Taylor's checkerspot (*Euphydryas editha taylori*) Captive Rearing and Translocation: South Puget Sound, Washington, 2016-2017

2017 Final Annual Report to:
US Fish and Wildlife Service (Cooperative Agreement #F15AC00515, F16AC00588), Joint Base Lewis-McChord (Contract #W911S8-17-2-0015), and the ACUB Technical Review Committee (Subaward #WA-S-2016-070-0)

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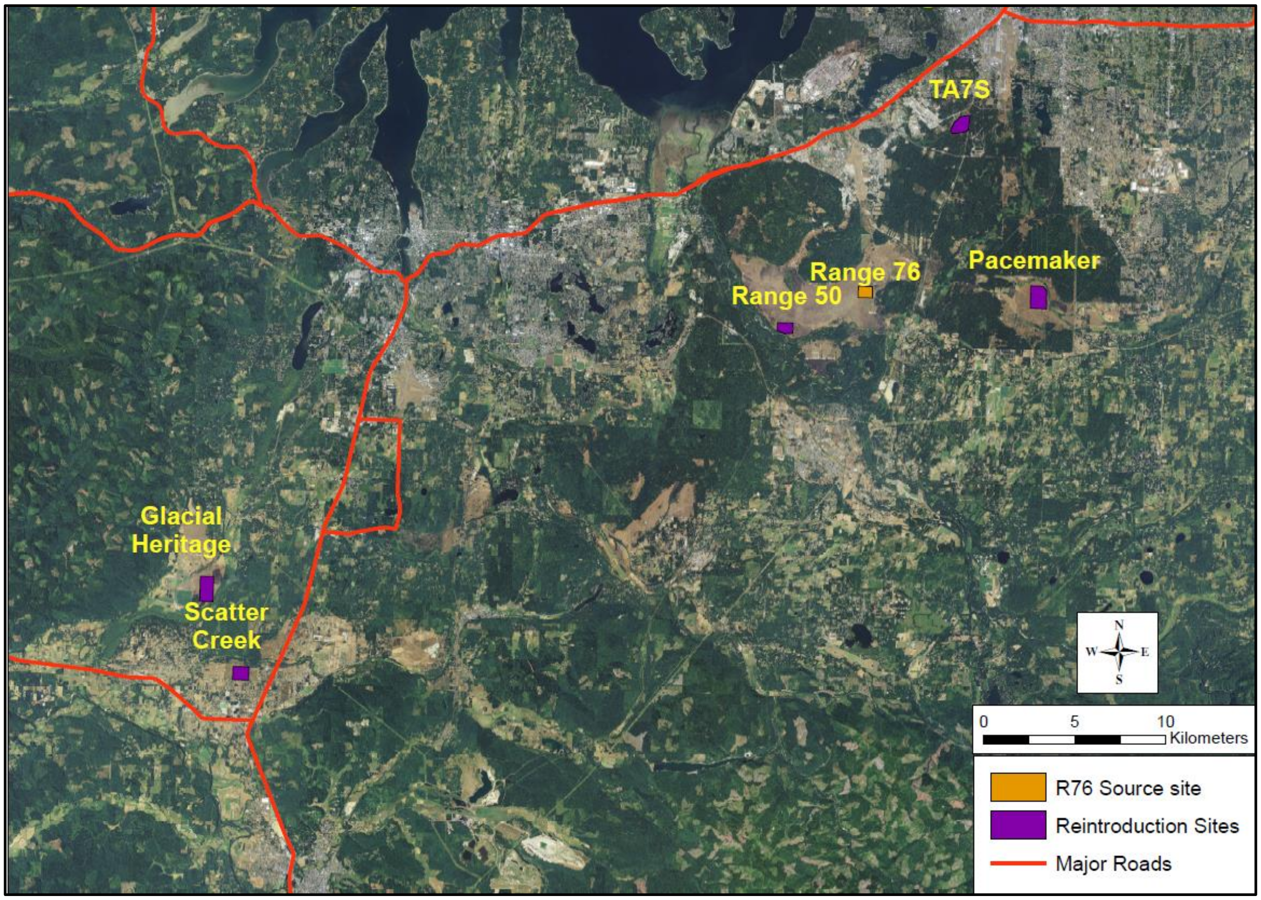
Project Sites

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; releases in 2007-14, 2016); Range 50, Joint Base Lewis-McChord (JBLM) (R50; releases in 2009-2011); Pacemaker Airstrip, JBLM (PCM; release in 2012); Glacial Heritage Preserve (GHP; releases in 2012-2017); Training Area 7 South (TA7S; releases in 2014-2017) and Range 76, JBLM (R76; population source for captive propagation). A second monitoring area at Scatter Creek South (Unit 2) was established to document potential colonization.



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Executive Summary

This report summarizes work on Taylor's checkerspot captive rearing and translocation conducted in July 2016-December 2017. The captive rearing section borrows heavily from annual reports produced by the Oregon Zoo (Lewis et al. 2017) and Mission Creek (Curry et al. 2017) programs. A total of 10,657 eggs were produced at the two facilities with 38.8 percent (4,139) of eggs produced by captive-mated females and 61.2 percent (6,518) by wild females. The latter were collected from the Range 76 (R76) source site on JBLM. Wild females at the Zoo produced 63.5 percent of eggs, with 36.5 percent produced by captive females between 19 April and 12 May 2016. At Mission Creek, wild females produced 52.9 percent of the eggs and with captive-mated females contributing the remaining 47.1 percent between 22 April and 14 May 2016. In all, 7,840 prediapause larvae (5,020 wild and 2,820 captive) were produced, with 6,965 of those (4,293 wild, 2,672 captive) surviving to diapause. In spite of a cold winter, high postdiapause mortality was again observed at the Oregon Zoo, ruling out warm winter nights as the likely cause; food quality and/or exposure to toxins are the next most likely causes. Mission Creek retained 288 larvae from twelve 2016 matrilines, as well as 41 second diapause larvae from three 2015 matrilines for breeding; all were offspring of wild females. At the Zoo, 189 larvae from eight 2016 matrilines and five larvae from three 2015 matrilines were retained for breeding**.**

Postdiapause larval Taylor's checkerspots were released at two existing reintroduction sites in 2017: Glacial Heritage Preserve (GHP; initiated in 2012) and Training Area 7 South (TA7S; initiated 2014); adults were also released at TA7S when their role in the captive rearing program was complete. Monitoring for establishment continued Scatter Creek South Unit 1 (SCS1; initiated in 2008), and at Range 50 (R50; initiated in 2009) in the event that site was needed for collection and to comply with JBLM's federal reporting requirements. A second monitoring unit at Scatter Creek South (Unit 2; SCS2) was also monitored in 2017 for evidence of colonization. Staff from Joint Base Lewis-McChord (JBLM) took over monitoring at Pacemaker (PCM; initiated in 2012) in 2016, as there were no sightings in 2015. A total of 1,843 postdiapause larvae from Mission Creek were released at GHP on 10 Mar 2017. In addition, 1,182 larvae were released from the Oregon Zoo on 23 Mar 2017. Larvae from both facilities were split about equally between the main monitoring area where *Castilleja hispida* has been inter-planted with *Plantago lanceolata,* and the area due west of the monitoring area where *C. levisecta* co-occurs with *Plantago*. Our intent was to quantify oviposition across the range of available host species. On 16 Mar 2017, we released 719 larvae from Mission Creek at TA7S in Plot D, with an additional 190 larvae added on 30 Mar 2017, once we determined they were not needed in the breeding colony. Of the 3,025 larvae released at GHP, 1,793 were offspring of wild females, 1,205 were offspring of captive-mated females, and 27 were of unknown (QC) origin; QC larvae became separated from their group at some point during the rearing process. Of 909 larvae released at TA7S, 758 were offspring of wild females, 150 were offspring of captive-mated females, and 1 as of unknown (QC) origin. One hundred forty-six adults and one Taylor's checkerspot pupa (88 females, 56 males and 1 unknown) were released at TA7S between 30 May and 2 Jun 2017. Of these, 67 came from the Oregon Zoo and 80 came from Mission Creek.

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), one potential colonization site (SCS2) and at R76 (source site). A total of 7,224 checkerspots were counted during distance surveys in 2017. Flight season initiation was one month later than in 2016 and was the second latest recorded since 2005. Peak counts were staggered from 10 to 29 May 2017. Peak day encounter rates varied among sites, with about 60 percent more butterflies observed at R50 (0.142 checkerspots/m) compared to SCS1 (0.090/m). Rates at R76 (0.053/m for transects 1-16; 0.048/m for transects 1-12) were about one third of those at R50 and half of what was observed at SCS1. Long-term

monitoring and population goals developed in fall 2012 were used to assess progress at R50 and SCS1. Based solely on natural reproduction, adult checkerspots were distributed across the majority of the 25-ha monitoring area at R50, and have increased their spread annually since 2014, with 20.9 ha acres occupied in 2017, the largest to date. The peak single day abundance estimate (4,244; 95% CI: 3,488-5,165) was also the highest recorded for that site and topped all Puget lowland sites for the second straight year. This population is officially established based on the project definition and continues to expand. Checkerspots at SCS1 met criteria in 2015 based solely on reproduction, but a larval release in spring 2016 to address concerns about *El Nino* weather on that small population restarted the five-year assessment period in 2017. Checkerspots at SCS1 occupied 61 percent of the 20-ha monitoring unit in 2017. The peak single day abundance estimate of adults in 2017 (2,298, 95% CI: 1,706-2,944; Table 17) suggests this site handily met its Year 1 establishment goal, is on a strong positive trajectory, and on track to complete the trial period for establishment by 2021.

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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 move toward species recovery. To accomplish this, WDFW and its partners intend to establish at least three new populations at three sites by 2022 via captive rearing and reintroduction. This report mainly summarizes 2016-2017 activities, but includes some longer-term summaries as well. Objectives for 2017 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 reintroduction success as follows:
    a. Evaluate release success using postdiapause larval and adult surveys at reintroduction sites (Glacial Heritage and Training Area 7S), and
    b. Evaluate reintroduction success based on presence, distribution and relative abundance of adults at two past reintroduction sites (Scatter Creek South Unit 1 and Range 50) and one potential colonization site (Scatter Creek South Unit 2).
5) Produce egg clusters to support research by Nate Haan at the University of Washington-Seattle.

We accomplished these objectives with joint funding from 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. For clarity and cohesion, this report covers all 2016-2017 captive propagation and reintroduction activities regardless of funding source.

The report is organized around five project objectives, including several smaller sub-tasks, which we demarcated by headings in throughout the document.

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 suitable for reintroduction (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 review habitat conditions, assess project progress and identify outstanding needs.

Final selection of reintroduction sites 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 values 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. In addition, at least one 5-ac block should be added annually for the next 3 years to total 25 acres of Reintroduction Ready habitat as well as 25 ac of additional habitat to support population expansion (Linders 2015a). Ideally all restoration would be complete prior to initiating reintroduction, but burn bans, seed shortages and other difficult-to-predict events have made this challenging. Some issues are within our ability to address, but others are weather related and/or beyond the control of our conservation collective. In addition to our baseline objectives in 2017, we wanted to release postdiapause larvae in habitat that contained abundant *Castillja levisecta* with the ultimate goal of examining whether adult checkerspots use both *Castilleja* species for oviposition when given a choice.

Results and Discussion

Five sites have been selected for reintroduction to date from the larger list of potential Taylor's checkerspot sites in the South Puget lowlands (Linders 2006, Fimbel and Dunn 2013). Four sites, SCS (2007-2014, 2016), R50 (2009-2011), GHP (2012-2017), and TA7S (2014-2017), have received multiple sequential releases consistent with the scheduled approach (Appendix A); one site (PCM) received a single release (2012), after which releases were restricted by JBLM.

Releases in 2017 occurred at GHP (6th year) and TA7S (4th year), consistent with our existing strategy (Linders et al. 2015). We established one release plot at TA7S, and two release areas, each with several release plots, at GHP (Table 1). The TA7S release plot was identified based on *Plantago lanceolata* abundance and plentiful nearby nectar, and was also adjacent to the plot where prediapause larvae were released in June 2016 (Appendix C, Fig. 5). Total plot area measures are listed in Table 1. Both GHP and TA7S exceeded the minimum habitat requirement (1,500 square meters) and provided more than 1 square meter of habitat per released larva (See Postdiapause larval release; Table 12).

We continued releases at GHP beyond the initial 5-year window because: 1) previous releases failed to result in a population large enough to be self-sustaining, and 2) this site supports two *Castilleja* species (*C. hispida* and *C. levisecta*) in geographically distinct areas of the site. Glacial Heritage Preserve is a unique example of where both Castilleja species reside within the context of abundant *Plantago lanceolata,* which provides a unique opportunity to examine whether adult checkerspots use both *Castilleja* species for oviposition when given a choice. To address this question we planned to first release postdiapause larvae within each of the areas where one of the *Castilleja* species co-occurred with *Plantago,* a strategy that would provide ample host plant for developing larvae and subsequent oviposition sites for emerging females. The *C. hispida* release plots (B, J and K) were located within the eastern portion of the larger monitoring area. They had been used in previous reintroductions but were

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Table 1. Size of release plots and total release area for Taylor's checkerspot larvae at two reintroduction sites, Training Area 7 South (TA7S) and Glacial Heritage Preserve (GHP), South Puget Sound, Washington, spring 2017. Larvae released at GHP were placed in either *Castilleja hispida* (CAHI) or *Castilleja levisecta* (CALE) dominated areas to observe if adult preferences were detected between host plant selection.

Site	Plot ID	Sq meters	Acres
GHP-CAHI			
	Plot B	191	0.047
	Plot C	275	0.068
	Plot J	393	0.097
	Plot K	150	0.037
GHP-CALE			
	Plot T	74	0.018
	Plot U	136	0.034
	Plot W	118	0.029
	Plot X	180	0.044
	Plot Y	224	0.055
	Plot Z	169	0.041
Site total		**1910**	**0.471**
TA7S	Plot D	**1237**	**0.305**

believed to be unoccupied at the time of release, and were close to Plot I, which hosted the existing population (Appendix C, Fig. 3). The *C. levisecta* release plots (T, U, W-Z) were located a minimum of 400 m west of the *C. hispida* area and had not been used previously (Appendix C, Fig. 3). In addition, forty 1x1-m survival plots were established in the vicinity of release plots to monitor larval survival. They were evenly split between the two *Castilleja* release areas (Appendix C, Fig. 3), with a buffer of at 3-75 meters between 1x1-m plots and concentrated release areas.

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

Our captive propagation objective is to produce 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.

Continue captive propagation at the Oregon Zoo and Mission Creek t 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, we collect wild females from the source population at R76 each year. A population size of at least 1000 adults at R76 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 at R76 often exceed 1000 adults (Appendix B). Should something happen to the wild

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population, 10 females per rearing facility annually is a minimum need 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 30 females (20 to Oregon Zoo and 10 to Mission Creek) were collected from R76 on 25 April 2016 and delivered to the rearing facilities on the same day following methods reported previously (Linders and Lewis 2013). All females were in good condition.

Captive mating

Methods

In total 126 adults from 9 maternal lines originating as eggs in 2015 (15FL) were available for inclusion in the captive breeding colony at the Oregon Zoo in 2016. Similarly, 299 adults from 13 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 (Lewis et al. 2015) whereby the first matriline entered the chain when 40% of postdiapause larvae pupated. Remaining matrilines were included as females from those lines eclosed, insuring males would be ready to mate when females are fresh. Adults were fit into 3 daisy chains between the two facilities, one at Mission Creek and two at the Zoo (e.g., Fig. 1, Table 3).

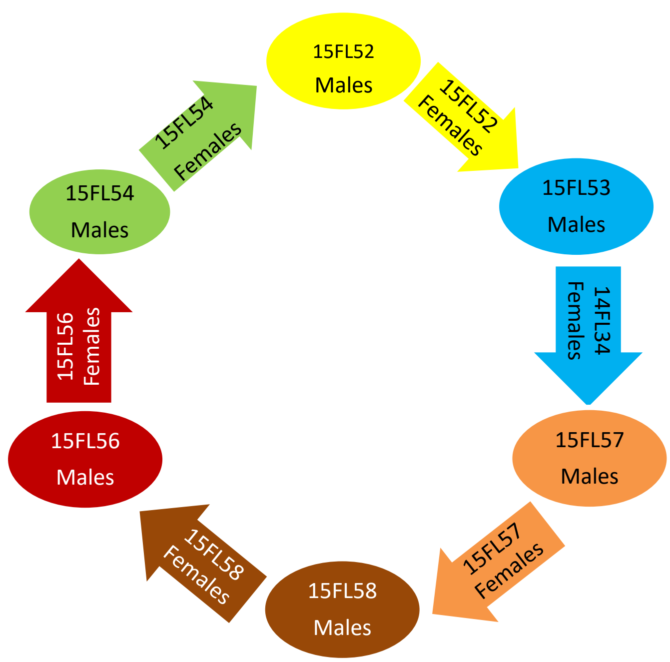

Figure 1. "Daisy chain" pairing strategies used to manage Taylor's checkerspot breeding introductions in 2016 at Mission Creek Corrections Center for Women, Belfair, Washington.

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Table 2. Number of adult Taylor's checkerspots retained for breeding by matriline and sex at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington; adults were later released in South Puget Sound, Washington, spring 2016.

Matriline	Females	Males	Released Females	Released Males
Oregon Zoo				
15FL01	5	8	3	6
15FL02	5	7	3	3
15FL05	3	4	2	2
15FL06	4	5	2	2
15FL07	11	10	9	5
15FL08	6	7	4	4
15FL09	6	5	4	4
15FL10	12	7	10	5
15FL13	15	6	13	2
OZ Total	**67**	**59**	**50**	**33**
Mission Creek				
15FL51	4	3	2	0
15FL52	11	18	7	3
15FL53	18	12	9	1
15FL54	9	11	5	1
15FL55	15	9	11	0
15FL56	15	14	11	3
15FL57	10	10	3	2
15FL58	14	13	13	3
15FL59	14	14	9	1
15FL67	15	13	11	9
15FL69	18	11	14	0
14FL21	9	6	8	2
14FL24	4	9	0	3
MC Total	**156**	**143**	**103**	**28**

Breeding introductions followed established protocols (Barclay et al. 2009) with recent modifications (Lewis et al. 2013, Hamilton et al. 2014). Our objective was to obtain two copulations from each mating dyad, to generate sufficient eggs for captive rearing and 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. We moved females to oviposition chambers once copulation was complete. Adults that were physically fit at the completion of the mating period were released (see Adult release).

Results and Discussion

Between 21 April and 2 May 2016, the Oregon Zoo conducted 47 planned breeding introductions, resulting in 17 copulations (Table 3), which was similar to the rate reported in 2015. Twenty females did not copulate despite six of those having multiple opportunities. Providing supplemental heat in advance of introductions and aging males for at least 3 days is believed to be improving male performance.

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Table 3. Summary of Taylor's checkerspot captive breeding introductions by matriline of origin (ID) and dyad, including number of introductions, unique females, copulations, copulation rate, and 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, spring 2016.

Female ID	Male ID	Intros	Females	Copulations	Copulation rate	Productive females
Oregon Zoo						
15FL01	15FL13	8	5	2	0.25	2
15FL02	15FL10	6	4	2	0.33	2
15FL05	15FL06	1	1	1	1.00	1
15FL06	15FL08	6	4	2	0.33	0
15FL07	15FL02	9	8	2	0.22	1
15FL08	15FL05	5	5	2	0.40	2
15FL09	15FL01	6	5	2	0.33	0
15FL10	15FL07	2	2	2	1.00	2
15FL13	15FL09	4	3	2	0.50	2
OZ Total		47	**37**	**17**		**12**
Mission Creek						
15FL52	15FL53	20	13	2	0.10	1
15FL53	15FL57	11	10	8	0.73	2
15FL57	15FL58	18	10	6	0.33	1
15FL58	15FL56	36	14	1	0.03	0
15FL56	15FL54	30	17	2	0.07	1
15FL54	15FL52	11	7	4	0.36	2
15FL59	15FL55	28	14	1	0.04	0
15FL55	15FL69	38	13	1	0.03	0
15FL69	15FL67	42	17	2	0.05	0
15FL67	14FL24	30	14	1	0.03	0
14FL24	14FL21	14	3	3	0.21	1
14FL21	15FL59	20	6	1	0.05	0
MC Total		**298**	**138**	**32**		**8**

At Mission Creek, the first copulation occurred on 19 April and the last on 3 May 2016. A total of 32 copulations resulted from 298 breeding introductions (Table 3); 106 females did not copulate. Copulation rates reported by Mission Creek from 2012-2015 were calculated using a different definition for "introduction" (Aubrey 2012, Hamilton et al. 2013, 2014, 2015), and cannot be compared to the current method. Copulations occurred among each of the recommended breeding dyads, although the overall rate was low. Poor weather is believed to have affected outcomes, but other factors may also be at play, including aging males and combining numbers to insure sufficient competition among males. Regardless of institution, all productive females except one copulated between 16 and 21 April 2016 (Table 5). As we have previously reported, sunlight affects the likelihood that adults will mate and the development rate of eggs (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. Both 2016 and 2017 have proved challenging in this regard.

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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 2016. We also wanted to produce egg clusters to support the UW-Seattle prediapause research. Oviposition and rearing methods followed Barclay et al. (2009). An official "hatch" count is obtained at 3rd instar when larvae are hardy enough to be manipulated individually. 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 record the number of larvae active in each cup during each diapause check (Lewis et al. 2016). 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 months.

Upon removal from cold diapause, larvae at both facilities 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 larvae at Mission Creek began to eat, a subset of larvae from wild females captured in 2016 were retained for the breeding colony, and the remaining larvae, including all offspring of captive-mated females, were released.

When postdiapause larvae at the Oregon Zoo exhibited increased mortality at and immediately following wake-up, sample larvae were sent to Mississippi State University for pathology testing. Results came back negative, with a recommendation to explore alternative food-related hypotheses including the nutritional quality of larval food and/or the potential that the food may contain toxins. They also suggested a test of Koch's postulates, which would detect a contagion if larvae were harboring one. The Oregon Zoo initiated a small-scale trial of Koch's postulates on 11 March 2017 using 89 postdiapause larvae; half came from Mission Creek (1 died during transport) and half from the Oregon Zoo. Two wild and four captive-bred female lines were included. Fifteen larvae from each of 6 matrilines (14 from 16MC54) were split into 3 cups of 5 larvae each, with one cup from each group randomly assigned to each of three conditions:

1. Super control - larvae housed in Taylor's checkerspot room with other healthy larvae and fed the same leaves
2. Control - larvae housed in the Oregon silverspot room and fed the same leaves as healthy larvae
3. Treatment - larvae housed in Oregon silverspot room and fed treated leaves (see text for details)

The treatment group received *Plantago* leaves dipped in a solution made from the macerated bodies of moribund larvae diluted in 25 ml of distilled H_2O. The treatment phase of the Koch's postulates trial concluded on 24 March 2017, at which point all larvae were again fed "clean" leaves. Surviving larvae were retained for follow-up monitoring and cared for alongside other larvae according to standard procedures. Surviving Zoo larvae not included in the trial were either retained (subset from wild females captured in 2016) for breeding or released (all offspring of captive females and those not needed for the breeding colony).

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Results and Discussion

At least 19 of 20 wild females at the Oregon Zoo laid eggs that hatched (i.e., were productive) in 2016 (Table 4); all eggs from one wild female were transferred to the UW for research and their outcome is unknown. In contrast, 7 of 10 wild females at Mission Creek laid eggs that hatched (Table 4). In addition, one female laid eggs that did not hatch and two died without laying eggs. At the Zoo, 12 of 17 captive-mated females produced eggs that hatched, whereas 8 or 32 females at Mission Creek did the same (Tables 3 & 5).

Table 4. Oviposition outcomes to diapause for 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 2016. Aggregate values for 6 egg clusters received from Nate Haan (P16FL-Haan), University of Washington-Seattle, are included, but were released as prediapause larvae. Number hatched is taken at 3rd instar but includes any mortality to that point. Hatch rates >1.0 result from undercounts of eggs.

Female ID	# eggs laid	# eggs retained	# hatched	Prop hatched	# to diapause
Oregon Zoo					
P16FL40	179	129	117	0.91	115
P16FL41	260	122	119	0.98	---
P16FL42	239	211	157	0.74	153
P16FL43	698	270	247	0.91	---
P16FL44	261	232	209	0.90	209
P16FL45	142	70	76	1.09	75
P16FL46	279	169	147	0.87	147
P16FL47	204	182	148	0.81	148
P16FL48	134	134	122	0.91	119
P16FL49	389	104	115	1.11	---
P16FL50	248	248	237	0.96	234
P16FL51	89	89	63	0.71	61
P16FL52	472	0	---	---	---
P16FL53	258	174	160	0.92	158
P16FL54	70	0	---	---	---
P16FL55	266	0	---	---	---
P16FL56	343	150	65	0.43	65
P16FL57	408	218	196	0.90	---
P16FL58	241	167	156	0.93	155
P16FL59	77	77	71	0.92	71
OZ Subtotal	**5257**	**2746**	**2405**	**---**	**1710**
Average	**262.9**	**137.3**	**141.5**	**0.88** [1]	**131.5**
SD	**149.0**	**81.00**	**57.1**	**0.14**	**54.3**
P16FL- Haan	374	374	337	0.90	---
OZ Total	**5631**	**3,120**	**2742**	**0.89**	**1710**
Mission Creek					
P16FL01	220	220	261	1.19	260
P16FL13	117	117	131	1.12	129
P16FL14	86	86	108	1.26	106
P16FL15	192	192	212	1.10	210
P16FL22	330	330	389	1.18	385
P16FL25	230	230	259	1.13	259
P16FL30	86	86	82	0.95	81
Subtotal	**1261**	**1261**	**1442**	**---**	**1430**
Eggs from OZ					
P16FL41		138	139	1.01	137

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Female ID	# eggs laid	# eggs retained	# hatched	Prop hatched	# to diapause
P16FL43		210	190	0.90	189
P16FL49		248	238	0.96	238
P16FL52		388	133	0.34	122
P16FL55		231	201	0.87	199
P16FL57		110	60	0.55	60
Subtotal		**1325**	**961**	**---**	**946**
Eggs from UW					
P16FL35		15	16	1.07	16
P16FL36		24	22	0.92	22
P16FL37		24	22	0.92	22
P16FL38		27	26	0.96	26
P16FL39		29	29	1.00	27
P16FL40		3	3	1.00	3
P16FL42		6	5	0.83	5
P16FL44		18	17	0.94	17
P16FL45		28	28	1.00	28
P16FL46		15	13	0.87	12
P16FL47		5	5	1.00	5
P16FL48		16	9	0.56	8
P16FL50		8	3	0.38	3
P16FL51		15	14	0.93	14
Subtotal		**233**	**212**	---	**208**
MC Total		**2819**	**2615**	**---**	**2583**
Ave		**104.4**	**96.9**	**0.92**[1]	**95.7**
SD		**112.1**	**105.9**	**0.23**	**105.2**

[1] Cases with hatch success >1.0 hatchling per egg laid omitted.

A total of 10,657 eggs were produced at the two facilities with 38.8 percent (4,139) of eggs produced by captive-mated females and 61.2 percent (6,518) by wild females. Wild females at the Zoo produced 63.5 percent of eggs (Tables 4) between 28 April and 12 May 2016, with 36.5 percent produced by captive females between 19 April and 11 May 2016 (Table 5). At Mission Creek, wild females produced 52.9 percent of the eggs between 29 April and 10 May 2016, with captive-mated females contributing the remaining 47.1 percent between 20 April and 14 May 2016 (Tables 4 and 5, respectively). About 1,325 eggs from the Oregon Zoo and 233 from the UW were transferred to Mission Creek on 10 May 2016 to supplement numbers; the UW also provided 374 eggs to the Zoo (Table 4). Conversely, 38 clusters totaling 1,184 eggs from wild females at the Zoo were transferred to the UW. Larvae from wild females at the Zoo hatched on 8-21 May 2017, with those from captive females hatching on 1-18 May. At Mission Creek, larvae from wild females hatched on 10-23 May, similar to those from captive females hatching on 8-23 May.

In all, 7,840 prediapause larvae (5,020 wild and 2,820 captive) were produced, with 6,965 of those (4,293 wild, 2,672 captive) surviving to diapause (Tables 4 and 5). Prediapause larvae from the 16FL cohort (young of the year) entered diapause at the Zoo between 31 May and 26 June, with all larvae at Mission Creek entering diapause by 13 July 2016. Diapausing larvae were moved outdoors for the start of cold diapause on 9 September at the Oregon Zoo, and on 7 September 2016 at Mission Creek. Once in diapause most larvae remain inactive, although some movement is normal, especially in late diapause. At the Zoo larval activity remained below 15 percent during diapause thru the last check on 12 February 2017. No mortalities were noted and larvae were removed from diapause on 19 February. Larvae at Mission Creek were removed from diapause on 17 February 2017.

Table 5. Date and duration of copulation, and oviposition outcomes by female for captive-mated Taylor's checkerspots at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2016. Number hatched is taken at 3rd instar but includes any mortality to that point.

Female ID	Date	Duration (min)	# eggs	# hatched	Prop hatched	# to diapause
Oregon Zoo						
15FL01-09	19-Apr	67	31	27	0.87	26[1]
15FL01-11	19-Apr	69	91	80	0.88	76
15FL02-09	17-Apr	45	310	209	0.67	201
15FL02-11	18-Apr	86	347	265	0.76	255
15FL05-02	18-Apr	85	391	349	0.89	336
15FL07-18	17-Apr	100	146	54	0.37	54
15FL08-10	18-Apr	60	152	134	0.88	132
15FL08-11	21-Apr	42	250	230	0.92	226
15FL10-07	16-Apr	139	241	123	0.51	112
15FL10-09	16-Apr	118	341	175	0.51	149
15FL13-06	20-Apr	99	238	176	0.74	123
15FL13-10	21-Apr	53	480	447	0.93	441[2]
OZ Total		**---**	**3018**	**2269**	**---**	**2131**
Ave		**80.3**	**251.5**	**189.1**	**0.75**	**177.6**
SD		**29.9**	**130.7**	**122.1**	**0.19**	**121.4**
Mission Creek						
15FL52-24	19-Apr	41	368	273	0.74	270
15FL53-11	19-Apr	43	43	10	0.23	10
15FL53-12	19-Apr	48	25	4	0.16	4
15FL54-10	20-Apr	66	177	149	0.84	146
15FL54-13	21-Apr	109	202	67	0.33	64
15FL56-26	21-Apr	50	125	1	0.01	1
15FL57-19	21-Apr	160	67	34	0.51	33
14FL24-09	3-May	130	114	13	0.11	13
MC Total		**---**	**1121**	**551**	**---**	**541**
Ave		**80.9**	**140.1**	**68.9**	**0.37**	**67.6**
SD		**45.9**	**110.9**	**96.1**	**0.30**	**94.9**

[1] One larva developed direct to adulthood

[2] Four larvae developed direct to adulthood

Forty-eight percent (1,904) of larvae that entered diapause at the Oregon Zoo in June 2016 failed to wake-up in February 2017, and an additional 641 (16 percent) larvae died in the two weeks prior to release (Table 6). These larvae were ascribed to diapause mortality (see Survival) as they appeared to have insufficient resources to make the physiological switch from diapause to feeding. This level of mortality was alarming because larvae appeared very healthy when they entered diapause and overwinter temperatures followed normal patterns compared to the previous two years (Linders et al. 2015, 2016), forcing us to abandon the hypothesis that warm nighttime temperatures might explain previous patterns of mortality.

Mission Creek retained 288 larvae from twelve 2016 matrilines, as well as 41 second diapause larvae from three 2015 matrilines for breeding (Table 6); all were offspring of wild females. At the Zoo, 189 larvae from eight wild 2016 matrilines and five larvae from three wild 2015 matrilines were retained for breeding (Table 6). Outcomes of postdiapause rearing, including progression to the adult stage, are presented in Table 7.

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Table 6. Numbers of Taylor's checkerspot larvae entering diapause, alive at wake-up, dead after wake-up, available for release, and retained for captive breeding or as part of Koch's postulates trial (see text for details) by matriline at the Oregon Zoo, Portland, Oregon and Mission Creek Corrections Center for Women, Belfair, Washington, 2016-2017.

Matriline	Diapause in	Diapause out	Dead pre-release	Released	Retained
Oregon Zoo					
15FL01	9	1	0	0	1
15FL02	13	3	0	0	3
15FL05	10	0	0	0	0
15FL06	15	0	0	0	0
15FL07	7	0	0	0	0
15FL08	13	0	0	0	0
15FL09	12	1	0	0	1
15FL10	4	0	0	0	0
15FL13	7	0	0	0	0
Subtotal	90	5	0	0	5
16OZ01	102	28	5	23	0
16OZ02	456	335	90	230	15[1]
16OZ05	336	234	47	172	15[1]
16OZ07	54	49	16	33	0
16OZ08	358	255	65	190	0
16OZ10	261	193	56	137	0
16OZ13	564	152	75	77	0
Subtotal	2131	1246	354	862	30
16FL40	115	111	21	66	24
16FL42	153	121	47	53	21
16FL44	209	147	54	58	35[1]
16FL45	75	51	25	5	21
16FL46	147	20	12	8	0
16FL47	148	37	10	4	23
16FL48	119	65	22	21	22
16FL50	234	115	35	56	24
16FL51	61	20	6	14	0
16FL53	158	50	30	1	19
16FL56	65	26	12	14	0
16FL58	155	16	4	12	0
16FL59	71	15	7	8	0
Subtotal	1710	794	285	320	189
16OZQC	33	15	2	13	0
OZ Total	**3964**	**2060**	**641**	**1195**	**224**
Mission Creek					
14FL21	1	1	0	1	0
15FL51	22	22	0	0	22
15FL54	9	9	0	0	9
15FL55	4	4	0	4	0
15FL57	10	10	0	0	10
15FL58	1	1	0	1	0
Subtotal	47	47	0	6	41
16FL01	260	259	3	232	24
16FL13	129	129	0	105	24
16FL14	106	106	1	81	24

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Matriline	Diapause in	Diapause out	Dead pre-release	Released	Retained
16FL15	210	209	6	179	24
16FL22	385	383	4	340	24[2]
16FL25	259	259	1	234	24
16FL30	81	81	0	57	24
16FL35	16	16	0	16	0
16FL36	22	22	0	22	0
16FL37	22	22	0	22	0
16FL38	26	26	0	26	0
16FL39	27	27	1	26	0
16FL40	3	3	0	3	0
16FL41	137	137	0	113	24
16FL42	5	5	0	5	0
16FL43	189	189	0	165	24
16FL44	17	17	0	17	0
16FL45	28	28	1	27	0
16FL46	12	12	0	12	0
16FL47	5	5	0	5	0
16FL48	8	7	0	7	0
16FL49	238	238	0	214	24
16FL50	3	3	0	3	0
16FL51	14	14	13	1	0
16FL52	122	122	1	97	24
16FL55	199	198	0	174	24
16FL57	60	60	1	59	0
Subtotal	2583	2577	32	2242	288
16MC52	270	270	2	253	0[2]
16MC53	14	14	0	14	0
16MC54	210	210	1	194	0[2]
16MC56	1	1	0	1	0
16MC57	33	33	0	33	0
16MC24	13	13	0	13	0
Subtotal	541	541	3	508	0
16MCQC	13	13		14	0
MC Total	**3184**	**3178**	**35**	**2770**	**329**

[1] Fifteen larvae were included in the test of Koch's postulates. See text for details.

[2] Fifteen larvae were transferred to the Oregon Zoo for the test of Koch's postulates. See text for details.

Only 3 larvae died during the two week test of Koch's postulates, all from the super control group (1 from MC and 2 from OZ). These results together with repeated pathological screening conducted by Mississippi State University in previous years (Linders et al 2014, 2015, 2016) offer strong support that mortality at wake-up in the Zoo colony is not the result of an infectious process.

Overall second diapause return rates were the same at both rearing facilities in 2017 (OZ – 37.8 percent; MC – 37.4 percent; Table 7), with differences among matrilines pronounced, especially at Mission Creek (Table 7). Multi-diapause larvae are retained and paired with other multi-diapause adults whenever possible because this trait is likely important for population persistence, especially in the face of climate changes, and in the wild, likely results in genetic mixing across years.

The first pupa formed at the Oregon Zoo on 23 April and the last on 24 May 2017, whereas larvae at Mission Creek pupated between 12 April and 4 May 2017. In both cases this is about one month later than in 2016, with Mission Creek larvae pupating earlier than those at the Oregon Zoo, but similar to those in the wild. Similarly, adults at the Oregon Zoo eclosed between from 6 to 31 May 2017, with

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those at Mission Creek eclosing from 30 April to 23 May 2017. In all, 258 adult checkerspots (143 males and 120 females) joined the 2016 captive breeding colony between facilities at The Oregon Zoo and Mission Creek (Table 7). Adults that were physically fit and no longer needed for breeding were provided to WDFW for release.

Table 7. Numbers of Taylor's checkerspots by life stage and matriline from stock reared and retained for breeding at the Oregon Zoo, Portland, Oregon and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2017. Improperly eclosed (IE) adults are a subset of all adults.

Matriline	Larvae	2nd diapause	Pupae	Adults	IE	Males	Females	Adult release
Oregon Zoo								
15FL01	1	0	1	1	0	0	1	1
15FL02	3	0	0	0	0	0	0	0
15FL09	1	0	1	1	0	0	1	1
Subtotal	5	0	2	2	0	0	2	2
16FL40	24	10	11[1]	11	0	6	4	3
16FL42	21	7	9[1]	9	1[2]	5	2	2
16FL44	22	11	8	8	1	6	2	5
16FL45	21	9	8	8	1	4	4	4
16FL47	23	6	9	9	1	4	5	4
16FL48	22	5	13	13	0	7	6	9
16FL50	24	3	13	13	2	7	6	10
16FL53	19	2	14	14	2[2]	6	7	9
Subtotal	176	53	89	85	8	45	36	46
16FL22T	15	10	3	3	0	1	2	3
16FL44T	13	4	4	4	1	2	2	3
16MC52T	14	9	2	2	0	1	1	2
16MC54T	14	10	2	1	0	0	1	1
16OZ02T	15	6	8	8	0	6	2	7
16OZ05T	15	9	6	6	2	2	4	3
Subtotal	86	48	25	24	3	12	12	19
OZ Total	**267**	**101**	**116**	**111**	**11**	**57**	**50**	**67**
Mission Creek								
15FL51	22	7	5	3	0	2	1	3
15FL54	9	0	6	4	1	4	0	1
15FL57	10	8	2	2	0	1	1	0
Subtotal	41	15	13	9	1	6	2	4
16FL01	24	3	21	21	1	12	9	10
16FL13	24	9	15	15	2	9	6	6
16FL14	24	8	14	14	0	9	5	5
16FL15	24	6	13	11	0	4	7	6
16FL22	24	15	7	5	0	4	1	2
16FL25	24	12	13	12	0	8	4	6
16FL30	24	8	11	11	0	8	3	6
16FL41	24	1	21	20	0	9	11	14
16FL43	24	20	4	2	0	2	0	0
16FL49	24	9	14	12	0	2	10	9
16FL52	24	1	22	21	0	9	12	10
16FL55	24	16	6	3	1	3	0	2
Subtotal	288	108	161	147	4	79	68	76
MC Total	**329**	**123**	**174**	**156**	**5**	**86**	**70**	**80**

[1] One pupa released prior to eclosion

[2] Unable to determine sex of one adult

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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 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, with conditions in Portland, Oregon considerably warmer than those in Belfair, Washington. The new targets also make it easier to compare actual conditions to targets. Targets will likely undergo additional modification as we 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, otherwise the lab is unheated; there is no mechanism to cool the facility at this time. Temperature and relative humidity (RH) were recorded every two hours using HOBO data loggers to obtain min/max data for during all rearing stages.

Table 8. Target temperature (°F) and relative humidity (% RH) conditions and methods used to achieve them 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, 2016-2017.

Life stage	Mission Creek	Oregon Zoo	Target		Mission Creek		Oregon Zoo	
	Location	Location	Temp (°F)	Ave min RH (%)	Heat/Cooling	Light	Heat	Light
Males	GH Main Room (Floor)	TCB room	≤85 78-85 for 2-6 h/day	≥50	Ice packs; evaporative cooler	Full shade[1]	40-watt bulb 8-9 AM & 1-1:30 PM	None
Females and Oviposition	GH Main Room	TCB room	50-90 78-90 for 4-8 h/day	≥50	40-watt bulb 10 AM-2 PM or outside	Partial shade[1]	40-watt bulb 10 AM-2 PM or outside	None
Egg & Prediapause	GH Main Room	TCB room	50-90; ave min ≤65	≤65	Ice packs; swamp cooler	None	None	7 AM - 7 PM
Warm diapause	GH Main Room	TCB room	50-90	≥40	Ice packs; swamp cooler	None	None	
Cold diapause	Shed	Building overhang	min ≤35 for ≥60 days	≥50; ave max ≥90	None	None	None	None
Postdiapause	GH Main Room	TCB room & Gazebo	≤45 night ≥60 daytime	≥50	None	None	None	7 AM - 7 PM
Pupation	GH Main Room	OSB room	≥50 night ≥65 daytime	≥65	Mist with water / humidifier	None	7 AM-7 PM	7 AM - 7 PM

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

Results and Discussion

Temperature (°F) and relative humidity (percent RH) metrics recorded at the Oregon Zoo and Mission Creek in 2016-2017 are in Table 9. Rearing conditions at the Oregon Zoo were generally on target from the adult life stage through warm diapause. However, minimum target temperatures were attained on fewer days than desired during cold diapause and postdiapause, and drier than target conditions during pupation. Given that postdiapause rearing at the Oregon Zoo extended well into May, it is less surprising that they were unable to maintain cool nighttime temperatures that late in the year. At Mission Creek, temperatures were within range during diapause and pupation, but veered from targets during other life stages. For both males and females at Mission Creek, temperatures were often outside the desired range, even though mean temperatures did meet targets. Similarly, temperatures during postdiapause typically failed to reach both a sufficient level of cooling at night, and warming during the day. The use of a heater at night to avoid the cold shock observed during pupation in 2016, likely explains the warm nighttime temperatures, with daytime temperatures moderated by sheets that were originally attached to the greenhouse roof to reduce over-heating during the prediapause phase, but have remained there ever since. Going forward, no heat will be used at night, and sunlight will be used to warm the greenhouse during the day, except as needed to meet protocol conditions.

Relative humidity was within target range at both facilities during nearly all life stages, except pupation. Females at Mission Creek also experienced drier conditions than desirable, and both facilities struggle with lower moisture levels than desired at times, which may reflect larger swings in ambient outdoor conditions, but may also be an artifact of using artificial forced-air heat (e.g., postdiapause at Mission Creek). Also, some target conditions may need to be modified (e.g., RH during pupation) as more data are evaluated both in captivity and in the field.

Table 9. Actual temperature (°F) and relative humidity (% RH) conditions and absolute range (range) during Taylor's checkerspot rearing by life stage, including ate range at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, 2016-2017. Numbers in **bold text** indicate measure was outside the target by more than 10 percent. Data for females at the Oregon Zoo are not available (NA).

Life stage	Date range		Temp (°F)			Ave min RH (%)		
	MC	OZ	Terms	Mission Creek	Oregon Zoo	Terms	Mission Creek	Oregon Zoo
Males	15 Apr – 11 May 2016	9 Apr - 2 May 2016	mean max (range) # days w/i range	78 (65-95) **12 of 26**	75 (61 - 80) 22 of 24	mean (range)	47 **(21-74)**	46 **(36-87)**
Females and Oviposition	19 Apr – 15 May 2016	9 Apr - 12 May 2016	mean (range) # days w/i range	64 (**41-100**) **15 of 27**	NA NA	mean (range)	**39** **(19-76)**	NA NA
Egg & Prediapause	22 Apr – 12 Jul 2016	19 Apr - 26 Jun 2016	mean (rng); ave min	65 (48-101); 57	71 (60 - 83); 65	mean (range)	50 (**21-76**)	53 (**32-100**)
Warm diapause	13 Jul – 6 Sep 2016	31 May - 8 Sep 2016	mean (range)	67 (52-89)	71 (62 - 83)	mean (range)	55 (**28-85**)	60 (38-100)
Cold diapause	7 Sep 2016 – 16 Feb 2017	9 Sep 2016 - 18 Feb 2017	# days min ≤35	68	**49**	mean (rng); ave max	84 (**37-97**); 91	82 (**40-99**); 89
Postdiapause	17 Feb – 4 May 2017	19 Feb - 23 May 2017	# nts ≤45 # days ≥60	**7 of 76** **53 of 76**	**65 of 93** 88 of 93	mean (range)	58 **(30-89)**	65 **(26-98)**
Pupation	12 Apr – 30 May 2017	23 Apr - 31 May 2017	# nts ≥50 # days ≥65	48 of 48 43 of 48	39 of 39 39 of 39	mean (range)	**50** **(28-83)**	**48** **(31-79)**

Cool spring conditions in 2017 led to a relatively late flight season, which is reflected in pupation dates that are about a month later than usual in captivity as well (Table 9). Given weather patterns are influencing Taylor's checkerspot rearing success in captivity, it is to be expected that effects on wild populations are also pronounced (Weiss and Weiss 1998). Understanding when and how weather influences Taylor's checkerspot population dynamics is fundamental to building and maintaining sustainable populations both in captivity and in the wild. Research on weather-related effects on Taylor's checkerspot populations remains a high priority that has gone unfunded for years, despite its well-documented importance (Thomas et al. 2011).

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 best estimate of egg numbers, but avoid counting larvae until they reach third instar. The number of hatched larvae at 3rd instar includes any larvae known to perish prior to that.

Results and Discussion

Stage specific survival rates for both rearing institutions appear in Table 10. As in years past, mortality was highest prior to 3rd instar (Linders 2010, 2011, 2012, Linders and Lewis 2013, Linders et al. 2014, 2015, 2016), except at the Oregon Zoo, where mortality at and following wake-up was high as previously discussed. Hatch rates for 2016 were good overall, and better than in 2015. This was also true for captive-mated females at Mission Creek, although these again lagged behind the rest (Table 10). As in 2015, hatch rates for wild females were higher than those for captive-mated females, but this difference was pronounced at Mission Creek. During all remaining life stages survival continues to meet or exceed the 90th percentile at both facilities (Linders 2012, Linders and Lewis 2013, Linders et al. 2014, 2015, 2016), and well above those expected in the wild (Moore 1989).

Unfortunately, we continue to struggle with high mortality following wake-up at the Zoo (Table 10). Having ruled out warm overwinter temperatures and disease as causal factors (see Captive rearing results for further discussion), we are seeking alternate explanations, primarily in the way of food quality. To this end, a feeding trial was initiated in spring 2017, whereby prediapause larvae were spilt into three groups and fed from either: 1) potted plants, 2) raised beds, or 3) potted plants with liquid fertilizer added. This trial is ongoing and will be discussed in the 2018 annual report.

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. We calculated hind wing area from photos using ImageJ ver. 1.46r (Schneider et al. 2012). Comparisons were made using an ANOVA worksheet developed by S. Knapp at WDFW. 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.

Table 10. Number of individuals and Kaplan-Meier (1958) survival (Surv) by life stage for Taylor's checkerspot butterflies at the Oregon Zoo (OZ), Portland, Oregon, and Mission Creek Corrections Center for Women (MC), Belfair, Washington, 2016-2017. Only offspring from females with eggs that hatched are included; number of hatched larvae recorded at 3rd instar. Larvae of unknown origin (QC) are not included. Column labels refer to rearing institution (OZ or MC), egg source (Wild - Joint Base Lewis-McChord (FL) or Captive-mated – (Captive), and year egg originated (e.g., 15=2015).

Life stage	**OZ Wild 16FL**		**OZ Captive 16OZ**		**OZ Koch's Postulates**		**MC Wild 16FL**		**MC Captive 16MC**		**MC Wild 15FL**	
	#	Surv	#	Surv	#	Surv	#	Surv	#	Surv	#	Surv
Eggs	3120	--	3018	--			2819	--	1121	--		
Egg to hatch[1]	2742	0.88	2269	0.75			2615	0.93	551	0.49		
Prediapause release	*1006*	--	--	--			--	--	--	--		
Hatch to diapause	1710	0.99	2131	0.94			2583	0.99	541	0.98		
Diapause to release	509	0.30	905	0.42			2545	0.99	538	0.99	47	--
Postdiapause held	176	--			86	---	288	--			41	--
Multi-diapause[2]	53	0.30			48	0.56	108	0.38			15	0.37
Pupation	89	0.72			25	0.66	161	0.89			13	0.50
Eclosion	85	0.96			24	0.96	147	0.91			9	0.69

[1] Hatch rates not adjusted for undercount of eggs.
[2] Proportion of postdiapause larvae that returned to diapause.

Results and Discussion

Animals at Mission Creek are heavier than those at the Oregon Zoo (Table 11). Pupal weights were significantly different between the facilities for both males ($p < 0.0001$) and females ($p < 0.0001$). This was also true for adult males ($p < 0.0001$) and females ($p < 0.0001$). On the other hand, wild females did not differ in weight between facilities ($p = 0.95$), indicating measures are being taken accurately. The difference in weight between wild females and captive females at the Oregon Zoo is statistically significant ($p = 0.027$), although not likely a real difference given weights in captivity are taken within 24 hrs of eclosion, whereas the age of wild females is unknown. Statistical differences in weight between Mission Creek females and those from the wild are significant ($p < 0.0001$), however wing area was not different ($p = 0.77$), indicating the captive females are more heavy-bodied than their wild counterparts. No concerns with regard to flight behavior have been noted in releasing captive adults from Mission Creek, but additional observation may be warranted.

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Table 11. Descriptive statistics for pupal and adult weights and wing area measurements for Taylor's checkerspot butterflies that pupated and those that successfully eclosed at Mission Creek Corrections Center for Women, Belfair, WA, and the Oregon Zoo, Portland, OR, 2016.

		Mission Creek			Oregon Zoo	
Group	**Statistic**	**Pupal wt (g)**	**Adult wt (g)**	**Wing area (cm2)**	**Pupal wt (g)**	**Adult wt (g)**
Captive males	N	143	143	118	54	54
	mean	0.2035	0.1017	1.1054	0.1563	0.0846
	var	0.0005	0.0005	0.0207	0.0001	0.0010
Captive females	N	154	154	124	65	65
	mean	0.2608	0.1700	1.4894	0.2093	0.1333
	var	0.0011	0.0007	0.0407	0.0002	0.0001
Wild females	N	---	10	10	20	20
	mean	---	0.1211	1.5377	0.2093	0.1240
	var	---	0.0009	0.0316	0.0002	0.0004

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 1,843 postdiapause larvae from Mission Creek were released at GHP on 10 Mar 2017. Of these, 894 were split between Plots B, K and 1x1-m survival monitoring plots #11-20 in the *C. levisecta* planting area, and 949 were split between Plots W-Z and 1x1-m survival monitoring plots #1-10 in the standard release area where *C. hispida* has been planted (Table 12). Our intent was to quantify oviposition across the range of available host species. Both release areas also contain ample amounts of *Plantago* (see Section I above). In addition, 1,182 larvae were released from the Oregon Zoo on 23 Mar 2017, including 560 in Plot J and 1x1-m survival monitoring plots #21-30 within the *C. hispida* area, with the remaining 622 larvae released within the *C. levisecta* area in Plots T, U, and 1x1-m survival monitoring plots 31-40 (Table 12). On 16 Mar 2017, we released 719 larvae from Mission Creek at TA7S in Plot D, with an additional 190 larvae added on 30 Mar 2017 (Table 12), once we determined they were not needed in the breeding colony. Fourteen larvae from Mission Creek and 10 larvae from the Oregon Zoo were dead on arrival and were not released. Of the 3,025 larvae released at GHP, 1,793 were offspring of wild females, 1,205 were offspring of captive-mated females, and 27 were of unknown (QC) origin; QC larvae became separated from their group at some point during the rearing process. Of 909 larvae released at TA7S, 758 were offspring of wild females, 150 were offspring of captive-mated females, and 1 as of unknown (QC) origin. Numbers reported may not match those in the captive rearing section, but discrepancies are small. Larvae were released in groups of 2-5 in the larger release plots, with 5 larvae placed in each 1x1-m survival plot. Larvae were placed on large and/or dense host plants/patches (*Plantago lanceolata or Castilleja spp*) within restored prairie. Due to persistent rain and cool temperatures in 2017, larvae were released under shelters constructed from an inverted plastic mesh berry basket and lid and secured to the ground using a landscape pin (Fig. 2), which provided protection from rain and allowed larvae to move freely in and out. Weather on release days was good with temperatures of 6.0 to 14.2°C, average wind speed of 0.0-8.5 mph, and clear to overcast skies; the only exception was the TA7S release, with an early temperature reading of 4.5 °C, which had climbed to 12.2°C by mid-day. At least 28 people assisted with releases, working from about 1000-1600 h each day to complete the task.

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Table 12. Numbers of wild and captive-bred larvae released in winter 2017 at Glacial Heritage Preserve, Thurston County, Washington, by rearing facility, plot ID/type, and *Castilleja* species (CAHI - *C. hispida*; CALE – *C. levisecta*; all plots included *Plantago lanceolata*; see text for details.

Oregon Zoo	*C. hispida*			*C. levisecta*					
Plot ID	J		1x1-m		T	U		1x1-m	Total
OZ Wild	136		11		92	78		0	317
OZ Captive	361		39		140	262		50	852
OZ QC					13				13
Total	497		50		245	340		50	1182
Mission Creek	*C. hispida*			*C. levisecta*					
Plot ID	B	K	1x1-m	W	X	Y	Z	1x1-m	
MC Wild	154	521	45	172	189	122	223	50	1476
MC Captive	168	0	5	0	105	0	75	0	353
MC QC	1					13			14
Total	323	521	50	172	294	135	298	50	1843

Adult release

One hundred forty-six adults (90 females, 55 males and 1 unknown) and one Taylor's checkerspot pupa were released at TA7S between 30 May and 2 Jun 2017. Of these, 65 came from the Oregon Zoo and 80 came from Mission Creek. All adults were offspring of wild females and were released once their roles in captive mating 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 from open cages or placed on nectar plants as needed. Release conditions were warm conditions (19-24.0 °C), with light winds (0.8-2.6 mph average), and broken clouds (2 Jun) or overcast skies (30 May).

Figure 2. Postdiapause Taylor's checkerspot were placed under shelters constructed from a berry basket with lid staked to the ground to provide protection from inclement weather during a reintroduction at Glacial Heritage Preserve, South Puget Sound, Washington, spring 2017.

Prediapause larval release

Due to a poor breeding season and limited production from wild females, no prediapause larvae were released in 2017. Cold and wet conditions delayed the flight season and continued through late spring, which appeared to affect productivity and survival in both the lab and the field.

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 exhibit normal behaviors (e.g. feeding, basking, mating, and egg-laying) immediately following release. We can also locate 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 minimum survival, and identify issues that may be cause for concern, we conducted surveys for postdiapause larvae in forty 1x1-m survival plots on at least two occasions in the weeks following release. Plots were split equally among larvae from the two rearing facilities (Oregon Zoo and Mission Creek) and within the two *Castilleja* release areas (see Postdiapause release above). We thoroughly searched the 1x1-m plot area first, then searched the surrounding 2x2-m area; we also noted larvae observed beyond this. We marked larval locations with a labeled pin flag, and marked forage locations with an alternate color in the event larvae were concealed nearby. We recorded number of larvae, instar, behavior and substrate, and mapped each location on a plot diagram.

Naïve survival was calculated by survey date for each set of 1x1-m survival plots (Table 13) by dividing the count total for a set of subplots by the by the total number of larvae released in the set; standard error was also calculated. We assumed that larvae present on the second survey were also present on the first survey. We did not run statistical tests by rearing facility or *Castilleja* host species due to low overall survival and irregularity in release dates and post-release sampling periods.

Results and Discussion

Results of survival plot surveys conducted at GHP in 2017 appear in Table 13. As in spring 2016, it was often challenging to find suitable survey conditions in spring 2017, so some surveys spanned more than one day. Survival was reasonably high 7-8 days post-release, but continued to decline with time since release. During initial follow-up surveys, some larvae were located up to 1.6 m outside of 1x1-m plot boundaries, and half of all 1x1-m plots were empty by the end of the monitoring period, which was unusual. Larvae dispersing too far from release areas at GHP risk starvation if they move beyond the distribution of host plant patches. Also unusual was that half (77 of 153) of all larvae observed at GHP between 30 March and 4 April were still in 5th instar; we also observed more larvae in their compact diapause skin and small head capsule (diapause condition) than in years past. Larvae also staggered their entry into pupation in 2017 with most larvae not pupating until 14 April, 3 weeks later than usual. Ratios of 5th to 6th instar larvae observed during post-release surveys at SCS1 and GHP2 in 2016 indicate that 5th instar larvae were prevalent during early surveys, with a shift to predominantly 6th instar larvae later in the feeding period. At the end of the 2017 survey period, however, larvae at GHP were mainly 5th instar, although some 6th instar larvae were observed in the main release plots, primarily on south and west-facing slopes. Collectively these observations suggest cool, wet weather, perhaps interacting with microclimate, had a stifling effect on checkerspot development in 2017. While larvae employ a variety of behaviors to maximize their exposure to sunlight (Fig. 3), in cold years, deep swales prevalent in the GHP release area may provide a limited number of warm microsites with sufficient food for survival. Similar effects might be expected where trees, slope or aspect reduce insolation.

Table 13. Results of post-release surveys for postdiapause Taylor's checkerspot larvae in two release areas (GHP2 – CAHI and GHP2L - CALE) at Glacial Heritage Preserve, South Puget Sound, Washington, spring 2017. Release plots were 1x1-m in size with a buffer totaling 2x2-m. *Castilleja* host species (CALE –*C. levisecta*; *CAHI-C. hispida*), source facility (MC-Mission Creek; OZ-Oregon Zoo), plot IDs, number of plots with at least one larva of total surveyed, larval counts inside (in) and outside (out) of 1x1-m plot, days (D) post-release, proportion surviving to that time, and standard error (SE). Highlighted cells indicate similar results at similar time periods.

	Release area	Larval source	Plot IDs	#plots >0	#larvae in	#larvae out	D post-release	Proportion surviving	SE
Survey 1									
16-Mar	CALE	MC	1-10	5 of 7	7	4	7	0.31	0.08
20-Mar	CAHI	MC	11-20	9 of 10	5	6	11	0.22	0.06
31-Mar	CAHI	OZ	21-30	6 of 10	7	8	8	0.30	0.06
31-Mar	CALE	OZ	31-40	9 of 10	13	7	8	0.40	0.07
Survey 2									
30-Mar	CALE	MC	1-10	4 of 10	3	1	21-24	0.08	0.04
31-Mar	CAHI	MC	11-20	3 of 10	2	1	22-24	0.06	0.03
3-Apr	CAHI	OZ	21-30	6 of 10	6	5	12	0.22	0.06
4-Apr	CALE	OZ	31-40	6 of 10	7	4	13	0.22	0.06

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 2017 flight season. Broad scale occupancy surveys were conducted by JBLM staff in 2017 using transects with a 100-m spacing, and included the former PCM release area. Distance sampling methods followed Linders and Olson (2014), except that all adults were recorded by 25-m segment. Distance surveys were conducted up to 3 times per week during the flight season. Survey transects at reintroduction sites included all release plots and

Figure 3. 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.

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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 added to the north in 2014 (Linders et al. 2014) were included in all 2017 surveys. In 2017, access to the dud hazard area in the southwest corner of the monitoring area was granted on all dates after EOD personnel cleared the lines on 4 May. Transect length and spacing by site and year is shown in Table 14. The closer transect spacing at reintroduction sites insures enough butterflies are detected to calculate an abundance estimate. All surveyors receive pre-season training annually and distance estimation skills are tested weekly throughout the flight season.

Data analyses. 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 in SAS. 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).

Table 14. Number and size (m) of distance sampling units, standard survey length and area for density estimation by site for extant (R76) and reintroduced Taylor's checkerspot populations, South Puget Sound, Washington, 2010-2017. Range 76 (R76), Scatter Creek South Unit 1 (SCS1), Range 50 (R50), Training Area 7 South (TA7S), Scatter Creek South Unit 2 (SCS2), Glacial Heritage Unit 2 (GHP2), and the associated *Castilleja levisecta* area (GHP2L).

Site	Year	# transects	Transect spacing	# segments	Segment length (m)	Transect length (m)	Survey length (m)	Survey area (ha)[1]
R76	2010-13	12	50	14	50	700	8400	42.0
R76	2014-15	12	50	28	25	700	8400	42.0
R76	2016-17	16	50	23-28	25	575-700	10900	54.5
SCS1	2010	11	25	11	50	550	6050	15.1
SCS1	2011-12	14	25	12	50	600	8400	21.0
SCS1	2013-15	16	25	14-24	25	350-600	5900-9100	14.8
SCS1	2016	16	25	16-20	25	400-500	7800	19.5
SCS1	2017	16	25	20	25	500	8000	20.0
R50	2011	16	25	9-11	50	450-550	8600	21.5
R50	2010	13	25	8	50	400	5200	13.0
R50	2012	16	25	9-13	50	450-650	9850	24.6
R50	2013	16	25	9-13	50	450-650	9900	24.8
R50	2014-17	16	25	18-26	25	450-650	9900	24.8
GHP2	2012	12	25	8	50	400	4800	12.0
GHP2	2013	12	25	8	50	400	4800	12.0
GHP2	2014-17	15	25	18	25	450	6750	16.9
TA7S	2014	14	25	6-13	25	150-325	2700	6.8
TA7S	2015	15	25	7-14	25	150-325	3600-3675	9.2
TA7S	2016-17	19	25	7-14	25	175-350	5125	12.8
SCS2	2017	12	25	18	25	450	5400	13.5
GHP2L	2017	12	25	13	25	325	3900	9.8

[1] Survey area for abundance estimation may be smaller than the total survey area and that used to map distribution.

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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 potential variability, 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 the O2 method were used to estimate 95% Confidence Intervals. In some cases, the Fewster variance estimates did not seem to adequately assess the uncertainty of the estimates due to a higher level of variation between observers in 2016, so the non-parametric bootstrap method of estimating variance recommended by Marques et al. (2007) was used to compute more appropriate confidence intervals. Data and density estimates for three reintroduction sites (SCS, R50, and GHP) plus R76 in 2016 are reported here, as well as estimates for R50 and R76 in 2017.

Results and Discussion

Adult abundance 2016

Strategies to reduce the impact of observer differences on data quality and detection functions were outlined by Linders and Olson (2014) and have worked well. Unfortunately, several of these guidelines were violated in 2016, affecting data quality and making analyses challenging. Six observers conducted surveys, only two of which had significant distance sampling experience and several new problems arose as a result of observer differences. Consistent and experienced observers are important because new observers cannot be trained on alternate species prior to the start of the flight season. Of the four new observers, three had some experience conducting butterfly surveys but not distance sampling, and the fourth had no butterfly experience. All observers attended a pre-season classroom training, were given handouts developed to reinforce the main training points, and passed field tests of their accuracy in distance estimation, but these steps were an insufficient substitute for field experience. Three conditions appeared in the resulting detection functions which can be described as 1) guarding the line (too many locations at or near the line), 2) avoiding the line (too few locations at or near the line), and 3) heaping of observations at favorite distances (i.e., lumps in the distribution curve). In addition, some observers varied in their recording of butterfly behaviors, which had confounding effects. Collectively this required more manipulation to fit curves to the data and also increased the 95 percent CIs.

Results are presented for three reintroduction sites (SCS, R50 and GHP) and the source site (R76). Only two checkerspots were observed at TA7S prior to releasing adults, so no density estimates were calculated. In some cases, the number of observations reported may differ from raw counts reported in Linders et al. (2016) 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.

<u>Scatter Creek South 1.</u> Eleven surveys were conducted by 2-4 observers in 2016 between 9 April and 24 May. No butterflies were observed on 9 April and 24 May (Linders et al. 2016). A total of 1,727 Taylor's checkerspots observations were included in this analysis, with the greatest number (499) seen on 21

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April. The analysis area was increased in 2016 (Table 14) due to the increase in numbers and spread of adults (Table 15, Appendix C, Fig. 1). The number of butterflies observed per survey date was sufficiently high on 6 days (19 April -6 May), to estimate detection functions separately by date, and by observer within date. Data were truncated at 8 m to eliminate outliers. On most dates, encounter rates were similar among observers even when allocation of effort differed. One notable anomaly occurred on 26 Apr, when a much larger proportion of butterfly behaviors were recorded as "perching" as opposed to "flying" by both observers in spite of protocol weather conditions. This appears to have had a dampening effect on the number of detections (Table 15), as perched butterflies are more difficult to observe at longer distances. The best models often varied by observer and survey date; for simplicity those details are omitted here. Daily density estimates with 95% Confidence Intervals are presented by date in Table 15 along with daily abundance estimates for the survey area.

Table 15. Number of distance sampling observations (#Obs) used to calculate density estimates (#/ha) and adult abundance estimates (#/site by date) including 95% Confidence Intervals for Taylor's checkerspot survey areas at Scatter Creek South (SCS1), Range 50 (R50), Glacial Heritage (GHP) Training Area 7 South (TA7S) and Range 76 (R76) in South Puget Sound, Washington, spring 2016. See text for details.

		Survey	Density			Abundance		
Date	**#Obs**	**area (ha)**	**#/ha**	**Lower CI**	**Upper CI**	**#/site**	**Lower CI**	**Upper CI**
SCS1								
19-Apr	260	19.50	38.0	24.1	59.9	741	471	1168
21-Apr	460	19.50	71.2	44.6	99.6	1388	869	1943
26-Apr[1]	181	19.50	31.6	6.8	78.2	616	132	1525
28-Apr	349	19.50	75.0	35.5	126.1	1463	692	2458
1-May	296	19.50	55.8	33.6	82.4	1088	655	1606
6-May	142	19.50	49.9	23.6	105.3	972	460	2054
10-May	39	19.50	9.8	5.5	17.2	190	107	336
R50								
16-Apr	377	24.75	35.4	21.6	58.0	876	534	1435
18-Apr	1051	24.75	105.3	67.8	163.5	2606	1678	4046
20-Apr	1133	24.75	137.0	86.6	216.8	3391	2143	5366
25-Apr	386	24.75	75.8	46.2	124.5	1877	1143	3081
30-Apr	613	24.75	78.7	46.1	134.2	1947	1141	3320
3-May	297	24.75	43.2	27.1	68.7	1069	672	1700
GHP								
17-Apr	10	16.88	1.8	0.8	4.1	31	14	69
19-Apr	46	16.88	8.3	3.7	18.6	140	63	314
21-Apr	80	16.88	14.6	9.8	21.3	246	166	359
26-Apr	50	16.88	9.0	5.7	14.3	152	96	241
1-May	14	16.88	2.5	1.3	5.0	43	22	84
3-May	2	16.88	0.4	0.1	1.5	6	2	25
R76								
16-Apr[2]	32	43.25	2.7	1.3	5.7	116	54	245
18-Apr[2]	152	43.25	18.5	9.1	32.2	800	395	1392
20-Apr[2]	250	43.25	30.3	16.0	49.6	1311	691	2144
25-Apr[2]	167	43.25	23.0	14.2	37.2	994	613	1611
30-Apr	107	54.50	11.0	6.4	19.0	601	348	1036
2-May[2]	86	43.25	12.9	8.5	17.7	557	369	764

[1] Estimates likely biased low for as a larger proportion of butterfly behaviors were recorded as "perching" as opposed to flying" in spite of protocol weather; appears to have had a dampening effects on the number of detections.
[2] Surveys excluded the EOD area and may not be comparable to other dates that area was included.

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Range 50. Eight surveys were conducted between 16 April and 12 May by five observers in 2016. Only one observation was recorded on 12 May (Linders et al. 2016). In all, 3,857 adult observations were used in the analyses, with the greatest number (1,133) recorded on 20 April (Table 14). All transects, totaling 9,900 m in length (Table 14), were surveyed on all dates except on 25 April when every fourth line was skipped due to time constraints, and 9 May, when only 8,600 m of transect length were logged due to a recorder malfunction; neither of these affected the resulting density estimates. There were 2-4 surveyors on any given date, but effort appeared to be distributed evenly across transects within and among survey days, with a range of 10,600 m to 18,050 m of effort per observer. Sample sizes were sufficient on all survey dates except 9 (n=12) and 12 May (n=1) to run date-specific detection function models. Data were truncated at 10 m to reduce un-necessary adjustment terms. The best models often varied by observer and survey date; for simplicity those details are omitted here. Daily density and abundance estimates with 95% Confidence Intervals are presented by date in Table 15.

Glacial Heritage Preserve. Seven surveys were conducted at GHP between 17 April and 10 May by 2-4 observers in 2016. No checkerspots were observed on 10 May, and only two were observed on 3 May. A total of 202 butterfly observations were used in the analyses, with a peak count of 80 on 21 April (Table 14). All transects were surveyed on all dates for a total of 6,750 m of effort per day (Table 14). Total survey effort was the same for two observers (JC and LTR) and greater for the remaining two (QR and MJL). All surveys were run under protocol weather conditions. Sample sizes were insufficient to estimate detection functions by observer, so data were pooled and truncated at 10 m. Daily density and abundance estimates with 95% Confidence Intervals are presented by date in Table 14.

Range 76. Eight surveys were conducted by 6 observers between 7 April and 9 May in 2016; no checkerspots were seen on 7 April, and only two were seen on 9 May. A total of 794 butterflies were used in the 2016 analysis, with a peak count of 250 on 20 April (Table 15). There were 2-4 observers present on each survey date and effort was fairly well-distributed among observers and dates. Daily survey effort (i.e., total line length surveyed) was 8,500 m for most dates, except 7 April (no checkerspots observed), 30 April and 9 May (less 400 m). April 30 was the only date when all segments on all transect lines were surveyed (a total of 10,900m); otherwise, a block of segments, effecting 6 lines (and a total of 2,400m), were not surveyed due to EOD restrictions. The number of butterflies observed per survey was sufficient to fit detection functions by observer and date, except on 16 April. The best models often varied by observer and survey date; for simplicity model details are omitted here. Daily density and abundance estimates with 95% Confidence Intervals are presented by date in Table 15.

Adult abundance 2017

From two to seven complete distance surveys were conducted at each of five occupied Taylor's checkerspot sites in 2017 (Table 16), and one partial survey each at R76, TA7S, and SCS2 was also conducted. The greatest amount of effort was expended on occupied sites, with 55.9 km surveyed at SCS1, followed by R76 (54.4 km), R50 (49.5 km), and SCS2 (30.6 km); effort on other sites was: GHP (33.8 km), GHP-CALE (15.6 km), and TA7S (16.3 km). The SCS1 survey area was adjusted slightly to total 20 ha.

Weather through the end of April 2017was challenging, with record-setting fall, winter and spring rains topping records set in 2016. Intermittent clouds eliminated shadows for portions of the survey period on 28 April at GHP, on 8 May at TA7S, on 18 May at R76, and on 19 May at R50. In addition, rain caused us to abort surveys at R76 on 4 May and at TA7S on 12 May. On 2 June at SCS2, we lacked the time for a complete survey, so skipped every fourth line, a systematic strategy we use to minimize the influence of incomplete surveys on density estimates; only one adult was observed on the remaining lines (Table 16).

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Weekday access to R76 was very good due to road maintenance work and new concerns about unexploded ordnance that kept the area closed to general training, which allowed us to just complete the five surveys (Table 16) necessary at this high priority site. However all JBLM sites are now closed to access weekends and holidays, restricting the number of available days with both access and protocol weather conditions. As a result, we were unable to complete the minimum of five protocol surveys needed at R50, where access was much more limited. Even at TA7S, where access was also good, we struggled to complete surveys, because we were forced to travel between JBLM and south Thurston County sites on the same day, wasting precious hours of protocol weather. In year's past, many surveys were completed on weekends, which also reduced conflicts with training.

Table 16. 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 Units 1&2; R50 = Range 50, GHP = Glacial Heritage Preserve, TA7S = Training Area 7S), South Puget Sound, Washington, spring 2017. R76: both standard (T1-12) and extended (T1-16) survey areas reported here (see Appendix C, Fig. 4).

Date	T1-16 R76	T1-12 R76	R50	SCS1	SCS2	GHP	TA7S	Comments
28-Apr						0		GHP: Stopped often to maintain protocol.
30-Apr				0				SCS1: Added segments T15:7-10 & T16:9-10.
3-May				2	0			
4-May	1	1	293					R50&76: EOD w/ea observer; R76: T1-4, then abort.
6-May						0		
8-May				410			1	TA7S: protocol in/out; SCS1: add segments T16:7-8.
9-May					0	0		
10-May	570	394	1410					
12-May							0	TA7S: Surveyed lines 1-3 and parts of 4-7, then rain.
18-May	582	406						R76: Marginal shadows at times.
19-May			1004				0	R50: Marginal shadows toward end.
21-May				717	15	0		
22-May	533	467	768					
23-May							0	
25-May	294	266			16			
26-May				422		0		
29-May				121	21			
31-May	1	1						
2-Jun				42	1			SCS2: Skipped lines 22, 26, 28, 32 due to time limit.
5-Jun			0					
Total	**1981**	**1535**	**3475**	**1714**	**53**	**1**[1]	**1**	

[1] One observed on non-survey day.

A total of 7,224 checkerspots were counted during distance surveys in 2017, with the greatest number observed at R50 (Table 16). Checkerspots comprised 6,870 groups with 6,545 recorded as single butterflies; 298 groups of two; 25 groups of three; and 2 groups of four observed. Flight season initiation was one month later than in 2016 and was the second latest recorded since 2005. Peak counts were staggered from 10 to 29 May 2017. Peak day encounter rates varied among sites, with about 60 percent more butterflies observed at R50 (0.142 checkerspots/m) compared to SCS1 (0.090/m). Rates at R76 (0.053/m for transects 1-16; 0.048/m for transects 1-12) were about one third of those at R50 and half of what was observed at SCS1. The shift away from R76 as the site with the highest encounter rates began in 2016. Only one checkerspot was observed at GHP, which was also true for TA7S (prior to release of adults). Staff from Joint Base Lewis-McChord continue to monitor PCM and adjacent parts of TA14 using transects with a 100-m spacing; these surveys are also conducted around the perimeter of 91st Division Prairie (the Artillery Impact Area) as well as on TA6; checkerspots were recorded at several locations where monitoring for colonization will continue as resources allow.

Only one adult checkerspot was observed at GHP in 2017 following release of 3,025 postdiapause larvae across several plots. These results were similar to TA7S where successive releases of 1,003 prediapause larvae in 2016 and 909 postdiapause larvae in 2017 occurred. These results are deeply concerning, and the causes are not clear. GHP has sustained small populations through one or more annual cycles in the past, as evidenced by the presence of eggs, pre- and postdiapause larvae, pupae and adults all in areas of the site independent of supplemental releases. So some other factor(s) (e.g., weather, microclimate, habitat, etc.) appear to be interfering with population persistence. We have less knowledge about the performance of checkerspots at TA7S, although both larval and adult food resources are abundant at the site (Waters et al. 2015). Other potential factors that could be influencing results at these sites include: 1) a failure to reproduce successfully due to conditions already mentioned, 2) low overwinter survival, 3) impacts from restoration and/or prescribed burn activities, and/or 4) unknown toxins or other environmental barriers; a table is being compiled for management and research consideration.

Analyzed data 2017

Distance data were analyzed for four sites (SCS1, SCS2, R50 and R76) with sufficient observations in 2017; results for are presented here and in Appendix B.

Scatter Creek South 1. Seven surveys were conducted at SCS 1 in 2017 between 30 April and 2 June; no butterflies were observed on 3 May. Of 1,573 observations used in the analysis, the greatest number (652) were collected on 21 May. All surveys were complete except on 30 April and 3 May, which omitted a few segments added at a later date. The number of observers per day varied from 2-4, with survey effort fairly evenly distributed over the season for 3 observers; a fourth observer had less than half the effort; encounter rates were similar among all observers when data were pooled over all survey dates. The number of butterflies was sufficiently high on 5 days (May 8-June 2) to estimate detection functions separately by date, and for most days also by observer within date. The best models had pooled observers on most dates, except on 26 May, when observer-specific models performed better. Daily density and abundance estimates with 95% Confidence Intervals are presented in Table 17.

Scatter Creek South 2. Six surveys were conducted at SCS 2 in 2017 between 3 May and 2 June; no butterflies were observed on 3 or 9 May, and only one butterfly was observed on 2 June. Full surveys were completed on all dates except 2 June, when 4 lines were systematically omitted due to a shortage of time (Table 16). Due to small sample sizes, the detection functions developed in the SCS1 monitoring area were used to estimate densities in the SCS2 area. Surveys were typically conducted in both areas by the same observers on the same dates, minimizing differences in detectability. Daily density and abundance estimates with 95% Confidence Intervals are presented in Table 17.

Range 50. Five surveys were conducted between 4 May and 5 June by four observers in 2017; no butterflies were recorded on 5 June. In all, 3,220 adult observations were used in the analyses, with the greatest number (1,312) on 10 May (Table 17). All transects were surveyed on all dates. Survey effort was fairly evenly distributed across observers, and sample sizes were sufficient on all days to run date-specific detection function models. All surveys were conducted under protocol conditions except on 19 May, when warm, muggy conditions appeared to dampen flight behavior as at R76 the previous day; these conditions were not found to influence on detectability. Data were truncated at 10 m to reduce

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unnecessary adjustment terms. Density estimates with 95% Confidence Intervals are presented in Table 17 along with daily abundance estimates for the entire survey area.

Table 17. Number of distance sampling observations (#Obs) used to calculate density estimates (#/ha) and adult abundance estimates (#/site by date) including 95% Confidence Intervals for Taylor's checkerspot survey areas at Scatter Creek South Unit 1 (SCS1), Scatter Creek South Unit 2 (SCS2), Range 50 (R50), and Range 76 (R76) in South Puget Sound, Washington, spring 2017. See text for details.

		Survey	Density			Abundance		
Date	**#Obs**	**area (ha)**	**#/ha**	**Lower CI**	**Upper CI**	**#/site**	**Lower CI**	**Upper CI**
SCS1								
8 May	366	20.00	67.82	48.8	94.27	1356	952	1838
21 May	652	20.00	114.91	87.47	150.95	2298	1706	2944
26 May	398	20.00	86.41	20.84	358.22	1728	406	6985
29 May	116	20.00	27.22	19.55	37.36	544	381	729
2 Jun	41	20.00	15.14	8.45	27.13	303	165	529
SCS2								
21 May	12	13.50	3.1	1.3	7.3	42	26	143
25 May	16	13.50	4.4	2.5	7.7	60	50	151
29 May	20	13.50	7.1	4.0	12.8	96	77	249
R50								
4-May	262	24.75	30.5	22.4	41.4	754	554	1025
10-May	1312	24.75	171.5	140.9	208.7	4244	3488	5165
19-May	928	24.75	103.3	52.5	203.7	2556	1300	5042
22-May	718	24.75	93.3	73.1	119.0	2309	1810	2946
R76								
10-May	505	54.50	73.2	32.7	164.3	3992	1780	8952
18-May	532	54.50	65.9	38.1	114.0	3594	2079	6213
22-May	502	54.50	50.5	35.4	71.9	2750	1929	3917
25-May	268	54.50	31.6	24.5	40.8	1723	1335	2224

Range 76. Six surveys were conducted by 4 observers from 4-31 May in 2017. One checkerspot was observed on 4 May, but the survey was aborted due to non-protocol conditions, and only one butterfly was observed on 31 May in spite of a complete survey. A total of 1,807 butterflies were used in the 2017 analysis, with the greatest number (532) recorded on 18 May (Table 17). There were 4 observers present on most survey dates (except 22 May) and effort was well-distributed among observers and dates. All transects (Table 14) were surveyed on all dates, except 4 May as indicated. Surveys on most other dates were conducted entirely in protocol conditions except 18 May, when warm, muggy conditions appeared to dampen flight behavior, as at R50 the following day; these conditions were not found to influence on detectability. The number of butterflies observed per survey was sufficient to fit detection functions separately by date and by observer within date, except on 4 and 31 May. Daily density and abundance estimates with 95% Confidence Intervals are presented by date in Table 15.

Other observations

Nectar observations have been recorded opportunistically during Taylor's checkerspot distance surveys since 2011. In addition to the data in Table 18, one observation each of: *Anaphalis margaritacea* (R76-2017); *Cytisus scoparius* (R76-2013), *Dodecatheon hendersoni* (R50-2015) and *Fritillaria camschatcensis* (R76-2015); Plantago lanceolata (R50-2017); Rumex acetosella (R76-2017); two observations on *Cerastium arvense* (R50-2012, TA7S-2014) have also been recorded. Sixteen other species were observed to be used across four survey areas in 2017, with a marked increase in the number of

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observations relative to 2017. Three plant species: *Balsamorhiza deltoidea*, *Lomatium triternatum* and *Saxifraga integrifolia (=Micranthes integrifolia)* made up 91.5 percent of nectar observations in 2017 and continue to account for the majority of all nectar observations recorded to date (89.1 percent). The continuing importance of these species across sites and years is likely because they provide good basking sites and roosting cover in addition to being quality sources of nectar.

Table 18. 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 Units 1&2, PCM = Pacemaker, GHP = Glacial Heritage Preserve, TA7S = Training Area 7 South) sites, South Puget Sound, Washington, 2011-2017. ACMI – *Achillea millefolium*; ARMA – *Armeria maritima*; BADE - *Balsamorhiza deltoidea*; CAHI – *Castilleja hispida; CAQI - Camassia quamash*; 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*.

Year	Site	ACMI	ARMA	BADE	CAHI	CAQU	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
	SCS1	0	2	2		1							1			4					10
Subtotal		**3**	**16**	**733**	**0**	**4**	**0**	**12**	**46**	**1**	**1**	**0**	**622**	**5**	**3**	**4**	**3**	**4**	**43**	**0**	**1500**
2012	R50			86	1	4			3			13	4						30		141
	R76		6	465		3			8			161	305	21				8	113		1090
	SCS1	1	2	6												1					10
	PCM			1								2	2					1			6
Subtotal		**1**	**8**	**558**	**1**	**7**	**0**	**0**	**11**	**0**	**0**	**176**	**311**	**21**	**0**	**1**	**0**	**9**	**143**	**0**	**1247**
2013	R50			67						2			6					1	17		93
	R76		1	219	1	1			2				48	6				1	3		282
	SCS1		1																		1
	GHP			2	1	5															8
Subtotal		**0**	**2**	**288**	**2**	**6**	**0**	**0**	**2**	**2**	**0**	**0**	**54**	**6**	**0**	**0**	**0**	**2**	**20**	**0**	**384**
2014	R50			35								1	2					1	8		47
	R76		6	484		12			8	1		14	35	1					35	4	600
	SCS1		1	6														1			8
	GHP					2															2
	TA7S		2	6			2					1		1							12
Subtotal		**0**	**9**	**531**	**0**	**14**	**2**	**0**	**8**	**1**	**0**	**16**	**37**	**2**	**0**	**0**	**0**	**2**	**43**	**4**	**669**
2015	R50			187	1				2				17					7	36		250
	R76		2	207		14			6				53	10				3	29		324
	SCS1			6		9	1									1					17
	GHP			1		4												1			6
	TA7S			1			1						3								5
Subtotal		**0**	**2**	**402**	**1**	**27**	**2**	**0**	**8**	**0**	**0**	**0**	**73**	**10**	**0**	**1**	**0**	**11**	**65**	**0**	**602**
2016	R50			80		3							7						21		111
	R76		1	12		6			1				14	4					2		40
	SCS1	1	3	6		6	4						1			10					31
	GHP					2							1								3
Subtotal		**1**	**4**	**98**	**0**	**17**	**4**	**0**	**1**	**0**	**0**	**0**	**23**	**4**	**0**	**10**	**0**	**0**	**23**	**0**	**185**
2017	R50			204		1				1		1	37	1		1		2	49	2	299
	R76	2	1	121		5	1	1		7	5		186	9			2		18	1	359
	SCS1	3	1	53		2										12		1			72
	SCS2			3																	3
Subtotal		**5**	**2**	**381**	**0**	**8**	**1**	**1**	**0**	**8**	**5**	**1**	**223**	**10**	**0**	**13**	**2**	**3**	**67**	**3**	**733**
Total		**10**	**43**	**2991**	**4**	**83**	**9**	**13**	**76**	**12**	**6**	**193**	**1343**	**58**	**3**	**29**	**5**	**31**	**404**	**7**	**5320**
Proportion		0.00	0.01	**0.56**	0.00	**0.02**	0.00	0.00	**0.01**	0.00	0.00	**0.04**	**0.25**	0.01	0.00	0.01	0.00	0.01	**0.08**	0.00	**1.00**

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As in years past, mardon skipper butterfly sightings were systematically recorded during 2017 surveys for Taylor's checkerspot at the three sites where they co-occur. In total, 48 mardon skippers were observed (Table 19), which is an increase from the all-time low observed in 2016 (Linders et al. 2016), even without inclusion of the SCS2 survey area. All observations were of lone mardon skippers, except for one group of two skippers observed at SCS1. Observation of five skippers at R76 was somewhat encouraging considering none were recorded there in 2016. Numbers of mardon skippers in the Puget lowlands have remained discouragingly low for the past decade and remain a source of real concern.

Table 19. Number of mardon skipper butterflies observed by date and site during distance surveys for Taylor's checkerspot in spring 2017 at three sites (R50 = Range 50; R76 = Range 76; SCS = Scatter Creek South), including two monitoring areas at Scatter Creek, South Puget Sound, Washington. "Zero" surveys are included nearest to dates when skippers were observed.

Date	R50	R76	SCS1	SCS2
8-May			0	
9-May				0
10-May	0	0		
18-May		0		
19-May	3			
21-May			1	
22-May	12	1		
25-May		4		3
26-May			10	
29-May			5	3
31-May		0		
2-Jun			5	1
5-Jun	0			
Total	**15**	**5**	**21**	**7**

Adult distribution

Methods

To illustrate the distribution of adults within survey areas at each site, all Taylor's checkerspot observations collected during 2017 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 (Table 14). 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

An index of area occupied by adult Taylor's checkerspots by site and year is summarized in Table 20. Trends at R50 and SCS follow a pattern of increase in recent years, which differs from R76. On sites with more than one observation (R76, R50 and SCS1 &2) in 2017, checkerspots were widespread and occupied the majority of sampling areas except at SCS2, where colonization appears to underway, as no releases have occurred. Checkerspots at R50 occupied 84.6 percent (335 of 396) of segments surveyed with high concentrations throughout the site center (Appendix C, Fig. 2). Checkerspots at SCS1 occupied 60.9 percent (195 of 320) of the segments surveyed, with 21.3 percent (46 of 216) of the segments occupied at SCS2.

At R76, checkerspots occupied 82.1 percent (276 of 336 segments) of the main monitoring area (transects 1-12) in 2017, a significant improvement over 2016. Contrary to results in 2016 when 47.9 percent (437 of 912) of checkerspots at R76 were observed on transects 13-16 (Linders et al. 2016), in 2017 just 22.5 percent (446 of 1981) of observations were recorded on survey lines at the north end of the site (see Appendix C, Fig. 4), with the overall distribution pattern also reversed. While some of this may be explained by improved access to the dudded area in 2017, this alone does not explain the differences in distribution and numbers. Reasons for shifts in occupancy in recent years are unclear, but site-specific factors such as habitat condition and/or military training impacts cannot be ruled out, and likely interact with larger scale drivers such as weather conditions.

We expect population expansion to continue at SCS, where hundreds of acres of potential habitat are being restored. A 2017 wildfire at that site likely impacted a portion of the population, but the opportunity to restore large portions of the 215 acres that burned is expected to increase available habitat significantly. Distribution at GHP through 2016 followed a pattern similar to SCS1 until 2016 (Table 20), then failed to maintain itself even with the large release of larvae in 2017. Ample winter and spring rains appeared to benefit habitat during the flight season, where conditions were good on all

Table 20. Index of area (ha) 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 – 2017. SCS = Scatter Creek South (1 = main reintroduction unit; 2 = second management unit in northwest corner of site), 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	2017
SCS1			0.2	5.8	4.3	4.3	4.3	3.9	5.4	10.6	12.2
SCS2											2.9
R50				6.5	13.6	18.8	17.5	11.2	15.3	18.4	22.4
PCM						3.0	0.0	0.0	0.0		
GHP						0.4	6.3	3.3	3.4	3.7	0.1
TA7S								2.1	3.4	0.3	0.1
R76	39.3	17.0	26.0	38.0	41.8	42.0	39.5	39.4	38.0	21.3	34.5
R76 T13-16								11.0	11.0	8.1	7.3
R76 Total	39.3	17.0	26.0	38.0	41.8	42.0	39.5	50.4	49.0	29.4	41.8

sites in May 2017. However persistent rain and cold well into April suppressed host plant growth and likely triggered many larvae to return to diapause both in the lab and in the field (M. Linders, pers. obs.).

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; focal surveys conducted by JBLM staff on the Artillery Impact Area are not included. A map of scattered sightings associated with the Artillery Impact Area shows small concentrations of adults in several locations (Appendix D, Fig. 2, upper right), although only a portion of this site can be covered in any given year. Numerous adults were also observed between survey units 1&2 at SCS (Appendix D, Fig. 1).

No adults were observed by JBLM staff at Pacemaker in 2017. Results from this site and others (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.

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. In turn, data from R76 were used to set population targets, evaluate population growth potential, and set long-term monitoring goals for 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 42-ha (100 ac) monitoring area at R76, which is about twice the size of most Puget lowland reintroduction units; these 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 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), because that site was roughly twice the size of the reintroduction sites. In that year it was difficult to find 2-3 butterflies together at R76 on any given day, yet the population was able to recover from that low point, giving us confidence that other populations could as well. Therefore, this is a minimum measure below which we believe populations are at high risk of extirpation. Furthermore, given the volatile nature of checkerspot population dynamics (Appendix B), functional populations are expected to be well above the peak single day abundance threshold of 250 adults in most years. A five-year monitoring window is a widely used standard for establishing butterfly occupancy and is a minimum duration for capturing the amplitude of the population cycle.

Methods

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Once releases are complete we use the existing distance sampling protocol (Linders and Olson 2014) to monitor for establishment. Results are evaluated annually against the above criteria using daily abundance estimates and distribution maps.

Results and Discussion

Based solely on natural reproduction, adult checkerspots were distributed across the majority of the 25-ha monitoring area at R50 (Appendix C, Fig. 2), and have increased their spread annually since 2014, with 20.9 ha acres occupied in 2017, the largest to date. In addition, the peak single day abundance estimate for 2017 (4,244; 95% CI: 3,488-5,165; Table 17) was the highest recorded for that site and topped all other Puget lowland sites for the second straight year. Peak single day abundance estimates have remained well above the minimum threshold of 250 adults every year since releases concluded in 2011. This population is officially established based on the project definition and continues to expand.

Checkerspots at SCS1 met these criteria in 2015 based solely on reproduction, but a release of larvae in spring 2016 to combat potential negative effects of *El Nino* weather on that small population meant the five-year assessment period restarted in 2017. Checkerspots at SCS1 occupied 61 percent of the 20-ha monitoring unit in 2017, with extensive restoration continuing to improve habitat across the site. The peak single day abundance estimate of adults in 2017 (2,298, 95% CI: 1,706-2,944; Table 17) indicates this site handily met its Year 1 establishment goal, is on a strong positive trajectory, and on track to complete the trial period for establishment by 2021.

Future Plans and Recommendations

Taylor's checkerspot reintroduction in the Puget lowlands

Fortunately, the declining trend (Appendix B) and apparent dispersal (Appendix D) of the R76 population in recent years did not persist into 2017. The increase in numbers at the R50 and SCS1 reintroduction sites is also very encouraging, and trends for these sites (Appendix B) are expected to continue, assuming that not all suitable habitat is occupied. In contrast, observance of only one adult at each of the two 2017 release sites (GHP and TA7S) is a reminder that many outstanding questions remain. However, our results were comparable to patterns across the species' geographic range in 2017, with many populations performing poorly, and others showing moderate returns based on data presented at the Rangewide Taylor's checkerspot Working Group meeting in Portland, Oregon, 7-8 November 2017. Given the dynamic nature of checkerspot populations, a strategic approach to recovery should include development of multiple high-density populations that act as sources, since these will be the most robust over time and produce the greatest number of emigrants (Thomas et al. 2011). Where high density populations are associated with high quality habitat patches (occupied and unoccupied), significant opportunity for successful colonization exists. This is the situation that appears to be unfolding at SCS (Appendix C, Fig. 1), where the population core continues to build in numbers, while the number of scattered sightings warrants more intensive monitoring. In contrast, where habitat quality is low (e.g., GHP), it is difficult to reach the critical densities necessary to sustain populations let alone produce a sufficient number of adults to foster growth. Consequently, the focus of reintroduction in SPS should be to:

1) Increase the species short and long-term viability through targeted habitat management,
2) Increase both the number and sizes of existing populations, and
3) Define then increase the number of source populations.

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Current reintroduction-related plans

This is the twelfth year of a multi-year Taylor's checkerspot recovery project. Consistent with previous recommendations, the robust response of checkerspots at SCS suggests no new releases are warranted there in 2018 in spite of the expansive 2017 wildfire (Appendix C, Fig. 1). In light of the large number of dispersed sightings and the wildfire, however, we are planning to survey the entire South Unit of Scatter Creek in 2018 to document checkerspot distribution, identify adult concentrations, and evaluate the effects of the fire. Surveys would employ a 100-m spacing, with the potential to follow up with 25-m transects where densities warrant. Population densities have already been calculated for both the burned and unburned areas from 2017 data for comparison with data collected post-burn in 2018. At a minimum, standard flight season monitoring within the SCS1 management unit will continue in 2018 and annually until 2021 to demonstrate that establishment criteria have been met. We also hope to continue monitoring SCS2, where numerous adults were observed in 2017 (Appendix C, Fig. 1) and where females were observed egg-laying in 2016.

Criteria for establishment at R50 were officially met in 2016, although monitoring in 2017 showed that population continuing to increase. Given 1) the need to secure existing populations, 2) establish multiple source populations, and 3) fully understand the habitat potential at that site, we recommend that monitoring at R50 continue.

The highest priority location for release in 2018 is Training Area 15 on Joint Base Lewis-McChord. A 2016 habitat assessment indicated the site hosts ten 5-ac "reintroduction-ready" units (Waters 2016), which is the greatest number of any site measured to date. Waiting to initiate releases until after habitat is restored is the recommended strategy throughout the conservation literature (e.g., Thomas 1989, Oates and Warren 1990, Stamps and Swaisgood 2007, Thomas et al. 2011,). This approach was highly successful at R50, where high quality habitat was already present, and only minimal release effort was required. The opportunity to repeat that success at TA15 is unparalleled and stands to shed light on the importance of habitat parameters in achieving reintroduction success (Thomas et al. 2011). We are currently awaiting approval from JBLM to initiate work at TA15, and are prepared to release larvae at TA7S as an alternative approach. While TA7S also performed poorly in 2017, far fewer larvae have been released there. The site does support three 5-ac "reintroduction-ready" units and while small, it is rich with checkerspot host and nectar resources as well as adult cover.

We are planning to discontinue releases at GHP at this time due to the poor response of checkerspots at that site to date, in spite of significant effort. Numerous factors may be contributing to these outcomes. 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 may be declining in places, rather than increasing. Potentially limiting is the lack of reintroduction-ready habitat including adult cover, patch isolation, and suitability of microsites, but herbicide use, pesticide drift, the scale and intensity of prescribed fire, and other factors may also be at play.

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, and at SCS based on the number and distribution of scattered sightings reported in 2017. 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 an alternative path to increasing the size and number of populations.

Some funding for the 2017-2018 field season and rearing year have been secured from JBLM and USFWS, although additional funds are being requested from JBLM. Funding for 2018-2019 is also partially in place from FWS, but detailed funding needs have not been calculated for several recent changes in project scope, including increases in the number of field monitoring sites, expansion of the rearing facility at Mission Creek, and post-fire monitoring at Scatter Creek. Additional funding will be needed to insure adequate coverage in FY 2019. We have also obtained funding 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 whenever possible. Sites are chosen to encompass a diversity of microsites to offset the influence of climatic perturbations on translocation success.

Access to JBLM sites is dynamic and unpredictable, and remains a source of concern due to the fundamental dependence of the project on the occupied sites in the AIA and the large amount of potentially habitat that occurs on base. Weekday access to R76 was good in 2017 due to road maintenance work and new concerns about unexploded ordnance that kept the area closed to general training. This allowed us to just complete the five surveys (Table 16) necessary at this high priority population source site. However all JBLM sites are now closed to access weekends and holidays, restricting the number of available days with both access and protocol weather conditions. As a result, we were unable to complete the minimum of five protocol surveys needed at R50, where access was much more limited. Even at TA7S, where access was also good, we struggled to complete surveys, because we were forced to travel between JBLM and south Thurston County sites on the same day, wasting precious hours of protocol survey weather. It is unclear what level of access we might expect in 2018, although unexploded ordnance clearing at R76 is likely to add significant new challenges Ultimately, the need for a consistent approach to scheduling that addresses the needs as they have been stated annually since 2008, is badly needed. In year's past, many surveys were completed on weekends, which reduced conflicts with training while providing sufficient flexibility to accommodate weather. This is just one example that should be considered to insure necessary surveys can be conducted.

Questions for Further Research

1) Does host plant patch quality drive population density and population growth of Taylor's checkerspot?

In reviewing data from 30 European butterfly species, Thomas et al. (2011) provide clear guidance for evidence-based conservation strategies derived from decades of work over large geographic scales. Studies required to compile sufficiently detailed life-tables for a key factor analysis typically require 3-7 years of intensive work and may have limited usefulness for conservation because they typically describe short-term fluctuations of a single, relatively stable population rather than identify factors that determine the carrying capacity of different sites or which drive long-term trends over larger geographic scales. Using data from the UK Butterfly Monitoring Scheme, Thomas et al. (2011) find that adult density in the largest populations of a given species are consistently about 100 times higher than densities in the smallest populations, which reflects the range of environmental quality that exists between optimum and low quality sites, that are nevertheless capable of supporting a predominantly closed population for at least 9-32 generations. They find that the main drivers of population change across species in order of influence are:
1) Within site quality of larval habitat: Optimum larval microsites are closely correlated with enhanced larval fitness in the wild.

2) Low adult dispersal: Insufficient dispersal limits colonization potential and the ability of populations to expand into available habitat over larger landscape scales.

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3) Shelter (all stages): Changes in the availability of shelter alters larval habitat by changing micro-climate, whereas for adults it affects their propensity to move or within-site, provides nighttime refugia and/or protection from inclement weather.

4) Climate change/weather: short-term synchronized changes across regions are widely accepted as being driven by weather. These are typically two orders of magnitude smaller than the consistent or long-term trends in population density between sites reported in Thomas et al. (2011).

Thomas et al. (2011) also considered population-scale effects from density-dependent factors such as parasitoids or resource interactions (e.g., food limitation), nectar resource abundance, and abiotic factors (aspect, soil depth, shelter and local climate), but conclude that these factors do not account for the 50-100 fold or greater variations that are the key drivers of population change. It is these major drivers that can convert a high quality site to a poor quality or extinct one, or vice-versa.

The scientific literature provides solid evidence for larval habitat quality as a primary driver of population growth for many species of butterflies across large geographic areas (Thomas et al. 2011, etc.). While short-term population fluctuations can be attributed to weather, larval habitat quality accounts for population changes that are 1-2 orders of magnitude larger than those explained by weather. Within-patch larval habitat quality is also important at the meta-population scale, because higher density populations supported by optimum habitat are less likely to go extinct, and are more likely to generate emigrants. In turn, emigrants that locate high quality patches are more likely to be successful at colonization.

Similar to results for other species that lay eggs in clusters, local research has shown that *Euphydryas editha taylori* selects host plants for oviposition where they occur in higher densities than in the surrounding area (Linders et al. 2009, Grosboll 2011, Severns and Grosboll 2011). Furthermore, Waters (pers. comm.) identified microsite as an important oviposition selection factor for Taylor's checkerspot butterflies at Scatter Creek Wildlife Area. As a follow up to this work we propose that larval nest densities could be correlated with host plant patch characteristics as a means of illustrating which habitat patches act as population sources. Larval nests can be readily located during first and second instar when clusters of webbed larvae are easy to spot. Patches that harbor large numbers of surviving larvae can be compared with those where larval densities and/or survival are relatively low. Existing variation within and between sites in both habitat patch quality and population growth suggest we already have a suitable range of sites on which to conduct this study. This approach allows us to build on the knowledge of generations of researchers that have successfully moved from creating suitable habitat, to creating optimum habitat, while avoiding the "shotgun" approach required to quantify all potential threats at all life stages.

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

The influence of weather on animal populations is not simply a matter of fate. Instead animals rely on the characteristics of their environment to help moderate temperature and humidity, provide protective cover, and supply the sustenance they need to endure and thrive in the midst of changing weather conditions. Weather is known to have dramatic impacts on the annual abundance of *Euphydryas editha* populations in California (Weiss and Weiss 1998) and we expect that to be true for *E. e. taylori* in Washington as well. Population monitoring in the Puget lowlands has documented order of magnitude changes over short, 1-3 year timelines that cannot be explained by changing vegetation. Understanding the role of weather in shaping population trends is fundamental to successful reintroduction, long-term management, and population recovery and persistence. Over time, this knowledge has the potential to elucidate questions about habitat quality relative to vegetation structure and composition; resource abundance and distribution; patch size and connectivity; microsite suitability and more.

The initial 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 in turn 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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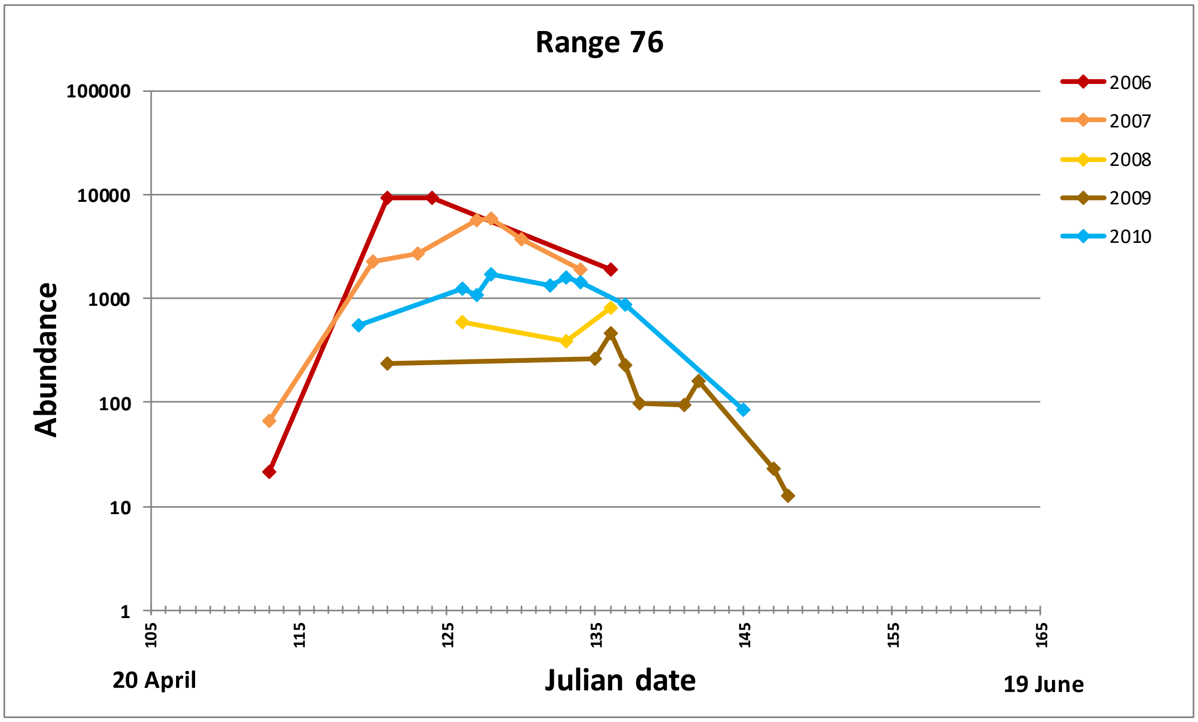
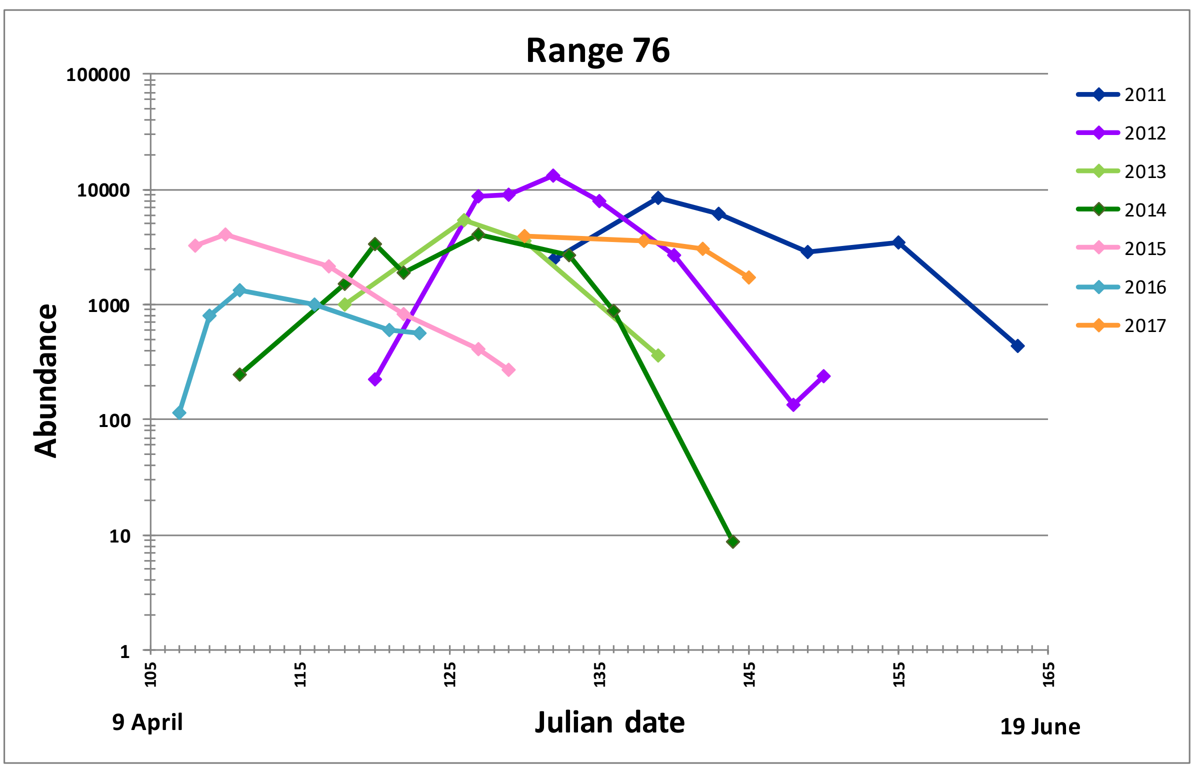
Appendix A. Approximate number of Taylor's checkerspot butterflies released by life stage (Pr = prediapause larvae; Ad = adult, Po = postdiapause larvae) and site (SCS = Scatter Creek South; R50 = Range 50; GHP = Glacial Heritage Preserve; PCM = Pacemaker Airstrip; SCN= Scatter Creek North; TA7S = Training Area 7 South in South Puget Sound, Washington, 2007-2017.

Site	**2007**	**2008**	**2009**		**2010**		**2011**			**2012**		**2013**		**2014**		**2015**		**2016**			**2017**	
	Po	Po	Po	Pr	Po	Ad	Po	Ad	Pr	Po	Ad	Po	Ad	Po	Ad	Po	Ad	Po	Ad	Pe	Po	Ad
SCS	199	340	747	2487	891	202	1109	167	1036		133	3250			56			1658				
R50				2956	1145		1141															
GHP										975		3372	107	1522		1693	226	2029			3024	
PCM			741							1565												
SCN			759																			
TA7S														1086		1102			231	1003	909	145

Appendix B. Estimated daily abundance of Taylor's checkerspots based on distance estimation, and timing of the flight season at: Range 76 (source site) in 2006-2010 (top), and in 2011-2017 (bottom); note log scale on the y-axis. Data for two reintroduction sites in South Puget Sound, Washington, Scatter Creek South Unit 1 (page 2 top) in 2010-2017, and Range 50 in 2010-2017 (page 2 bottom), are also shown; note linear scale on the y-axis. Area surveyed varies by site and year; see Table 5 and text for details.





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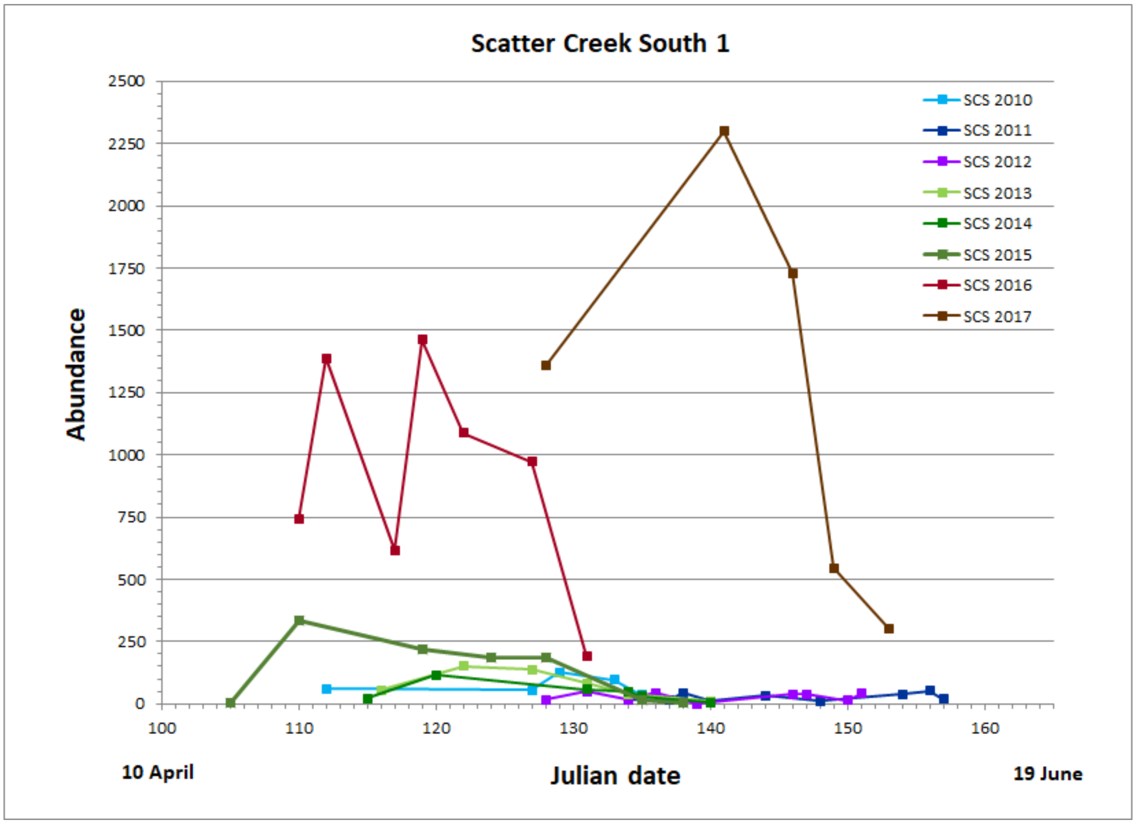
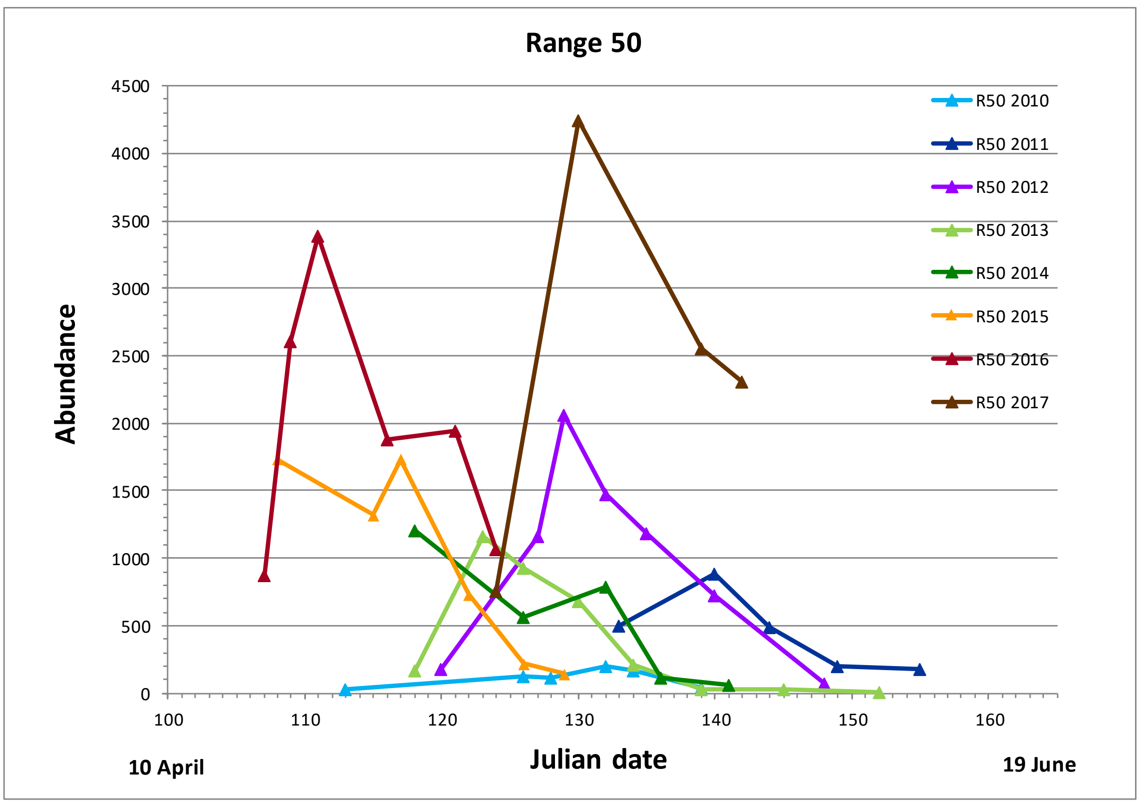

Scatter Creek South 1
2500
2250
2000
1750
1500
1250
1000
750
500
250
0
Abundance
100
110
120
130
140
150
160
10 April
Julian date
19 June
SCS 2010
SCS 2011
SCS 2012
SCS 2013
SCS 2014
SCS 2015
SCS 2016
SCS 2017


Range 50
4500
4000
3500
3000
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
R50 2016
R50 2017

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Appendix C. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at two reintroduction sites (SCS and R50) and one extant site (R76) in South Puget Sound, Washington, spring 2017.

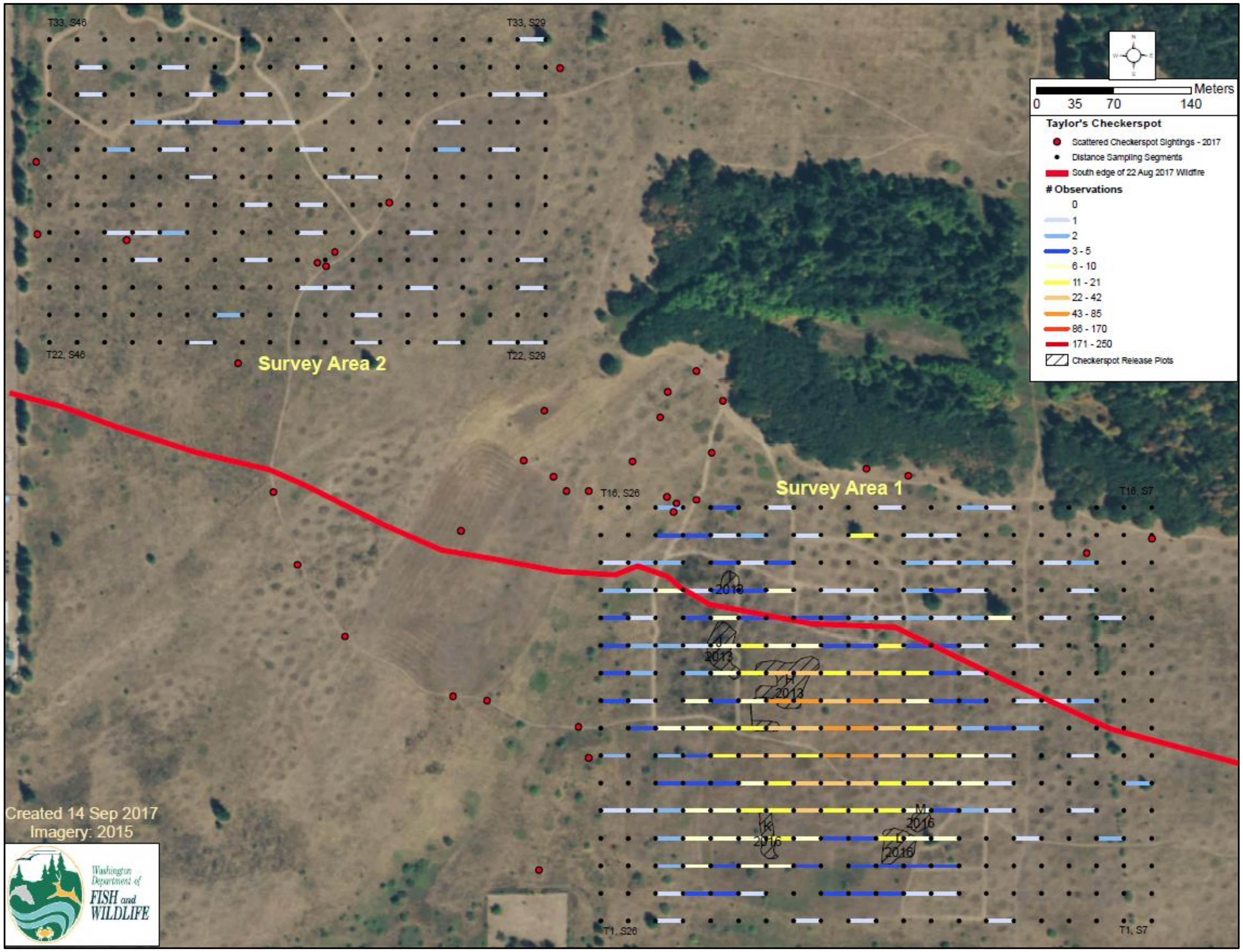

Figure 1. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Scatter Creek South in survey areas 1 and 2 combined across all survey dates in spring 2017, South Puget Sound, Washington; scattered sightings located in surrounding areas are also shown.

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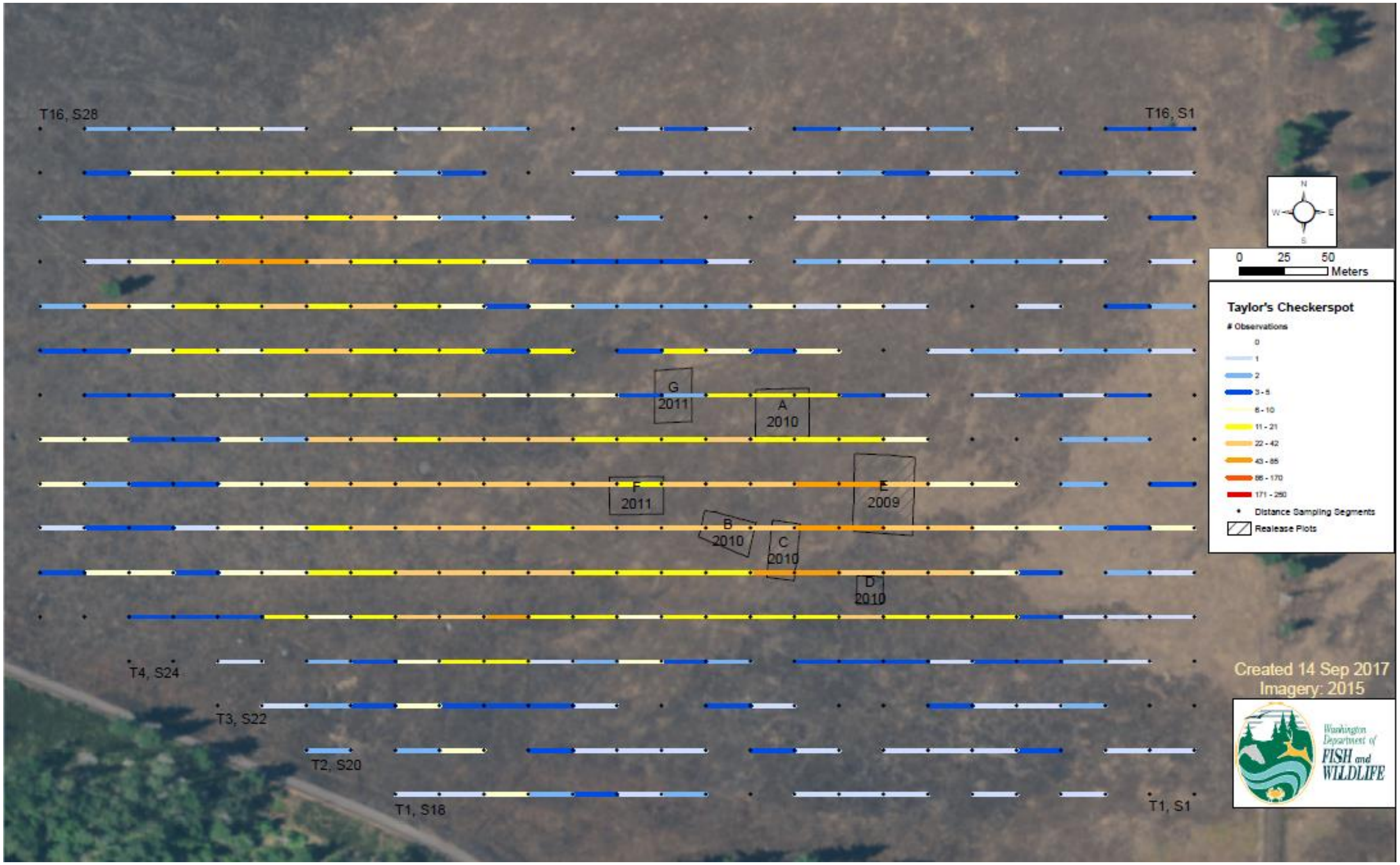

Figure 2. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Range 50, combined across all survey dates in spring 2017, South Puget Sound, Washington.

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Figure 3. Two distance sampling areas were delineated at Glacial Heritage Preserve in 2017 based on the prevalence of *Castilleja hispida* (GHP2, right) and *C. levisecta* (GHP2L, left); releases occurred in all plots (hashed polygons and yellow X's); Plot I was expected to be occupied by existing larvae. Only one adult Taylor's checkerspot was observed at the sites in spring 2017, South Puget Sound, Washington

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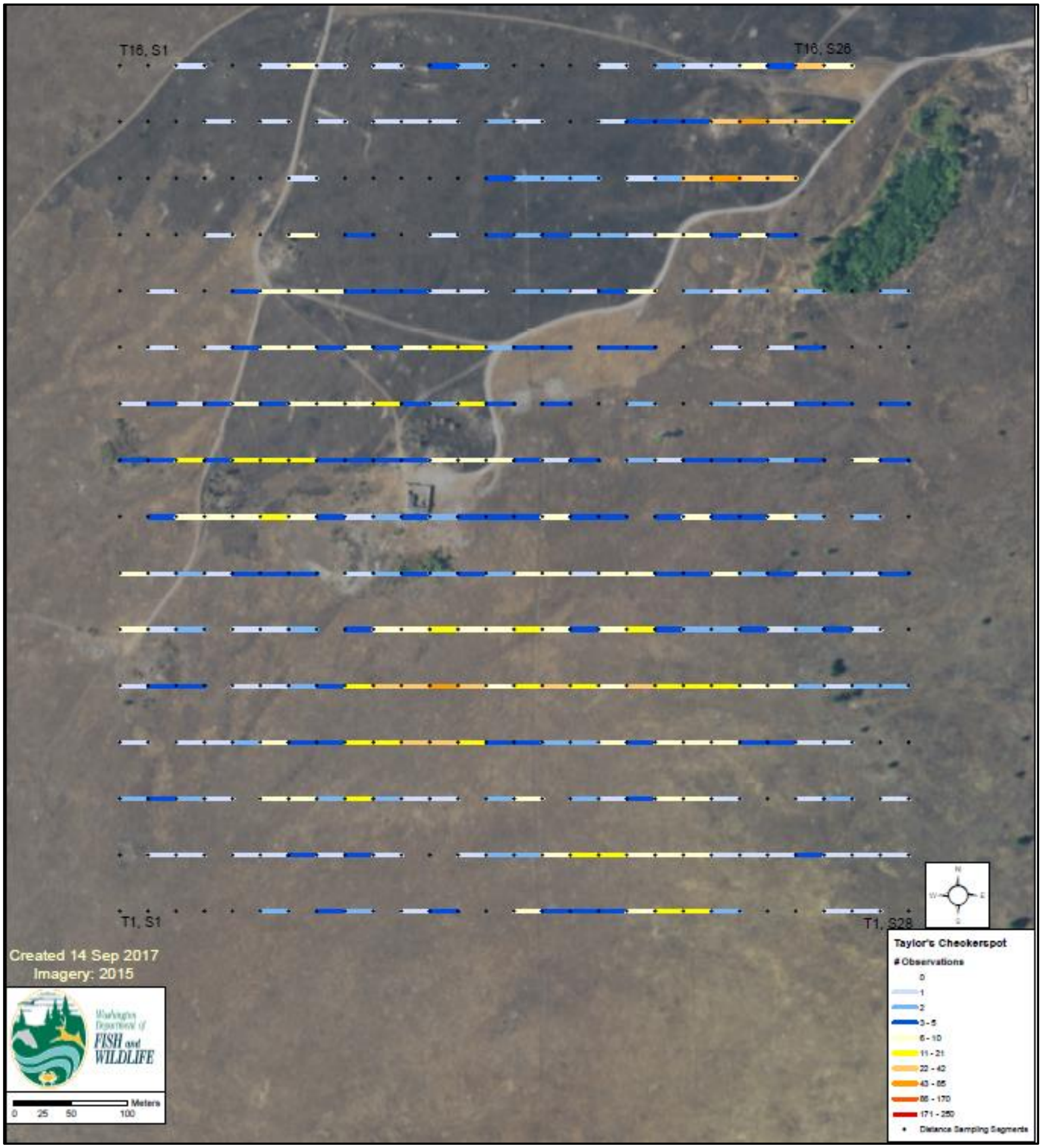

Figure 4. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Range 76, combined across all survey dates in spring 2017, South Puget Sound, Washington.

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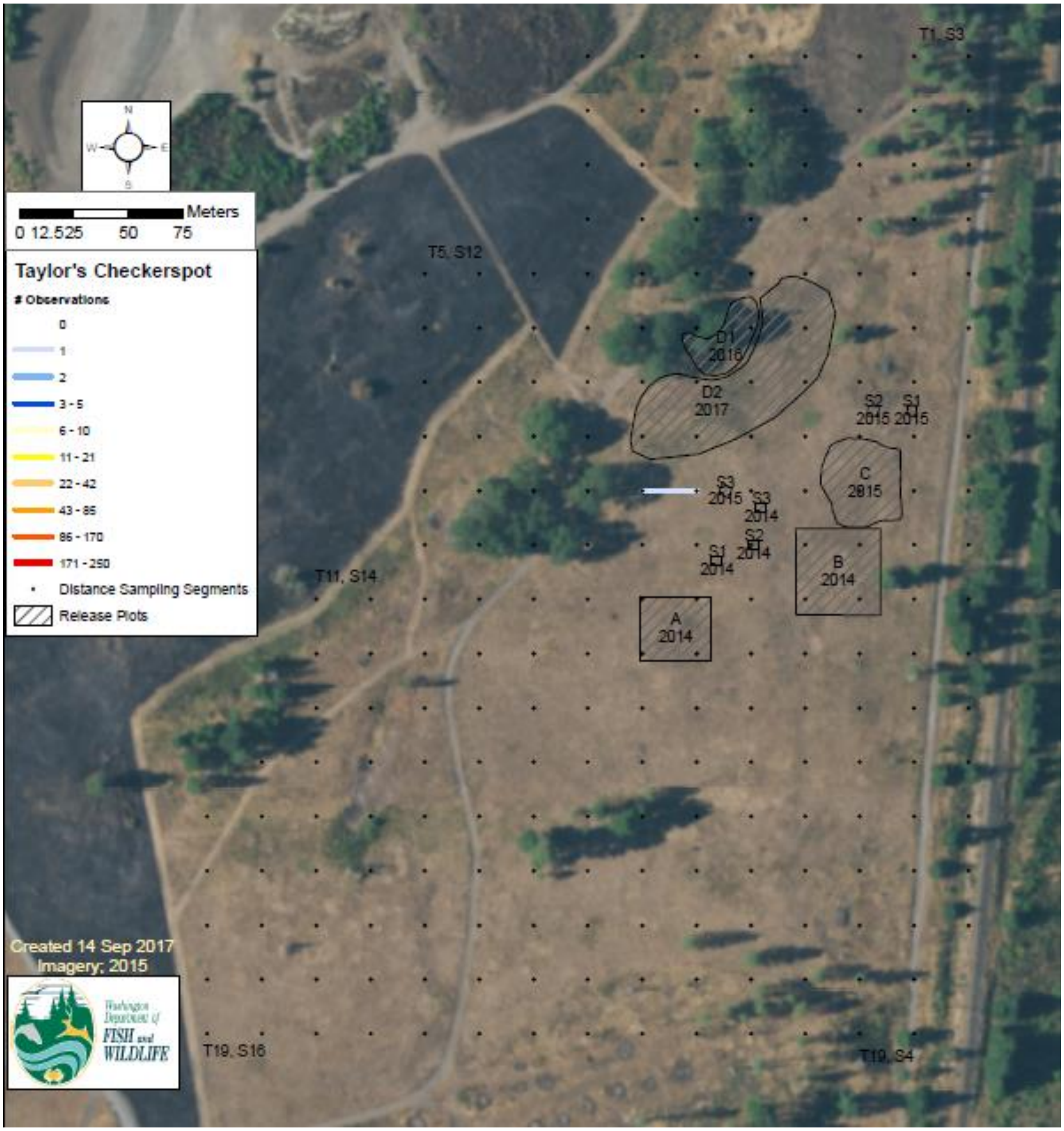

Figure 5. Only one adult Taylor's checkerspot was observed during distance sampling surveys at Training Area 7S, combined across all survey dates in spring 2017, South Puget Sound, Washington.

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Appendix D. Scattered sightings (2012-2017) of Taylor's checkerspots outside of distance survey areas.

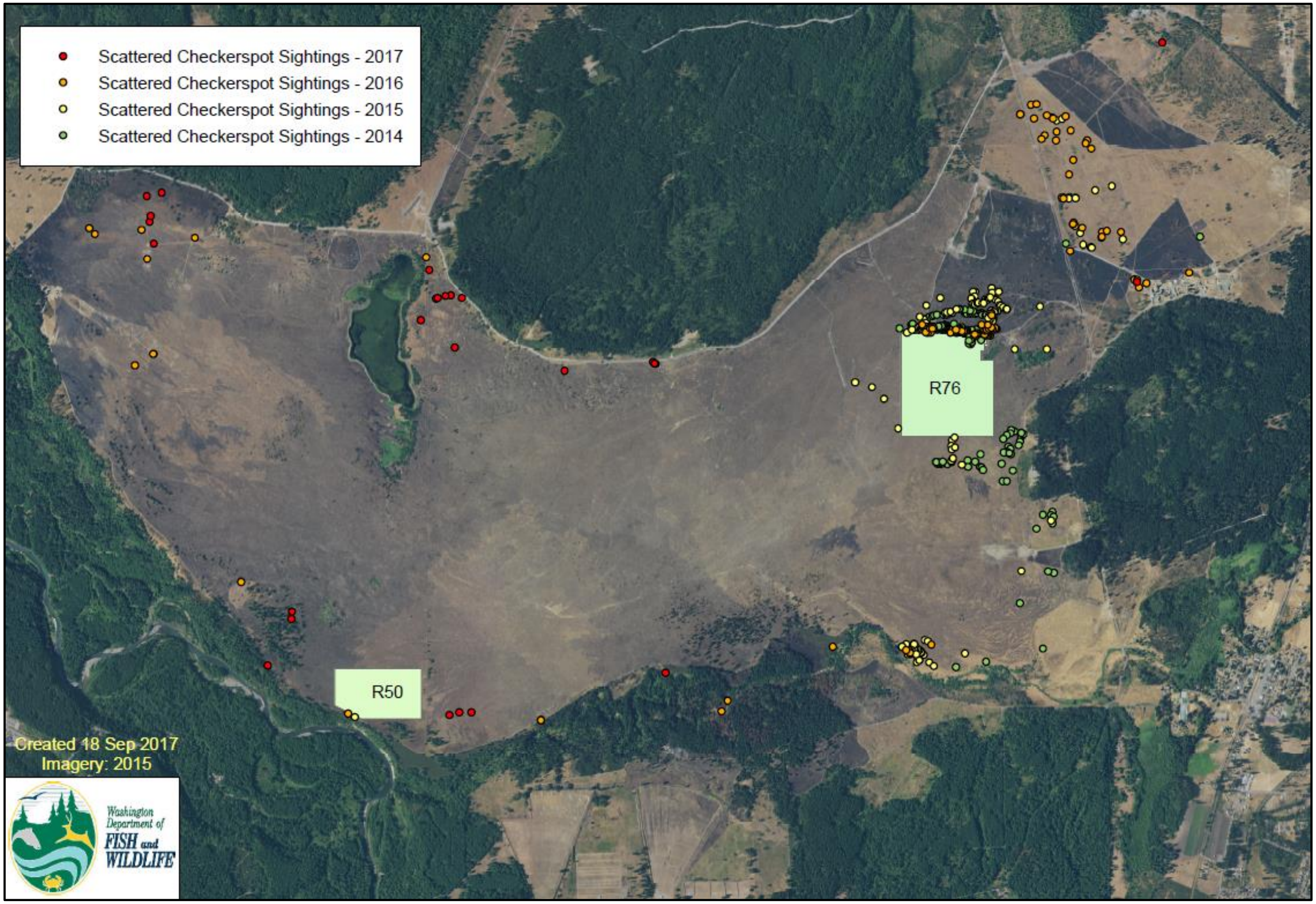

Figure 1. Scattered sightings of adult Taylor's checkerspot on the Artillery Impact Area outside of distance survey area (yellow shade), Joint Base Lewis-McChord, Washington, 2014-2017. Additional sightings (not shown) were recorded in areas searched systematically by JBLM staff (e.g., near Nisqually Lake).

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