30 times more toxic than the Chlorothalonil itself. We recently identified Synergistic Pesticide Lab in Portland, Oregon as a lab capable of doing a larval assay with a lower threshold limit than many of the larger labs, but we are as yet uncertain whether they can also test for the metabolite.

In all, 5963 prediapause larvae (4,295 wild, 1,668 captive) were produced, with 5,397 of those (3,844 wild and 1,553 captive) surviving to diapause (Tables 4 and 5). Prediapause larvae from the 15FL cohort (young of the year) entered diapause at the Zoo between 4 June and 31 July, with all larvae at Mission Creek entering diapause between 15 June and 6 July 2015.

Diapausing larvae were moved outdoors on 13 August at the Oregon Zoo, and on 11 September 2015 at Mission Creek. Once in diapause most larvae remain inactive, although some movement is normal, especially in late diapause. About 8-10 percent of larvae at the Zoo were active in diapause between 4 December 2015 and mid-January 2016, followed by a sharp increase to 30 percent movement on 30 January. As of 6 February 2016, 27 percent of larvae were still active and 100 larvae had died, so all overwintering larvae were removed from diapause on 12 February 2016; larvae at Mission Creek were also removed from diapause that day. Larvae at Mission Creek had showed increased levels of movement starting on 17 January 2016 and continued to increase through the end of the month, which follows an increase in nighttime temperatures (Fig. 4). Warm nighttime temperatures were also thought to play a role in the unusual late diapause mortality observed at the Zoo in 2015; data from Mission Creek in 2016 appear to corroborate that hypothesis. None of the food placed in diapause cups at the Oregon Zoo in winter 2016 was ever consumed, suggesting larvae were warm enough to be active but not warm enough to eat, and thus burning energy reserves they could not replenish.

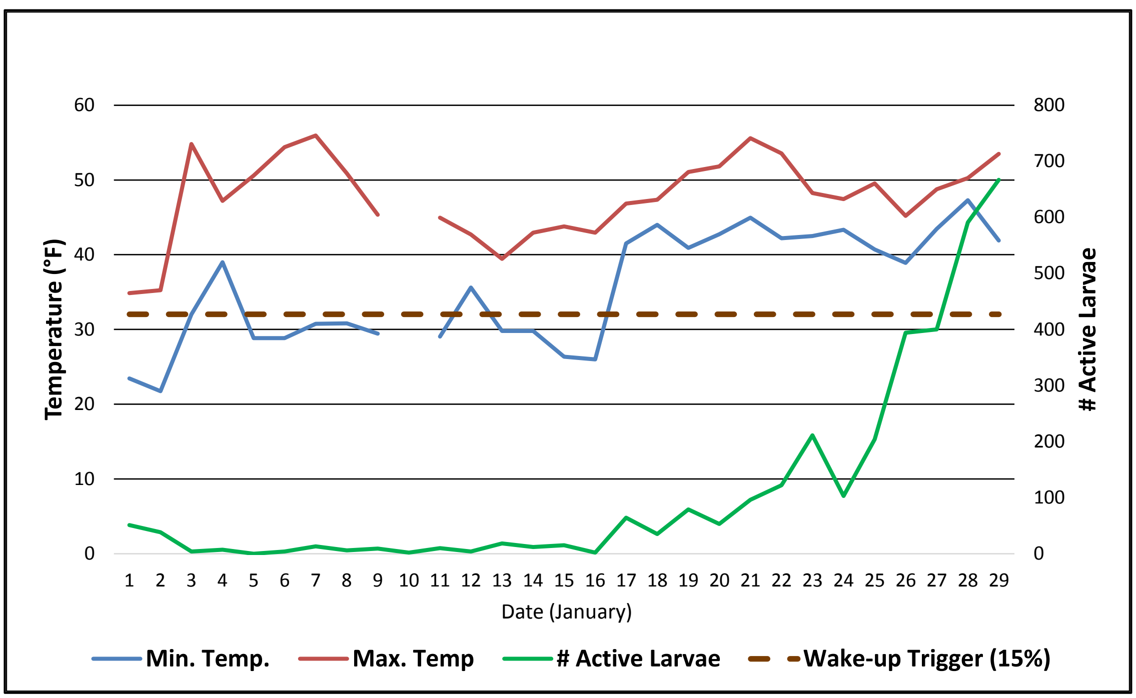


Figure 4. Number of larvae active relative to minimum and maximum temperatures by day at the Mission Creek Corrections Center for Women, Belfair, Washington, in January 2016 (courtesy of S. Dorman).

Seventeen percent (445) of larvae that entered diapause at the Oregon Zoo in 2015 died prior to wake-up and an additional 581 (22%) larvae died in the two weeks prior to release (Table 6); these larvae were

Table 6. Numbers of Taylor's checkerspot larvae surviving diapause and available for release by matriline at the Oregon Zoo, Portland, Oregon and Mission Creek Corrections Center for Women, Belfair, Washington, Summer 2015 to spring 2016 (from Dorman et al. (2016) and Lewis et al. (2016).

Matriline	Diapause in	Diapause out	Dead pre-release	Release
Oregon Zoo				
14FL22	10	3	3	0
14FL23	5	2	2	0
14FL25	4	1	1	0
14FL73	2	2	2	0
14FL77	10	5	5	0
14FL81	5	0	0	0
Subtotal	36	13	13	0
15FL01	178	152	56	66
15FL02	93	68	24	14
15FL04	3	3	1	2
15FL05	227	126	72	24
15FL06	158	152	11	111
15FL07	207	139	52	57
15FL08	190	157	45	82
15FL09	229	201	82	89
15FL10	168	166	15	121
15FL13	147	135	27	78
15FL14	35	29	3	26
15FL15	4	4	0	4
15FL16	14	13	2	11
15FL18	4	1	0	1
15FL19	1	4	0	4
Subtotal	1658	1350	390	690
15OZ22	27	22	15	7
15OZ23	65	42	23	19
15OZ25	227	186	58	128
15OZ28	195	158	66	92
15OZ73	39	39	4	35
15OZ77	106	106	1	105
15OZ80	211	210	9	201
15OZ81	30	30	0	30
QC[1]	16	9	2	7
Subtotal	900	793	176	617
OZ Total[1]	**2,610**	**2,165**	**581**	**1,314**

Matriline	Diapause in	Diapause out	Dead pre-release	Release
Mission Creek				
14FL21	19	18	0	0
14FL24	15	15	0	0
14FL30	6	6	0	6
14FL31	1	1	0	1
14FL34	8	8	0	8
Subtotal	49	48	0	15
15FL51	114	104	0	74
15FL52	266	266	0	236
15FL53	204	149	0	119
15FL54	147	147	0	117
15FL55	226	223	1	192
15FL56	176	176	1	145
15FL57	126	126	0	96
15FL58	150	150	0	120
15FL59	162	160	0	130
15FL61	73	73	0	73
15FL63	31	31	1	30
15FL64	70	70	0	70
15FL65	17	17	0	17
15FL66	61	60	0	60
15FL67	108	104	0	74
15FL68	62	62	1	61
15FL69	193	193	0	163
Subtotal	2186	2111	4	1777
15MC21	303	298	1	297
15MC24	18	18	0	18
15MC26	7	7	0	7
15MC30	111	108	0	108
15MC34	198	193	0	193
QC[1]	26	97	5	92
Subtotal	637	624	1	623
MC Total[1]	**2898**	**2880**	**10**	**2507**

[1] QC larvae included in facility totals only; 65 of the QC larvae in "Diapause out" from either 15FL53 or 15FL54.

ascribed to diapause mortality (see Survival). While this mortality was disappointing, it was a significant improvement over diapause survival in 2015 (Lewis et al. 2015), when mortality was thought to be the result of warm winter conditions during diapause (Linders et al. 2015), particularly warm nighttime temperatures. Similar conditions were observed in 2016 (see Rearing conditions below). While food contamination during late prediapause may also have contributed to higher diapause mortality rates in 2016, both captive and wild groups experienced higher than usual diapause mortality, even though only offspring of wild females appeared to have consumed the contaminated food.

On 23 February 2016, 740 from the Oregon Zoo and 999 larvae from Mission Creek were provided to WDFW for release at Scatter Creek South. In addition, 576 larvae from the Oregon Zoo and 1478 larvae from Mission Creek were provided to WDFW for release at Glacial Heritage Preserve on 25 Feb 2016. The Oregon Zoo retained 270 of 960 wild larvae for breeding from nine 2015 matrilines, with Mission Creek retaining 330 of 2,107 larvae from eleven 2015 matrilines, as well as 33 second diapause larvae from two 2014 matrilines for breeding (Table 7). Outcomes of postdiapause larval rearing, including progression to the adult stage, are presented in Table 7.

Table 7. Numbers of Taylor's checkerspots by life stage and matriline from stock retained for breeding at the Oregon Zoo, Portland, Oregon and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2016 (from Dorman et al. (2016) and Lewis et al. (2016). Improperly eclosed (IE) adults are a subset of all adults.

Matriline	Larvae	2^{nd} diapause	Pupae	Adults	IE	Males	Females	Adult release
Oregon Zoo								
15FL01	30	9	13	13	1	5	8	9
15FL02	30	13	12	12	1	5	7	6
15FL05	30	10	8	7	1	3	4	4
15FL06	30	15	9	9	1	4	5	4
15FL07	30	7	21	21	0	11	10	14
15FL08	30	13	13	13	0	6	7	8
15FL09	30	12	11	11	2	6	5	7
15FL10	30	4	22	19	1	12	7	15
15FL13	30	7	22	21	0	15	6	15
OZ Total	**270**	**90**	**131**	**126**	**7**	**67**	**59**	82
Mission Creek								
15FL51	30	23	7	7	1	3	4	2
15FL52	30	0	30	29	1	18	11	10
15FL53	30	0	30	30	0	13	17	10
15FL54	30	9	21	20	1	11	9	6
15FL55	30	4	26	25	3	10	15	11
15FL56	30	0	30	30	2	14	16	14
15FL57	30	10	20	20	1	10	10	5
15FL58	30	1	27	27	1	13	14	16
15FL59	30	0	30	28	2	14	14	10
15FL67	30	0	30	27	0	13	14	20
15FL69	30	0	29	29	0	11	18	14
14FL21	18	1	17	15	0	6	9	10
14FL24	15	0	13	13	2	9	4	3
MC Total	**363**	**48**	**310**	**300**	**14**	**145**	**155**	**131**

Second diapause return rates varied considerably between institutions in 2016, with one third (90 of 270) of larvae returning to second diapause at the Oregon Zoo, compared to 48 of 363 larvae (13.2 percent) at Mission Creek (Table 7). The rate at Mission Creek was lower than in both 2014 and 2015 (Linders et al. 2014, Linders et al. 2015), whereas rates of diapause return at the Oregon Zoo were similar to 2014 and higher than in 2015 (Linders et al. 2014, 2015). Multi-diapause larvae are the offspring of wild females; they are retained and paired with other multi-diapause adults whenever possible because this trait is likely important for population persistence and genetic mixing across years.

Similar to 2015, the first pupa was found at the Oregon Zoo on 30 March and the last on 19 April 2016, whereas larvae at Mission Creek pupated between 12 March and 3 April 2016. Adults at the Oregon Zoo eclosed between 9 and 28 April 2016, whereas those at Mission Creek eclosed between 15 and 28 April 2016. A total of 426 adult checkerspots (214 females and 212 males) were produced for the 2016 captive breeding colony between facilities at The Oregon Zoo and Mission Creek (Table 7).

Rearing conditions

Methods

Our initial strategy with respect to identifying suitable environmental conditions for captive propagation was to mimic ambient conditions as much as possible, because instituting environmental controls assumes that detailed knowledge exists on which conditions maximize survival. In an effort to gain more clarity on factors affecting survival and improve our success during mating and diapause, we revised our target environmental conditions in late fall 2015 based on existing data, expert opinion and field observation. These refined targets (Table 8) attempt to quantify values that were previously described as "ambient" and exclude conditions that may be detrimental. This was necessary because ambient conditions at the Oregon Zoo and Mission Creek were sometimes markedly different, and the warm weather that has predominated since 2014 has presented new and unexpected challenges. The new targets also improve our ability to make comparisons between desired conditions and those actually observed. We expect that these targets will undergo additional modifications as we have the opportunity to delve more deeply into existing data.

Supplemental light and heat are needed at the Oregon Zoo to achieve target conditions, whereas shade and cooling are required in the Mission Creek greenhouse to achieve those same targets (Table 8). At the Zoo heat and basking lamps are used to increase temperatures, but otherwise the rearing facility is unheated; there is currently no mechanism to actually cool the facility, although there are plans to upgrade this system. Temperature and relative humidity (RH) during all rearing stages were measured as min/max data with HOBO data loggers set to take a reading every two hours. These devices were kept with the animals at all times to document microclimate conditions, except that sensors were placed outside of oviposition chambers when they were moved outdoors at the Oregon Zoo.

Results and Discussion

Temperature (°F) and relative humidity (percent RH) metrics recorded at the Oregon Zoo and Mission Creek in 2015-2016 are presented in Table 9. Rearing conditions at the Oregon Zoo were generally on target from the adult life stage through warm diapause, although RH was lower than desired during the adult period. Heat lamps used during the day may have contributed to the reduced RH values for adults and yet were suboptimal for maintaining temperature increases during specified periods (e.g., 78-85 for 2-6 h for males), so new techniques will be considered. Temperature conditions during cold diapause and postdiapause were warmer than desired at night, with daytime temperatures during postdiapause a bit cooler than desired on 10 of 47 days, although RH during this period was generally suitable. Finally, target temperature conditions were maintained during the pupation period, although RH was quite low

(Table 9). Low RH values for pupal and adult stages may be reflective of the warm dry spring we experienced in 2016.

Rearing conditions at Mission Creek mirrored those at the Zoo. The reported average duration of daily temperatures of 78-85° F for males at Mission Creek (Table 9) was below the target, but as with the Zoo, this value is misleading since most days temperatures were either above or below this range.

Table 8. Target temperature (°F) and relative humidity (% RH) conditions for Taylor's checkerspot rearing by life stage and rearing location at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, 2015-2016. Supplemental heat and light was provided only at the Oregon Zoo.

Life stage	Location	Temp (°F)	RH (%)	Temp treatment	Light treatment
Oregon Zoo					
Males	East room	≤85; 78-85 for 2-6 h/day	≥50	40-watt bulb 8 - 9 AM and 1:00-1:30 PM	None
Females and Oviposition	East room	50-90; 78-90 for 4-8 h/day	≥50	40-watt bulb: 10 AM - 2 PM or outside	None
Cooler females	Cooler	55-70	≥50	None	None
Egg & Prediapause	East room	50-90; ave min ≤65	≤65	None	7 AM - 7 PM
Warm diapause	East room	50-90	≥40	None	None
Cold diapause	Outdoor	≤35 for ≥60 days	≥50; ave max ≥90[3]	None	None
Postdiapause	Gazebo West room	≥45° night; ≥65° day	≥50	None	7 AM - 7 PM
Pupa	West room	≥50° night; ≥65° day	≥65	Heat: 7 AM - 7 PM	7 AM - 7 PM
Mission Creek					
Males	Main Room	≤85; 78-85 for 2-6 h/day	≥50	Ice packs	Full shade[1]
Females and Oviposition	Main Room	50-90; 78-90 for 4-8 h/day	≥50	40-watt bulb: 10 AM - 2 PM or outside	Partial shade[1]
Cooler females	Camp Cooler Main	55-70	≥50	None	None
Egg & Prediapause	Main Room	50-90; ave min ≤65	≤65	Ice packs; evaporative cooler	None
Warm diapause	Main Room	50-90	≥40	Ice packs; evaporative cooler	None
Cold diapause	Small Room/Shed	≤35 for ≥60 days	≥50; ave max ≥90	None	None
Postdiapause	Main Room	≥45° night; ≥65° day	≥50	None	None
Pupa	Main Room	≥50° night; ≥65° day	≥65	Mist with spray bottle; humidifier	None

[1] Sheets used to create full or partial shade.

Table 9. Target and actual temperature (°F) and relative humidity (% RH) conditions during Taylor's checkerspot rearing by life stage, including time frame and rearing location at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, 2015-2016. **Bold text** under actual conditions indicates measure was outside the target by more than 10 percent.

		Target conditions		**Actual conditions**			
Life stage	**Date range**	**Temp (°F)**	**Ave min RH (%)**	**Ave temp (°F)**	**Temp range (°F)**	**Ave min RH (%)**	**RH range (%)**
Oregon Zoo[1]							
Males	13 Apr - 6 May 2015	≤85; 78-85 for 2-6 h/day	≥50	86[2]; ave 2.5h/d at 78-85 on 6d	61-**92**	**18**	**12**-85
Females and Oviposition	17 Apr - 21 May 2015	50-90; 78-90 for 4-8 h/day	≥50	69; ave 2.2h/d at 78-85	57-87	**40**	**21**-100
Cooler females	26 Apr - 6 May 2015	55-70	≥50	NA	52-**75**	**36**	**32**-79
Egg & Prediapause	20 Apr - 30 Jul 2015	50-90; ave min ≤65	≤65	71; 67	58-88	57	39-**96**
Warm diapause	31 Jul - 12 Aug 2015	50-90	≥40	76	68-89	46	**28**-94
Cold diapause	13 Aug 2015 - 11 Feb 2016	≤35 for ≥60 days	≥50; ave max ≥90	**21 days ≤35**	26-**86**	78; 89	**35**-100
Postdiapause	26 Feb - 18 Apr 2016	≤45 night; ≥65 day	≥50	**10 of 47 nts ≤45; 37 of 47 d ≥65**	38-**68**; **54**-83	49	**27**-100
upa	30 Mar - 28 Apr 2015	≧50 night; ≥65 day	≥65	30 of 30 nts ≥50; 30 of 30 d ≥65	58-67; 72-80	**43**	**28**-68
Mission Creek							
Males	10 Apr – 14 May 2015	≤85; 78-85 for 2-6 h/day	≥50	74[2]; **ave 1.5 h/d at 78-85**	46-87	**42**	**18**-99
Females and Oviposition	18 Apr – 16 May 2015	50-90; 78-90 for 4-8 h/day	≥50	63; **ave 3 hrs/d at 78-90**	46-87	**42**	**18**-99
Cooler females	NA	55-70	≥50	NA	NA	NA	NA
Egg & Prediapause	7 May – 6 Jul 2015	50-90; ave min ≤65	≤65	67; **59**	55-**95**	52	28-**89**
Warm diapause	15 Jun - 11 Sep 2015	50-90	≥40	70	51-**98**	49	**30**-92
Cold diapause	11 Sep 2015 – 12 Feb 2016	≤35 for ≥60 days	≥50; ave max ≥90	**45 days ≤35**	26-**75**	87; 92	57-99
Postdiapause	2 Feb – 2 Apr 2016	≤45 night; ≥65 day	≥50	**11 of 51 nts ≤45; 24 of 51 d ≥65**	37-**62**; **54**-85	53	**26**-98
Pupa	12 Mar – 3 Apr 2016	≧50 night; ≥65 day	≥65	**8 of 23 nts ≧50; 10 of 23 d ≥65**	**37**-53; **58**-80	**49**	**26**-70

[1] Dates for which data are available vary but are within 7 days of date range presented, except for Males: 24 Apr – 6 May 2015.
[2] Mean maximum temperature.

As this is the first year we are testing these refined targets, we will likely modify this metric in the future to better reflect actual and desired conditions. Unlike conditions at the Zoo, both daytime and nighttime temperatures during pupation at Mission Creek were often cooler than desired, which may have been responsible for poor longevity and reproductive fitness of adults (Dorman et al. 2016). An experimental release of pupae during the very cool spring conditions prevalent in 2008 resulted in a prolonged 6-week eclosion period, with many weak adults and pupae that turned black and failed to eclose (Linders, pers. obs.). The cool conditions at Mission Creek appear to have been an over-correction of the warm nighttime conditions prevalent during the postdiapause period, which led to the first pupa forming on March 12, 8 days earlier than in 2015, and at least three weeks earlier than would be expected in the wild. The prevalence of warmer winter nights but average daily temperatures that have characterized the past several years is consistent with climate change predictions and observed trends in several national studies (Snover et al. 2013, Tebaldi et al. 2013, Arndt 2015). Unusually sunny spring conditions in the past several years have also led to earlier flight seasons and dry spring conditions (see also Appendix B). Such change in weather patterns can have profound effects on a species like Taylor's checkerspot, which is highly responsive to its environment. Weather has been shown to affect populations of the Bay checkerspot (Weiss and Weiss 1998), a related subspecies. Taylor's checkerspot is also highly likely to be affected by changes in weather, though not necessarily in the same manner as for Bay checkerspot because of differences in their phenology and life cycle. Research on weather-related effects on Taylor's checkerspot populations remains a high priority because we continue to observe changes in both the field and the lab that appear to coincide with changing weather patterns. While we cannot alter the weather, it is imperative that we identify environmental factors that drive population change for Taylor's checkerspot, as doing so will improve our ability to manage the species for long-term persistence by predicting significant population changes, especially declines.

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

Survival of captive animals

Methods

We used stage-specific Kaplan-Meier (1958) survival rates to track success within and between years and identify areas for improvement. Survival rates for captive stock were calculated between the following life stages: egg, hatching (3rd instar), diapause, postdiapause, pupa, and adult. We also calculated the rate at which postdiapause larvae returned to second diapause, averting development to the adult stage. Only offspring from females with eggs that hatched are included in egg counts and hatch rates because females can lay eggs without copulating and/or when copulation is inadequate. 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. The number of hatched larvae at 3rd instar includes any larvae that were known to perish prior to that point.

Results and Discussion

Stage specific survival rates for both rearing institutions are presented in Table 10. As in years past, mortality was highest prior to 3rd instar (Linders 2010, 2011b, 2012, Linders and Lewis 2013, Linders et al. 2014, 2015). Hatch rates for 2015 are on the lower end of those we've observed, but are not as low as those observed in 2010. Hatch rates for wild females were notably higher than those for captive-mated females, a factor which has also varied by year. During all remaining life stages survival is typically at or above the 90th percentile at both facilities (Linders 2012, Linders and Lewis 2013, Linders et al. 2014, 2015), except for the high mortality seen at the Zoo following removal from diapause in 2015, which we associated with warm winter temperatures in Portland (Njus 2015). For the most part this pattern was observed again in 2015-2016 (Table 10), although mortality following removal from diapause at the Zoo was considerably lower in 2016 than in 2015. To date, all larvae tested for pathogens have come back negative, and insect disease specialists (A. Lawrence, Research Associate, Mississippi State University, pers. comm.) at Mississippi State University have suggested this mortality is far more likely to be the result of environmental conditions (See Captive rearing results for further discussion). All survival rates remain higher than those expected in the wild (Moore 1989).

Table 10. 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, 2014-2015. Only offspring from females with eggs that hatched are included; number of hatched larvae recorded at 3rd instar. Column labels refer to rearing institution, egg source (Wild - Joint Base Lewis-McChord (FL) or Captive-mated: OZ or MC), and year of origin as an egg (e.g., 15).

Life stage	OZ Wild 15FL		OZ Captive 15OZ		MC Wild 15FL		MC Captive		All 2015 stock[2]	
	#	Survival	#	Survival	#	Survival	#	Survival	#	Survival
Eggs	3094	-	2246	-	2772	-	2205	-	10317	-
Egg to hatch[1]	2070	0.669	933	0.415	2225	0.803	735	33.3	5963	0.578
Hatch to diapause	1658	0.801	900	0.965	2186	0.982	637	86.7	5423	0.909
Diapause to release	960	0.579	617	0.686	2107	0.964	623	97.8	4406	0.812
Postdiapause held	270	---			363	-			633	-
Multi-diapause[3]	90	0.333			48	0.132			138	0.218
Pupation	131	0.728			310	0.984			441	0.891
Eclosion	126	0.962			300	0.968			426	0.966

[1] Hatch rates not adjusted for low egg counts.
[2] QC larvae included here.
[3] Proportion of postdiapause larvae that returned to diapause.

Adult measurements

Methods

Pupae and adults from each captive female line were measured and weighed using standardized procedures (Barclay et al. 2009) for comparison with measures from wild adults. Adult weight and ventral hind wing (left side) photos were also collected on all incoming wild adults at the Oregon Zoo. Hind wing area was calculated from the photos using ImageJ ver. 1.46r (Schneider et al. 2012). 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.

Results and Discussion

Pupal and adult data have been slow to come in from both facilities, however all past data are being compiled for analyses between facilities and relative to available data for wild adults. We expect to complete these comparisons in winter 2017.

III. Release captive and associated wild stock

Postdiapause larval release

Postdiapause larvae are the primary stage for release because they are robust and nearly mature. Larvae were brought to the field housed in labeled deli containers with freshly-cut leaves of *Plantago*; containers were packed in coolers without ice or heat. A total of 1,658 postdiapause larvae were released at SCS on 23 Feb 2016 (327 in Plot K, 789 in Plot L, and 542 in Plot M). On 25 Feb 2016, a total of 2,029 larvae were released at Glacial Heritage Preserve (343 in Plot B, 792 in Plot C, and 894 in Plot J). Of the 3,687 larvae released, 2,377 were offspring of wild females, 1,210 were offspring of captive-mated females, and 100 were of unknown (QC) origin; the latter larvae became separated from their group at some point during the rearing process. Four larvae from Mission Creek and 105 larvae from the Oregon Zoo were dead on arrival and were not released. Numbers reported may not match those in the captive rearing section, but discrepancies are small. Larvae were released in groups of 2-5 on large and/or dense host plants/patches (*Plantago lanceolata or Castilleja hispida*) within restored prairie. Weather on release days was excellent, with temperatures of 8.0 to 16.3 °C, average wind speed of 1.5-4.8 mph, and clear skies. At least 18 people assisted with releases, working from about 1000-1600 h each day to complete the task.

Adult release

Eighty-two adult Taylor's checkerspots (50 females and 32 males) were obtained from the Oregon Zoo on 2 May 2016 for release at TA7S; 2 died in transit with 80 released. On 12 May 2016, 131 adults (103 females and 28 males) from Mission Creek were released at TA7S. All adults released from the rearing facilities were offspring of wild females and were released once their roles in mating trials and oviposition were complete. As in previous years, males and females were co-housed in net enclosures to encourage mating in transit, and released directly into the environment by opening the cage or placing them on nectar plants. In addition, 20 of 21 wild females from R76 that were brought to the University of Washington for oviposition were released at TA7S once egg targets were met; 10 females were released on both 5 and 9 May 2016. Adults on 2 May were released under warm conditions (26.0 °C), 2.4 mph winds and high thin clouds with a distinct shadow. Many adults remained near the release area, although at least 15 of them quickly moved ~50 m west to the oak edge, where annual host plants predominated. During releases on 5 and 9 May, skies were partly cloudy and cooler (18.0 °C) with a light breeze; no one behavior pattern dominated. Weather on the 12 May release was clear and warm (23.0 °C) with a light wind (~3.5 mph); many adults flew west on this day.

Prediapause larval release

In 2016, we collected additional wild females to support prediapause research at the University of Washington, which resulted in an abundance of prediapause larvae at rearing facilities. Consequently, 1,003 prediapause larvae were released at TA7S on 8 Jun 2016 under overcast skies with faint to no shadow. Temperatures were moderate (22.0 °C) with moderate winds (6 mph), which helped insure host plants were in a suitable condition. Many of the released larvae were ready to enter diapause.

IV. Monitor success of the reintroduction

Documenting presence and abundance through various life stages provides near-term measures of persistence and increases the likelihood of detecting factors that affect success. Population targets and monitoring goals are used to evaluate success in population establishment (see below) and demonstrate progress on the way to species recovery.

Document postdiapause larval presence and abundance in release areas

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

Methods

To confirm site occupancy, quantify larval abundance and minimum survival, and identify issues that may be cause for concern, we conducted surveys for postdiapause larvae in 2016 release plots on two occasions in the weeks following release. Larval search methods were the same as those used previously (Linders 2011a). Sampling plots were laid out along transects established in ArcGIS by placing the first transect near the northern border of the release plot with space for at least one plot measuring 2 m wide x 5 m long, then placing each subsequent transect 8 m from the last. The first sampling plot was randomly located 1-3 m from the edge of the release plot 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 with a 1 m separation. In 2016, about one third of these subplots were sampled be a second observer to assess differences in detectability between observers. Dual observer plots were surveyed in succession (within 1 hour), with all larvae counted and mapped, including descriptive clues (e.g., foraging on *Plantago*) to determine how many animals were seen by one vs. both observers. Our objective was to sample about 10 percent of each release plot. At times more than one day may be required to survey a site due to the short duration of suitable weather in March.

Abundance estimates were calculated by survey date for each release area by multiplying the count total for all subplots by the ratio of potential subplots to sampled subplots (Thompson 2012). Survival was calculated by dividing the abundance estimate by the number of larvae released; 90 percent confidence intervals were also calculated. This method is somewhat different that previous calculations, and will be applied to data collected in previous years as time allows.

Results and Discussion

Results of standard release plot surveys conducted at GHP and SCS in 2016 are presented in Table 11. Survey conditions were often challenging in spring 2016, with several surveys spanning more than one day. Double observer plot data for 2016 (Table 12) showed no consistent difference in detectability between observers at GHP, although LTR did observe more larvae in release plots at SCS. This is in contrast to results from 2013-2015 (Linders, *unpub. data*, Linders et al. 2014, 2015), and is likely the result of several factors. Mapping larvae for comparison has shown that they are typically quite sedentary, but can also move quite quickly when they want to. They also employ a variety of behaviors to adjust their exposure to sunlight and wind, which can greatly affect their detectability. For example, on a hot day larvae have been observed basking vertically on the north side of a stem or on the underside of a leaf, whereas in colder weather larvae adjust their basking location to take advantage of warmer microclimates (Fig. 5). Collectively, these results suggest that the number of larvae observed is affected more by the number visible (i.e., not concealed) at any given time than on observer detectability.

Larval survival at SCS was successful based on our threshold of ≥50 percent (0.50), although some of the lower confidence limits do dip below this point (Table 9). While Plot M appears to be an exception based on the second survey, the fact that two high count subplots surveyed on the first date were not re-surveyed on the second date confounds this comparison; we intended to survey eight sampling plots in Plot M. In contrast, larval survival at GHP was poor by all measures, with none of the estimates exceeding the desired threshold. Larvae were released in the best available locations based on *Plantago*

Table 11. Results of post-release surveys for postdiapause Taylor's checkerspot larvae on two reintroduction sites in South Puget Sound, Washington, spring 2016. Release plot size, number of potential 2 x 2-m subplots, larval counts, number of subplots surveyed, abundance and survival estimates and 90 percent confidence intervals; **bold text** indicates survival ≥ 0.50). SCS = Scatter Creek South; GHP = Glacial Heritage Preserve.

Release location	Area sq m	Potential plots	Release #	Survey date	Larval count	#sample plots	Abundance	Survival	90% CI low	90% CI hi
SCS										
Plot K	495	124	327	5 Mar	28	12	289	**0.88**	0.44	**1.33**
Plot L	753	188	789	3 Mar	58	26	420	**0.53**	0.38	**0.69**
Plot M	238	60	542	3,5 Mar	31	6	307	**0.57**	0.21	**0.92**
Plot K				17 Mar	24	12	248	**0.76**	**0.52**	**1.00**
Plot L				17-18 Mar	59	26	427	**0.54**	0.41	**0.67**
Plot M				17-18 Mar	9	6	89	0.16[1]	0.07	0.26
GHP										
Plot B	315	79	343	6-7 Mar	9	8	89	0.26	0.13	0.38
Plot C	560	140	792							
Plot J	795	199	894	11 Mar	19	18	210	0.23	0.14	0.33
Plot B				16 Mar	4	8	39	0.11	-0.02	0.25
Plot C				16 Mar	24	16	210	0.27	0.12	0.41
Plot J				18 Mar	10	19	105	0.12	0.04	0.20

[1] SCS Plot M: Plot Subplot 19A & B (count = 20) not surveyed on second date.

abundance and vegetation condition (Fig. 6), which also turned out to be within the footprint of a 2015 burn unit. We have generally avoided releasing into recently burned areas due to concerns about sufficient cover; however *Plantago* within the GHP burn unit was in good condition and as a result of ample rain and warm temperatures prior to release, had put on significant fresh growth compared to most years. In contrast, *Plantago* on other parts of the site had been heavily impacted by the *Plantago* pathogen and was inappropriate for release. It is not clear what other agent(s) of mortality may have been a factor at GHP in 2016, but these could include both vertebrate and invertebrate predation and disease. It is also conceivable that larvae dispersed beyond the bounds of the survey area, which at this site, could lead to starvation if they move beyond the distribution of host plants.

Contrary to many past years, fewer larvae were detected in later surveys than on earlier ones (Linders 2011, Linders 2012, Linders et al. 2013, 2015) in 2016 (Table 9), although this was similar to observations at TA7S in 2014 (Linders et al. 2014) and consistent with the standard assumption that mortalities increase over time. Lower counts on earlier surveys in the past was assumed to have resulted from larvae concealing themselves while molting and thereby not available to be counted. In addition to

Table 12. Number of subplots in which larval counts by one observer were higher vs. number of ties, during double observer surveys for postdiapause Taylor's checkerspot larvae at two reintroduction sites in South Puget Sound, Washington, spring 2016. SCS = Scatter Creek South; GHP = Glacial Heritage Preserve.

Site	LTR more	MJL more	Tie
SCS	14	6	11
GHP	3	2	13

Figure 5. Taylor's checkerspot larvae adjust their basking location in colder weather to reap thermal benefits of (left to right) being in a group of black bodies, basking on decaying vegetation, or adjusting their angle to maximize the thermal absorption, South Puget Sound, Washington, 2014.

count data, we also noted what instar each larva was in, so we may be able to plot the ratio of 5^{th} to 6^{th} instar larvae for each survey to see if this theory is in line with the data. In addition, methods used to calculate survival for 2016 differed somewhat from methods used previously, which also did not include confidence intervals. As time allows, all previous survival estimates will be re-evaluated using consistent methods so that survival can be compared across sites and years.

Figure 6. Habitat conditions within release Plot C at Glacial Heritage Preserve (GHP), Thurston County, Washington, spring 2016.

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

Adult presence and relative abundance

Methods

We used line transect sampling to estimate daily density and population size, and to illustrate the distribution of adults within the sampling area. Four reintroduction sites (SCS, R50, GHP, and TA7S) plus one extant site (R76) were surveyed for adult checkerspots during the 2016 flight season; surveys at PCM in 2016 were conducted by JBLM staff using different methods. 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 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. Transects at R76 covered the majority of the occupied area to which we had access. Four additional transects (Linders et al. 2014) were included north of the main monitoring area and were surveyed on all but the first date, 7 April, when no butterflies were observed. Data for R76 have become confounded by irregular access to the dud hazard zone in the southwest corner of the monitoring area, and in some years by lack of access near the end of the flight season. In 2016, access to the dud hazard area was granted on only two occasions: 7 and 30 April. Transect length and spacing by site and year is shown in Table 13. The closer transect spacing at reintroduction sites insures enough butterflies are detected to calculate an abundance estimate. As in year's past, all surveyors received pre-season training and distance estimation skills were tested weekly throughout the flight season.

<u>Data analyses.</u> Analyses were conducted using Program Distance, Version 6.2 (Thomas et al. 2010) with density estimates computed for each survey date because population numbers can change daily due to eclosion and mortality of individuals. 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 date, and 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).

Previous analyses identified observer as a consistent source of variation in detectability, so a suite of models were developed for analysis to account for this dependent on the number of observations collected (sample size) at each site and date. The greatest factor affecting precision of the estimates is the high variability in encounter rates among transect lines. The best detection functions were chosen based on Akaike's Information Criterion (AIC), goodness-of-fit tests, and additional model criteria recommended for MCDS analyses (Marques et al. 2007). Truncation of the data at distances less than the maximum observed were considered for each analysis set if it appeared outliers were present and their inclusion led to overfitting of models at the tails of the distribution.

After determining the detection function(s) to use, density estimates were computed by date. Variance estimates of density were calculated using the method of Fewster et al. (2009) that takes advantage of the sequential (evenly spaced) layout of transects to reduce variance estimates over those that assume transects are placed randomly. Of the two methods of this type available in Program Distance, we used 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

Table 13. Number and sizes (m) of distance sampling units, and standard area for density estimation by site for extant (R76) and reintroduced Taylor's checkerspot populations, Puget Trough, Washington, 2010-2015.

Site	# transects	Transect spacing	# segments	Segment spacing (m)	Transect length (m)	Total line length per survey(m)	Analysis 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]	16	25	7-12	50	350-600	5900-7100	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[3]	16	25	14-24	25	350-600	5900-7100	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[3]	14	25	6-13	25	150-325	2675-2700	6.75
2015							
SCS[3]	16	25	14-24	25	350-600	5900-9100	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[3]	15	25	6-13	25	150-325	3600-3675	9.20
GHP Eric	13	25	4-9	25	100-225	2625	6.60
2016							
SCS	16	25	16-20	25	400-500	7800	19.50
R50	16	25	18-26	25	450-650[2]	9900	24.75
GHP	15	25	18	25	450	6750	16.88
R76	16	50	23-28	25	575-700	10900	54.50
TA7S	19	25	7-14	25	175-350	5125	12.81

[1] Two short transects measure 450 m each.
[2] Four short transects measure 450, 500, 550 and 600 m, respectively.
[3] Survey area for abundance estimation may be smaller than the total survey area and that used to map distribution.

the O2 method were used to estimate 95% Confidence Intervals. Data and density estimates for 2015 for four reintroduction sites (SCS, R50, GHP and TA7S) plus R76 are reported here.

Results and Discussion

In some cases the number of observations reported here may differ from the raw counts reported in Linders et al. (2015) because 1) the distance analysis area may be smaller than the total survey area, and 2) some observations were excluded from distance analysis due to truncation, weather constraints, etc.

Adult abundance 2015

S<u>catter Creek South.</u> Ten surveys were conducted by three observers in 2015 between 2 April and 27 May. No butterflies were observed on 2 April, 21 May and 27 May (Linders et al. 2015). A total of 285 Taylor's checkerspots observations were included in this analysis, with the greatest number (117) seen on 20 April. The analysis area has been reduced since 2013 with results applied to the zone where adults were most likely to be observed (Linders and Lewis 2013, Linders et al. 2014, 2015). For the purpose of this analysis there were 13 transect lines, four of which were 600 m long and 9 that were 350 m long, for a total line length of 5,900 m of effort for each survey day except 15 April. On that day, two lines (totaling 950 m) surveyed in non-protocol weather conditions, were removed from the analysis for a total effort of 4,950 m on that day. Summary statistics showed a slightly uneven distribution of effort with LTR doing more surveys (22,200 m total) than LMH (17,800), and MJL (18,050). Daily allocation of effort was spread fairly evenly among observers and there appeared to be no consistent patterns of observer assignments that would likely cause observer differences to be confounded with other potential effects on detectability, especially survey day. The number of observers per day varied from 2-3, but all surveys during the main flight season employed all 3 observers The number of butterflies observed per survey date was sufficiently high on 4 days (20 April, 29 April, 3 May, and 8 May) to consider estimating separate detection functions by date in CDS models, and including date as a covariate in MCDS models. Therefore CDS models were fit with both a global DF with observers pooled and a DF by date with observers pooled and these were compared to MCDS models with a global detection function and either a date covariate or an observer covariate, with both w and σ scale parameter options. The best model based on AICc, was the CDS model with the detection function estimated by date. There appeared to be very different detection functions among dates, so separate detection functions were fit to each of four dates (Apr 20, Apr 29, May 3, and May 8). Although these were the best models based on AICc, goodness-of-fit test results were poor and the shape of the detection function did not follow the expected pattern for distance sampling data (see the Range 50 analysis discussion below, for a similar situation). To improve model fit and better meet distance shape criteria, the data were manually grouped into 2 m intervals. For the remaining dates with non-zero observations (April 15, May 15, and May 18), a model with a global detection function was fit to all data and applied to the date-specific encounter rates to derive density estimates; these data were also grouped into 2 m intervals for consistency. All data were truncated at 10 m to eliminate outliers. Daily density estimates with 95% Confidence Intervals are presented by date in Table 14 along with daily abundance estimates for the survey area.

<u>Range 50.</u> Eight surveys were conducted between 12 April and 26 May by three observers in 2015. No butterflies were observed on 26 May (Linders et al. 2015). In all, 1,750 adult observations were used in the analyses, with a peak single day count of 543 on 27 April (Table 14). There were 16 transect lines, 12 of which were 650 m in length and the remaining 4 were 600, 550, 500, and 450 m, for a total effort of 9,900 m. All transects were surveyed on all dates except on 12 April when only 3,800 m total transect length was surveyed. Most of that survey was conducted under non-protocol weather conditions, so no distance analyses were conducted for that date. Some non-protocol weather conditions were also recorded on 25 April which may have affected the number of butterflies observed, as the total count on

that day was lower than on 18 April and 27 April. Effort among observers varied from 28,350 m (LTR) to 21,700 (MJL). Usually there were 3 surveyors on any given date but occasionally 2; there was no apparent confounding between observers and other potential influences on detectability. Sample sizes were sufficient on most survey dates to consider running date-specific detection function models, yet global detection functions (pooled over all dates) with and without covariates were also considered. Although samples sizes were fairly large for most dates, modeling detection functions proved to be challenging because the data were not optimally distributed for distance sampling estimation. To smooth out some of the variability, CDS models were also fit to data that were manually grouped into 2 m intervals. Since it is inappropriate to use AIC to compare these models with those based on ungrouped data, selection of a best detection function model was based on goodness-of-fit tests provided by Program Distance. Analyses from all dates combined indicated a strong preference for date-specific detection functions, so models were run separately by date. For the dates with the largest sample sizes, April 18, 25, and 27, CDS models with grouped data fit very well. For May 6 and May 9, grouping was not required. Data were truncated at 10m to reduce unnecessary adjustment terms and to satisfy criteria associated with MCDS models. Density estimates with 95% Confidence Intervals are presented by date in Table 12 along with daily abundance estimates for the entire survey area.

Glacial Heritage Preserve. Eight surveys were conducted at GHP between 2 April and 20 May (Linders et al. 2014) by three observers in 2015. No checkerspots were observed 2 April or 20 May, and only one was observed on 28 May. A total of 184 butterflies were used in the analysis for 2015, with a peak count of 62 on 17 April (Table 14). There were 14 transects, each 450 m in length, for a total of 6,750 m of effort per day; all transects were surveyed on all dates. Total survey effort was the same for LTR and LMH (17,100 m each), with a greater level of effort by MJL (19,800 m); all 3 observers participated in surveys on all dates with >1 observation except 17 April, which had only 2 observers. Surveys were generally run under protocol weather conditions except on 30 April, when 4 transects were recorded as having non-protocol conditions. Distance sampling estimates generated for this date included non-protocol samples, so these may be biased low as a result. Only data from 17 April to 8 May were analyzed due to samples sizes. Density estimates with 95% Confidence Intervals are presented by date in Table 12 along with daily abundance estimates for the entire survey area. Sample sizes were sufficient to run CDS detection function models by date, so these were run along with a global DF model with and without date and observer covariates. Truncation at 10 m did not improve model fit, so was not applied. The global detection function model without a covariate was chosen as estimates were nearly identical to the global model with an observer covariate, but it had fewer parameters.

Training Area 7 South. Eight surveys were conducted between 8 April and 19 May by four observers in 2015. No butterflies were observed on 8 April or 19 May; only one was seen on 14 May and four on 7 May, so these dates were excluded from analysis. All observations were made under protocol weather conditions except one of the many observations on April 22. There were 15 transects with line lengths varying from 175-325m, and a total length of 3,675 m, but no butterflies were observed on the five most southerly transect lines (T 15-19). These lines were deleted from the analysis to reduce variability in encounter rates, under the assumption that they were outside the occupied area. Density estimates were projected over the remaining area to estimate abundance. Total line lengths for this subset were 2,075 m on 16 April, and 2,325 m on all other dates. There were 2-3 observers on each date, but one observer surveyed only on 8 April, when no butterflies were observed. Distribution of effort was somewhat uneven, with MJL doing fewer surveys (4,250 m total) than LMH (6,225 m), and LTR (6,600 m), but no systematic patterns in distribution of effort were evident. Sample sizes precluded running CDS detection function models by date, so only global detection function models were run on data truncated at 8 m and untruncated data. A hazard rate key function with no adjustment terms was

Table 14. Raw counts, density estimates (#/ha) and adult abundance estimates (#/site by date) including 95% Confidence Intervals for Taylor's checkerspot survey areas at Scatter Creek South (SCS), Range 50 (R50), Glacial Heritage (GHP) Training Area 7 South (TA7S) and Range 76 (R76) in South Puget Sound, Washington, Spring 2015. See text for details regarding derivation of density estimates.

		Survey	Density			Abundance		
Date	RawCnt	area (ha)	#/ha	Lower CI	Upper CI	#/site	Lower CI	Upper CI
SCS								
15 Apr[1]	2	12.38	0.50	0.17	1.48	6	2	18
20 Apr	130	14.75	22.80	16.69	31.14	336	246	459
29 Apr	87	14.75	14.94	9.97	22.39	220	147	330
3 May	58	14.75	12.54	7.24	21.75	185	107	321
8 May	37	14.75	12.62	7.27	21.90	186	107	323
15 May	4	14.75	0.84	0.36	1.97	12	5	29
18 May	2	14.75	0.42	0.11	1.61	6	2	24
R50								
18 Apr	453	24.75	69.9	54.0	90.6	1730	1337	2242
25 Apr	430	24.75	53.4	40.4	70.5	1322	1000	1745
27 Apr	622	24.75	69.9	53.8	90.7	1730	1332	2245
2 May	309	24.75	29.6	24.6	35.7	733	609	884
6 May	29	24.75	8.7	6.1	12.5	215	151	309
9 May	14	24.75	5.7	4.2	7.8	141	104	193
GHP								
17 Apr	69	16.88	11.24	7.64	16.54	190	129	279
22 Apr	52	16.88	7.95	2.90	21.82	134	49	368
30 Apr[2]	54	16.88	8.47	4.62	15.54	143	78	262
3 May	17	16.88	2.77	1.51	5.06	47	25	85
8 May	13	16.88	2.08	1.01	4.25	35	17	72
TA7S								
16 Apr	10	5.19	9.83	3.67	26.32	51	19	137
22 Apr	59	5.81	40.35	19.02	85.58	235	111	497
27 Apr	27	5.81	19.30	9.40	39.62	112	55	230
1 May	28	5.81	20.18	10.78	37.77	117	63	220
7 May	4	5.81	3.51	0.99	12.48	20	6	73
R76								
18 Apr	472	42.00	77.05	61.96	95.81	3236	2602	4024
20 Apr	652	42.00	95.91	79.6	115.56	4028	3343	4854
27 Apr[3]	305	30.75	70.64	57.58	86.66	2172	1771	2665
2 May[3]	192	30.75	27.02	21.12	34.57	831	649	1063
7 May	106	42.00	9.65	7.39	12.61	405	310	530
9 May[3]	82	30.75	8.95	5.91	13.57	275	182	417

[1] Survey area reduced as non-protocol weather prevented completion.
[2] Estimates are likely biased low for this date as some observations occurred during non-protocol weather conditions.
[3] Surveys excluded the EOD area and may not be comparable to other dates that included the EOD area due to inconsistencies in the density of butterflies between those areas.

selected as the best CDS model for this analysis. Density estimates with 95% Confidence Intervals are presented by date in Table 14 along with daily abundance estimates for the survey area.

Range 76. Ten surveys were conducted by 4 observers between 8 April and 30 May in 2015; no checkerspots were seen on 30 May, and only one was seen on 8 April. A total of 1,675 butterflies were used in the 2015 analysis, with a peak count of 652 on 20 April (Table 14). Three observers were present on most remaining dates, except on 8 April and 7 May when there were 2. Effort was evenly distributed among observers both within and among dates. Non-protocol weather dominated the 4 May survey, which was excluded from the analyses. Daily survey effort (i.e., total line length surveyed) varied considerably by date. Normally the survey area at R76 consists of 12 transect lines, each 700 m in length, for a total of 8,400 m of effort. But in 2015, only 3 dates (20 Apr, 4 May, and 7 May) had 8,400 m of effort. All other dates had less, ranging from 3,025 m on 30 May to 8,225 m on 18 April (175 m of data were lost due to equipment malfunction); no two dates were equal. On 8 April, 27 April, 2 May, and 9 May, surveys excluded the EOD restricted area in the southwest corner of the sample area. The EOD area covered (very) roughly 10ha, but the area avoided was slightly different on these dates, leading to variable effort (from 6125m to 7075m).

The main concern regarding the EOD area was whether the daily density estimates obtained from the remaining survey area were representative of the entire area. To investigate this, we analyzed data from two dates for which the entire area was sampled (20 April and 7 May), and from one date (18 April) where only a small portion (175 m) of the data was missing. Mean counts per transect (T 1-6) from inside the EOD area were compared to those outside the EOD area by date. While lines were slightly longer (1.07x) inside the EOD area, EOD means were much higher (24.6 vs. 11.9 counts/transect) for all dates combined. This difference also varied by date, so that on 18 April counts/transect were similar (EOD: 23.5, outside EOD 20.17) even though part of one transect outside the EOD area was missing. Analysis of variance on these data confirmed these results, with date, area, and the interaction between date and area all highly significant factors (G. Olson, unpub. data). To examine the effect of excluding the EOD area on overall density estimates for the entire survey area and see if a "fudge factor" could be developed for the surveys that excluded the EOD area, data for each of the three full survey dates was used to estimate density first using all data, and second using a subset that excluded the EOD area. The standard analysis strategy was used for these three dates and data were truncated at 10 m. Both CDS models and MCDS models were run with a date-specific detection function and with and without an observer covariate. The best model for the comparison of estimates was an MCDS model with date-specific detection functions and an observer covariate (G. Olson, unpub data). Consistent with the ANOVA analysis of the count data, these results indicated that the effect of excluding the EOD area varied substantially by survey date. The lack of a consistent relationship also makes it difficult to identify a suitable adjustment factor for dates when the EOD area was not surveyed, so density estimates (and associated abundance estimates) from partial surveys are not comparable to those based on data from the entire survey area. Analyses for the remaining survey dates (27 April, 2 May, and 9 May) were run as a separate group, following the same procedures as the previous analyses. The best model was the CDS model with date-specific detection functions and no covariates. Density estimates with 95% Confidence Intervals are presented by date in Table 14 along with daily abundance estimates for the survey area.

Adult abundance 2016

From six to eleven complete distance surveys were conducted at each of five sites occupied by Taylor's checkerspot in 2016 (Table 15). Several partial surveys were also conducted, particularly at R76 where access to the dud hazard area (EOD) was restricted on all but two dates. Effort varied by site with the greatest effort expended at SCS (85.8 km) and R50 (84.6 km); effort on other sites was: R76 (76.0 km), GHP (52.7 km), and TA7S (30.8 km). We adjusted the survey area at SCS in 2016 to eliminate poor quality habitat to the east and include higher quality habitat to the west where active restoration is underway. This strategy minimizes inclusion of low density areas and limits impacts to mardon skipper.

A total of 7,402 checkerspots were counted during distance surveys in 2016, including those on transects 13-16 at Range 76; the greatest number was observed at R50. Checkerspots comprised 6,858 groups of 1-4 individuals each with 6,368 single butterflies observed; 441 groups of two; 44 groups of

Table 15. 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, GHP = Glacial Heritage Preserve, TA7S = Training Area 7S), South Puget Sound, Washington, spring 2016. R76: standard survey area reported here; four northern transects surveyed on all but first date; EOD access as shown (see Appendix C, Fig. 4).

Date	R76	SCS	R50	GHP	TA7S	Comments
7-Apr	0					R76: T. 1-12 only
8-Apr					0	
9-Apr		0				
11-Apr			1			R50: T5-16, time limited; 4 Eet off survey
15-Apr				1		GHP: T4-15, lost weather on T16-18
16-Apr	28		424			R76: No EOD
17-Apr		8		10		
18-Apr	96		1151		1[1]	R76: No EOD; TA7S: Start 1645, protocol weather
19-Apr		296		57		
20-Apr	114		1246		0	R76: No EOD
21-Apr		539		85		
25-Apr	74		438			R50 & R76: time limit, skipped T1,5,9,13; R76: No EOD
26-Apr		194		54	0	
28-Apr		396				
30-Apr	100		685			
1-May		348		14		
2-May	61				0	R76: No EOD; TA7S: Survey then release 80 adults
3-May			318	2		
6-May		161			4	TA7S: released 10 adults 5 May 2016
9-May	2		14			R50: T10,14 data lost; R76: No EOD; TA7S: release 10 adults
10-May		40		0		
12-May	0		1			R76: No EOD; TA7S: 131 release adults
17-May		2				
24-May		0				
Total	**475[2]**	**1984**	**4278**	**223**	**5**	

[1] Also one observed on road outside survey.
[2] 437 additional checkerspots were recorded on T. 13-16, for a site total of 912; peak count on 20 Apr 2016 was 261.

three; and 5 groups of four. Peak counts occurred on 20-21 April (Table 15), but encounter rates varied among sites, with twice as many butterflies observed at R50 (0.126 checkerspots/m) compared to SCS (0.069/m). Numbers were substantially lower at both R76 (0.024/m for transects 1-16; 0.014/m for transects 1-12) and GHP (0.013/m) compared to both R50 and SCS. This is a significant shift from peak dates in 2015, when R76 had the highest encounter rate (0.078 checkerspots/m for transects 1-12), followed by R50 (0.063/m), SCS (0.017/m) and GHP (0.010/m).

Nearly half (437 of 912) of checkerspots observed at R76 were counted on transects 13-16 at the north end of the site (see Appendix C, Fig. 4), continuing the northward shift in distribution we have been tracking since 2014. Reasons for this trend are not clear, but site-specific factors such as habitat condition and/or military training impacts cannot be ruled out since we do not see similar trends at other sites. Either way, decline or loss of the R76 population could endanger both reintroduction and recovery efforts in South Puget Sound.

Only two checkerspots were counted at TA7S prior to adult releases. As no new postdiapause larvae were released in 2016, we assume the low numbers are due to one or more of the following: failure to successfully reproduce, 2) low overwinter survival, and/or 3) impacts from restoration and/or prescribed burn activities. Habitat assessment using data from 2013-2014 indicated the site was suitable (Waters et al. 2015) then, and most changes in the 2016 raw data appear positive (Linders, unpub. data). Completion of the habitat assessment using data from spring 2016 is scheduled for fall 2016.

Inconsistencies in the data appear in counts at R76 and R50 on 25 April and at SCS on 26 April (Table 15). In these instances, counts were lower than they were during surveys before and after those dates. Due to limited access to R76 and R50 on 25 April, transects 1, 5, 9 and 13 were omitted at both sites. Survey conditions were otherwise suitable, so we expect the influence of the missed transects on density estimates to be nominal. At SCS on 26 April, limited sun may have created somewhat marginal survey conditions, and a lowered count (Table 15). A closer examination of the ratio of perched to flying adults may reveal this to be the case.

As in years past, nectar observations were recorded opportunistically during Taylor's checkerspot distance surveys (Table 16); one observation each on *Cytisus scoparius* (R76-2013), *Dodecatheon hendersoni* (R50-2015) and *Fritillaria camschatcensis* (R76-2015) are omitted from the table. Ten species were observed to be used at four sites in 2016, with the total number of observations the fewest to date even though there were more butterfly observations in 2016 than in all other years reported in Table 16, except 2012 (Linders 2011b, 2012; Linders and Lewis 2013; Linders et al. 2014, 2015). We employed three new surveyors in 2016 that may have been more reluctant to report nectar observations, but other factors may also be at play, such as the warm, droughty springs that have been prevalent since 2013. Similar to previous years, three plant species: *Balsamorhiza deltoidea*, *Lomatium triternatum* and *Saxifraga integrifolia (=Micranthes integrifolia),* accounted for the majority (77.8 percent) of nectar observations in 2016. Warm and dry spring weather also appears to be influencing flowering phenology and duration, such that the availability of nectar by species has become more variable from one year to the next. For example, *Camassia quamash* was previously considered an early season species, but in recent years it has been flowering midway to late in the flight season.

These changes coincide with a northward shift of adults at R76 to an area near the wetlands where *C. quamash* flowers persist well into the season. Vegetation monitoring using the RHA protocol was conducted in the main survey area at R76 in 2013-2014 (Waters et al. 2015), but has not been done since, so it is not clear whether other habitat factors may also be contributing to this shift. These data could also shed light on habitat conditions at reintroduction sites and improve our understanding of the relationship between population size, distribution and habitat condition.

Table 16. Number and nectar species used by Taylor's checkerspots recorded during surveys on extant (R76 = Range 76) and reintroduction (R50 = Range 50, SCS = Scatter Creek South, PCM = Pacemaker, GHP = Glacial Heritage Preserve, TA7S = Training Area 7 South) sites, South Puget Sound, Washington, 2011-2016. ACMI – *Achillea millefolium*; ARMA – *Armeria maritima*; BADE - *Balsamorhiza deltoidea*; CAHI – *Castilleja hispida; CAQI - Camassia quamash*; CEAR – *Cerastium arvense* ; COPA – *Collinsia parviflora*; *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**	**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**	**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				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**		**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**	**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**	**8**	**1**	**0**	**16**	**37**	**2**	**0**	**0**	**0**	**2**	**43**	**4**	**670**
2015																					
R50			**187**	**1**					**2**				**17**					**7**	**36**		**251**
R76		**2**	**207**		**14**				**6**				**53**	**10**				**3**	**29**		**325**
SCS			**6**		**9**		**1**									**1**					**17**
GHP			**1**		**4**													**1**			**6**
TA7S			**1**				**1**						**3**								**5**
Subtotal	**0**	**2**	**402**	**1**	**27**	**0**	**2**	**0**	**8**	**0**	**0**	**0**	**73**	**10**	**0**	**1**	**0**	**11**	**65**	**0**	**604**
2016																					
R50			**80**		**3**								**7**						**21**		**111**
R76		**1**	**12**		**6**				**1**				**14**	**4**					**2**		**40**
SCS	**1**	**3**	**6**		**6**		**4**						**1**			**10**					**31**
GHP					**2**								**1**								**3**
TA7S																					**0**
Subtotal	**1**	**4**	**98**	**0**	**17**	**0**	**4**	**0**	**1**	**0**	**0**	**0**	**23**	**4**	**0**	**10**	**0**	**0**	**23**	**0**	**185**
Total	**5**	**41**	**2608**	**3**	**70**	**2**	**8**	**12**	**76**	**4**	**1**	**192**	**1120**	**48**	**3**	**16**	**3**	**28**	**337**	**4**	**4584**
Proportion	0.00	0.01	**0.57**	0.00	**0.02**	0.00	0.00	0.00	**0.02**	0.00	0.00	**0.04**	**0.24**	0.01	0.00	0.00	0.00	0.01	**0.07**	0.00	

As in years past, mardon skipper butterfly sightings were systematically recorded during 2016 surveys for Taylor's checkerspot at the three sites where they co-occur. In total, only 18 mardon skippers were observed (Table 17), by far the lowest number to date (Linders 2011b, 2012; Linders and Lewis 2013; Linders et al. 2014, 2015). All observations were of lone mardon skippers, and no skippers were observed at R76, where they were once readily observed and widely distributed (Linders, unpub, data). While trends are encouraging elsewhere in the species range, numbers of mardon skippers in the Puget lowlands have remained discouragingly low for the past decade and are a source of real concern. Updated genetics work is badly needed to determine whether the application of a single subspecies designation to all coastal and montane populations from Washington to California is appropriate, especially considering regional isolation and barriers to dispersal.

Table 17. Number of mardon skipper butterflies observed by date and site during distance surveys for Taylor's checkerspot in spring 2016 at three sites (R50 = Range 50; R76 = Range 76; SCS = Scatter Creek South), South Puget Sound, Washington. "Zero" surveys are included nearest to dates when skippers were observed.

Date	R50	R76	SCS
19-Apr			0
20-Apr	0	0	
21-Apr			0
25-Apr	1	0	
26-Apr			0
28-Apr			0
30-Apr	1	0	
1-May			0
2-May		0	
3-May	4		
6-May			3
9-May	1	0	
10-May			6
12-May	2	0	
17-May			0
Total	**9**	**0**	**9**

Adult distribution
Methods

To illustrate the distribution of adults within survey areas at each site, all Taylor's checkerspot observations collected during 2016 distance sampling surveys were spatially joined to a GIS layer of transects and sections that run through the centers of underlying 25 x 25-m grid cells. Observations for all survey dates were combined for each site and mapped using standardized category classes. Category classes were scaled so the midpoint of each successive bin increased by a factor of two. Observations were overlaid on 2015 National Agriculture Imagery Program (NAIP) color aerial photos with 1-m resolution. To date, we have summarized the checkerspot distribution as the number of segments where butterflies were observed (occupied) as a proportion of all segments in the survey area. However changes in segment length and total survey area over time, limit the usefulness of these comparisons. To improve comparisons of distribution between years and sites, we used the number of segments where butterflies were observed within each survey area to create an index of occupied area by assuming segment data are representative of the underlying 25 x 25-m grid cells. This assumes that patterns of occupancy and detectability near the transect line are similar to more distant areas. This assumption is likely to hold true if habitat conditions are random relative to the placement of transect lines. Both assumptions are consistent with those of distance sampling. There is more potential to violate these assumptions at R76, where transects are spaced 50 m apart (i.e., less of the area is sampled), than at reintroduction sites where a 25-m spacing is employed. In addition, the effect of such a violation may be more pronounced at lower occupancy levels (i.e., fewer segments with observations and/or fewer butterflies per segment) than at higher ones, meaning the index may have a stronger upward bias when populations are low.

Results

Habitat occupancy varied markedly by site (R76 vs. R50) on the Artillery Impact Area at JBLM in 2016. As in years past, adult checkerspots were distributed across the majority of the sampling area at R50 (Table 18), occupying 74.2 percent (294 of 396) of segments surveyed with high concentrations throughout the sites center and a nearly contiguous distribution along three sides of the monitoring area (Appendix C, Fig. 2). However at R76, checkerspots occupied only 50.6 percent (170 of 336 segments) of the main monitoring area (transects 1-12) in 2016, compared to 90.4 percent (304 of 336 segments) of the same area in 2015. Occupancy within the four northern lines was 65 percent (65 of 100 segments) in 2016 vs. 88 percent (44 of 50 segments) in both 2014 and 2015 (Linders et al. 2014, 2015). Large sections of the main monitoring area were unoccupied in 2016 (Appendix C, Fig. 4) even if we account for the limited surveys within the EOD area (Table 15). The only notable concentration of checkerspots was in the far northeast corner of the site, associated with a hilltop where heavy training is ongoing. In fact, in 2015 we reported observing >5 checkerspots per segment across 83.9 percent (156 of 386 segments) of the survey area (Linders et al. 2015), whereas in 2016 we observed such concentrations on just 8.5 percent of the same area (37 of 446 segments; see Table 13 for change in segment length). When the EOD area, surveyed only once in 2016, is excluded from the assessment the concentration area decreases to 42.5 percent in 2015, with no change in percentage for 2016. Given the highly gregarious nature of Taylor's checkerspot, it is difficult to view these findings in a positive light.

Habitat occupancy increased at both ACUB sites in 2016. At SCS adult distribution doubled from 2015 to 2016 (Table 18), with checkerspots occupying 54.5 percent (87 of 236) of the segments surveyed. Adults continued to concentrate near the 2013 release area (Plot H), but also reached high concentrations to the south and east, linking up with the area of the 2016 release (Appendix C, Fig. 1). Several prescribed burns have taken place within this large concentration area since 2010 and, are believed to have been a driving factor in this restoration success. Checkerspots at GHP were mainly concentrated in the vicinity of the 2015 release area, although some sightings were also associated with the 2016 release areas (Appendix C, Fig. 3). Adults were distributed across 21.9 percent (59 of 270) of the segments surveyed at GHP (Appendix C, Fig. 3), a slight increase from 2015. We also surveyed the area of Glacial Heritage Preserve known as "Eric's Plots" in the far northwest corner of the Preserve, where small releases of postdiapause larvae occurred in 2014 and 2015 (Linders et al. 2014, 2015). Surveys occurred under protocol conditions on two occasions (17 April and 1 May) in 2016, but no checkerspots were observed.

Other sites at JBLM fared poorly. Very few checkerspots were sighted at TA7S overall. We observed only two naturally-occurring adults, both in the southern portion of the site over 200 m from the 2015 release areas (Appendix C, Fig. 5), prior to several adult releases later in the flight season. Adults occupied just 2.0 percent (4 of 205 segments) of the survey area, although post-release surveys for adults were a relatively low priority. No sightings of Taylor's checkerspots were reported by JBLM personnel, who conducted surveys at PCM in 2016.

An index of area occupied by adult Taylor's checkerspots by site and year is summarized in Table 18 and Fig. 7. Trends at R50 and SCS are similar in recent years, although these sites may diverge in the future as restoration and recovery continue at SCS, where hundreds of acres of potential habitat are being restored. Distribution at GHP is similar to trends in occupancy at SCS until 2015, when habitat improvements appeared to foster population growth (Appendix B) and expansion (Table 18, Fig. 7). We suspect habitat at GHP may be failing to support and concentrate checkerspots. Habitat data collected in 2016 will be assessed using established criteria in fall 2016 (Waters 2016). Trends in occupancy (Fig. 7) and abundance (Appendix B) at R76 and R50 appear similar in 2010-2015, but diverged sharply in 2016 (Fig. 7 and Table 15). Weather (e.g., heavy rain in the winter of 2016) is an unlikely explanation, because numbers increased at other Puget lowland sites (e.g., SCS and GHP) and sites in Oregon where numbers were considerably higher in 2016 compared to other years (D. Ross, pers. comm.). Occupancy at TA7S and PCM also appear to follow similar tracks, due in part to limited release efforts, but other factors such as prescribed fire and other restoration activities may also be at play.

Table 18. Index of area (ac) occupied by adult Taylor's checkerspot butterflies based on distance sampling surveys by site and year for reintroduction sites and one extant site (R76 = Range 76) in South Puget Sound, Washington, 2007 – 2016. SCS = Scatter Creek South, R50 = Range 50, PCM = Pacemaker, GHP = Glacial Heritage Preserve, TA7S = Training Area 7 South and R76 = Range 76.

Site	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
SCS			2.0	17.4	10.5	10.5	10.5	9.6	13.4	26.3
R50				15.9	33.7	46.3	43.2	27.6	37.7	45.4
PCM						7.4	0.0	0.0	0.0	
GHP						0.9	15.4	8.0	8.5	9.1
TA7S								5.3	8.5	0.6
R76 T1-12	97.0	42.0	64.2	93.9	103.2	103.8	97.6	97.3	93.9	52.5
R76 T13-16								27.2	27.2	20.1
R76 Total	97.0	42.0	64.2	93.9	103.2	103.8	97.6	124.5	121.1	72.6

We also collated and mapped scattered sightings of Taylor's checkerspots outside standard survey areas to document dispersal and identify potential colonization areas. This effort was largely opportunistic except on the Artillery Impact Area, where some focal surveys were conducted by JBLM staff. A map of scattered sightings locations associated with releases at SCS and GHP is located in Appendix D, Fig. 1, with those from the Artillery Impact Area, including R50 and R76) located in Fig. 2. Numerous observations of adults were reported from Training Area 6 by JBLM staff in 2016 (Appendix D, Fig. 2, upper right). These and other widespread sightings, together with the low numbers remaining at R76 suggest dispersal is primarily occurring from this site. Scattered sightings are SCS (Appendix D, Fig. 1) included observations of two females egg-laying in habitat on the west-central edge of the site, which is undergoing active restoration for checkerspots; RHA data collected in 2015 indicate this management unit has three 5-ac blocks that qualify as reintroduction-ready.

No adults were observed by JBLM staff at Pacemaker in 2016, although one potential observation was reported a few kilometers to the west in TA 13. Results from this site (Linders and Lewis 2013, Linders et al. 2014, 2015) appear to corroborate our experience that reintroductions based on a single release have a low likelihood of persistence beyond the first year.

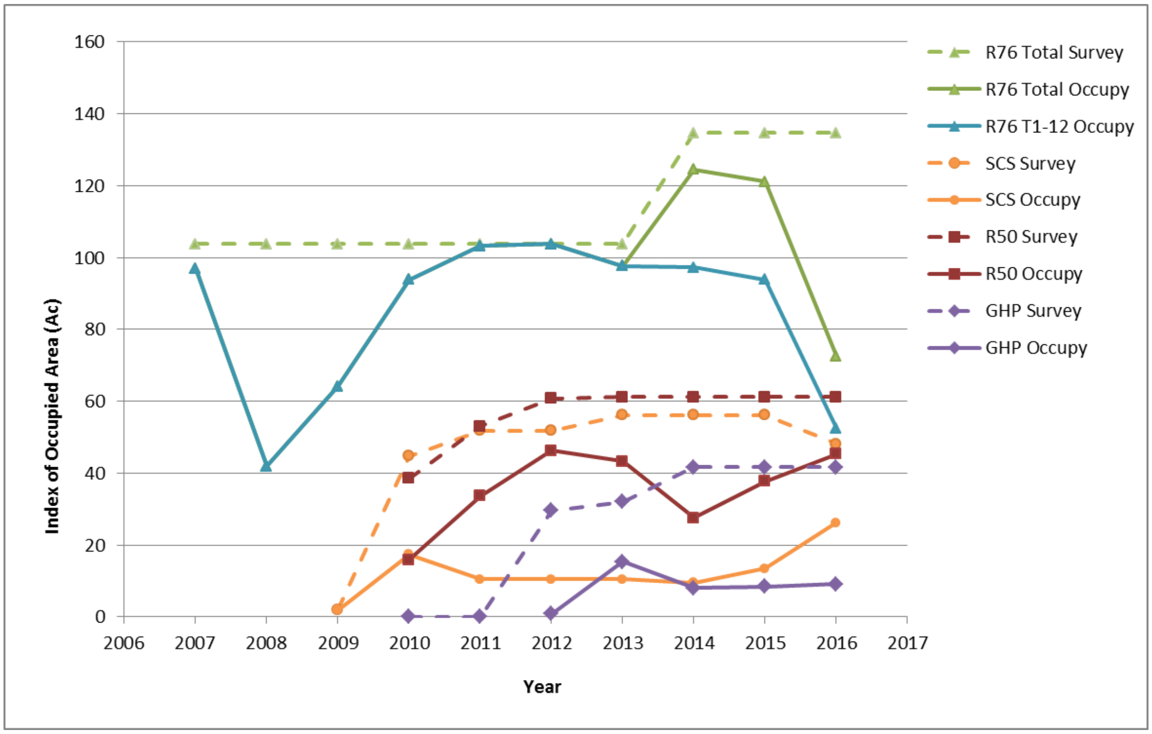


Figure 7. Change in index of occupied area and survey area (ac), by site and year at extant (R76 = Range 76) and reintroduction (R50 = Range 50, SCS = Scatter Creek South, PCM = Pacemaker, GHP = Glacial Heritage Preserve, TA7S = Training Area 7 South) sites, South Puget Sound, Washington, 2007-2016. The survey area at R76 expanded to the north when an increase in butterflies was noted there in 2013.

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

A key step in establishing a naturally-occurring population is documenting local reproduction of released animals. In the past we have done this by searching for larval clusters in and around release plots on one site annually using methods described previously (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 3rd instar, a sign of successful reproduction. In a collaborative effort to maximize search area, number of oviposition sites located, and collectively increase our knowledge base in 2016, we teamed up with Susan Waters at the Center for Natural Lands Management to survey plots for eggs and prediapause larvae. Susan's work was focused on larval predation (Waters 2016 b), but was compatible with our objectives of documenting successful reproduction.

Methods

To identify search areas and to assure >5m separation between plots required for Susan's work, we randomly selected one of the two 2 x 2-m subplots from each 2 x 5-m belt transect used to survey for postdiapause larval survival in 2016 (total of 24 for GHP and 26 for SCS). We then added more 2 x 2-m plots further out along these transects to increase our sample size to 50 plots per site. The spatial spread of the resulting 100 plots covered all three 2016 larval release areas south of the road at Scatter Creek, with all area between also covered. At Glacial Heritage, three swales were covered, including all 2016 larval release areas (Waters 2016b).

We also located larval clusters opportunistically by searching between plots as we moved across the site and by following distance sampling transects. These larval clusters were used to increase the number we monitored for survival. Plots were searched during two life stages, egg and early (1^{st}-2^{nd} instar) prediapause. Data collected included the height of the eggs and/or larval cluster, otherwise they were the same as those described previously (Linders and Lewis 2013). Larval sites were revisited about once each week to record number by larval instar.

Results and Discussion

Survey data from GHP and SCS are still being entered and will be summarized in winter 2017. Parameters used to describe survival will be similar to those reported by Haan (2016). More than 40 larval clusters were located at each site in 2016 (Fig. 8).

Figure 8. Larval masses, webbing, and feeding damage found during prediapause larval searches at Scatter Creek South and Glacial Heritage Preserve indicate Taylor's checkerspots are reproducing on site, South Puget Sound, Washington, summer 2016.

V. Conduct long-term monitoring and evaluate progress towards meeting establishment criteria

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) we might expect for other Taylor's checkerspot populations in south Puget Sound. Data from R76 were in turn, used to set population targets, evaluate population growth potential, and set long-term monitoring goals for the reintroduced populations. Long-term monitoring is used to measure progress toward population establishment in the five years after releases are 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 the following criteria are met:*
>
> 1) *Adults are widely distributed across a monitoring area at least 20 ha (50 ac) in size,*
> 2) *they occupy the site solely through natural reproduction,*
> 3) *they achieve a single day abundance estimate of at least 250 adult butterflies, and*
> 4) *these conditions are met each year for five consecutive years.*

The rationale for these criteria come from the main monitoring area at R76, which covers about 42 ha (100 ac), which is about twice that of most reintroduction units, which are relatively large compared to sites elsewhere in the species range. In the absence of alternatives, data from this naturally occurring population forms the basis for our expectations on reintroduction sites. For example, the minimum single day abundance estimate referenced above is about half of the peak single day estimate at R76 in 2009 (Appendix B), adjusted for site size. 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 low point, giving us confidence that other populations could as well. Finally, a five-year monitoring window is a widely used standard for establishing occupancy by butterflies.

Methods

The monitoring scheme and distance sampling protocol already developed (Linders and Olson 2014) form the basis for monitoring reintroduction sites. We evaluate reintroduction sites where releases are complete against the above criteria annually using daily abundance estimates and distribution maps.

Results and Discussion

Referencing the above criteria, adults were distributed across the majority of the 22-ha monitoring area at R50 in 2016 (Appendix C, Fig. 2), and increased relative to 2014 and 2015 (Linders et al. 2014, 2015 and Table 18). Checkerspots were also observed regularly along three sides of the monitoring area at R50, and may also be moving beyond the boundaries of the survey area. Larval distribution surveys in 2015 also show that checkerspots are reproducing throughout the site (Linders et al. 2015). Using the abundance criteria above, R50 exceeded the target of 250 adults on a single day based solely on reproduction for the fourth consecutive year in 2015 (Appendix B), with a peak single day abundance estimate of 1,730 adults on both 18 and 27 April (range 878-1663 and 603-1017, respectively; Table 14). The peak raw count for 2016 (1,246; Table 15) was double that in 2015, suggesting 2016 will also exceed the single day target for a fifth consecutive year when data are analyzed in winter 2017. Meeting this final milestone will mean this site can be considered an officially established population based on the definition above.

Using the same definition, checkerspots at SCS were distributed across just over half of the 19.50 ha surveyed, and extended to the edges along parts of three sides (Appendix C, Fig. 1). Analyzed distance data show that SCS also exceeded the target of 250 adults in a single day abundance estimate in 2015 with a single day abundance estimate of 336 (range 246-459; Table 14), which was based solely on reproduction that year. While the release of larvae in spring 2016 will restart the five-year assessment period beginning in 2017, the robust counts recorded there in 2016 (Table 15) suggest this site is now on a very positive trajectory toward establishment.

Future Plans and Recommendations

The future of Taylor's checkerspot reintroduction and recovery in the Puget lowlands is at risk

The low numbers (Table 15) and apparent dispersal (Appendix D, Fig. 2) of the R76 population make it unlikely we can collect the requisite adults needed for captive rearing and reintroduction in 2017. In addition, the uncertain fate of this last extant Puget lowland population has critical implications for the future of this project and for recovery of Taylor's checkerspot as a whole. Currently, we monitor the R76 population to provide:

1) the basis for take as the only source of animals for captive rearing and reintroduction,
2) a reference point for reintroduction sites in terms of population phenology, trends and dynamics, and

3) the basis for our definition of a population and for expectations about population persistence and stability.

Consequently, loss or a significant reduction in numbers at R76 threatens the foundation on which checkerspot recovery in the Puget lowlands is built, by:

1) reducing the number and size of populations,
2) eliminating our only population source, and/or
3) reducing the species short and long-term viability through genetic bottlenecking.

If we cannot collect adults from R76, we have but two options: try to maintain a captive colony with the animals in captivity, or collect from R50 if that population can support take. Both are poor choices that are likely to further the risk of inbreeding in the near future. The lack of baseline data on the genetic diversity at R76, the proportion of that transferred to R50, and knowledge about the make-up of larvae in captivity also means we cannot inform our approach to captive breeding to reduce or minimize the likelihood of inbreeding.

What can we do to insure that captive propagation and reintroduction continues?

- Continue to population monitoring at R76, including access to the EOD area to insure the best possible comparisons with data from previous years.
- Conduct well-designed and extensive searches across the Artillery Impact Area to determine whether dispersing adults are congregating at a new site.
- Continue population monitoring at R50 in the event we need to collect adults from that population.
- Conduct genetic assessments to maximize the transfer of unique genetic material to reintroduction sites and to minimize the likelihood of inbreeding as a result of uninformed breeding strategies in captivity.

What can we do to secure the population at R76?

- Immediately reduce and/or eliminate all potential impacts to the Taylor's checkerspot population and its habitat at R76 that may result from range maintenance and military training, including wildfire and associated actions.
- Implement previously recommended habitat improvements that include increasing the abundance of host and nectar resources, as well as their overlap, particularly in portions of the site where training impacts are lowest and habitat structure is most suitable.
- Conduct habitat monitoring in 2017 using the RHA method, which includes larval distribution to determine whether and how habitat degradation may be contributing to the decline and dispersal of this population.

Current reintroduction-related plans

This is the eleventh year of a multi-year Taylor's checkerspot recovery project. Analyses of 2016 distance sampling data are scheduled for completion in winter 2017. We also anticipate generating annual population estimates from existing data at that time. At this time, reintroduction plans for 2017 include continuing releases at Glacial Heritage Preserve (Thurston County) and Training Area 7S (JBLM). We expect that 5-ac habitat assessments will be completed in fall 2016 using data collected in spring 2016 (Waters 2016). In the event those results indicate that reintroduction-ready habitat is lacking, decisions about where releases will occur in 2017 may be revisited. Habitat condition remains a concern at GHP, as the population there has failed to build after 5 years of releases and it appears host plants are declining in places, rather than increasing. The Butterfly Habitat Enhancement team visited this site in summer 2016 and identified several strategies that might be tried to increase the resource base while also improving structure and microsite diversity. If habitat quality does not improve there soon, we may need to abandon work in the current reintroduction area in favor of a site that can demonstrate that habitat is reintroduction-ready (see Waters 2016a).

Given the robust response of the population at SCS no further releases are recommended there at this time, although flight season monitoring will need to continue until 2021 to demonstrate that establishment criteria have been met. We also hope to initiate monitoring in management unit 2 at SCS where females were observed ovipositing in summer 2016.

It appears we met our criteria for establishment at R50 in 2016, which should trigger the end of monitoring at that site. However for the reasons listed above we assume that monitoring will continue in 2017.

Finally, we hope to continue periodic broad-scale searches in areas outside of our standard monitoring grids to search for adults and identify potential colonization areas. This work is particularly important on the Artillery Impact Area, at SCS and at GHP based on scattered sightings reported in 2016. Documenting new breeding populations is a key element of recovery that will improve our understanding of checkerspot demography and ultimately reduce the expense of captive propagation and reintroduction by providing another path for increasing the size and number of populations.

Funds for the 2016-2017 field season and rearing year have been secured from ACUB and USFWS, and are being requested from JBLM. Funding for 2017-2018 will be requested from ACUB and JBLM in winter 2017, and funds have already been received from the USFWS. The USFWS has also provided additional funding to increase the number of larvae produced and so that prediapause releases will be added to the reintroduction strategy. We have also obtained funds to write a reintroduction plan that will lay out a long-term approach for this project, including additional site selection criteria. The plan will be completed in collaboration with our conservation partners. Our objectives are to continue relatively large releases, while maintaining more than one release site, each of which encompasses a diversity of microsites to offset the influence of climatic perturbations on translocation success. Additional sources of funding and new conservation partnerships are also being explored.

Questions for Further Research

1) What is the genetic make-up of Taylor's checkerspot butterflies at Range 50 compared to the Range 76 source site and how successful have we been transferring that diversity via captive?

Now that the R50 population has met objectives for establishment, it is critical to understand what proportion of the genetic material present at R76 has been transferred to this reintroduction site. The primary objective of this genetic management project is to characterize the level of genetic relatedness between three populations: 1) the Taylor's checkerspot source population at Range 76 on Joint Base Lewis-McChord, 2) individuals surviving at the reintroduction site (Range 50), and 3) brood stock from the Oregon Zoo used for reintroduction. In addition, the relationship between these populations will inform the following conservation actions: 1) whether additional releases are needed at Range 50 to increase (i.e., match) genetic diversity relative to the Range 76 source site, 2) whether the number of "maternal lines" collected and used for reintroduction annually should be increased, and 3) identification of a strategy for preservation of genetic material in captivity that will contribute to future genetic monitoring needs. This work is particularly important because no other suitable population source exists (Severns et al. 2013) and because that population faces ongoing threats from live-fire and ground-based military training as well as habitat threats from exotic species invasions, and loss of host and nectar resources. The low numbers and scattered distribution of adults at this site indicate we are rapidly losing our opportunity to capture the genetic diversity of that population, especially if the population already went through a bottleneck in 2009 (Appendix B). If R50 is found to require a genetic boost, this could be accomplished by collecting larvae from across R76 and moving them directly to R50, then resampling the genetics after a readjustment period. This work would also shed light on the rate of genetic transfer from captive reared stock to reintroduced populations, which could be increased if needed, by increasing the number of maternal lines used in captive breeding. Capturing all of the genetic diversity from R76 is critical to maximizing the likelihood of species persistence through the trials of reintroduction-related mortality, inclement weather, change in habitat quality, diversifying microsites, and ultimately surviving in the face of a changing climate. The loss of thousands of larvae during diapause at the Oregon Zoo in winter 2015 underscores the potential for bottlenecking that can occur with small imperiled populations facing an uncertain future. As such, it is imperative that we take a cautious approach now to conserve opportunities for Taylor's checkerspot to repopulate suitable sites in the future as habitat across a broader range of sites becomes suitable through restoration.

2) What are the effects of weather and climate on the population dynamics of Taylor's checkerspot and how might they be influence reintroduction success?

Weather is known to have dramatic impacts on the annual abundance of *Euphydryas editha* populations in California (Weiss and Weiss 1998). Similarly, population monitoring of Taylor's checkerspot has documented order of magnitude changes over short, 1-3 year timelines that cannot be explained by changing vegetation. While we cannot change the weather, we can work to design and build habitat that improves the likelihood of successful reintroduction and recovery. The objectives of this project would be to determine whether 1) adult abundance is correlated with weather-related factors, 2) flight season phenology is correlated with weather-related factors, 3) adult emergence is correlated with cumulative heat indices (e.g., degree days), and 4) flight season phenology is correlated with climate indices (e.g., oscillation indices) that reflect regional climatic conditions. These data would improve our understanding of abiotic influences on population size and elucidate the role of habitat quality in reintroduction success. Understanding the influence of weather and a changing climate on population size will inform recovery planning, timelines and the suitability of proposed actions. Multi-year population data obtained via distance estimation are in hand for two extant checkerspot sites (Range 76 on JBLM and Graysmarsh in Clallam County) in Washington and some weather data have already been compiled. A similar approach has been used by others to examine the influence of climate and weather on spotted owl population trends (Glenn et al. 2010, Dugger et al. 2016).

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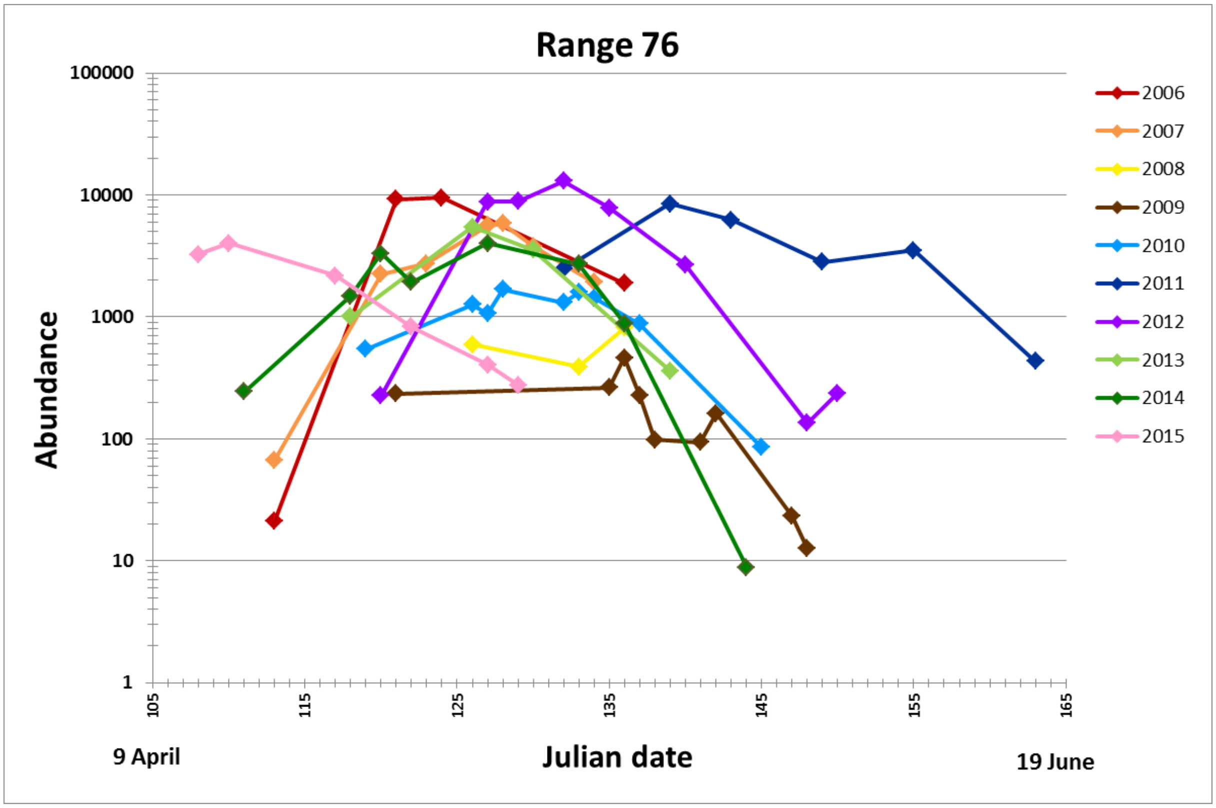
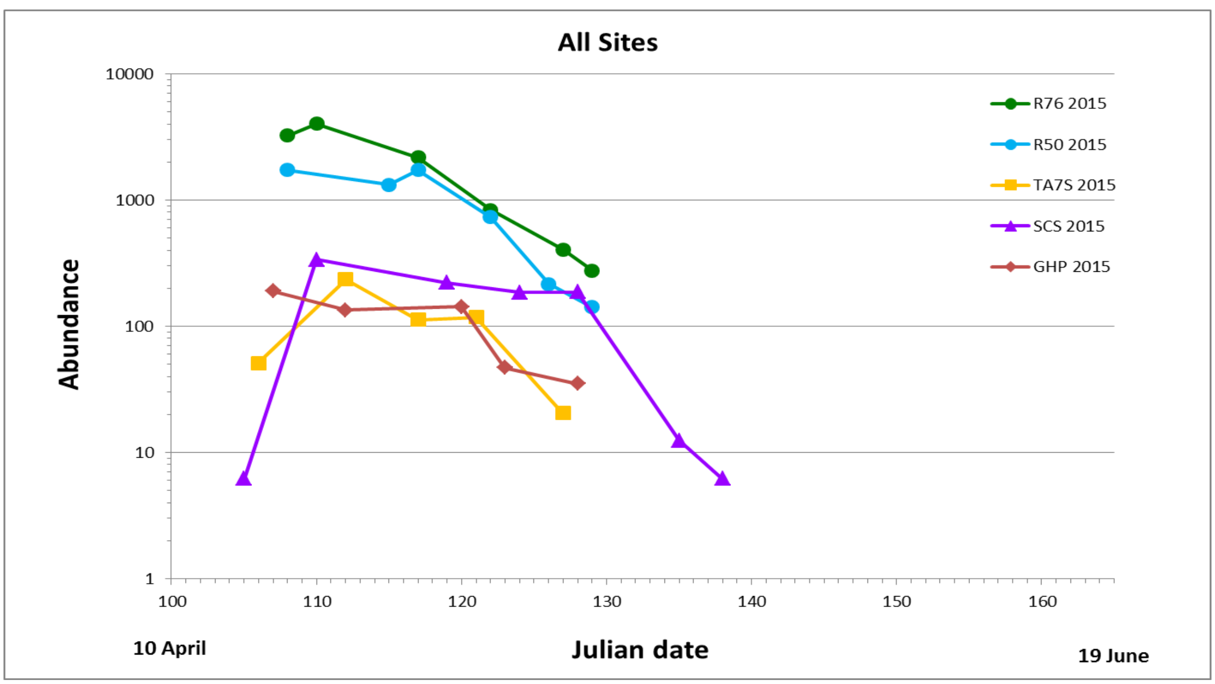
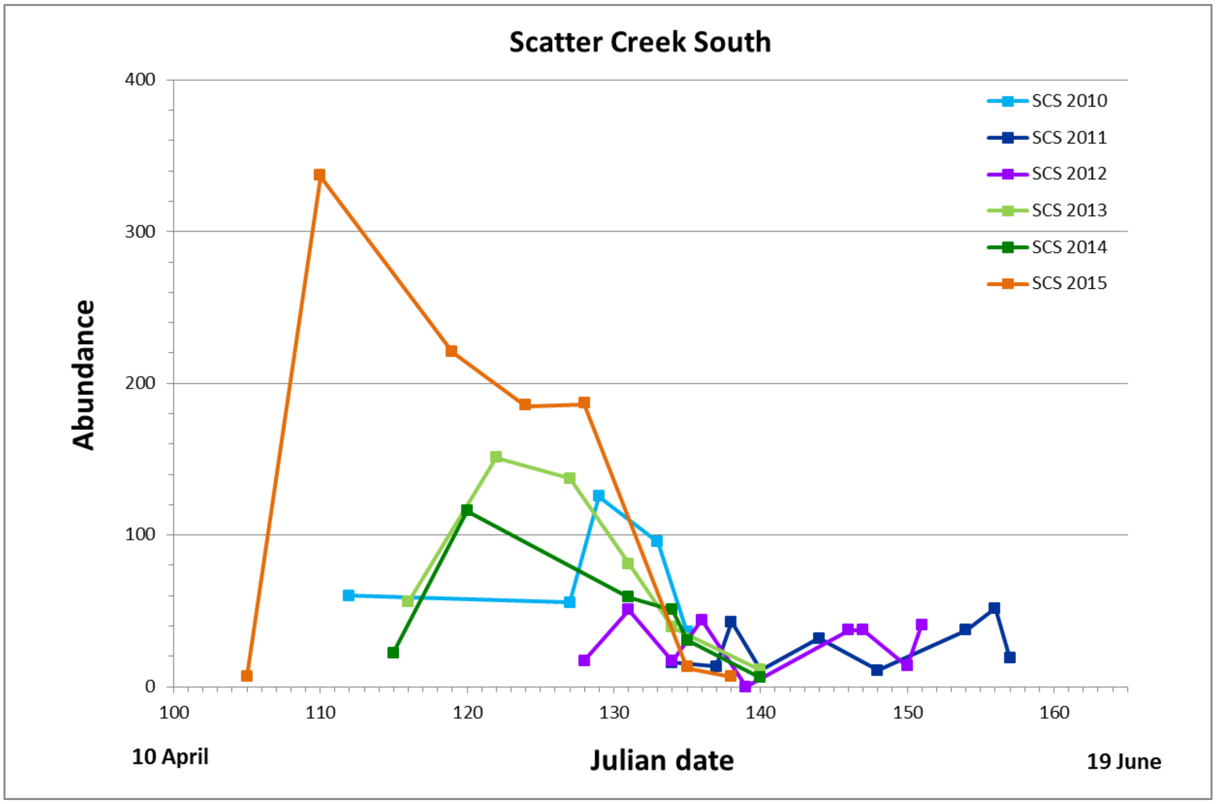
Appendix A. Approximate number of Taylor's checkerspot butterflies released by life stage (Pre = prediapause larvae; Ad = adult, Post = postdiapause larvae) and site (SCS = Scatter Creek South; R50 = Range 50; GHP = Glacial Heritage Preserve; PCM = Pacemaker Airstrip; TA7S = Training Area 7 South; SCN=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

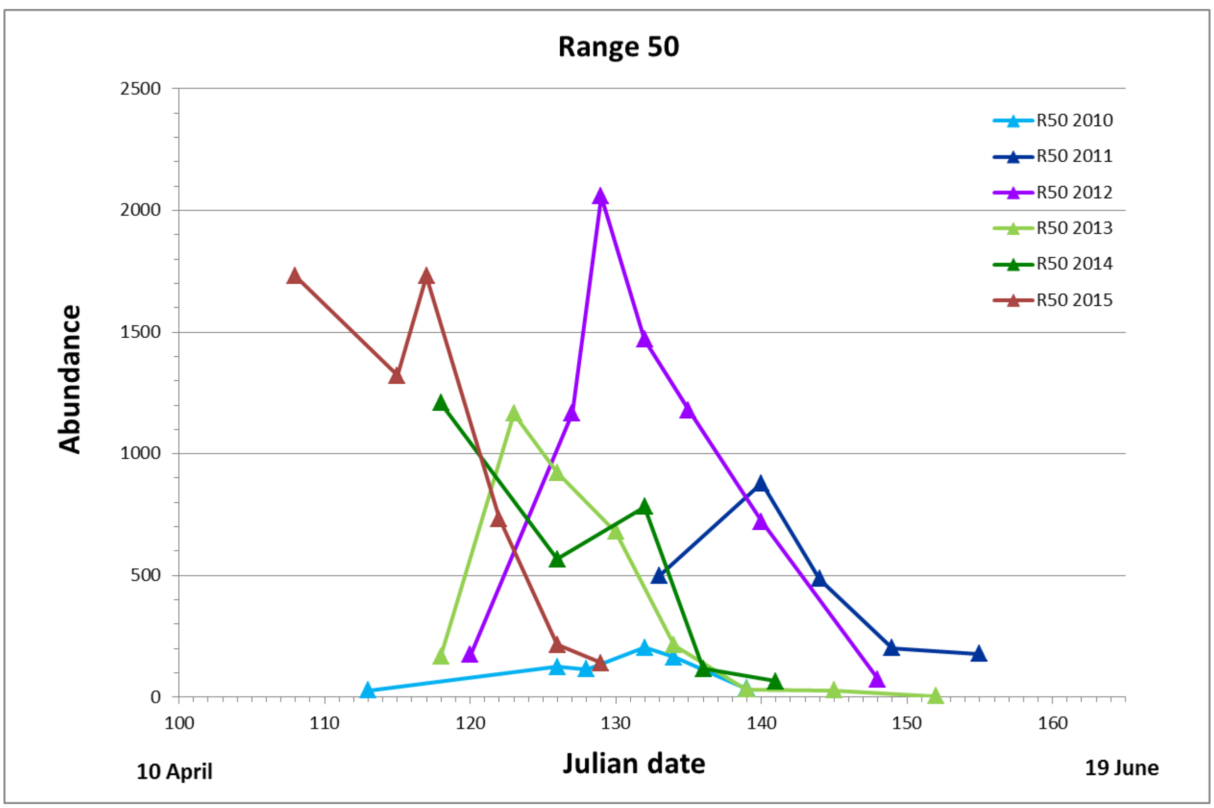
	2007	2008	2009		2010		2011			2012		2013		2014		2015		2016		
Site	Post	Post	Post	Pre	Post	Ad	Post	Ad	Pre	Post	Ad	Post	Ad	Post	Ad	Post	Ad	Post	Ad	Pre
SCS	199	340	747	2487	891	202	1109	167	1036		133	3250			56			1658		
R50				2956	1145		1141													
GHP										975		3372	107	1522		1203	226	2029		
PCM			741							1565										
TA7S														1086		1102			231	1003
SCN			759																	
TNQ																				
WRP																				
T15																				
BHP																				
MMP																				
WHI																				

Appendix B. Estimated daily abundance of Taylor's checkerspots based on distance estimation, and timing of the flight season at Range 76 (R76, source site) in 2006-2015 (top), and for 2015 relative to four reintroduction sites (bottom): Scatter Creek South (SCS), Range 50 (R50), Training Area 7 South (TA7S), and Glacial Heritage preserve (GHP) in South Puget Sound, Washington. Note log scale on the y-axis. Area surveyed varies by site and year; see text for details. Daily abundance estimates for 2010-2015 on two long running reintroduction sites (SCS and R50) are shown on the next page.






Scatter Creek South
400
300
200
100
0
Abundance
100
110
120
130
140
150
160
10 April
Julian date
19 June
SCS 2010
SCS 2011
SCS 2012
SCS 2013
SCS 2014
SCS 2015


Range 50
2500
2000
1500
1000
500
0
Abundance
100
110
120
130
140
150
160
10 April
Julian date
19 June
R50 2010
R50 2011
R50 2012
R50 2013
R50 2014
R50 2015

Appendix C. Distribution of adult Taylor's checkerspots observed during distance sampling surveys at four reintroduction sites (SCS, R50, GHP, and TA7S) and one extant site (R76) in South Puget Sound, Washington, spring 2016.

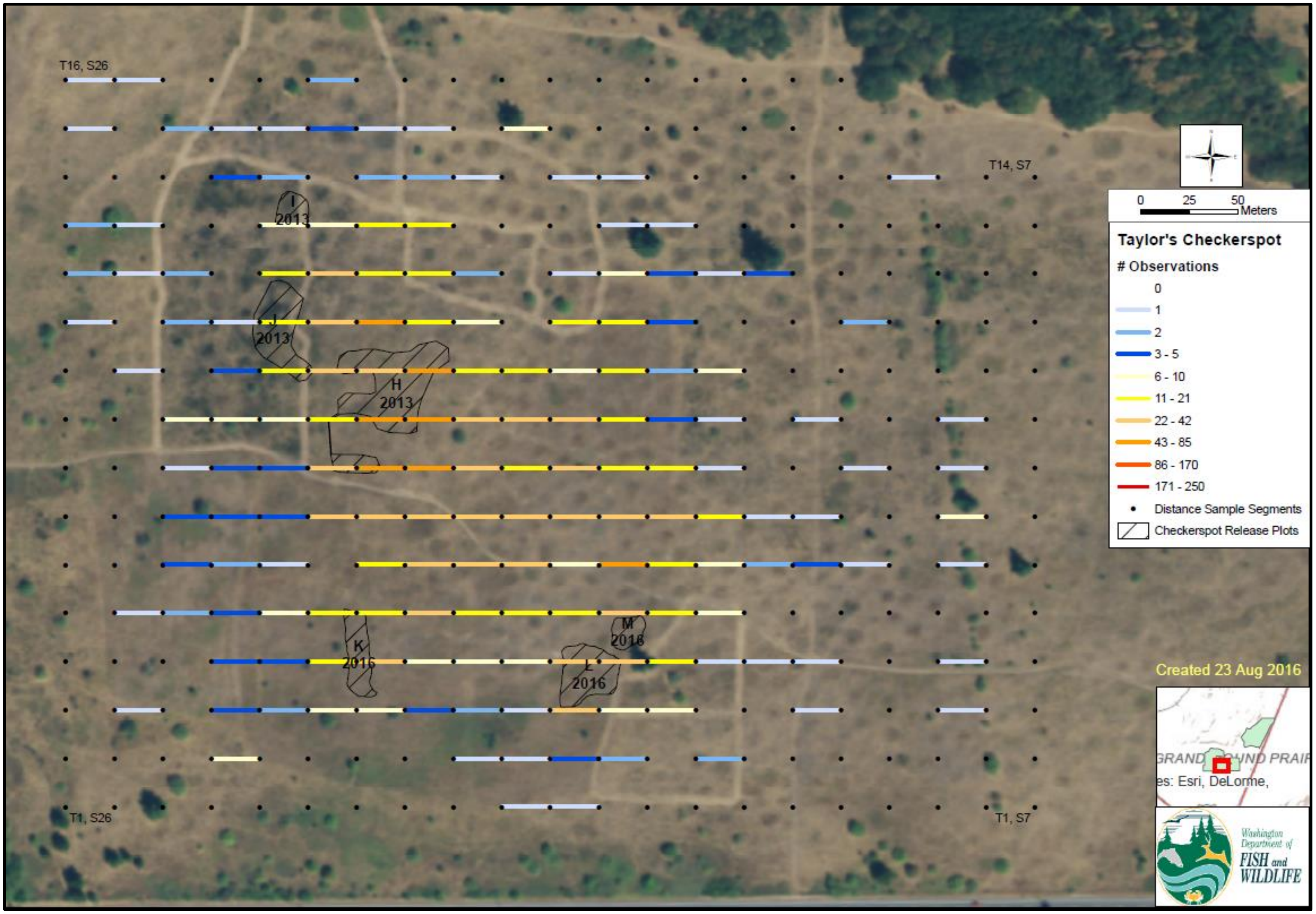


Figure 1. Distribution of adult Taylor's checkerspots observed during distance sampling surveys at Scatter Creek South combined across all survey dates in spring 2016, South Puget Sound, Washington.

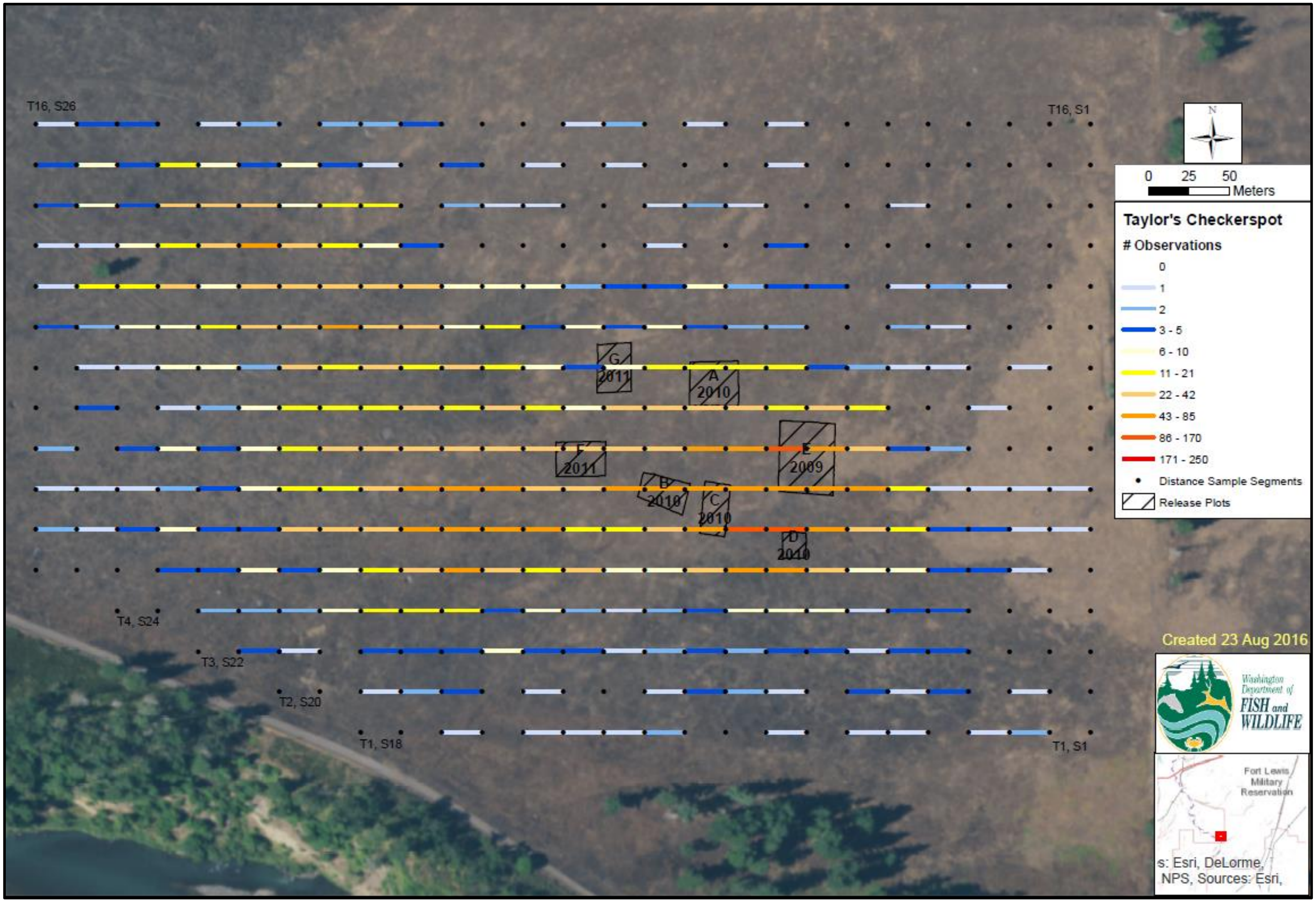


Figure 2. Distribution of adult Taylor's checkerspots observed during distance sampling surveys at Range 50, combined across all survey dates in spring 2016, South Puget Sound, Washington.

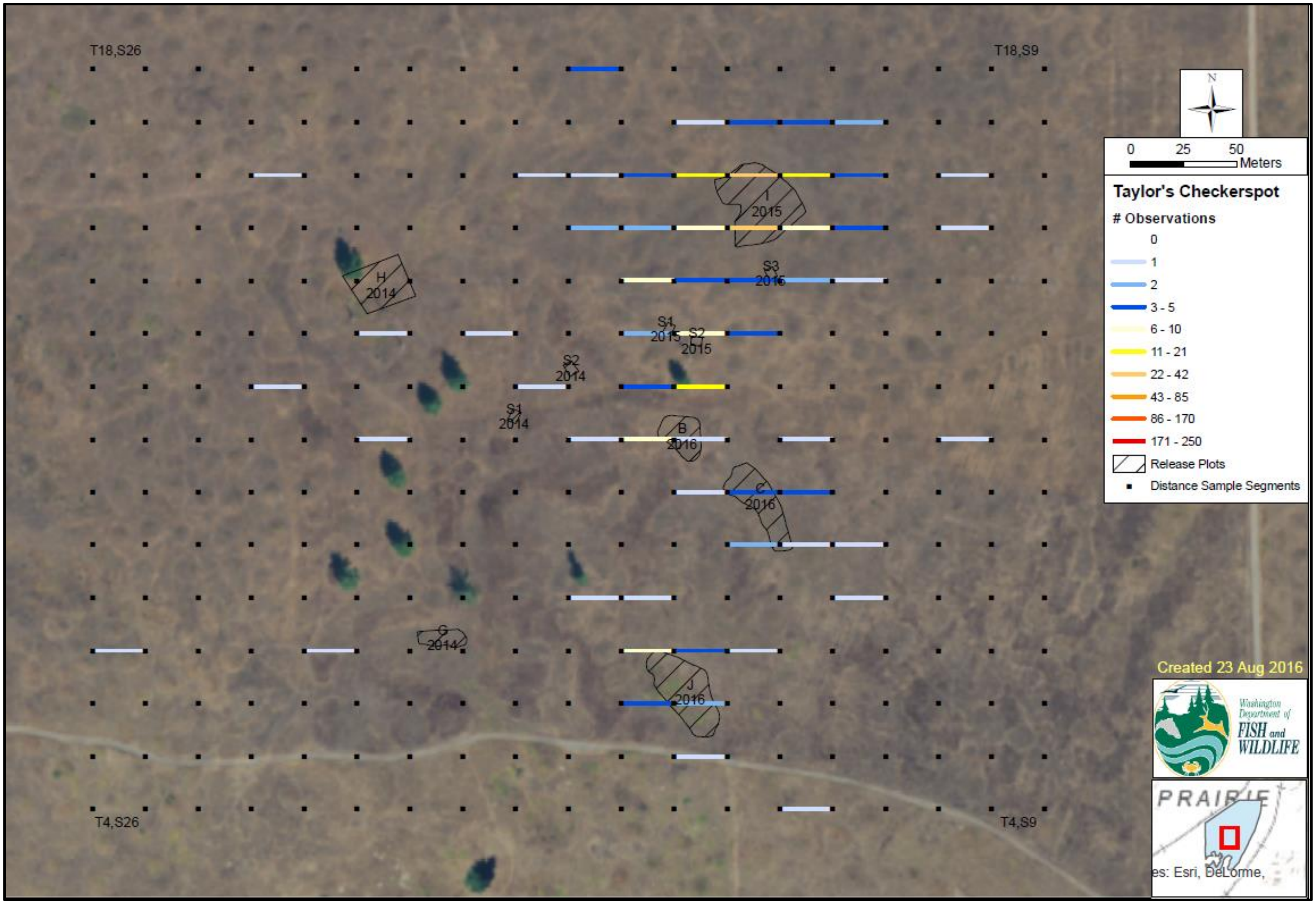


Figure 3. Distribution of adult Taylor's checkerspots observed during distance sampling surveys at Glacial Heritage Preserve, combined across all survey dates in spring 2016, South Puget Sound, Washington.

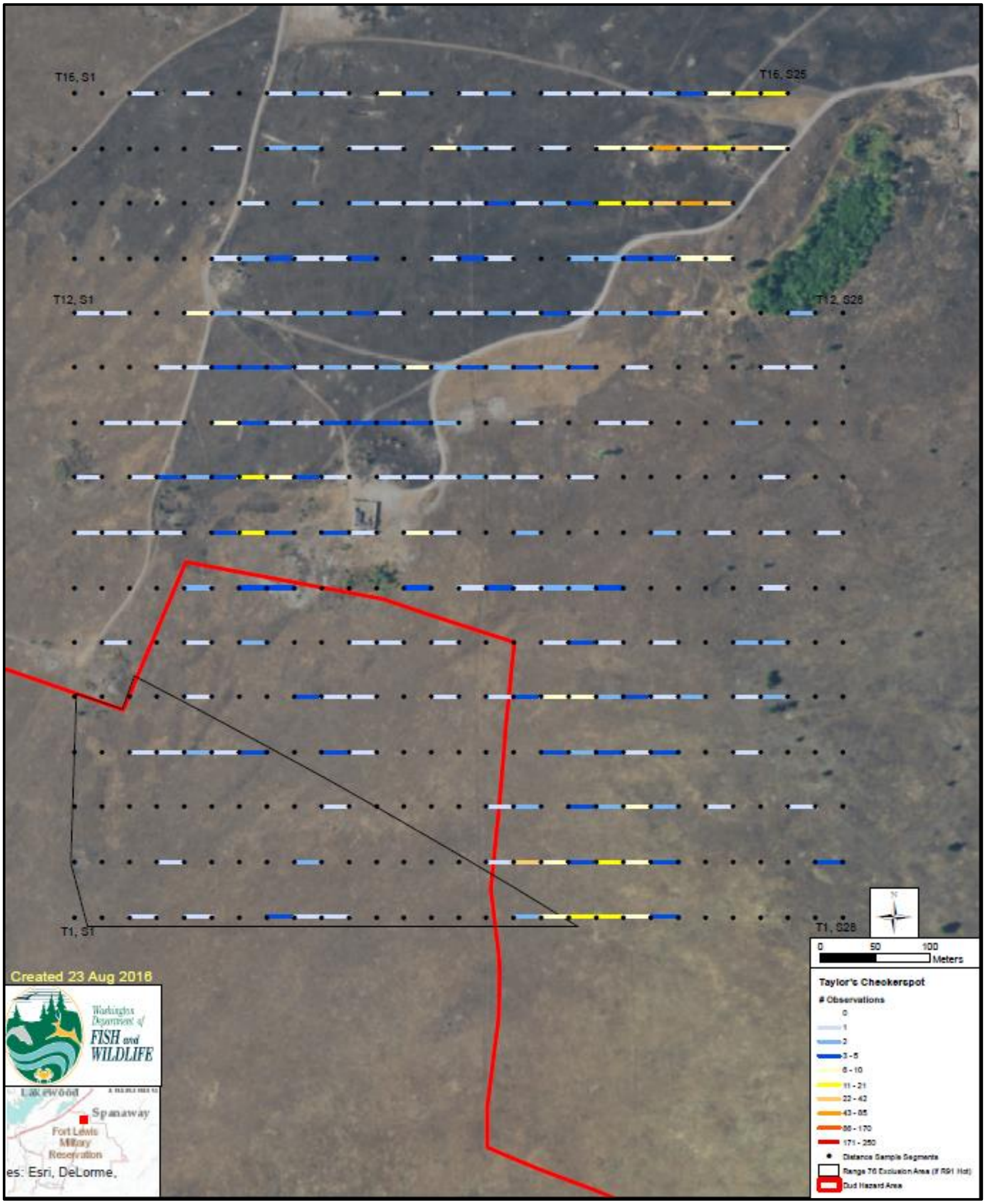


Figure 4. Adult Taylor's checkerspots observed during distance sampling surveys at Range 76, combined across all survey dates in spring 2016, South Puget Sound, Washington.

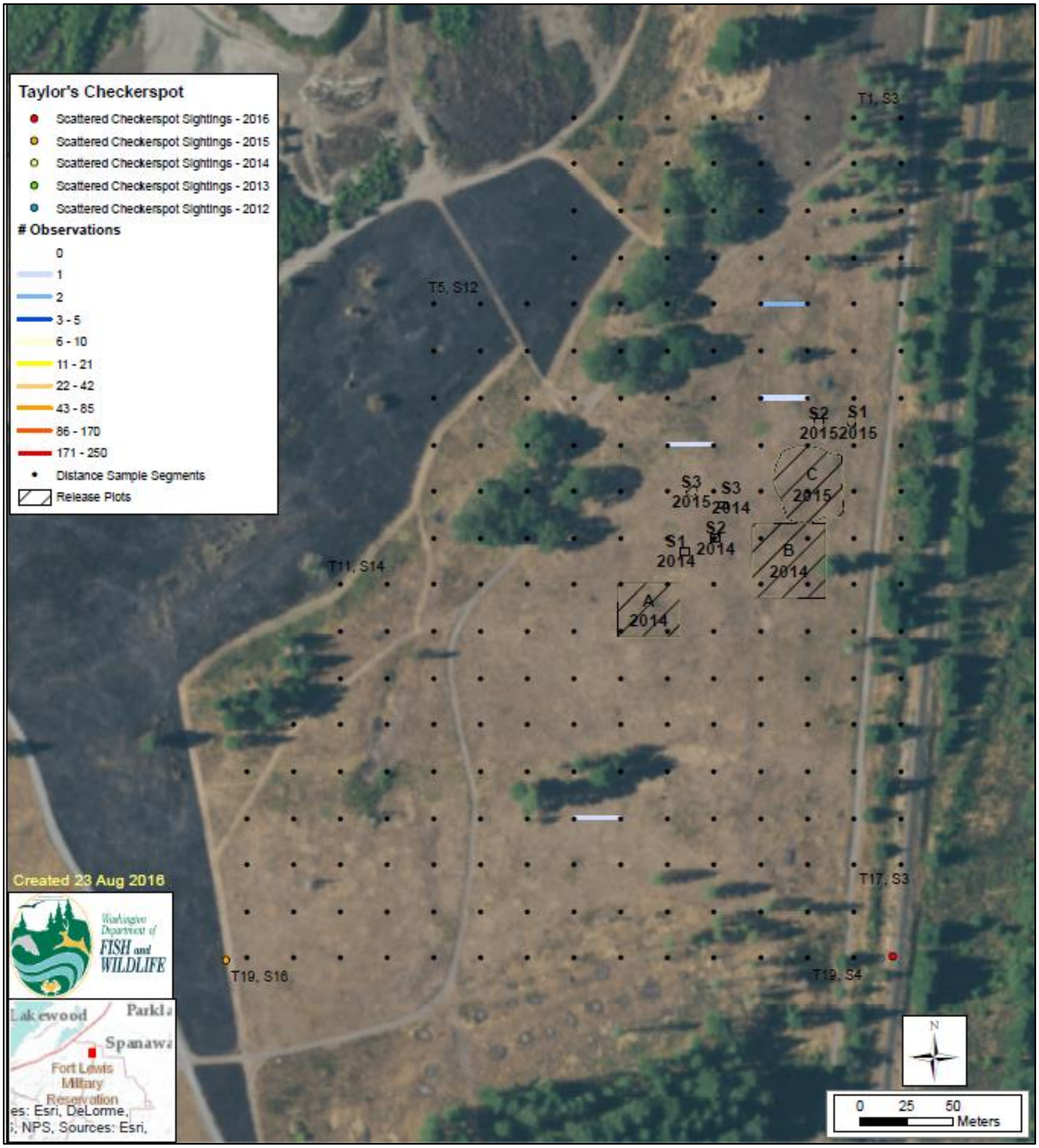


Figure 5. Distribution of adult Taylor's checkerspots observed during distance sampling surveys and scattered sightings at Training Area 7S, combined across all survey dates in spring 2016, South Puget Sound, Washington.

Appendix D. Scattered sightings (2012-2016) of Taylor's checkerspots outside of distance survey areas.

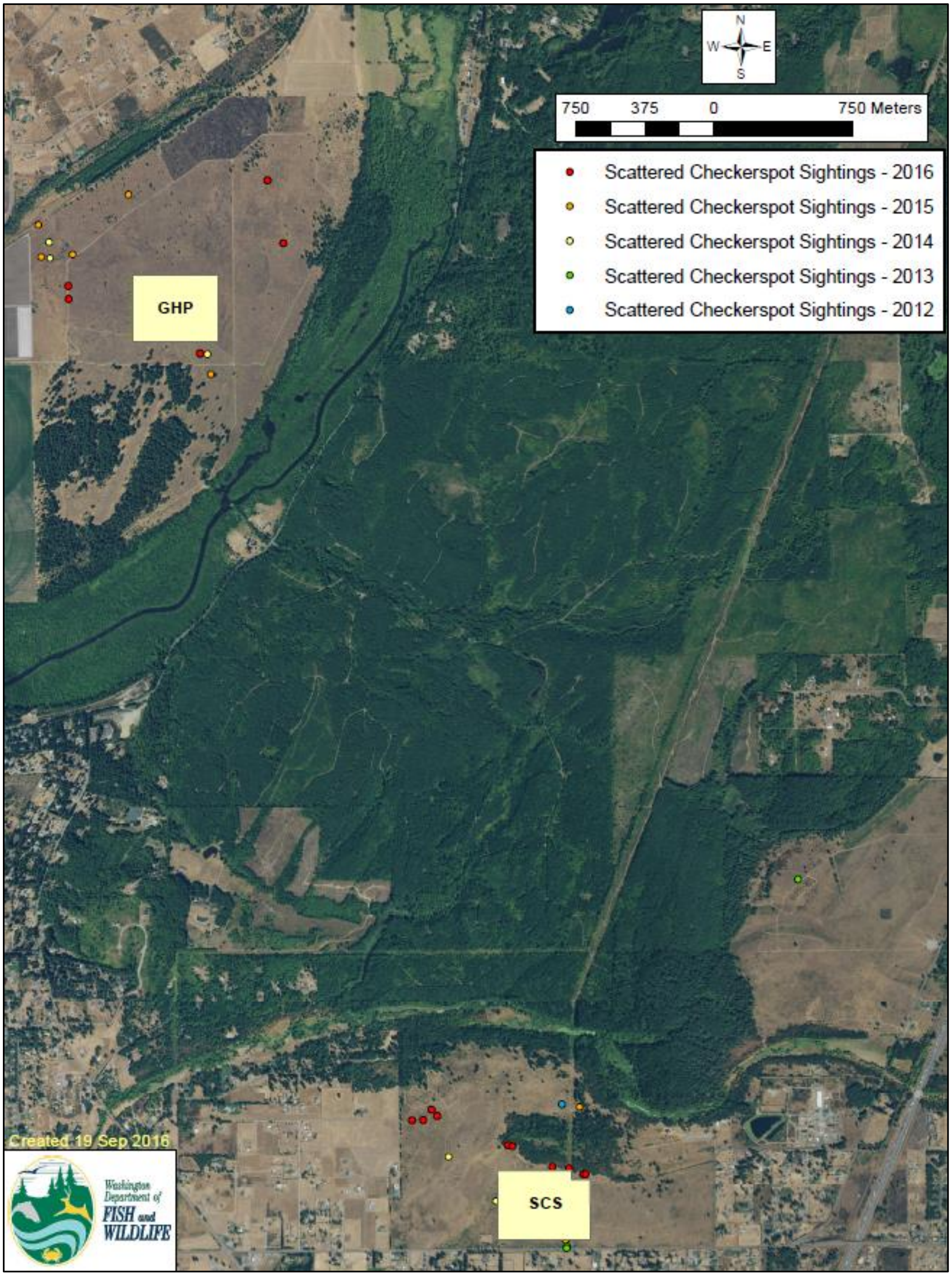


Figure 1. Scattered sightings of adult Taylor's checkerspot observed at Glacial Heritage Preserve (top) and Scatter Creek South (bottom), outside of distance survey areas (yellow shade), South Puget Sound, Washington, 2012-2016.

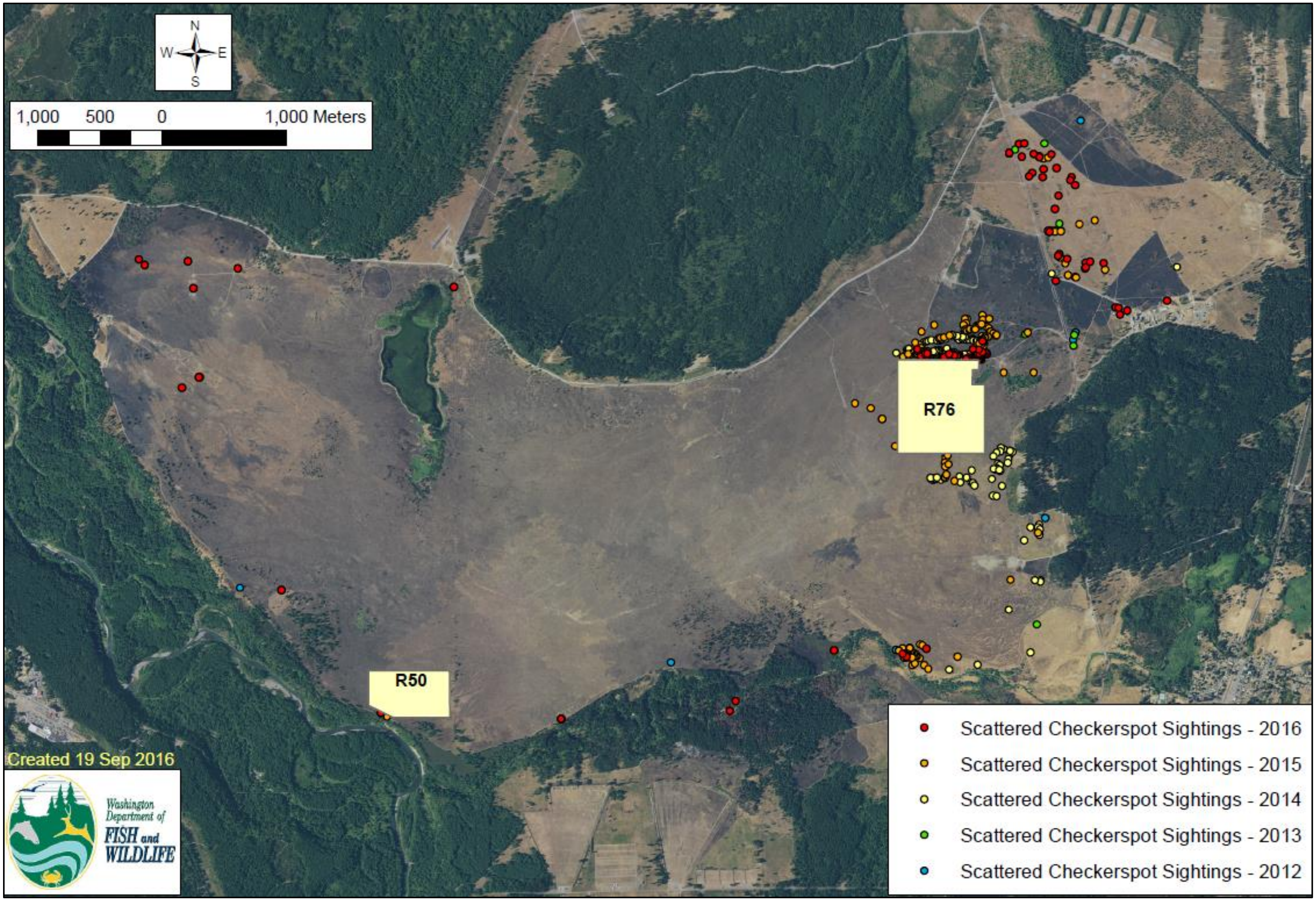


Figure 2. Scattered sightings of adult Taylor's checkerspot on the Artillery Impact Area outside of distance survey area (yellow shade), Joint Base Lewis-McChord, Washington, 2012-2016. Some areas (e.g., north of R76 survey area) were searched systematically by JBLM personnel.