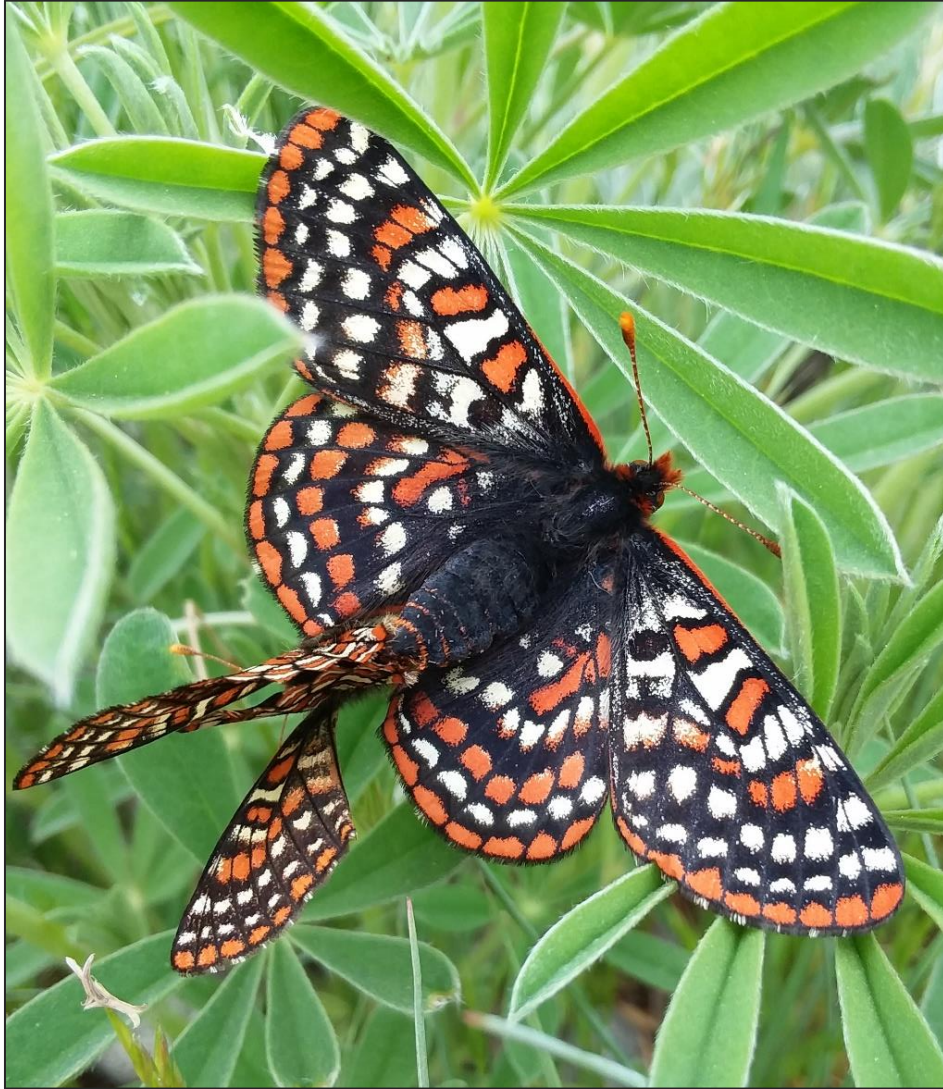


**Taylor's checkerspot (*Euphydryas editha taylori*)
Captive Rearing and Translocation:
South Puget Sound, Washington, 2017-2018**



**2018 Final Annual Report to:
US Fish and Wildlife Service (Cooperative Agreement # F16AC00588) and
Joint Base Lewis-McChord (Contract #W911S8-12-H-0001, W911S8-18-2-0008)**

10 March 2019

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Table of Contents

List of Tables	ii
List of Figures	iii
Executive Summary.....	1
Project Goals and Objectives	3
I. Select areas for the release of Taylor’s checkerspot larvae in South Puget Sound, Washington	3
II. Produce larvae for release via collection of wild stock and captive propagation	5
Continue captive propagation at the Oregon Zoo and Mission Creek to achieve target numbers of eggs, larvae and adults for translocations	5
Collection of wild checkerspots	5
Captive mating	6
Captive rearing, oviposition to adult	7
Rearing conditions	16
Assess the efficacy of the captive propagation program and identify opportunities for improvement	18
Survival of captive animals.....	18
Adult measurements.....	20
III. Release captive and associated wild stock	20
Postdiapause larval release.....	20
Adult release	22
Prediapause larval release	22
IV. Monitor success of the translocation	22
Document postdiapause larval presence and abundance in release areas.....	22
Evaluate translocation success based on adult presence, relative abundance and distribution	24
Adult presence and relative abundance	25
Adult distribution	33
V. Conduct long-term monitoring and evaluate progress towards meeting establishment criteria	35
Future Plans and Recommendations	36
Questions for Further Research	39
Literature Cited	42

List of Tables

Table 1. Size and area of release and survival plots for Taylor’s checkerspot larvae by life stage and translocation site: Training Areas 7 South (TA7S) and 15 (TA15), South Puget Sound, Washington, spring and summer 2018.	5
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; surviving adults were released in South Puget Sound, Washington, spring 2017.....	6
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., eggs that hatched) conducted at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2017. Number hatched taken at 3rd instar; includes prior mortality.	8
Table 4. Oviposition outcomes to diapause by female for wild-caught Taylor’s checkerspots collected at Range 76 (R76) or Scatter Creek (SC) and reared at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2017. Number hatched taken at 3rd instar; includes prior mortality.	10
Table 5. Prediapause outcomes to diapause for wild-collected Taylor’s checkerspot egg clusters reared at the Oregon Zoo, Portland, OR, spring 2017. Number hatched taken at 3rd instar; includes prior mortality.	11
Table 6. 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. ..	12
Table 7. Numbers of Taylor’s checkerspot larvae entering diapause, alive at wake-up, alive at release, retained for captive breeding, and available for release by matriline at Mission Creek Corrections Center for Women, Belfair, Washington, 2017-2018.	13
Table 8. Numbers of Taylor’s checkerspot larvae entering diapause, alive at wake-up, alive at release, retained for captive breeding, and available for release by matriline at the Oregon Zoo, Portland, Oregon, 2017-2018.....	14
Table 9. Numbers of Taylor’s checkerspots by life stage and matriline reared and retained for breeding at the Oregon Zoo, Portland, Oregon and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2018. Improperly eclosed (IE) adults are a subset of all adults.	16
Table 10. Target temperature (°F) and relative humidity (% RH) conditions by life stage and rearing location, including methods to achieve them for Taylor’s checkerspot rearing at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, 2017-2018.	17
Table 11. Actual average temperature (°F) and relative humidity (% RH) conditions and absolute range (range) by Taylor’s checkerspot by life stage and date range during captive rearing at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, 2017-2018. Numbers in bold text indicate measures outside the target by more than 10 percent.	18
Table 12. 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, 2017-2018. Only offspring from females with eggs that hatched are included; number of hatched larvae recorded at 3 rd 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 Scatter Creek (SC) <u>or</u> Captive-mated – (Captive), and year egg originated (e.g., 16=2016).....	19
Table 13. Descriptive statistics for pupal and adult weights, and wing area measurements for Taylor’s checkerspot butterflies that pupated and eclosed at the Oregon Zoo, Portland, OR, and Mission Creek Corrections Center for Women, Belfair, WA, 2017; only weights are included for adults whose wings eclosed improperly.	21

Table 14. Survey results pooled by date for post-release assessment of postdiapause Taylor’s checkerspot larvae from two captive rearing facilities, placed in thirteen 4x4-m survival plots (Mission Creek, n = 10 plots; Oregon Zoo, n = 3 plots) at Training Area 15, South Puget Sound, Washington, spring 2018. Number of days post-release, ratio of 5 th to 6 th instar larvae observed, detection method (single/double observer), detection rate (p-hat), larvae counted, abundance estimate (n-hat), proportion surviving (s-hat), standard error (SE) and 95% Confidence Intervals (CI) are reported. See text for release details and calculations.	24
Table 15. 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, 2010-2018. 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).	25
Table 16. Number of Taylor’s checkerspots counted by site and date during distance sampling surveys at extant (R76= Range 76) and translocation sites (SCS = Scatter Creek South Units 1&2; R50 = Range 50, GHP = Glacial Heritage Preserve, TA7S = Training Area 7S, TA15 = Training Area 15), South Puget Sound, Washington, spring 20198. Surveys were conducted prior to adult release where applicable. R76: both standard (T1-12) and extended (T1-16) survey areas reported here (see Appendix C, Fig. 6).	27
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 (CI) for Taylor’s checkerspot survey areas at Scatter Creek South Unit 1 (SCS1), Range 50 (R50), Range 76 (R76) and Training Area 15 (TA15) in South Puget Sound, Washington, spring 2018. See text for details.	29
Table 18. Number and species of nectar used by Taylor’s checkerspots during surveys on extant (R76 = Range 76) and translocation sites (R50 = Range 50, SCS = Scatter Creek South Units 1&2, PCM = Pacemaker, GHP = Glacial Heritage Preserve, TA7S = Training Area 7 South, Training Area 15 = TA15), South Puget Sound, Washington, 2011-2018. ACMI – Achillea millefolium; ARMA – Armeria maritima; BADE - Balsamorhiza deltoidea; CAHI – Castilleja hispida; CAQI -Camassia quamash; COSP – Collinsia species; ERLA – Eriophyllum lanatum; FRVI – Fragaria virginiana; HYRA – Hypochaeris radicata; LOSP – Lomatium species; LOTR- Lomatium triternatum; LOUT-Lomatium utriculatum; PLCO- Plectritis congesta; POGR - Potentilla gracilis; RAOC - Ranunculus occidentalis; SAIN - Saxifraga integrifolia; ZIVE Zigadenus venenosus.	31
Table 19. Number of mardon skipper butterflies observed by date and site during distance surveys for Taylor’s checkerspot in spring 2018 at three sites (R50 = Range 50; R76 = Range 76; SCS = Scatter Creek South Units 1&2), including two monitoring areas at Scatter Creek, South Puget Sound, Washington. “Zero” surveys are included nearest to dates when skippers were observed.	33
Table 20. Index of area (ha) occupied by adult Taylor’s checkerspot butterflies based on distance sampling surveys by site and year for translocation sites and one extant site (R76 = Range 76) in South Puget Sound, Washington, 2007 – 2018. SCS = Scatter Creek South (1 = main translocation unit; 2 = second management unit in northwest corner of site), Range 50 = R50, Pacemaker = PCM, Glacial Heritage Preserve = GHP, Training Area 7 South = TA7S, Training Area 15 = TA15, and Range 76 = R76.	34

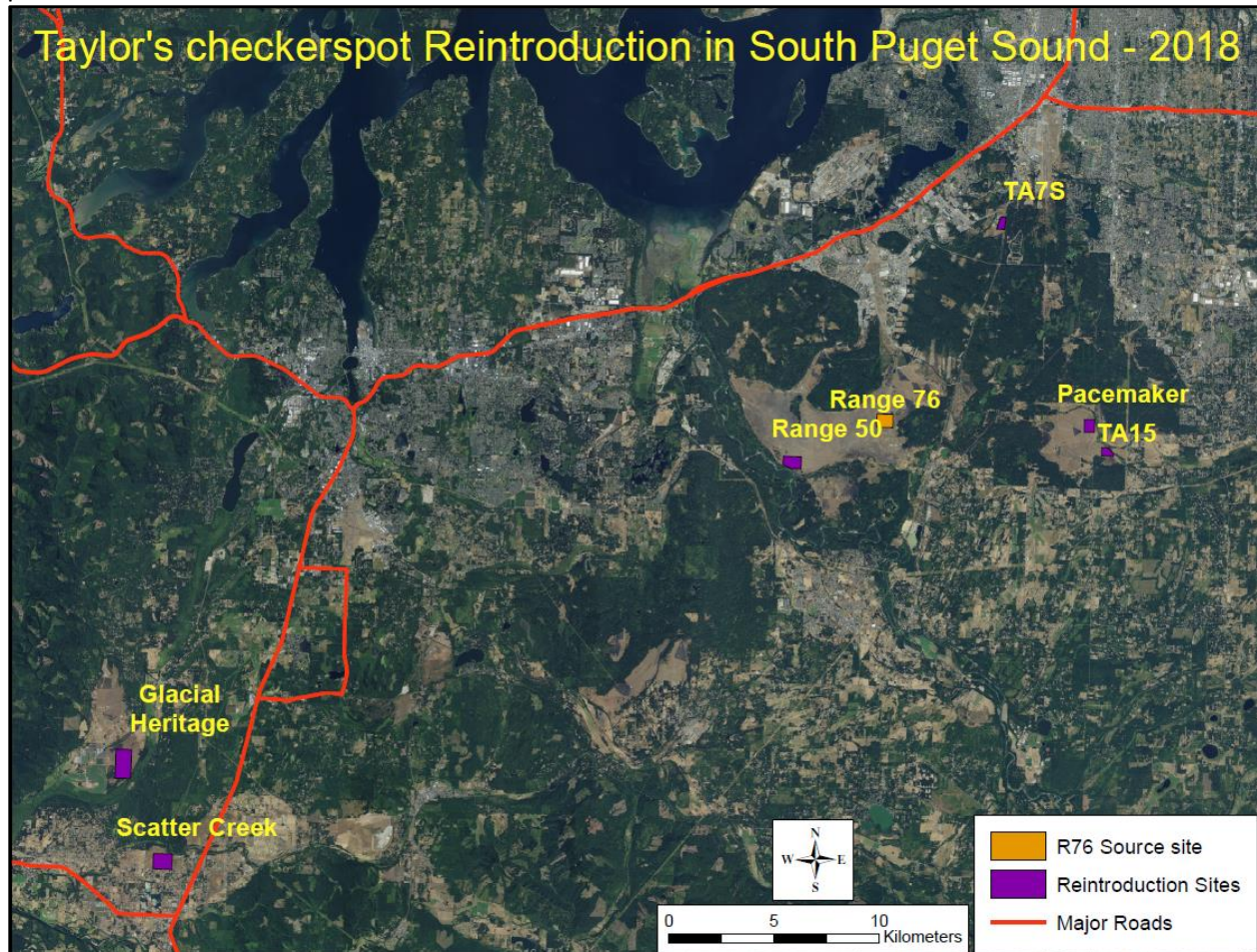
List of Figures

Figure 1. Percent of diapausing Taylor’s checkerspot larvae by rearing institution that moved their location within the diapause cup between checks, winter 2018. CC = Coffee Creek Corrections Center, OZ = Oregon Zoo, Portland, Oregon; MC = Mission Creek Corrections Center, Belfair, Washington. Larvae at CC were removed from diapause on 14 Feb 2018, whereas those for OZ and MC were removed on 2 Mar 2018.	12
Figure 2. Release of prediapause Taylor’s checkerspot larvae at Training Area 15, Joint Base Lewis-McChord, Washington, spring 2018.	23
Figure 3. Encounter rates (ER) by day of flight season for two portions (burned and unburned) of the Scatter Creek South 1 (SCS1) monitoring unit prior to the August 2017 wildfire, and in 2018, after the fire burned a portion of the area. Encounter rate is the number of observations divided by line length surveyed in meters.	30

Appendix A. Approximate number of Taylor’s checkerspot butterflies released by life stage (Po = postdiapause larvae; Ad = adult; Pr = prediapause 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; TA15 = Training Area 15) in South Puget Sound, Washington, 2007-2018.	46
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 2011-2018 (bottom); note log scale on the y-axis. Data for three translocation sites in South Puget Sound, Washington, are also shown with linear scales on the y-axis: Scatter Creek South Unit 1 (page 2 top) 2010-2018, Range 50 2010-2018 (page 2 bottom), and Training Area 15 2018 (page 3). Area surveyed varies by site and year; see Table 14 and text for details.	47
Appendix C. Distribution and number of adult Taylor’s checkerspots observed during distance sampling surveys at extant site (R76) and translocation sites in South Puget Sound, Washington, spring 2018.	50
Figure 1. Distribution and number of adult Taylor’s checkerspots observed during distance sampling surveys at Range 50 combined across all survey dates in spring 2018, South Puget Sound, Washington.	50
Figure 2. 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 2018, South Puget Sound, Washington.	51
Figure 3. Two distance sampling areas surveyed at Glacial Heritage Preserve in 2018 based on the prevalence of <i>Castilleja hispida</i> (GHP2, right) and <i>C. levisecta</i> (GHP2L, left); only two adult Taylor’s checkerspot were observed at the sites in spring 2018, South Puget Sound, Washington.	52
Figure 4. No adult Taylor’s checkerspots were observed during distance sampling surveys at Training Area 7S prior to release of adults, data combined across all survey dates in spring 2018, South Puget Sound, Washington.	53
Figure 5. Distribution and number of adult Taylor’s checkerspots observed during distance sampling surveys at Training Area 15, combined across all survey dates in spring 2018, South Puget Sound, Washington.	54
Figure 6. Distribution and number of adult Taylor’s checkerspots observed during distance sampling surveys at Range 76, combined across all survey dates in spring 2018, South Puget Sound, Washington.	55
Appendix D. Scattered sightings of Taylor’s checkerspots and 100-m transect survey results.	56
Figure 1. Adult Taylor’s checkerspot observations (scattered sightings and JBLM’s transect survey results) on the Artillery Impact Area (AIA) relative to standard monitoring areas (light green shade), Joint Base Lewis-McChord, Washington, 2018. North-south transect surveys with a 100-m spacing around the periphery of the AIA were implemented by JBLM staff and partners, with 25-m east-west transects employed at MP13 (lower right).	56
Figure 2. Adult Taylor’s checkerspot observations (scattered sightings and 100-m transect survey results) at Scatter Creek South relative to standard monitoring areas (blue boundaries). Wandering observations were recorded in route to start and end points.	57

Project Sites

Captive rearing facilities are located at the Oregon Zoo, Portland, OR, and Mission Creek Corrections Center for Women in Belfair, WA. Translocation and monitoring sites included Scatter Creek Wildlife Area – South Unit, (SCS1; 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, JBLM (TA7S; releases in 2014-2018); Training Area 15, JBLM, (TA15; releases in 2018) and Range 76, JBLM (R76; population source for captive propagation). A second monitoring area at Scatter Creek South (SCS2) was established to document potential colonization.



Executive Summary

This report summarizes work on Taylor's checkerspot captive rearing and translocation conducted in July 2017–December 2018. The captive rearing section borrows heavily from annual reports produced by the Oregon Zoo (Lewis et al. 2018) and Mission Creek (Curry et al. 2018) programs. A total of 5,514 eggs were produced at the two facilities with 32.1 percent (1,772) of eggs produced by captive-mated females and 67.9 percent (3,742) by wild females. More than 371 eggs were transferred from Mission Creek to the Oregon Zoo on 1 June 2017 to supplement numbers. In addition, due to poor reproduction at the Zoo and failure of wild, late-season females to produce many eggs, 25 clusters totaling 885 eggs were collected from R76. There were far fewer eggs produced in 2017 than in years past, which was intentional in part, as the Oregon Zoo was focusing its effort on trying to identify the cause of increased diapause mortality that has prevailed since 2013. In all, 4,730 prediapause larvae (4,573 wild and 156 captive) were produced, with 3,765 of those (3,615 wild, 150 captive) surviving to diapause. In keeping with our typical pattern, less than 1 percent of 3365 of larvae died during or immediately after diapause at the Mission Creek facility in 2018. However, 46 percent of 1577 larvae that entered diapause at the Oregon Zoo in June 2017 failed to wake-up in February 2018, and an additional 3 percent (43 larvae) died in the two weeks prior to release, which were ascribed to diapause mortality (see Survival) as they appeared to have insufficient resources to make the physiological switch from diapause to feeding. This is a slight improvement over results from 2017, but not the results we hoped for.

All available postdiapause larval Taylor's checkerspots were released at one new translocation site (TA15) in 2018, with excess adults split between TA15 and 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 2018 for evidence of colonization. A total of 2,747 postdiapause larvae from Mission Creek were released at TA15 on 9 Mar 2018. In addition, 589 larvae were released on 15 Mar 2018. Of the 3,336 larvae released at TA15, 3,108 were offspring of wild females, 11 were offspring of captive-mated females, and 77 were of unknown (QC) origin; QC larvae became separated from their group at some point during the rearing process. One hundred fifty-eight adults (89 females, 69 males) were released at TA15 on 11 and 22 May 2018, with 152 adults (90 females, 62 males) were released at TA7S on 12 and 24 May 2018. Of these, 116 came from the Oregon Zoo and 194 came from Mission Creek. A relatively warm and dry spring resulted in good breeding conditions in captivity and good production from wild females, which led to release of 5,175 prediapause larvae in 2018. Of these, 3,262 were released at TA15 on 11 Jun and 1,913 were released at TA7S on 14 Jun 2018.

To confirm site occupancy, quantify minimum survival, and identify issues that may be cause for concern, we conducted surveys for postdiapause larvae in thirteen 4x4-m survival plots at TA15 (10 with Mission Creek Larvae and 3 with Oregon Zoo larvae) on three occasions in the weeks following release. Daily post-release survival (Mission Creek = 0.932; Oregon Zoo = 0.976) was not different between rearing facilities ($z = 0.5464$; $p = 0.5823$), confirming lab results that suggest the increased mortality at the Zoo is the result of larvae failing to transition from diapause to wake-up (see Survival of Captive Animals, above). Survival for larvae from both facilities was similar to that reported in the literature (Moore 1989) for *E. editha* in California.

We used distance sampling to quantify daily population density, daily population size, and to illustrate the distribution of adults at five translocation sites (R50, SCS1, GHP TA7S and TA 15), one potential colonization site (SCS2) and at R76 (source site). A total of 19,773 checkerspot observations were recorded during distance surveys in 2018, setting a new annual record, with the greatest number (7,305)

observed at R50. Flight season initiation was intermediate in its timing relative to past years, with peak counts recorded on 2-12 May. Peak day encounter rates were high, with about 30 percent more butterflies observed at R50 (0.29 checkerspots/m) compared to SCS1 (0.20/m), but both sites harbored about twice as many butterflies as in 2017. Rates at R76 (0.19/m for transects 1-16; 0.22/m for transects 1-12) were about four times higher than in 2017. The peak day encounter rate at TA15 (0.03 checkerspots/m), was also respectably high for a first year site. Only two checkerspot were observed at GHP, neither of which was confirmed during transect surveys, and adults were observed on TA7S only after captive adults were released. 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 entire 25-ha monitoring area at R50 for the first time. In addition, the peak single day abundance estimate for 2018 (8,832; 95% CI: 6,857-11,377) was the highest recorded for that site, but did not surpass R76 (14,263; 95% CI: 9,801-20,755), which covers 55 percent more area and also had a strong year. This population is officially established based on the project definition and continues to expand. Checkerspots at SCS1 occupied 76.9 percent of the 20-ha monitoring unit in 2018, and extensive restoration continues to improve habitat across the site. The peak single day abundance estimate of adults in 2018 (4,188, 95% CI: 3,527-4,973) indicates this site far exceeded its Year 2 goal in spite of the 2017 wildfire, and is on track to meet all establishment criteria by 2021.

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. Initially WDFW and its partners intend to establish at least three new populations at three sites by 2022 via captive rearing and translocation, with additional sites to follow as a more comprehensive plan is developed. This report mainly summarizes 2017-2018 activities, but includes some longer-term summaries as well. Objectives for 2018 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 translocation sites (Training Areas 7S and 15),
- 4) Monitor translocation success as follows:
 - a. Evaluate release success using postdiapause larval and adult surveys at translocation sites (Training Areas 7S and 15), and
 - b. Evaluate translocation success based on presence, distribution and relative abundance of adults at three past translocation sites (Scatter Creek South Unit 1 and Range 50) and one potential colonization site (Scatter Creek South Unit 2).

We implemented this work 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 2017-2018 captive propagation and translocation 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 translocations
- 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 translocation (Fimbel and Dunn 2013). Priority host and nectar species have been identified (Linders 2014) and restoration targets identified (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 translocation 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 2016). This

protocol insures standardized baseline data is used for comparison with 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 translocation. Project partners also visit a few sites annually to review habitat conditions, assess project progress and identify outstanding needs.

Final selection of translocation sites occurs on an as-needed basis with input from 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 Translocation 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 translocation, 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 2018, we wanted to release postdiapause larvae in habitat that contained abundant *Castilleja levisecta* with the ultimate goal of examining whether adult checkerspots use both *Castilleja* species for oviposition when given a choice.

Within release plots, we flagged locations for larval release where *Plantago* or *Castilleja* plants were expected to be at least 1000 cm³ at the time of release (i.e., large enough to provide food and cover for 2-5 released larvae. Release plants also had to have at least 3 similarly-sized plants (or abundant smaller plants) in the surrounding half meter, with comparable conditions predominating in the surrounding area to form a patch of at least 250 sq m.

Results and Discussion

Six sites have been selected for translocation 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), TA7S (2014-2018), and TA15 (2018) have received multiple sequential releases (Appendix A); two sites (SCN and PCM) were included in a research-related release in 2009 which failed to produce more than a few adults and were subsequently dropped, although one site (PCM) received a single release three years later (2012), after which releases were restricted by JBLM.

In 2018 releases occurred at TA15 (1st year) and TA7S (5th year), consistent with our existing strategy (Linders et al. 2015b), but we did not meet our objective for a postdiapause release at TA7S due to restoration concerns and a preference for maximizing the number of larvae when initiating the translocation at TA15. We established three postdiapause and two prediapause release plots at TA15 (Appendix C, Fig. 5), and one prediapause release plot at TA7S (Appendix C, Fig. 4). The TA15 postdiapause release plots were identified based on the size and abundance of *Plantago lanceolata*, but also considered associated nectar and vegetation structure in an effort to accommodate all life stages of the butterfly in the vicinity of release. Total plot areas are listed in Table 1 and include numbers of 4x4-m plots used to monitor larval survival. Prediapause release plots were based on similar criteria, but also considered the freshness of foliage to insure plentiful food until larvae reached diapause. Prediapause release plots are typically located near postdiapause plots to promote population cohesion; this was hard to achieve at TA15 (Appendix C, Fig. 5) because while abundant, much of the *Plantago* closer to postdiapause plots was too small size to support larvae. TA15 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). Prediapause larvae were provided with 0.85 and 0.77 sq m/larvae of habitat at TA15 and TA7S, respectively.

We discontinued releases at GHP because: 1) previous releases over the past 6 years failed to result in a significant population, 2) of four 5-ac units evaluated in the 2016 Rapid Habitat Assessment, only one qualified as translocation-ready, and 3) concern that there may be other factors at play (e.g., inappropriate microsites, lack of roost cover for adults, toxic residues, etc.).

Table 1. Size and area of release and survival plots for Taylor's checkerspot larvae by life stage and translocation site: Training Areas 7 South (TA7S) and 15 (TA15), South Puget Sound, Washington, spring and summer 2018.

Site	Plot ID	Sq meters	Acres
TA 15 - postdiapause	A	5185	1.28
	B	273	0.67
	C	528	0.13
	Survival plots 1-13	208	0.05
Postdiapause total		6194	2.13
TA 15 - prediapause	D	2213	0.55
	E	456	0.11
	Survival plots 14-19	96	0.02
Prediapause total		2764	0.68
TA7S - prediapause	E	1417	0.35
	Survival plots 1-4	64	0.02
Prediapause total		1481	0.37

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 release as proposed (Linders 2015b). Captive propagation methods were developed at the Oregon Zoo (Barclay et al. 2009) and adapted to the rearing facility at Mission Creek Corrections Center for Women in Belfair, Washington, under the supervision of the Sustainability in Prisons Project - The Evergreen State College.

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

Collection of wild checkerspots

Methods

To increase the number of founders contributing to translocation and reduce the potential influence of captivity on founders, 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 population, 10 females per rearing facility annually is the minimum needed 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 23 wild females were collected from R76 with same day delivery to Mission Creek (13 on 17 May; 10 on 23 May 2017, respectively) following methods reported previously (Linders and Lewis 2013); all females were in good condition. Initially, we opted not to collect wild females for the Oregon Zoo because we expected population levels at R76 to be low and because Oregon Zoo larvae would be part of a feeding trial aimed at identifying the source of diapause-related mortality that has prevailed at the Zoo since 2013. However when it became evident that captive-bred females would not produce the needed offspring, we collected 10 females on 31 May 2017 from R76 and delivered them to the Oregon Zoo the same day. When these late season females also produced few eggs, 7 more females (the last of the season) were collected from SCS1 on 4 June 2017, in addition to >885 eggs in 25 wild-collected egg clusters found at R76 on 5 June 2017.

Captive mating Methods

In all, 87 adults from 8 maternal lines originating as eggs in 2016 (16FL) were available for inclusion in the captive breeding colony at the Oregon Zoo in 2017. Similarly, 156 adults from 15 maternal lines were included in Mission Creek's breeding colony (Table 2). Following eclosion, adults were processed and housed according to established procedures (Barclay et al. 2009, Lewis et al. 2013). As in the past, a daisy chain was used to assign pairing of males and females by matriline (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 enters the chain when 40% of postdiapause larvae pupated. Remaining matriline were added once females eclosed, which ensures that males, which eclose first, will be ready to mate while females are fresh.

Breeding introductions followed established protocols (Barclay et al. 2009) with recent modifications (Lewis et al. 2013, Hamilton et al. 2014). Males were aged for three days prior to being included in breeding tents, and 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).

Table 2. Number of adult Taylor's checkersspots retained for breeding by matriline and sex at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington; surviving adults were released in South Puget Sound, Washington, spring 2017.

Matriline	Adults	Females	Males	Females Released	Males Released
Oregon Zoo					
16FL40	10	4	6	1	1
16FL42 ¹	8	2	5	1	0
16FL44/44T	12	4	8	1	7
16FL45	8	4	4	2	2
16FL47	9	5	4	3	1
16FL48	13	6	7	5	4
16FL50	13	6	7	6	4
16FL53 ¹	14	7	6	3	6
OZ Total	87	38	47	22	25

Mission Creek					
15FL551	3	1	2	1	2
15FL554	4	0	4	0	1
15FL557	2	1	1	0	0
16FL601	21	9	12	7	3
16FL613	15	6	9	5	1
16FL614	14	5	9	4	1
16FL615	11	7	4	5	1
16FL622	5	1	4	1	1
16FL625	12	5	7	3	3
16FL630	11	4	7	3	3
16FL641	20	11	9	9	5
16FL643	2	0	2	0	0
16FL649	12	10	2	9	0
16FL652	21	12	9	8	2
16FL655	3	0	3	0	2
MC Total	156	72	84	55	25

¹Sex could not be determined for one adult.

Results and Discussion

Between 19 and 28 May 2017, the Oregon Zoo conducted 41 planned breeding introductions, resulting in 14 copulations (Table 3) between 19 and 26 May. Eighteen females did not copulate despite four of those having multiple opportunities. At Mission Creek, copulations occurred between 6 and 22 May, from introductions spanning 6-25 May 2017. A total of 7 copulations resulted from 91 breeding introductions; 50 females did not copulate (Table 3). Copulation rates at both facilities were unusually low. At the Zoo, poor results may have been related to 1) lower overall health of postdiapause larvae animals remaining from the test of Koch's postulates (see Survival), 2) fewer larvae retained per matriline combined with a relatively high rate of return to diapause (Lewis et al. 2017). At Mission Creek this may have been the result of: 1) misunderstandings that altered male-female ratios in breeding tents, 2) fewer larvae retained per matriline combined with a relatively high rate of return to diapause (Curry et al. 2017), 3) the order that matriline were placed in daisy chains creating a three day delay in breeding, and/or 4) efforts to locate an appropriate breeding location. Copulation behavior is very sensitive to temperature and sunlight, so several different greenhouse locations were used. Based on these results and past successes, we will: 1) retain 30 larvae per matriline for breeding, 2) combine males from more than one line if there are 2 or fewer males per line, 3) age males for three days prior to introductions, and 4) use all available males in breeding tents.

Captive rearing: oviposition to adult Methods

Taylor's checkerspot readily oviposit in captivity. We aimed to collect about 250 eggs from each gravid wild and captive female to meet our captive rearing target of 7,500 eggs in 2017. This was lower than usual because the focus of the Zoo's efforts was on identifying the source of larval mortality that has reduced productivity since 2013. 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). A female's choice

of host plant species for oviposition is a genetically derived trait that is not affected by the species on which they fed as larvae (Singer 2004).

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., eggs that hatched) conducted at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2017. Number hatched taken at 3rd instar; includes prior mortality.

Female ID	Male ID	Intros	Females	Copulations	Copulation rate	Productive females
Oregon Zoo						
16FL40	16FL53	3	3	3	1.00	0
16FL42	16FL40	4	2	1	0.25	0
16FL44/44T	16FL48	3	3	3	1.00	0
16FL45	16FL44/44T	9	4	2	0.22	1
16FL47	16FL42	3	3	1	0.33	0
16FL48	16FL45	7	7	0	0.00	0
16FL50	16FL47	6	5	1	0.17	0
16FL53	16FL50	6	5	3	0.50	1
Total	NA	41	32	14	0.34	2
Mission Creek						
16FL615	16FL601	4	3	1	0.25	1
16FL614	16FL615	12	5	1	0.08	1
16FL613	16FL614	9	5	0	0.00	0
16FL641	16FL613	10	8	1	0.10	0
16FL601	16FL641	10	9	1	0.10	0
16FL625	16FL622	9	2	0	0.00	0
16FL649	16FL625	15	9	1	0.07	0
16FL630	16FL643	2	1	0	0.00	0
16FL649	16FL630	2	2	0	0.00	0
16FL622	16FL649	1	1	0	0.00	0
16FL652	16FL630	6	6	2	0.33	0
15FL551	16FL652	4	1	0	0.00	0
15FL557	15FL551	3	1	0	0.00	0
16FL622	15FL557	1	1	0	0.00	0
16FL630	15FL554	2	2	0	0.00	0
16FL622	16FL643	1	1	0	0.00	0
Total	NA	91	57	7	0.08	2

Larvae were checked periodically during diapause (Barclay et al. 2009) and the number of active larvae recorded (Lewis et al. 2016). Upon removal from diapause, larvae were placed in a high humidity environment and cared for according to established procedures (Barclay et al. 2009; Lewis et al. 2012; Lewis et al. 2013). Once they began to eat, a subset of larvae from wild females were retained for breeding, and the remaining larvae, including all offspring of captive-mated females, were released.

Due to the persistent pattern of diapause mortality at the Oregon Zoo over the past several years (Linders et al. 2015, 2016, 2017), we initiated a feeding trial in spring 2017 to determine whether

cultivation method (*Plantago* grown in pots with and without fertilizer, vs. plants grown in the ground) may be affecting larval survival through diapause. To date we've ruled out both disease and warm overwinter temperatures as potential causes of elevated diapause mortality, leading us to suspect the Oregon Zoo's food supply (Lewis et al. 2018), which has been drawn from a variety of sources since 2013. All Zoo larvae were assigned to one of three groups for the feeding trial (Lewis et al. 2018):

- FERT - *Plantago* plants grown in 1-gallon pots at the Oregon Zoo and fertigated twice/week; "experimental condition", based on potential concern, but with an exaggerated dose of fertilizer to increase the likelihood of showing results;
- POTS - *Plantago* plants grown in pots at OZ and watered twice/week without added fertilizer; most similar to what was fed to prediapause larvae in 2014-2016; and
- LCC - *Plantago* plants grown in raised beds at Larch Corrections Center; "control" in being most similar to plants previously available at the Zoo and currently raised at Mission Creek.

Variables to compare between groups include: stage-specific mortality; rate of larval development (# days to each instar/between instar); larval weight at diapause; rate of return to diapause, and wing size. While we recognize that controlling for both matriline and egg cluster would provide the best study design, this would require altering the standard rearing procedures, and doing so could have other unpredictable outcomes. The purpose of this management study is to identify the best approach to cultivation from the three methods available. It is not a definitive study of the effects of fertilizer on larval growth and development. Data presented in this report are not split by feeding trial because no clear or consistent results were evident in these data. However we are still evaluating outcomes, especially as they may relate to differences in nutrient content relative to *Plantago* collected at field sites. We hope to have a complete report on our findings by summer 2019.

Results and Discussion

At Mission Creek, 18 of 23 wild females laid eggs that hatched, whereas 9 of 15 wild females at the Oregon Zoo laid eggs that hatched (i.e., were productive) in 2017 (Table 4). Wild females collected for the Zoo were taken very late in the season, because we were expecting a poor flight season, and hoped to use only captive-bred offspring to test determine potential causes of mortality. In addition, due to poor reproduction at the Zoo and failure wild late-season females to produce many eggs, 25 clusters totaling 885 eggs (Table 5) were collected from R76 on 5 June 2017 and transferred to the Zoo for rearing. At the Zoo, 2 of 14 captive-mated females produced eggs that hatched, and 2 of 7 captive females at Mission Creek did the same (Tables 3 and 6).

At least 5,514 eggs were produced at the two facilities with 32.1 percent (1,772) of eggs produced by captive-mated females and 67.9 percent (3,742) by wild females. Egg counts at Mission Creek are undercounts based on hatch proportions. Wild females at the Zoo produced eggs (Tables 4) between 22 May and 10 June 2017, with captive females laying between 21 May and 20 June 2017 (Table 6). At Mission Creek, wild females produced eggs between 19 May and 5 June 2017, with captive-mated females laying from 10 May to 10 June 2017 (Tables 4 and 6, respectively). More than 371 eggs were transferred from Mission Creek to the Oregon Zoo on 1 June 2017 to supplement numbers (Table 5). Eggs collected at R76 and sent to the Zoo, hatched on 4-10 June 2017. Larvae from wild females at the Zoo hatched 2-22 May 2017, with those from captive females hatching on 15-20 June. At Mission Creek, larvae from wild females hatched 29 May–12 June, similar to those from captive females hatching on 27 May–17 June.

Table 4. Oviposition outcomes to diapause by female for wild-caught Taylor's checkerspot collected at Range 76 (R76) or Scatter Creek (SC) and reared at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2017. Number hatched taken at 3rd instar; includes prior mortality.

Female ID	Eggs	Hatched ¹	Hatch proportion	Diapause in
Oregon Zoo				
P17FL724	34	22	0.65	21
P17FL726	1	1	1.00	1
P17FL727	14	12	0.86	12
P17FL728	21	20	0.95	20
P17FL729	21	18	0.86	18
P17FL732	25	22	0.88	21
P17SC734	13	12	0.92	10
P17SC735	62	60	0.97	58
P17SC736	59	32	0.54	30
Total	250	199	NA	191
Ave	27.8	22.1	0.85	21.2
St Dev	20.6	16.6	0.15	16.1
Eggs to Oregon Zoo from Mission Creek				
P17FL705	197	220	1.12	213
P17FL710	174	189	1.09	188
Total	371	409	NA	401
Mission Creek				
P17FL701	265	283	1.07	286
P17FL702	151	118	0.78	118
P17FL703	378	437	1.16	434
P17FL704	45	28	0.62	28
P17FL705	217	239	1.10	239
P17FL706	40	15	0.38	0
P17FL707	7	0	0.00	0
P17FL708	115	133	1.16	103
P17FL709	272	354	1.30	349
P17FL710	123	169	1.37	169
P17FL711	11	0	0.00	0
P17FL712	252	230	0.91	229
P17FL713	117	123	1.05	123
P17FL714	0	0	0.00	0
P17FL715	53	45	0.85	43
P17FL716	108	145	1.34	145
P17FL717	46	0	0.00	0
P17FL718	0	0	0.00	0
P17FL719	143	161	1.13	160
P17FL720	244	260	1.07	257
P17FL721	95	80	0.84	80
P17FL722	107	89	0.83	88
P17FL723	149	174	1.17	172
Total	2938	3083	NA	3023
Ave ²	127.7	134.0	0.74	131.4
St Dev¹	102.3	122.6	0.19	122.7

¹ Hatch rates >1.0 indicate undercounts of eggs. ² Cases with hatch success >1.0 omitted.

In all, 4,729 prediapause larvae (4,574 wild and 155 captive) were produced, with 4,640 of those (4,490 wild, 150 captive) surviving to diapause (Tables 4-6). Prediapause larvae from the 17FL cohort (young of the year) entered diapause at the Zoo between 29 June and 20 July, with all larvae at Mission Creek entering diapause on 10-20 July 2017.

Table 5. Prediapause outcomes to diapause for wild-collected Taylor's checkerspot egg clusters reared at the Oregon Zoo, Portland, OR, spring 2017. Number hatched taken at 3rd instar; includes prior mortality.

Cluster ID	Eggs	Hatched	Hatch proportion	Diapause In
E17FL741	18	18	1.00	18
E17FL742	27	29	1.00	29
E17FL746	27	25	0.93	24
E17FL747	8	8	1.00	8
E17FL751	35	43	1.00	43
E17FL752	32	25	0.78	24
E17FL753	11	12	1.00	12
E17FL754	13	13	1.00	13
E17FL755	35	34	0.97	34
E17FL761	54	54	1.00	54
E17FL762	13	14	1.00	14
E17FL763	28	29	1.04	29
E17FL764	133	141	1.06	140
E17FL765	26	25	0.96	25
E17FL766	34	34	1.00	32
E17FL767	45	34	0.76	34
E17FL768	132	123	0.93	120
E17FL769	48	53	1.10	53
E17FL770	31	36	1.16	36
E17FL771	9	9	1.00	9
E17FL772	29	32	1.10	32
E17FL773	13	13	1.00	13
E17FL774	28	26	0.93	26
E17FL775	31	30	0.97	30
E17FL776	25	23	0.92	23
Total:	885	883	NA	875
Avg	35	35	0.98	35
SD	31.52	31.62	0.09	31.17

¹ Cases with hatch success >1.0 omitted.

Diapausing larvae were moved outdoors for the start of cold diapause on 23 October 2017 at the Oregon Zoo, and on 13 September 2017 at Mission Creek. Once in diapause most larvae remain inactive, although some movement is normal, especially in late diapause. At the Zoo, larval activity during diapause remained low (10-13 percent) until mid-January, when 68 percent of larvae were found to have moved (Fig. 1). Movement levels again declined but rose to a second peak in mid-February before a week long cold snap reduced activity to 9 percent in late February. No molts and only a few larval mortalities were recorded at the Zoo during diapause. At Mission Creek, larval movement was only 8 percent in mid-January, then increased steadily to peak in mid-February, with only a moderate

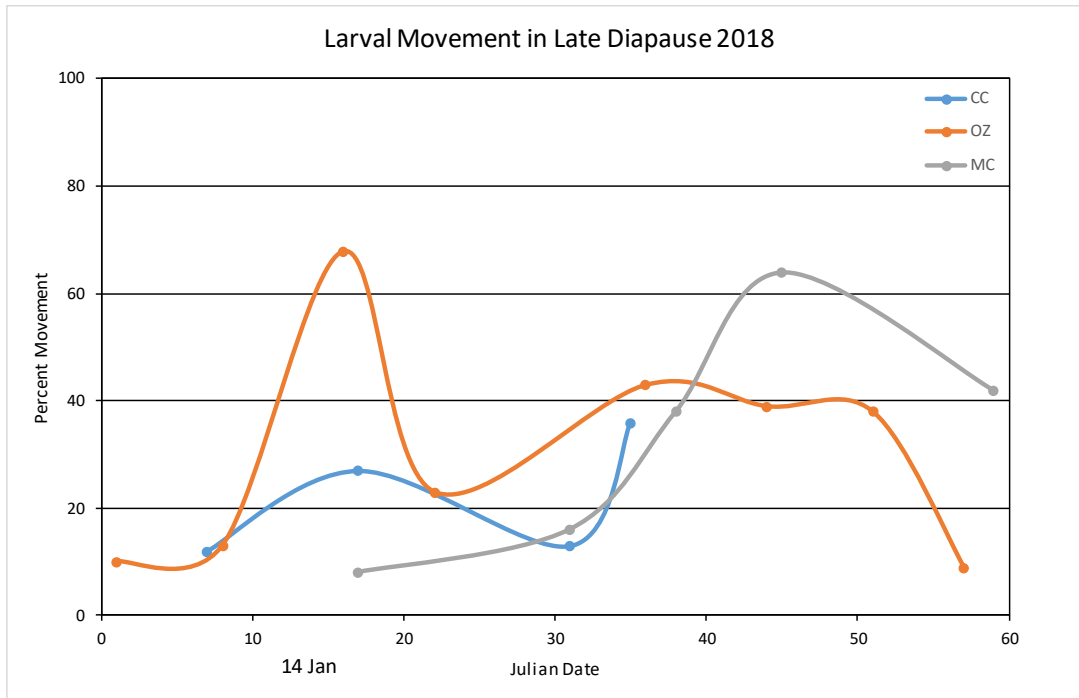


Figure 1. Percent of diapausing Taylor’s checkerspot larvae by rearing institution that moved their location within the diapause cup between checks, winter 2018. CC = Coffee Creek Corrections Center, OZ = Oregon Zoo, Portland, Oregon; MC = Mission Creek Corrections Center, Belfair, Washington. Larvae at CC were removed from diapause on 14 Feb 2018, whereas those for OZ and MC were removed on 2 Mar 2018.

drop in activity during the cold snap (Fig. 1). Movement of checkerspot larvae from an Oregon site being reared at Coffee Creek Corrections Center is less dramatic, but follows a similar pattern to those at the Zoo; these data are included for reference (Fig. 1). Movement patterns at the Zoo track both the record warming observed in mid-January (Mass 14 Jan 2018) and the record cold that set in by President’s Day (Mass 19 Feb 2018). Tracking movement during diapause may assist us in understanding how checkerspots at the Zoo are responding to their environment relative to other facilities. All larvae from both facilities were removed from diapause on 2 March 2018, about 10 days later than usual.

Table 6. 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	Hatch proportion	Diapause In
Oregon Zoo						
16FL40-8	19-May	60	56	0	0.00	0
16FL44-5	19-May	105	112	0	0.00	0
16FL40-2	21-May	60	179	0	0.00	0
16FL45-3	21-May	127	77	0	0.00	0
16FL40-9	26-May	65	141	0	0.00	0
16FL44-7	26-May	65	24	0	0.00	0
16FL45-8	26-May	195	178	3	0.02	3
16FL53-12	26-May	165	189	1	0.01	1
16FL53-8	26-May	80	207	0	0.00	0
Total		NA	1163	4	NA	4
Ave		102.4	129.2	0.4	NA	0.4
St Dev		50.0	65.3	1.0	NA	1.0

Mission Creek						
16FL615-01	6-May	overnight	94	67	0.71	67
16FL641-15	15-May	185	392	0	0.00	0
16FL652-02	16-May	106	0	0	0.00	0
16FL601-17	17-May	152	0	0	0.00	0
16FL649-13	18-May	58	38	0	0.00	0
16FL652-18	22-May	132	2	0	0.00	0
16FL614-14	22-May	62	83	84	1.01	79
Total	NA	NA	609	151	NA	146
Ave	NA	115.8	87.0	21.6	NA	20.9
St Dev	NA	50.4	140.2	37.2	NA	35.8

Mission Creek retained 300 larvae from ten 2017 matriline, as well as 14 third diapause larvae from two 2015 matriline and 94 second diapause larvae from eight 2016 matriline for breeding (Table 7); all were offspring of wild females. At the Zoo, 384 larvae from nine wild 2017 matriline were retained for breeding (Table 8); each egg cluster collected from the wild is assumed to be from a unique female as most were collected from disparate locations.

Table 7. Numbers of Taylor's checkerspot larvae entering diapause, alive at wake-up, alive at release, retained for captive breeding, and available for release by matriline at Mission Creek Corrections Center for Women, Belfair, Washington, 2017-2018.

Matriline	Diapause in	Diapause out	# at Release	Retained	TA15 Release
15FL551	7	7	7	7	0
15FL557	7	7	7	7	0
16FL601	3	3	2	0	2
16FL613	9	9	9	9	0
16FL614	8	7	7	7	0
16FL615	5	5	5	0	5
16FL622	15	14	14	14	0
16FL625	12	11	11	11	0
16FL630	8	8	8	8	0
16FL641	1	1	1	0	1
16FL643	20	20	20	20	0
16FL649	9	9	9	9	0
16FL655	16	16	16	16	0
<i>Subtotal</i>	<i>120</i>	<i>117</i>	<i>116</i>	<i>108</i>	<i>8</i>
17FL701	286	286	285	30	255
17FL702	118	118	118	0	118
17FL703	434	433	433	30	403
17FL704	28	28	28	0	28
17FL705	239	239	238	30	208
17FL708	103	103	103	30	73
17FL709	349	346	346	30	316
17FL710	169	169	168	0	168
17FL712	229	228	228	30	198
17FL713	123	123	123	0	123

17FL715	43	43	43	0	43
17FL716	145	145	145	30	115
17FL719	160	159	159	30	129
17FL720	257	257	257	30	227
17FL721	80	80	80	0	80
17FL722	88	87	87	0	87
17FL723	172	172	172	30	142
<i>Subtotal</i>	<i>3023</i>	<i>3016</i>	<i>3013</i>	<i>300</i>	<i>2713</i>
17MC614-14	79	79	79	0	79
17MC615-01	67	67	67	0	67
<i>Subtotal</i>	<i>146</i>	<i>146</i>	<i>146</i>	<i>0</i>	<i>146</i>
QC	76	76	77	0	77
Total	3365	3355	3352	408	2944

Outcomes of postdiapause rearing, including progression to the adult stage, are presented in Table 9. Second diapause return rates had been nearly identical in 2017 (Linders et al. 2017) but were markedly different between rearing facilities in 2018 (OZ – 39.8 percent; MC – 27.0 percent), with differences among matriline pronounced, especially at Mission Creek (Table 9). 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. Mission Creek reared the largest group of multi-diapause larvae to date, with excellent results (see Survival).

Table 8. Numbers of Taylor’s checkerspot larvae entering diapause, alive at wake-up, alive at release, retained for captive breeding, and available for release by matriline at the Oregon Zoo, Portland, Oregon, 2017-2018.

Matriline	Diapause in	Diapause out	At release	Retained	TA15 Release
16FL40	10	1	1	0	1
16FL42	7	0	0	0	0
16FL44	11	3	2	0	2
16FL45	9	3	3	0	3
16FL47	6	0	0	0	0
16FL48	5	2	2	0	2
16FL50	3	0	0	0	0
16FL53	2	0	0	0	0
<i>Subtotal</i>	<i>53</i>	<i>9</i>	<i>8</i>	<i>0</i>	<i>8</i>
16MC52T	9	1	1	0	1
16MC54T	10	5	5	0	5
16OZ02T	6	1	1	0	1
16OZ05T	9	1	1	0	1
16FL22T	10	0	0	0	0
16FL44T	4	2	2	0	2
<i>Subtotal¹</i>	<i>48</i>	<i>10</i>	<i>10</i>	<i>0</i>	<i>10</i>
E17FL741	18	2	2	0	2
E17FL742	29	24	24	24	00
E17FL746	24	19	19	0	19
E17FL747	8	5	5	0	5

E17FL751	43	12	10	0	10
E17FL752	24	12	10	0	10
E17FL753	12	3	3	0	3
E17FL754	13	7	7	0	7
E17FL755	34	22	22	22	0
E17FL761	54	50	49	30	19
E17FL762	14	1	1	0	1
E17FL763	29	13	12	0	12
E17FL764	140	80	78	30	48
E17FL765	25	3	3	0	3
E17FL766	32	3	3	0	3
E17FL767	34	33	33	0	33
E17FL768	120	96	96	30	66
E17FL769	53	52	52	30	22
E17FL770	36	6	4	0	4
E17FL771	9	6	6	0	6
E17FL772	32	14	14	0	14
E17FL773	13	4	3	0	3
E17FL774	26	2	1	0	1
E17FL775	30	17	12	0	12
E17FL776	23	21	2	0	2
<i>Subtotal</i>	<i>875</i>	<i>507</i>	<i>471</i>	<i>166</i>	<i>305</i>
17FL705	213	102	102	96	6
17FL710	188	72	72	68	4
17FL724	21	17	13	0	13
17FL726	1	0	0	0	0
17FL727	12	9	8	0	8
17FL728	20	18	17	0	17
17FL729	18	12	12	0	12
17FL732	21	3	3	0	3
<i>Subtotal</i>	<i>494</i>	<i>233</i>	<i>227</i>	<i>164</i>	<i>63</i>
17SC734	10	9	9	0	9
17SC735	58	54	54	54	0
17SC736	30	25	25	0	25
<i>Subtotal</i>	<i>98</i>	<i>88</i>	<i>88</i>	<i>54</i>	<i>34</i>
17OZ45	3	3	3	0	3
17OZ53	1	0	0	0	0
<i>Subtotal</i>	<i>4</i>	<i>3</i>	<i>3</i>	<i>0</i>	<i>3</i>
QC	5	1	1	0	1
Total	1577	851	808	384	424

¹ These from test of Koch's postulates.

The first pupa at the Oregon Zoo formed on 3 April and the last on 1 May 2018, whereas larvae at Mission Creek pupated 8-25 April 2018; this is earlier than in 2017. Adults at the Zoo eclosed between from 18 April and 13 May 2018, with those at Mission Creek eclosing from 25 April to 10 May 2018. In all, 418 adult checkerspots (208 males and 205 females) made up the 2018 captive breeding colony (Table 9). Adults that eclosed improperly were included in the breeding colony so long as their wing deformities were not debilitating. Improper eclosion is typically the result of an imbalance of heat, humidity and air flow during the period when the wings are drying, rather than a genetic defect. Adults that were physically fit and finished with breeding were sent to WDFW for release.

Table 9. Numbers of Taylor's checkerspot by life stage and matriline reared and retained for breeding at the Oregon Zoo, Portland, Oregon and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2018. Improperly eclosed (IE) adults are a subset of all adults.

Matriline	Larvae	2nd diapause	Pupae	Adults	IE	Males	Females	M release	F release
Oregon Zoo									
17FL705	96	27	62	59	3	35	24	12	15
17FL710	68	29	31	30	3	15	15	7	13
17SC735	54	13	32	30	6	10	20	2	15
<i>Subtotal</i>	<i>218</i>	<i>69</i>	<i>125</i>	<i>119</i>	<i>12</i>	<i>60</i>	<i>59</i>	<i>21</i>	<i>43</i>
E17FL742	24	15	7	7	0	4	3	2	1
E17FL755	22	19	0	0	0	0	0	0	0
E17FL761	30	26	4	4	1	2	2	1	1
E17FL764	30	3	26	25	1	7	18	3	14
E17FL768	30	13	17	16	0	3	13	1	11
E17FL769	30	7	23	22	4	6	16	2	16
<i>Subtotal</i>	<i>166</i>	<i>83</i>	<i>77</i>	<i>74</i>	<i>6</i>	<i>22</i>	<i>52</i>	<i>9</i>	<i>43</i>
Total	382	152	202	193	18	82	111	30¹	86
Mission Creek									
15FL551	7	0	2	2	0	1	1	1	0
15FL557	7	0	4	3	0	2	1	2	0
16FL613	9	0	3	3	0	1	2	1	1
16FL614	7	0	7	7	0	5	2	5	1
16FL622	14	1	2	2	0	0	2	0	0
16FL625	11	2	3	3	0	3	0	3	0
16FL630	8	1	6	5	0	2	3	2	2
16FL643	20	2	13	13	1	5	7	5	5
16FL649	9	0	6	5	0	2	3	2	3
16FL655	16	4	4	4	0	1	3	1	2
QC-1FL	0	3	0	0	0	0	0	0	0
<i>Subtotal</i>	<i>108</i>	<i>13</i>	<i>50</i>	<i>47</i>	<i>1</i>	<i>22</i>	<i>24</i>	<i>22</i>	<i>14</i>
17FL701	30	7	22	21	0	13	8	6	8
17FL703	30	4	26	24	0	14	10	9	10
17FL705	30	8	21	21	0	13	8	8	8
17FL708	30	25	5	5	0	4	1	4	0
17FL709	30	9	19	18	0	8	10	6	9
17FL712	30	2	20	18	2	11	5	11	3
17FL716	30	12	15	15	1	10	4	9	4
17FL719	30	6	21	20	0	12	8	11	6
17FL720	30	4	26	26	1	15	11	15	9
17FL723	30	20	10	10	1	4	5	3	5
<i>Subtotal</i>	<i>300</i>	<i>97</i>	<i>185</i>	<i>178</i>	<i>5</i>	<i>104</i>	<i>70</i>	<i>82</i>	<i>62</i>
Total	408	110	235	225	6	126	94	104¹	76

¹ OZ: Also one male of unknown origin; MC: also one male 17MC-01.

Rearing conditions

Methods

Our rearing strategy has focused on mimicking ambient conditions to the greatest degree possible, because instituting environmental controls assumes that detailed knowledge exists on which conditions maximize survival. In fall 2015 based on existing data, expert opinion and field observation, we refined our targets (Table 10) in order to exclude potentially detrimental conditions and increase consistency between the Oregon Zoo and Mission Creek. The new targets are also easier to compare between institutions.

Differences in climate and facility design require that supplemental light and heat be used 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 10). To track rearing conditions at both facilities, temperature and relative humidity (RH) were recorded every two hours using HOBO data loggers to obtain min/max data during all rearing stages.

Table 10. Target temperature (°F) and relative humidity (% RH) conditions by life stage and rearing location, including methods to achieve them for Taylor's checkerspot rearing at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, 2017-2018.

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	≥50	Ice packs; wet towels	Full shade ¹	40-watt bulb 8-9 AM & 1-1:30 PM	None
			78-85 for 2-6 h/day					
Females and Oviposition	GH Small Room	TCB room	50-90	≥50	40-watt bulb 10 AM-2 PM or outside	Partial shade ¹	40-watt bulb 10 AM-2 PM or outside	None
			78-90 for 4-8 h/day					
Egg & Prediapause	GH Main Room	TCB room	50-90; ave min ≤65	≤65	Ice packs; wet towels	None	None	7 AM - 7 PM
Warm diapause	GH Main Room	TCB room	50-90	≥40	Ice packs; wet towels	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	≤45 night	≥50	None	None	None	7 AM - 7 PM
			≥60 daytime					
Pupation	GH Main Room	OSB room	≥50 night	≥65	Misting/ humidifier	None	7 AM-7 PM	7 AM - 7 PM
			≥65 daytime					

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 2017-2018 are listed in Table 11. For the most part, rearing conditions at both facilities followed similar patterns across the rearing year. In most cases temperature and humidity levels met target levels, with some values falling outside the desired range, or falling short of the target for the specified duration. For example, both facilities reported they met target temperatures for adults and oviposition, but the number of days when temperatures were 78-85 °F for 2-6 h/day was below the desired number. At Mission Creek this was likely the result of too much shading, whereas it's not clear

what happened at the Zoo. Similarly, both facilities reported fewer nights below 35°F during cold diapause and below 45°F during postdiapause than desired. In this case, results reflect several periods when ambient outdoor temperatures were simply warmer overnight, than the target specified. The number of days targeted was a best guess, but could be better informed by review and summary of actual overwinter conditions over the past 12 years.

Table 11. Actual average temperature (°F) and relative humidity (% RH) conditions and absolute range (range) by Taylor’s checkerspot by life stage and date range during captive rearing at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, 2017-2018. Numbers in **bold text** indicate measures outside the target by more than 10 percent.

Life stage	Date range		Temp (°F)			Ave min RH (%)		
	MC	OZ	Terms	Mission Creek	Oregon Zoo	Terms	Mission Creek	Oregon Zoo
Males	30 Apr – 31 May 2017	7 - 30 May 2017	mean max (rng)	75 (52-91)	92 (60 - 99)	mean	72	25
			# days w/i rng	11 of 32	8 of 24	(rng)	(32- 80)	(23 -58)
Females and Oviposition	7 May – 10 June 2017	19 May - 20 Jun 2017	mean (rng)	67 (52-99)	71 (55-99)	mean	61	44
			# days w/i rng	16 of 26	7 of 32	(rng)	(23 -100)	(23- 92)
Egg & Prediapause	10 May – 19 Jul 2017	23 May - 13 Jul 2017	mean (rng); ave min	68 (55-93); 59	74(60 - 80); 67 ¹	mean (rng)	63 (28- 98)	53 (38- 88) ¹
Warm diapause	20 Jul – 12 Sep 2017	14 Jul - 22 Oct 2017	mean (rng)	71 (58-93)	68 (49 - 86)	mean (rng)	62 (26 -84)	71 (28-95)
Cold diapause	13 Sep 2017 – 1 Mar 2018	23 Oct 2017 - 1 Mar 2018	# days min ≤35	47 of 171	38 of 129 ²	mean (rng); ave max	85 (31 -99); 91	80 (31 -94); 85
Postdiapause	2 Mar – 25 Apr 2018	2 Mar - 1 May 2018	# nts ≤45	14 of 55	3 of 61	mean	55	60
			# days ≥60	50 of 55	56 of 61	(rng)	(22 -89)	(36 -93)
Pupation	8 Apr – 10 May 2018	23 Apr - 1 Jun 2018	# nts ≥50	32 of 33	40 of 40	mean	64	63
			# days ≥65	28 of 33	40 of 40	(rng)	(34 -89)	(36 -96)

¹ Data missing for 21 days when logger failed. ² Data missing for 17 days when logger failed.

Relative humidity met targets at both facilities during nearly all life stages, except for adults and oviposition at the Zoo, when humidity was lower than desired. The Zoo also reported that males had good activity and longevity levels, so there was no indication that that this became a problem. Some target conditions may be modified as more data are evaluated both in captivity and in the field.

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, deferring 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 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 applied, whereby

we get a best estimate of egg numbers, but avoid counting larvae until they reach 3rd instar, and including any larvae known to perish prior to that point.

Results and Discussion

Stage specific survival rates for both rearing institutions appear in Table 12. 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, 2017), and is under-reported due to undercounts of eggs in some cases. Hatch rates for wild-sourced eggs were high (Table 12). When only productive females are considered hatch rates for eggs from captive females were poor at the Zoo, but good at Mission Creek, although the small number of productive reduces the importance of this discrepancy. Survival to eclosion of 47 multi-diapause larvae (MC Wild 16/15-1FL; Table 12) at Mission creek is a notable accomplishment. With so many of these larvae surviving in captivity, this will be the first time they contribute in breeding in a significant way. 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, 2017), and well above those expected in the wild (Moore 1989).

Table 12. 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, 2017-2018. 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 Scatter Creek (SC) or Captive-mated – (Captive), and year egg originated (e.g., 16=2016).

Cohort	OZ Wild 17FL/17SC		OZ Wild E17FL		OZ Captive 17OZ		OZ Wild 16FL		OZ Mixed origin Koch's Postulates	
Life stage	#	Survival	#	Survival	#	Survival	#	Survival	#	Survival
Eggs	621		885		367					
Egg to hatch ¹	608	0.98	883	1.00	4	0.01				
Hatch to diapause	592	0.97	875	0.99	4	1.00	53		48	
Diapause to release	315	0.53	471	0.54	3	0.75	8	0.15	10	0.21
Postdiapause	218		166							
Multi-diapause ²	69	0.32	83	0.50						
Pupation	125	0.84	77	0.93						
Eclosion	119	0.95	74	0.96						
Cohort	MC Wild 17FL		MC Captive 17MC		MC Wild 16/15-1FL					
Life stage	#	Survival	#	Survival	#	Survival				
Eggs	2938		177							
Egg to hatch ¹	3083	1.05	151	0.25						
Hatch to diapause	3023	0.98	146	0.97	120					
Diapause to release	3013	1.00	146	1	116	0.97				
Postdiapause	300				108					
Multi-diapause ²	97	0.32			13	0.12				
Pupation	185	0.91			50	0.53				
Eclosion	178	0.96			47	0.94				

¹ Hatch rates not adjusted for undercount of eggs.

² Proportion of postdiapause larvae that returned to diapause.

Mortality at the Oregon Zoo at diapause and immediately following wake-up was still higher than normal, as previously discussed. To date we’ve ruled out warm overwinter temperatures and disease as

causal factors. In addition, feeding trials did not provide a clear solution, although larvae fed from either fertilized potted plants or from plants grown in the ground generally fared a bit better than those fed from unfertilized plants grown in pots in terms of both weight gain going into diapause and survival during diapause. One other notable detail was that many more larvae fed from fertilized plants continued their progression to the adult stage (16 percent), compared to those in the other two groups, which returned to diapause at the same rate (52 percent).

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, with alpha set at 0.05. This is in keeping with best management practices (Crone et al. 2007) and allows us to determine whether adults produced in captivity are undersized relative to their wild counterparts.

Results and Discussion

As in 2016 (Linders et al. 2017), both male ($p < 0.0001$) and female ($p = 0.0037$) pupae at Mission Creek were heavier than those at the Oregon Zoo (Table 13). This was also true for captive-reared adult males ($p < 0.0001$) and females ($p < 0.0180$). Wild females at Mission Creek were heavier than those taken to the Oregon Zoo in 2017 ($p < 0.0001$). While this would be unusual in most years, in 2017 females sent to the Zoo were the last of those available in the wild (collected at R76 on 31 May, and at SCS on 4 June) whereas collections for Mission Creek (10 and 23 May) overlapped the peak of the flight season (Linders et al. 2017). The difference in weight between wild and captive females at the Oregon Zoo is statistically significant ($p < 0.0001$), although no difference was found between the weight of captive females at the Zoo and wild females at Mission Creek ($p = 0.7023$). Captive females at Mission Creek were significantly heavier than Mission Creek's wild females ($p = 0.0004$), however wild females had a larger wing area on average ($p = 0.0112$); no difference in wing size was reported in 2016 (Linders et al. 2017). There was no difference in wing area of wild females between rearing facilities ($p = 0.0735$), but as at Mission Creek, wild females were larger than those from the Zoo ($p < 0.0001$). Finally, wing area of captive males at Mission Creek was larger than at the Zoo ($p = 0.0189$), but was not different between captive females ($p = 0.1021$) at the two facilities. Some refinements to the protocol for wing area measures are under investigation at the Zoo, which should reduce variance between observers and facilities, and increase our confidence in the reported results. Cross-year analyses of wild and captive adult measures would also increase our understanding of natural variance and trends.

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. Larvae were placed on large and/or dense host plants/patches (*Plantago lanceolata* or *Castilleja* spp) within restored prairie. Larvae were released in groups of 2-5 in the larger release plots, with 25 larvae placed in each 4x4-m survival plot.

Table 13. Descriptive statistics for pupal and adult weights, and wing area measurements for Taylor's checkerspot butterflies that pupated and eclosed at the Oregon Zoo, Portland, OR, and Mission Creek Corrections Center for Women, Belfair, WA, 2017; only weights are included for adults whose wings eclosed improperly.

Units of Analysis	Statistic	Pupal Weight (g)	Adult Weight(g)	Wing Area (cm ²)
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Oregon Zoo				
Captive males	N	58	56	37
	mean	0.1604	0.0781	0.9428
	sd	0.0220	0.0167	0.1573
	var	0.0005	0.0003	0.0248
	median	0.1575	0.074	0.942
	range	0.121 - 0.23	0.055 - 0.13	0.539 - 1.412
Captive females	N	50	48	37
	mean	0.2085	0.1317	1.2635
	sd	0.0397	0.0388	0.2153
	var	0.0016	0.0015	0.0463
	median	0.2085	0.125	1.303
	range	0.116 - 0.37	0.05 - 0.311	0.779 - 1.9
Wild females	N		17	16
	mean		0.0685	1.6466
	sd		0.0143	0.1770
	var		0.0002	0.0313
	median		0.065	1.6275
	range		0.052 - 0.095	1.352 - 1.903
Mission Creek				
Captive males	N	84	84	45
	mean	0.1872	0.1001	1.0334
	sd	0.0240	0.0226	0.1248
	var	0.0006	0.0005	0.0156
	median	0.1865	0.0960	1.0460
	range	0.1400-0.2600	0.0620-0.1630	0.7600-1.3280
Captive females	N	72	72	54
	mean	0.2319	0.1484	1.3479
	sd	0.0352	0.0246	0.1549
	var	0.0012	0.0006	0.0240
	median	0.2280	0.1480	1.3400
	range	0.1232-0.3220	0.0770-0.2030	0.9670-1.7770
Wild females	N	---	23	13
	mean	---	0.1243	1.4963
	sd	---	0.0234	0.1471
	var	---	0.0005	0.0216
	median	---	0.1200	1.4680
	range	---	0.0910-0.1720	1.2520-1.7490

A total of 2,747 postdiapause larvae from Mission Creek were released at TA15 on 9 Mar 2018 (Plots A and Survival Plots 1-10). In addition, 589 larvae (165 into Plot B from Mission Creek; 424 into Plots C and survival plots 11-13 from the Oregon Zoo) were released on 15 Mar 2018. To assess survival by rearing institution, 25 larvae were released into each of thirteen 4x4-m survival plots (except Plots 6, 7, and 2 received 24, 26, and 28 larvae, respectively). Fifteen larvae from Mission Creek were dead on arrival and were not released. Of the 3,336 larvae released at TA15, 3,108 were offspring of wild females, 11

were offspring of captive-mated females, and 77 were of unknown (QC) origin as they were separated from their sibling groups at some point in the rearing process. Numbers reported may not match those in the captive rearing section exactly, but discrepancies are small. All larvae available for release were placed at TA15 to maximize the likelihood that a population would take hold. Weather on release days was good with temperatures 10.0-11.7°C, average wind speed 0.0-3.5 mph, and clear to partly cloudy skies; weather in the days following both releases was generally dry, warm and mostly sunny. At least 18 people assisted with releases, working from about 1000-1700 h each day to complete the task.

Adult release

One hundred fifty-eight adults (89 females, 69 males) were released at TA15 on 11 and 22 May 2018, with 152 adults (90 females, 62 males) were released at TA7S on 12 and 24 May 2018. Of these, 116 came from the Oregon Zoo and 194 came from Mission Creek. Two males and five females died in route and were not released. Most adults were the offspring of wild females, but 17 wild females were also 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 (15.5-23.2 °C), with light winds (2.5-6.7 mph average), and clear or overcast skies (24 May).

Prediapause larval release

The timing of the 2018 flight season was fairly typical, with relatively warm and dry conditions through late spring. This had a positive effect on productivity and survival in both the lab and the field, which favored breeding conditions in captivity and production from wild females, and led to release of 5,175 prediapause larvae in 2018 (Fig. 2). Of these, 3,262 prediapause larvae were released at TA15 on 11 Jun 2018 (1,461 from the Oregon Zoo into Plots D, E, and Survival Plots 14-16; 1,801 from Mission Creek into Plots D, E and Survival Plots 15, 17-19). In addition, 1,913 prediapause larvae were released at TA7S on 14 Jun 2018 (1,192 from Mission Creek into Plots E, Survival Plots 1 and 4; and 721 from the Oregon Zoo into Plots E, and Survival Plots 2 and 3). To assess survival, 40 larvae were placed into each of eight 4x4-m survival plots, except Plot 16, which received 41. Seven larvae from Mission Creek and three from the Oregon Zoo were dead on arrival and were not released. Of larvae released at TA15, 2,981 were offspring of captive-mated females and 281 were offspring of wild females. Similarly, 1,591 of the larvae released at TA7S were offspring of captive-mated females and 322 were offspring of wild females.

IV. Monitor success of the translocation

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. The fact that larvae can be found in and around release areas in the days, months and years following release, even when no successive releases have occurred, indicates checkerspot are surviving and reproducing on site.



Figure 2. Release of prediapause Taylor's checkerspot larvae at Training Area 15, Joint Base Lewis-McChord, Washington, spring 2018.

Methods

To confirm site occupancy, quantify minimum survival, and identify issues that may be cause for concern, we conducted surveys for postdiapause larvae in thirteen 4x4-m survival plots at TA15 on three occasions in the weeks following release. Ten survival plots were established for larvae from Mission Creek and three for Oregon Zoo larvae (see Postdiapause release). We used both single and double observer strategies to maximize coverage and measure detectability, respectively. A dependent double observer approach was used for a subset of plots on each of three survey dates to measure detectability and to maximize the likelihood that we were accurately reconciling observations of individuals (Pierce et al. 2012). Two observers cannot survey the same square meter simultaneously without getting in the other's way, so the primary observer, going first, flagged and labeled each larval group encountered. The secondary observer, following in close proximity, verified the presence of flagged larvae, then flagged and recorded any additional larvae observed. Larvae within 10 cm of one another were considered part of the same group. On occasion, larvae moved away from their flagged location prior to confirmation by the secondary observer, so we assumed larvae within 15 cm of a flagged location were already counted unless they were in excess of those already recorded. We thoroughly searched each 4x4-m plot area, also noting any larvae within one meter of the perimeter. For each group we recorded number of larvae, instar, behavior and substrate, and mapped each location on a plot diagram to reconcile differences between observers. Single observer plots were surveyed and marked in the same manner as double observer plots.

To estimate abundance, we first generated detection probabilities by observer within date and rearing institution (Oregon Zoo and Mission Creek) using double observer plot data. We then used an online calculator (Stangroom 4 Feb 2019) for the two-tailed z-test of proportions (Zar 1999) at a significance level of $\alpha = 0.05$, to compare detection rates between observers. On some survey dates only the single observer method was used (Table 14). In these cases we either 1) employed the pooled detection rate from 19 March to all subsequent dates (Oregon Zoo), or 2) after using the z-test to compare detection rates between dates, we pooled individual detection rates from the double observer data for Mission Creek plots and applied it to data collected on 12 March (Table 14). Detection rates were applied to counts of larvae pooled across survival plots by rearing institution and date (Nichols 2000) to

estimate abundance. Survival estimates were calculated by dividing abundance estimates by the total number of larvae released into each set of survival plots (10 Mission Creek plots = 230 larvae; 3 Oregon Zoo plots = 75 larvae) for each survey date; standard error and 95 percent Confidence Intervals (CIs) for survival were also calculated (Zar 1999). We also calculated daily survival rates (Powell 2007) by institution and to evaluate whether larvae released from the Zoo were surviving at a similar rate to those from Mission Creek. To compare survival rates between rearing facilities we calculated 95 percent CIs and checked for overlap.

Results and Discussion

Results of post-release surveys conducted for postdiapause Taylor's checkerspot larvae at TA15 in 2018 appear in Table 14. Due to good weather we were able to complete three rounds of surveys for each set of survival plots grouped by rearing institution. As noted on previous occasions (e.g., Linders and Lewis 2013, Linders et al. 2014, 2015), the number of larvae available to be counted may decline coincident with molt from 5th to 6th instar (Table 14), a time when larvae may hide due to increased vulnerability, then increase again in 6th instar. Ratios of 5th to 6th instar larvae were high during early surveys, shifting to predominantly 6th instar by the 3rd survey (Table 14). Unlike results from 2017 (Linders et al. 2017), nearly all larvae were in 6th instar at the close of the survey period, suggesting a relatively low rate of return to diapause, which is what we might expect given the sunny and warm conditions prevalent in spring 2018 (Mass 30 Dec 2018).

Detection rates ($\hat{p}$) were relatively high (Table 14), with no significant differences between observers or dates ($z = 0.1069-0.7987$; $p = 0.424-0.912$), which allowed us to pool detection rates within or among dates, as appropriate, and generate survival rates by date. Daily post-release survival was high in both cases (Mission Creek = 0.931, range: 0.925-0.937; Oregon Zoo = 0.956, range: 0.951-0.961), but was significantly higher for Oregon Zoo larvae based on a lack of overlap in the 95% CIs. This supports results from the lab that suggest increased mortality at the Zoo is the result of larvae failing to transition from diapause to wake-up (Table 12). The higher daily survival for Zoo larvae is likely because they were released later than those from Mission Creek and after this period of mortality had passed. At the end of the survey period, survival for larvae from both facilities (Table 14) was similar to the weighted average survival (0.476) calculated from (Moore 1989) for *E. editha* in California across three years and two subpopulations.

Table 14. Survey results pooled by date for post-release assessment of postdiapause Taylor's checkerspot larvae from two captive rearing facilities, placed in thirteen 4x4-m survival plots (Mission Creek, $n = 10$ plots; Oregon Zoo, $n = 3$ plots) at Training Area 15, South Puget Sound, Washington, spring 2018. Number of days post-release, ratio of 5th to 6th instar larvae observed, detection method (single/double observer), detection rate ($\hat{p}$), larvae counted, abundance estimate ($\hat{n}$), proportion of released larvae surviving ($\hat{s}$), standard error (SE) and 95% Confidence Intervals (CI) are reported. See text for release details and calculations.

Date	Days	5:6	Method	$\hat{p}$ -hat	Count	$\hat{n}$ -hat	$\hat{s}$ -hat	SE	95%CI Low	95%CI-Hi
Mission Creek										
3/12	3	1.00	Single	0.677 _{pa}	130	192	0.759	0.031	0.699	0.819
3/16	8	0.91	Sgl/dbl	0.652 _{pi}	60	92	0.364	0.016	0.333	0.394
3/19-20	10/11	0.17	Sgl/dbl	0.703 _{pi}	81	115	0.455	0.016	0.424	0.487
Oregon Zoo										
3/19	4	0.57	Double	0.916 _{pd}	45	49	0.655	0.022	0.612	0.698
3/21	6	0.56	Single	0.916 _{pd}	30	33	0.437	0.018	0.402	0.472
3/30	15	0.06	Single	0.916 _{pd}	35	38	0.509	0.019	0.472	0.547

_{pa} –pooled detection rate for Mission Creek plots across observers and dates

_{pi} – average detection rate of observers within survey date

_{pd} - detection rate from dependent double observers on 19 March.

Evaluate translocation 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. Five translocation sites (SCS, R50, GHP, TA7S, and TA15), one colonization site, and one extant site (R76) were surveyed for adult checkerspots during the 2018 flight season. 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 until counts were at or near zero. Survey transects at translocation sites included all release plots and a buffer of sufficient size (up to 200 m) to capture the anticipated adult use area. Transects at R76 covered the majority of the occupied area to which we had access. Four additional transects added to the north in 2014 (Linders et al. 2014) are now routinely surveyed. Transect length and spacing by site and year is shown in Table 15. The closer transect spacing at translocation 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.

Table 15. 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, 2010-2018. 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	2016-18	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	2017-18	16	25	20	25	500	8000	20.0
R50	2010	13	25	8	50	400	5200	13.0
R50	2011	16	25	9-11	50	450-550	8600	21.5
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	2014-18	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	2014-18	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	2016-18	19	25	7-14	25	175-350	5125	12.8
SCS2	2017-18	12	25	18	25	450	5400	13.5
GHP2L	2017-18	12	25	13	25	325	3900	9.8
TA15	2018	14	25	6-17	25	150-425	4425	11.1

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

Data analyses. Analyses were conducted using Program Distance, Version 7.1 (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 MDCS analyses, we also computed univariate statistics and plots of distance data for potential covariates, such as observer, butterfly behavior, survey protocol, and weather, as recommended by Marques et al. (2007).

Previous analyses identified observer as a consistent source of variation in detectability, so a suite of models were developed for analysis to account for this potential variability, dependent on the number of observations collected (sample size) for 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 MDCS 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 were calculated using the method of Fewster et al. (2009) that takes advantage of the systematic transect spacing to reduce variance estimates over those that assume transects are placed randomly. Of two such methods 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 were used to estimate 95% CIs.

In 2018, 5 observers conducted surveys, although one conducted only a few surveys when observations were rare. All observers had distance sampling experience, although the amount varied widely. As in previous years, there were notable differences among observers, but greater survey experience was not related to the collection of distance data that was well-fit by Distance models. Data and density estimates for three translocation sites (SCS, R50, and TA15) plus R76 are reported here for 2018; there were an insufficient number of observations at GHP, TA7S and SCS2 to generate density estimates.

Results and Discussion

From six to nine complete distance surveys were conducted at each of seven occupied Taylor's checkerspot sites in 2018 (Table 16); one partial survey each at R50 and SCS1 was also conducted. The greatest amount of effort was expended on sites with the largest populations (i.e., R76, R50, SCS1), with about half as much effort expended on newer sites (i.e., TA7S, SCS2, and TA15). A total of 79.2 km were surveyed at R50, 76.3 km at R76, and 72.0 km at SCS1, with 63.9 km surveyed at GHP & GHP2L combined, 39.8 km at TA15, 37.8 km at SCS2, and 30.8 km at TA7S.

Rain in early April ended what was otherwise a dry and fairly warm winter in 2018, with the exception of one snowy week in February; dry conditions returned in late April. Intermittent clouds created marginal shadow conditions for portions of the survey period on 1 May at TA15, and on 4 May at TAs 7S and 15. In addition, we aborted surveys due to weather at SCS1 on 6 May, and at R50 on 11 May. Range access coupled with periodic clouds made it challenging to complete JBLM surveys efficiently.

Except when 3-hr midday check-fires were granted, access to R76 and R50 was primarily limited to Mondays, with a few added days in May when concerns about completing surveys began to mount. In 2018, access to the dud hazard area in the southwest corner of the monitoring area was granted on all dates, facilitating reliable calculations of daily population estimates. Check-fire access was not granted

consistently in spite of prior agreements and at times was poorly implemented. For example, surveyors had to enter and exit the AIA multiple time while conducting surveys, which reduced survey efficiency and effectiveness across sites. Ultimately we achieved the minimum of five qualifying surveys at high priority AIA sites and others (Table 16), by working significantly more hours over the course of the 5-week flight period; fortunately the weather in 2018 was accommodating.

Table 16. Number of Taylor's checkerspots counted by site and date during distance sampling surveys at extant (R76= Range 76) and translocation sites (SCS = Scatter Creek South Units 1&2; R50 = Range 50, GHP = Glacial Heritage Preserve, TA7S = Training Area 7S, TA15 = Training Area 15), South Puget Sound, Washington, spring 2018. Surveys were conducted prior to adult release where applicable. R76: both standard (T1-12) and extended (T1-16) survey areas reported here (see Appendix C, Fig. 6).

Date	T1-16	T1-12	R50	SCS1	SCS2	GHP	TA7S	TA15	Comments
	R76	R76							
18-Apr							0		
19-Apr				0	0	0			
20-Apr			0						
23-Apr	0	0	101					0	
24-Apr				13	0	0			
25-Apr	19	16					0	0	
26-Apr				173	0	0			
1-May								7	TA15: Shadows marginal at times.
2-May	1291	1164	2862						
3-May				1077	2				
4-May							0	81	TA7S: Stopped often; TA15: Shadows marginal at times.
5-May			2660						R50: No shadows at times, checkerspots active
6-May				8		0			SCS1: 12 segments; GHP: possible TCB
7-May	2117	1924							
8-May				1630				112	
11-May			547					44	R50: T9-16 protocol in/out, abort; TA15: Release 145 adults
12-May					21		0		TA7S: Release 139 adults
13-May				1097		0			
14-May	790	723	1055						
15-May							2	37	TA15: T 25-26 added for distribution
19-May					6	0			
21-May			68						
22-May				78			0	8	TA15: Release 13 adults
23-May	8	8	11						TA7S: Release 6 adults
24-May				9					
29-May				0	0				
30-May	1	1	0					0	
Total	4226	3836	7305	4086	29	0	2	289	

Density and abundance estimates 2018

Distance data were analyzed for four sites (R50, R76, SCS1, and TA15) in 2018; results are presented here and in Appendix B. A total of 19,773 checkerspot observations were recorded during distance surveys, setting a new annual record, with the greatest number observed at R50 (Table 16). Group size

ranged from one to eight, with 14 groups comprised of four or more adults. Flight season initiation was intermediate in its timing relative to past years, with peak counts recorded on 2-12 May. Peak day encounter rates were high, with about 30 percent more butterflies observed at R50 (0.29 checkerspots/m) compared to SCS1 (0.20/m), but both sites harbored about twice as many butterflies as in 2017. Rates at R76 (0.19/m for transects 1-16; 0.22/m for transects 1-12) were about four times higher than in 2017. The peak day encounter rate at TA15 (0.03 checkerspots/m), was also respectably high for a first year site. Only two checkerspot were observed at GHP, neither of which was confirmed during transect surveys, and adults were observed on TA7S only after captive adults were released. Staff from Joint Base Lewis-McChord are also monitoring parts of TAs 6, 7S, 14, 15 and the perimeter of 91st Division Prairie (the Artillery Impact Area) using transects with a 100-m spacing; they will report on these data separately, although they are displayed in Appendix D.

SCS 1. Ten surveys were conducted at SCS 1 in 2018 between 19 April and 29 May; no butterflies were observed on either of those dates. Of 3,534 observations used in the analysis, the greatest number (1,373) were recorded on 8 May. All surveys were complete except on 6 May, when the survey was aborted due to poor weather, resulting in 7 qualifying surveys (Linders and Olson 2014). The number of observers per day varied from 2 to 4, with effort and encounter rates more variable than desired. For dates with sufficient sample sizes (3, 8, and 13 May), observer-specific detection functions were modeled. Data for 8 and 13 May presented many difficulties in spite of large sample sizes, with densities somewhat lower (8 May) or higher (13 May) than expected based on relative counts (Table 16); the reasons for this are not clear. For dates with insufficient data to estimate a date-specific detection function (24 April, 22 and 24 May), data were pooled with an adjacent date. Data were truncated at 8m to eliminate outliers. Daily density and abundance estimates with 95% CIs are presented in Table 17. See next section for results related to the 2017 wildfire.

Range 50. Nine surveys were conducted between 20 April and 30 May by five observers in 2018. No butterflies were recorded on 20 April and 30 May. On any given date, survey effort was fairly evenly distributed among observers. All were complete surveys (Table 15) except on 11 May when only 5200 m of line length was surveyed due to non-protocol conditions; all other surveys were conducted under protocol conditions for a total of 6 qualifying surveys (Linders and Olson 2014). In all, 6,073 adult observations were used in the analyses, with the greatest number (2,638) on 2 May (Table 17). Detection functions were date-specific except that data from May 21 and 23 were combined to estimate a single joint detection function for both dates due to a smaller sample size. For 2, 5 and 14 May, detection functions were also either observer-specific or were from a multiple covariate model with observer covariates. Data for 5 and 14 May were truncated at 8m, data for 23 April and 2 May were truncated at 10m to reduce unnecessary adjustment terms; data for 21 and 23 May were not truncated. Density estimates with 95% CIs (CI) are presented in Table 17 along with daily abundance estimates and 95% CI for the entire survey area.

Range 76. Seven surveys were conducted by 5 observers from 23 April to 30 May 2018. No butterflies were observed on Apr 23 and only 1 was recorded on May 30, for a total of 6 qualifying surveys (Linders and Olson 2014). Four observers were present on most survey dates, with effort well-distributed among observers and dates. All transects (Table 15) were surveyed on all dates. Surveys were conducted entirely in protocol conditions. A total of 3,580 adult observations were used in the analyses, with the greatest number (1816) observed on 7 May (Table 17). Sufficient observations were recorded on 2, 7, and 14 May to fit detection functions. Detection functions were best modeled separately by date and observer on 2 and 7 May, but best pooled over all observers on 14 May. Data were truncated at either 8 m (2 and May 14) or 10 m (7 May), depending on which was best for detection function estimation. Daily density and abundance estimates with 95% CIs are presented by date in Table 17.

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 (CI) for Taylor's checkerspot survey areas at Scatter Creek South Unit 1 (SCS1), Range 50 (R50), Range 76 (R76) and Training Area 15 (TA15) in South Puget Sound, Washington, spring 2018. See text for details.

		Survey	Density			Abundance		
Date	#Obs	area (ha)	#/ha	Lower CI	Upper CI	#/site	Lower CI	Upper CI
SCS1								
24-Apr	173 ¹	20.00	1.9	0.9	4.1	37	17	81
26-Apr	160	20.00	23.4	17.5	31.4	468	349	628
3-May	970	20.00	148.2	125.6	174.9	2964	2511	3498
8-May	1373	20.00	162.5	104.2	253.4	3250	2084	5067
13-May	933	20.00	209.4	176.4	248.7	4188	3527	4973
22-May	85 ²	20.00	32.9	17.9	60.4	658	358	1207
24-May	85 ²	20.00	3.9	1.8	8.5	78	36	170
R50								
23-Apr	91	24.75	11.6	7.4	18.1	286	183	447
2-May	2638	24.75	344.3	299.8	516.0	8521	7419	12770
5-May	2343	24.75	356.9	277.0	459.7	8832	6857	11377
14-May	922	24.75	103.3	88.6	120.4	2555	2192	2979
21-May	68	24.75	7.1	5.2	9.8	176	128	243
23-May	11	24.75	1.2	0.7	1.9	28	18	46
R76								
2-May	1138	54.50	202.48	132.7	309.0	11035	7231	16842
7-May	1816	54.50	261.7	179.8	380.8	14263	9801	20755
14-May	626	54.50	85.05	67.5	107.1	4635	3680	5838
TA15								
1-May	6	11.1	1.5	0.58	4.02	17	6	45
4-May	64	11.1	16.3	10.09	26.26	181	112	291
8-May	96	11.1	24.1	17.97	33.16	268	199	368
11-May	37	11.1	9.4	5.46	16.22	104	61	180
15-May	34	11.1	7.3	3.51	14.98	80	39	166
22-May	8	11.1	2.0	0.8	5.18	23	9	57

¹ Due to small sample size, one detection function, derived from combined data for 22 and 24 May, was applied to both dates.

² Detection function was fit to pooled data for these dates; sample size given is the pooled value.

TA15. Nine surveys were conducted at TA15 in 2018 between 23 April and 30 May by 4 observers; no butterflies were seen on 23 and 25 April, or on 30 May; 6 of these were qualifying surveys (Linders and Olson 2014). Full surveys (Table 15) were completed on all dates (Table 16). The greatest number of observations (96) were collected on 8 May, with 245 total detections used in the analyses (Table 17). Total line length surveyed was similar among 3 of 4 observers, but encounter rates differed greatly, indicating the potential bias in daily estimates due to observer differences, especially because sample sizes were insufficient to estimate date- and observer-specific detection functions. All estimates were generated from a single multiple covariate model with observer covariates with observations pooled over all dates. Data were truncated at 10 m. Daily density and abundance estimates with 95% CIs are presented in Table 17.

Other observations

Scatter Creek Wildfire

On 22 August 2017, after the flight season had ended, a wildfire swept through Scatter Creek Wildlife Area's South Unit, burning a portion of the SCS1 monitoring area and all of SCS2 (Appendix C, Fig. 2). To estimate the effects of the wildfire on the checkerspot population, we generated separate encounter rates for the burned and unburned areas in both 2017 and 2018 based on the location of each 25-m monitoring segment relative to the fire line (Appendix C, Fig. 2). Checkerspot observations were collated for each 25-m segment by date and burn designation. We calculated encounter rates based on the total line length in each burn designation.

Encounter rates were higher and more variable in the "unburned" area compared to the "burned" area in both 2017 and 2018 (Fig. 3). However in 2017, encounter rates were comparable between the two areas by day 22 of the flight season, presumably due to adult dispersal from higher density areas. In 2018, encounter rates in the burned area stayed well below those in the unburned area, and dissimilar until day 28 of the flight season (Fig. 3), suggesting adults were slow to disperse into the burned area. In addition, the peak encounter rate in the unburned area increased by a factor of 2.2, compared to an increase of 1.7 in the unburned area in 2018, which was a good year for checkerspots overall.

Evidence suggests checkerspots were eliminated in the burned area following the 2017 wildfire. During surveys, observers noted that checkerspots were absent in the burned area 10 m or more from the fire line early in the flight season. This can also be seen in the data for SCS2, where no checkerspots were observed until the 10th day of the flight season (Table 16). The only butterflies observed deep within the burned area early on were silvery blues. Puget blues were also observed in the burned area at the start of their flight season (A. Potter, pers. comm.), suggesting diapause locations for these species are better protected from fire than those occupied by checkerspots and some other butterfly species. Fortunately, high population levels in 2018 (Table 17; Appendix B, Scatter Creek South 1) clearly aided dispersal of checkerspots into the burned area (Appendix C, Fig. 2, Appendix D, Fig. 2) later in the flight season. Extensive restoration of the 215 burned acres of prairie should significantly improve habitat quality, and foster colonization across the remainder of the site.

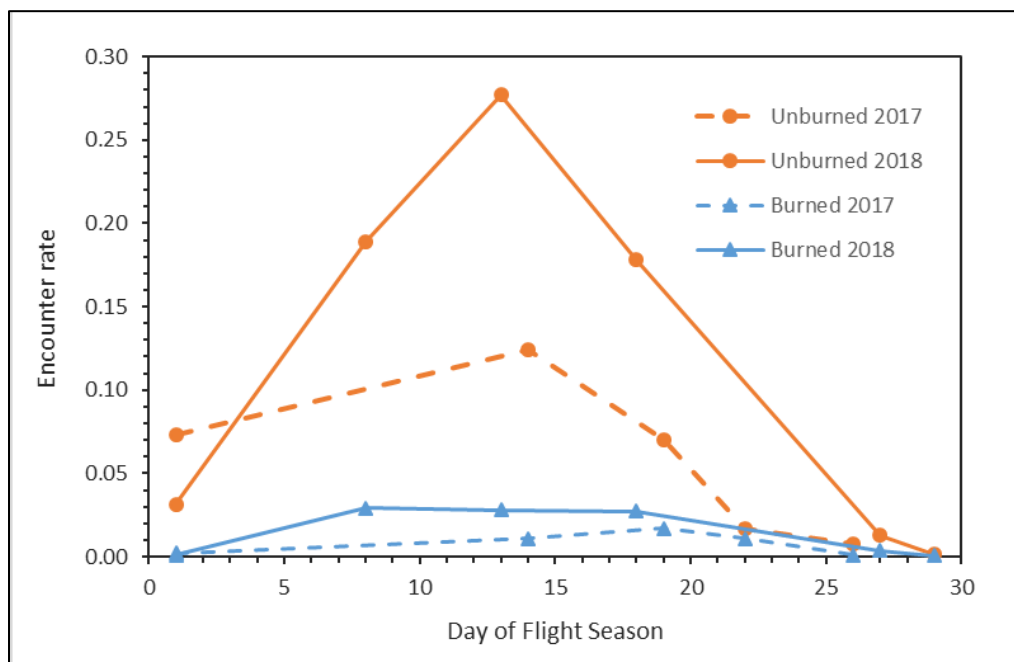


Figure 3. Encounter rates (ER) by day of flight season for two portions (burned and unburned) of the Scatter Creek South 1 (SCS1) monitoring unit prior to the August 2017 wildfire, and in 2018, after the fire burned a portion of the area. Encounter rate is the number of observations divided by line length surveyed in meters.

Nectar

Nectar observations have been recorded opportunistically during Taylor's checkerspot distance surveys since 2011 (Table 18). Nectar observations were recorded on 26 species across five survey areas in 2018, with a marked increase in observations relative to recent years. Three species: *Balsamorhiza deltoidea*, *Lomatium triternatum* and *Saxifraga integrifolia* (= *Micranthes integrifolia*) accounted for 83.1 percent of nectar observations in 2018 and the majority of all observations to date (87.9 percent). The importance of these species across sites and years is likely two-fold: 1) consistent nectar production across years, and 2) provision of basking and/or roosting sites. Both functions likely increase longevity and fitness (Murphy et al. 1983, Dover et al. 1997). In addition to tabled data, 41 nectaring observations have been recorded from 17 other species: *Amalanchier alnifolia* (SCS-2018); *Anaphalis margaritacea* (R76-2017); *Castilleja levisecta* (SCS1-2018); *Cerastium arvense* (R50-2012, TA7S-2014, R50-2018, 3 at SCS-2018); *Crataegus* spp. (2 at SCS1-2018); *Cytisus scoparius* (R76-2013, 3 at SCS-2018); *Dodecatheon hendersoni* (R50-2015); *Fritillaria camschatcensis* (R76-2015); *Lepidium campestre* (R76-2011, 5 in 2017); *Lupinus Lepidus* (3 at R76-2011); *Marah oreganus* (R76, SCS 1&2-2018); *Plantago lanceolata* (R50-2017, R76-2018); *Prunus virginiana* (2 at TA15-2018); *Rumex acetosella* (R76-2017); *Teasdalea nudicaulis* (R50-2018, 2 at R76-2018, 3 at SCS-2018), and *Vicia sativa* (R50—2018) have also been recorded.

Table 18. Number and species of nectar used by Taylor's checkerspots during surveys on extant (R76 = Range 76) and translocation sites (R50 = Range 50, SCS = Scatter Creek South Units 1&2, PCM = Pacemaker, GHP = Glacial Heritage Preserve, TA7S = Training Area 7 South, Training Area 15 = TA15), South Puget Sound, Washington, 2011-2018. ACMI – *Achillea millefolium*; ARMA – *Armeria maritima*; BADE - *Balsamorhiza deltoidea*; CAHI – *Castilleja hispida*; CAQI - *Camassia quamash*; COSP – *Collinsia* species; ERLA – *Eriophyllum lanatum*; FRVI – *Fragaria virginiana*; HYRA – *Hypochaeris radicata*; LOSP – *Lomatium* species; LOTR- *Lomatium triternatum*; LOUT-*Lomatium utriculatum*; PLCO- *Plectritis congesta*; POGR - *Potentilla gracilis*; RAOC - *Ranunculus occidentalis*; SAIN - *Saxifraga integrifolia*; ZIVE *Zigadenus venenosus*.

Year	Site	ACMI	ARMA	BADE	CAHI	CAQU	COSP	ERLA	FRVI	HYRA	LOSP	LOTR	LOUT	PLCO	POGR	RAOC	SAIN	ZIVE	Total
2011	R50	0	0	113													6		119
	R76	3	14	618		3		12	46	1		621	5		3	4	37		1367
	SCS1	0	2	2		1						1		4					10
Subtotal		3	16	733	0	4	0	12	46	1	0	622	5	4	3	4	43	0	1496
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	176	311	21	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	54	6	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	16	37	2	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	73	10	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	23	4	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		186	9		2		18	1	354
	SCS1	3	1	53		2								12		1			72
	SCS2			3															3
Subtotal		5	2	381	0	8	1	1	0	8	1	223	10	13	2	3	67	3	728
2018	R50	1		303	1	6			1		1	7	1		1	2	201	1	526
	R76			144	1	13	10			3	5	91	3		1	4	106		381
	SCS1		10	91	2	46	14				6	14		31	1	3			218
	SCS2	1		2			2					1							6
	TA15		2			2						2							6
Subtotal		2	12	540	4	67	26	0	1	3	12	115	4	31	3	9	307	1	1137
Total		12	55	3531	8	150	35	13	77	15	205	1458	62	60	8	40	711	8	6447
Proportion		0.00	0.01	0.54	0.00	0.02	0.01	0.00	0.01	0.00	0.03	0.22	0.01	0.01	0.00	0.01	0.11	0.00	1.00

Mardon skipper

As in years past, state endangered mardon skipper (*Polites mardon*) butterfly sightings were systematically recorded during 2018 surveys for Taylor's checkerspot at the three sites where they co-occur. In total, 108 mardon skippers were observed on surveys, with an additional 31 observations made off transect; the majority were observed at R50 (Table 19). This is the first time in many years that sufficient observations were collected at a single site to make calculation of a density estimate possible. Any observations at R76 and SCS are encouraging given the low numbers observed in recent years (Linders et al. 2016, 2017) combined with the effects of wildfire at SCS in 2017 and a long history of fire at R76. Fire may be particularly damaging to this species given its habit of hiding in and feeding from bundles of dead fescue, and actively feeding through fall. All initial observations were recorded as lone skippers, except for one group of two skippers observed at R50. However several groups of multiple skippers were observed at R50 in the course of our work there. This is the most encouraging report we've been able to make for mardon skippers in the Puget lowlands in many years, and their status remains a source of concern.

Table 19. Number of mardon skipper butterflies observed by date and site during distance surveys for Taylor’s checkerspot in spring 2018 at three sites (R50 = Range 50; R76 = Range 76; SCS = Scatter Creek South Units 1&2), including two monitoring areas at Scatter Creek, South Puget Sound, Washington. “Zero” surveys are included nearest to dates when skippers were observed.

Date	Distance surveys				Off transect		
	R50	R76	SCS1	SCS2	R50	R76	SCS
25-Apr		0					
2-May	0	1					
3-May			0	0			
5-May	2						
7-May		1					
8-May			2				3
11-May	8						
12-May				2			
13-May			1				
14-May	58	8			10	1	
19-May							2
21-May	16				14		
23-May	8	1			2	2	
24-May							
29-May			0	0			
30-May	0	0					
Grand Total	92	11	3	2	26	3	5

Adult distribution

Methods

To illustrate the distribution of adults within survey areas at each site, all Taylor’s checkerspot observations collected during 2018 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. Data for all survey dates were combined for each site and mapped using standardized classes. Classes were scaled so the midpoint of each successive bin increases by a factor of two. Observations were overlaid on 2017 National Agriculture Imagery Program (NAIP) color aerial photos with 1-m resolution. To compare distribution of checkerspots between years and sites, we used the number of segments with checkerspot observations in each survey area as an index of occupied area. This assumes that patterns of occupancy and detectability near the transect line are representative of the underlying grid cell and that placement of transects is random relative to habitat conditions; both assumptions are consistent with those of distance sampling. It is important to note that cell size changes by site and year as a result of segment length and transect spacing (see Table 15). These assumptions are more likely to be violated where transects are widely spaced (i.e., less area sampled), and violations may be more pronounced at lower occupancy levels compared to higher ones, (i.e., stronger upward bias when populations are low).

To document dispersal and identify potential colonization areas, we collated and mapped scattered sightings of Taylor’s checkerspots recorded outside of standard surveys. In addition, we conducted a pilot test of occupancy surveys across all of SCS in 2018 using transects spaced 100 m apart with an east-west orientation. We recorded the exact location of each checkerspot if near the transect line, or the point on the line perpendicular to the butterfly if more than 5 m off the line. Transects were extensions of the existing grid, with distance sampling areas excluded. Survey timing targeted the flight period peak to capture the greatest abundance and distribution. Similar surveys were implemented by JBLM,

WDFW, and CNLM staff around the periphery of the AIA, and in several Training Areas where checkerspots were likely to be found, including the former release area at PCM in TA 14.

Results

An index of area occupied by adult Taylor's checkerspots by site and year is summarized in Table 20. Occupied area at R50 and SCS has increased every year since 2014, while occupied area at TA7S and GHP have been declining since 2015 despite repeated releases (Appendix A). At R76, occupancy declined from 2014-2016, rebounding in 2017-2018. Checkerspots at R50 occupied all survey segments for the first time, with high concentrations all along the south and west edges of the monitoring unit (Appendix C, Fig. 1). At SCS1 checkerspots occupied 76.9 percent (246 of 320) of the segments surveyed (Appendix C, Fig. 2), up from 60.9 percent in 2017. In contrast, occupancy in SCS2 declined by half in 2018, to 10.6 percent (23 of 216) of segments surveyed (Appendix C, Fig. 2). This was clearly the result of the 2017 wildfire, which eliminated checkerspots from the northern portion of the site, as evidenced by the lack of observations in SCS2 for the first 10 days of the flight season (Table 16). Occupancy during the first year of release at TA15 appears good both in terms of its size (Table 20) and connectivity (Appendix C, Fig. 5), and shows the expected pattern of concentrations in and around release areas that indicates habitat acceptance. At R76, checkerspots occupied 97.9 percent (329 of 336 segments) of the main monitoring area (transects 1-12) in 2018, the greatest spread since 2012. On transects 13-16 (Appendix C, Fig. 6), occupancy continues to lag behind the maximum, observed in 2014-2015. In addition to better habitat to the south, improved access to the duded area, and/or military training impacts to the north are also likely to influence checkerspot distribution.

Table 20. Index of area (ha) occupied by adult Taylor's checkerspot butterflies based on distance sampling surveys by site and year for translocation sites and one extant site (R76 = Range 76) in South Puget Sound, Washington, 2007 – 2018. SCS = Scatter Creek South (1 = main translocation unit; 2 = second management unit in northwest corner of site), Range 50 = R50, Pacemaker = PCM, Glacial Heritage Preserve = GHP, Training Area 7 South = TA7S, Training Area 15 = TA15, and Range 76 = R76.

Site	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018
SCS1			0.2	7.1	4.3	4.3	4.3	3.9	5.4	10.6	12.2	15.4
SCS2											2.9	1.4
R50				6.5	13.6	18.8	17.5	11.2	15.3	18.4	22.4	24.8
PCM						3.0	0.0	0.0	0.0	-	-	-
GHP						0.4	6.3	3.3	3.4	3.7	0.1	0.1
TA7S								2.1	3.4	0.3	0.1	0.0
TA15												5.3
R76	39.3	17.0	26.0	38.0	41.8	42.0	39.5	39.4	38.0	21.3	34.5	41.1
R76 T13-16								11.0	11.0	8.1	7.3	9.1
R76 Total	39.3	17.0	26.0	38.0	41.8	42.0	39.5	50.4	49.0	29.4	41.8	50.3

A map of scattered sightings and 100-m occupancy survey results for the AIA shows a significant increase in the number and distribution of dispersing checkerspots in 2018, with concentrations of adults in several disparate locations (Appendix D, Fig. 1). One location (Mortar Point 13) was also resurveyed using 25-m transects oriented east-west, to better reveal density. Surveyors visited all transects around the periphery of the AIA in 2018, which is rare due to weather and access restrictions. No confirmed sightings of adults were reported from PCM in 2018, corroborating our experience that without multiple consecutive releases, there is a low likelihood of population persistence.

Occupancy surveys at SCS revealed numerous adults west and north of monitoring unit 1, and confirmed that for all practical purposes the entire unit can be considered occupied (Appendix D, Fig. 2). In fact the encounter rate on the occupancy surveys (0.0029 checkerspots/m), was only about 25 percent lower

than that in SCS 2 (0.0039) on the same day (12 May). The encounter rate on SCS1 (0.1371) the following day (13 May) indicates the peak flight season had passed, so these figures should be considered minimums. This pilot test indicates that while overall numbers were low, they may be high enough to warrant using distance sampling methodology, as it requires a similar level of effort but yields more data. Also, because we were able to survey the whole prairie in less than 2 hours, the cost of collecting these data a few times each year would be minimal, while the data gained would be useful for monitoring colonization and informing habitat enhancement efforts.

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 translocation 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 translocation 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 translocation 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), resilient 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

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 entire 24.8-ha monitoring area at R50 (Appendix C, Fig. 1) for the first time, having increased their spread annually since 2014. In addition, the peak single day abundance estimate for 2018 (8,832; 95% CI: 6,857-11,377; Table 17) was the highest recorded for that site, but did not surpass R76 (14,263; 95% CI: 9,801-20,755), which also had a strong year, and covers 55 percent more area. Peak single day abundance estimates at R50 have remained well above the minimum threshold of 250 adults every year since releases concluded in 2011. This population has met or exceeded criteria 1-4 and is officially established based on the project definition. In addition, it has continued to expand such that dispersing butterflies are congregating in surrounding areas with the potential for colonization (Appendix D, Fig. 1).

Checkerspots at SCS1 met criteria 1-3 in 2015, but a release of larvae in spring 2016 to combat potential negative effects of *El Nino* weather, restarted the five-year assessment period in 2017. Checkerspots at SCS1 occupied 76.9 percent of the 20-ha monitoring unit in 2018, and extensive restoration continues to improve habitat across the site. The peak single day abundance estimate of adults in 2018 (4,188, 95% CI: 3,527-4,973; Table 17) indicates this site far exceeded its Year 2 goal, and is on track to meet all establishment criteria by 2021. Similar to R50, checkerspots at SCS 1 are also dispersing into surrounding habitat (Appendix D, Fig. 2), where colonization potential is high.

Glacial Heritage failed to produce more than a few adult checkerspots in 2018, in spite of a sizeable release of postdiapause larvae in 2017 (Linders et al. 2017). This indicates that even larvae that may have returned to diapause in 2017, did not survive in sufficient numbers to take advantage of the warm and generally favorable conditions in 2018. Given the level of effort we have put into this site, we have opted to discontinue releases at GHP until we have a better understanding of factors that may be affecting our success. Factors may include the condition of food plants, availability of microsites, pesticide residues or other unidentified factors. See next section for a discussion of research related to larval host plant availability and microsite.

Future Plans and Recommendations

Captive Propagation

Based on the cumulative results of captive breeding efforts across years and facilities, we will: 1) retain 30 larvae per matriline for breeding, 2) combine males from more than one line if two or fewer males are available per line, 3) age males for three days prior to breeding introductions, and 4) use all available males from a given line in the breeding tents.

Larvae at the Oregon Zoo that survived wake-up did so at a comparable rate to those at Mission Creek, suggesting larvae are not making the physiological transition from hibernation to feeding. Work related to food quality at the Oregon Zoo is ongoing in an effort to reverse this issue, and includes assessment of nutrients in food plants from field sites as well as both rearing facilities.

Both rearing facilities reported fewer nights than desired below 35°F during cold diapause and below 45°F during postdiapause, which reflects several periods of warm ambient outdoor temperatures overnight. The target for the specified number of days below these benchmarks was a best guess that could be better informed by review and summary of actual data on overwinter conditions over the past 12 years. This would be an excellent student project as all of the data are either already in hand or can be easily compiled from historic records.

Conditions in spring 2018 were fairly typical (near-normal precipitation combined with relatively warm overwinter and spring temperatures) once we moved past the warm/cold swings that marked late winter 2018, which appears to have produced favorable conditions for checkerspots. Results from this project suggests that weather appears to be influencing Taylor's checkerspot rearing success in captivity

and in the wild, as had been demonstrated elsewhere in the species range (Weiss and Weiss 1998). Understanding when and how weather influences 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). See Questions for Further Research (below) for an extended discussion on this topic.

Taylor's checkerspot translocation in the Puget lowlands

The significant increase in numbers at R76, R50 and SCS1 to record highs and the favorable response to initiation of postdiapause releases at TA 15 (Appendix B), together with follow-up releases of prediapause larvae in 2018, all bode well for the status of Taylor's checkerspot in the Puget lowlands. In addition, dispersal from core sites (R76, R50, and SCS1) into surrounding habitat (Appendix D) suggests this trend is likely to continue in the near future. Where congregating adults meet with suitable habitat, as is likely at SCS1 and parts of the AIA, colonization can be expected so long as habitat is of sufficient quality to sustain populations through the year-to-year variability that can be expected based on our experience to date. As in 2017 (Linders et al. 2017), our results were comparable to patterns across the species' geographic range as reported at the Rangewide Taylor's checkerspot Working Group meeting in Olympia, Washington, 4-5 December 2018, with many populations performing well in 2018. In contrast, a varied performance was reported in 2017, with many of the stronger sites performing well, while marginal sites reported more tepid results. Given the lack of new releases at GHP and TA7S in 2018, observance of 2 and 0 adults, respectively, is not surprising, and serves as a reminder that many outstanding questions remain.

Given the dynamic nature of checkerspot populations, a strategic approach to recovery should include development of multiple high-density populations that can function as sources for adult dispersal. When surrounded by satellite sites harboring high quality habitat, colonization events would have a high likelihood of success (Thomas et al. 2011), that could lead to a network of occupied sites that function as a mega- or metapopulation. These are the conditions that appear to be developing at both SCS and on the AIA (Appendix D). Occupancy surveys being conducted in both locations are doing a good job of delineating checkerspot distribution and delineating concentration areas, but more effort will be needed to demonstrate colonization of these sites and to evaluate whether habitat conditions are likely to lead to long-term persistence. In contrast, where habitat quality is low (e.g., GHP), populations do not appear to reach the critical densities necessary to for persistence, let alone produce a sufficient numbers to foster population growth. Consequently, the focus of translocation efforts in the Puget lowlands should be to:

- 1) Increase the species short and long-term viability through targeted habitat management, including restoration of satellite sites within dispersal distance of source locations,
- 2) Define and then increase the number of source populations, and
- 3) Increase the number and size of populations through colonization and active translocation.

Current translocation-related plans

This is the thirteenth year of a multi-year Taylor's checkerspot recovery project. The robust response of checkerspots at SCS in spite of the 2017 wildfire reinforces the recommendation that additional releases are not needed and the site is on track to meet or exceed the establishment target by 2021 (Table 17 and Appendix C, Fig. 2). Furthermore, checkerspots were found broadly distributed across the 300-ac site (Appendix D, Fig. 2), although this was not the case until later in the flight season, suggesting the fire eliminated any existing checkerspots. Overall the species is expected to respond positively to post-fire restoration efforts, which have been significant. However full restoration will require many more years,

as funding and seed availability was inadequate to mitigate all of the fire-related impacts. Occupancy surveys using a 100-m spacing were quick and easy to implement. We recommend a similar strategy be employed in 2019, although with a closer transect spacing (e.g., 50 m) and using distance sampling if this method can be done without flagging the site ahead of time. We hope to continue monitoring SCS2 in 2019, although funding for work off of JBLM is perilously tight.

The doubling of the population at R50 in 2018 was a surprise, but is consistent with the findings of Thomas et al. (2011) that suggest where habitat quality is high, populations increase by increasing density. This is in keeping with the outward push of adults from the monitoring area that is evident in from the JBLM occupancy surveys (Appendix D, Fig. 1). While the R50 population met establishment criteria in 2016, 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.

Training Area 15 on Joint Base Lewis-McChord should continue to be the highest priority site for release, both because of abundant “reintroduction-ready” habitat (Waters 2016) and to reinforce the positive results obtained following initial releases in 2018 (Table 17 and Appendix B). This site represents our best opportunity to repeat the success observed at R50 and SCS, and with further research on larval habitat quality, could highlight habitat parameters critical to achieving translocation success (Thomas et al. 2011).

While TA7S has not performed well to date, far fewer larvae were released there than at GHP. In addition, the site supports three 5-ac “reintroduction-ready” units and while small, it is rich with checkerspot host and nectar resources as well as adult cover. Unlike most sites, the restoration challenge at TA7S is one of too much vegetation, even if much of it is native. This may be impacting suitability by reducing the number of suitable basking sites, especially for developing larvae. New approaches to the use of fire and herbicides appear to be increasing the amount of open ground on this site, which we hope will increase the likelihood that checkerspots will remain, lay eggs and that larvae will prosper. This site is another reminder that waiting to initiate releases until after habitat is fully restored is the best strategy (e.g., Thomas 1989, Oates and Warren 1990, Stamps and Swaisgood 2007, Thomas et al. 2011), and should be employed whenever possible. Unfortunately restoration has been hard to predict on most sites, and now faces further setbacks due to funding shortfalls.

No new releases are planned at GHP at this time due to our inability to maintain checkerspots at that site to date in spite of significant effort. Numerous factors may be contributing to these outcomes, but a better understanding of larval habitat quality, specifically pertaining to host plant abundance and microsite conditions can only shed light on the suitability of this site. 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 subsequent releases. In addition, like TA7S, the site was occupied historically, suggesting it is not wholly unsuitable and worth a more careful evaluation.

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 2018. 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 translocation by providing an alternative path to increasing the size and number of populations.

Funding for both captive rearing and translocation is in short supply, with current monies projected to last only until September 2019 unless new funds are secured from DoD, JBLM and/or the USFWS.

Additional funding has already been provided by JBLM to cover captive rearing costs in 2018-2019. A DoD Legacy grant was also submitted in January 2019 that could provide project support and enable some much needed research on larval habitat quality and improvements to program efficacy if funded. Funding is badly needed from DoD's ACUB program, which was the initial impetus for significant and widespread restoration off base, which sought to prepare sites for translocation of Taylor's checkerspot. Success at Scatter Creek owes in large part to this program, but may be difficult to maintain or replicate if the program does not resume very soon. Expansion of the rearing facility at Mission Creek, funded by the USFWS was designed to provide the additional animals needed for scaling up translocations, which are now restricted to JBLM due to funding availability.

A translocation plan for the Puget lowlands is being developed that will lay out a long-term approach for this project, including additional site selection criteria. The plan will be completed in collaboration with 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, especially those on the AIA, remains dynamic and unpredictable, and is a source of constant concern due to the fundamental dependence of the project on these occupied sites and the large amount of potential habitat on base. Access to R76 was sufficient to just complete the five surveys required at this high priority population source site, but we nearly missed out on a much needed opportunity to collect the females needed for captive rearing in 2018. It is unclear what level of access we might expect in 2019, although recent conversations suggest it is likely to be poor. This could add significant new challenges in the face of the captive rearing facility expansion, which is designed to relieve the conservation burden on DoD by expanding the number of translocation sites. The need for a consistent scheduling approach to address the stated needs has been a challenge every year since 2008, and its importance cannot be emphasized enough. The project has gone to great lengths to accommodate this uncertainty, even at a cost to monitoring on other sites. A more predictable management agreement is critical to reduce the time and effort required to meet the survey requirements as contracted.

Questions for Further Research

- 1) Does the availability of preferred larval habitat 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. They report that 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. This is because these studies typically describe short-term fluctuations of a single, relatively stable population rather than identify factors 1) that determine the carrying capacity of different sites, or 2) which drive long-term trends over larger geographic scales. Using data from the UK Butterfly Monitoring Scheme, Thomas et al. (2011) found 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: Preferred 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.

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

4) Climate change/weather: short-term synchronized changes in population size 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), which are identified as factors regulating population size or driving population change.

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. This research question was included in the DoD Legacy grant proposal submitted in January 2019.

2) What are the effects of weather and climate on the population dynamics of Taylor's checkerspot and how might they influence translocation 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 translocation, 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 translocation 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 Sequim 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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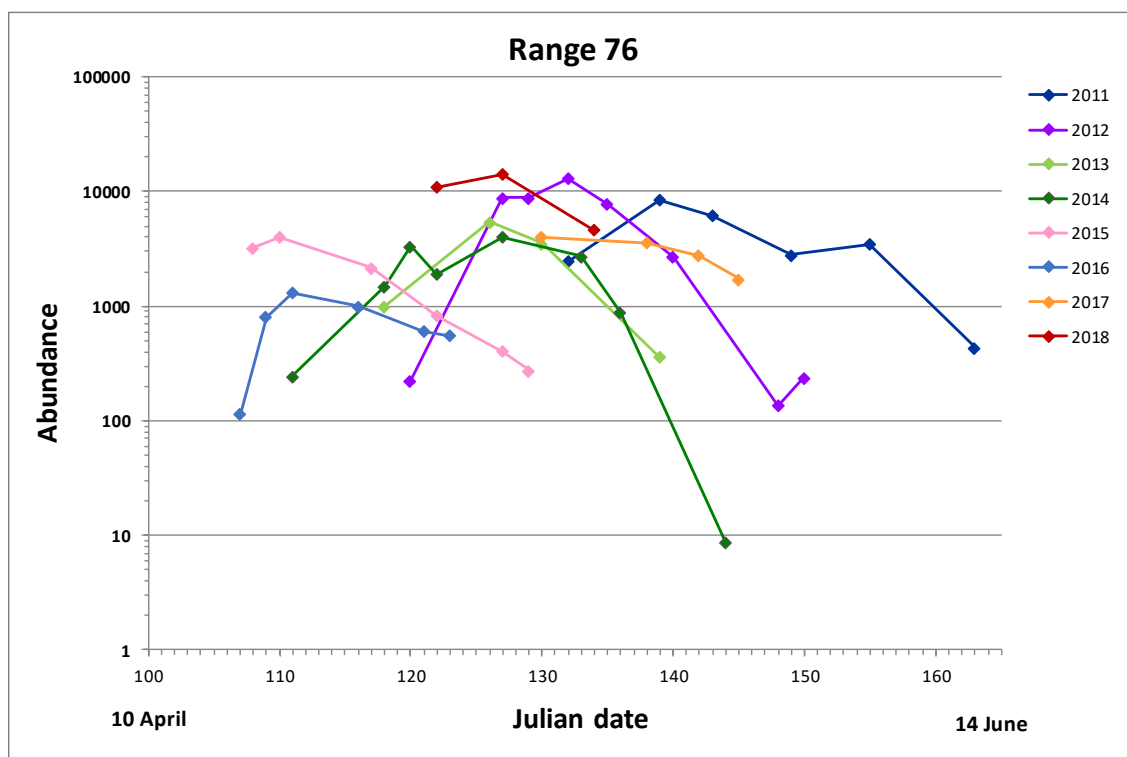
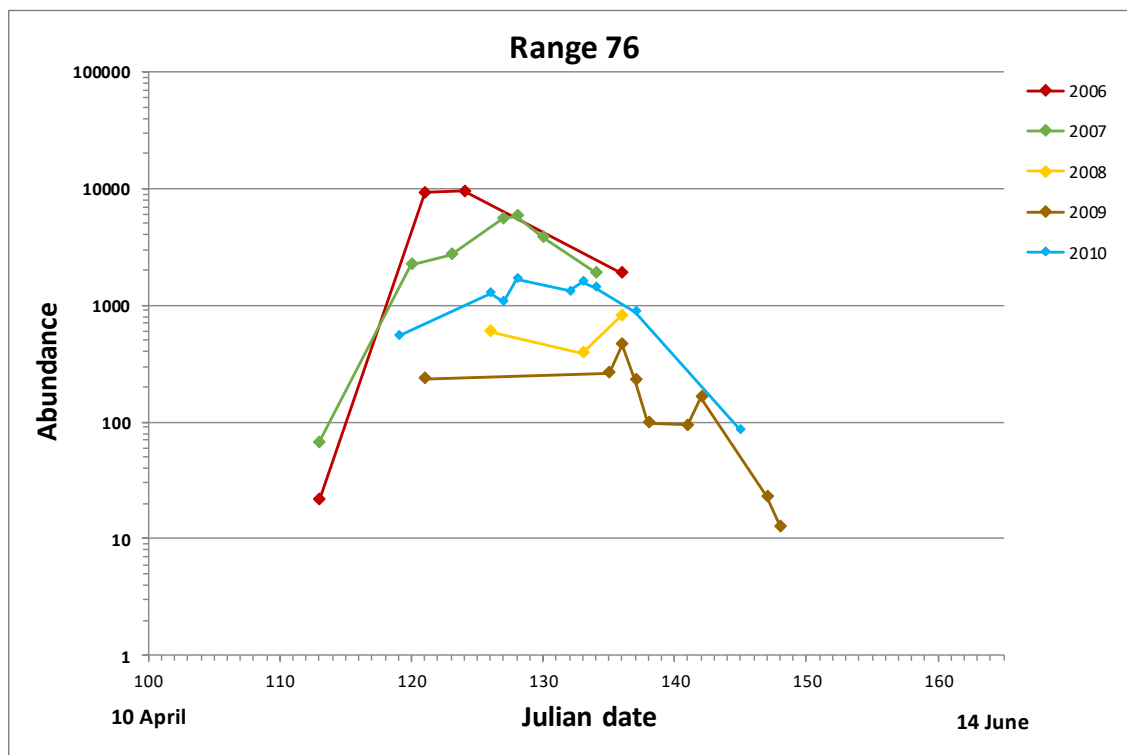
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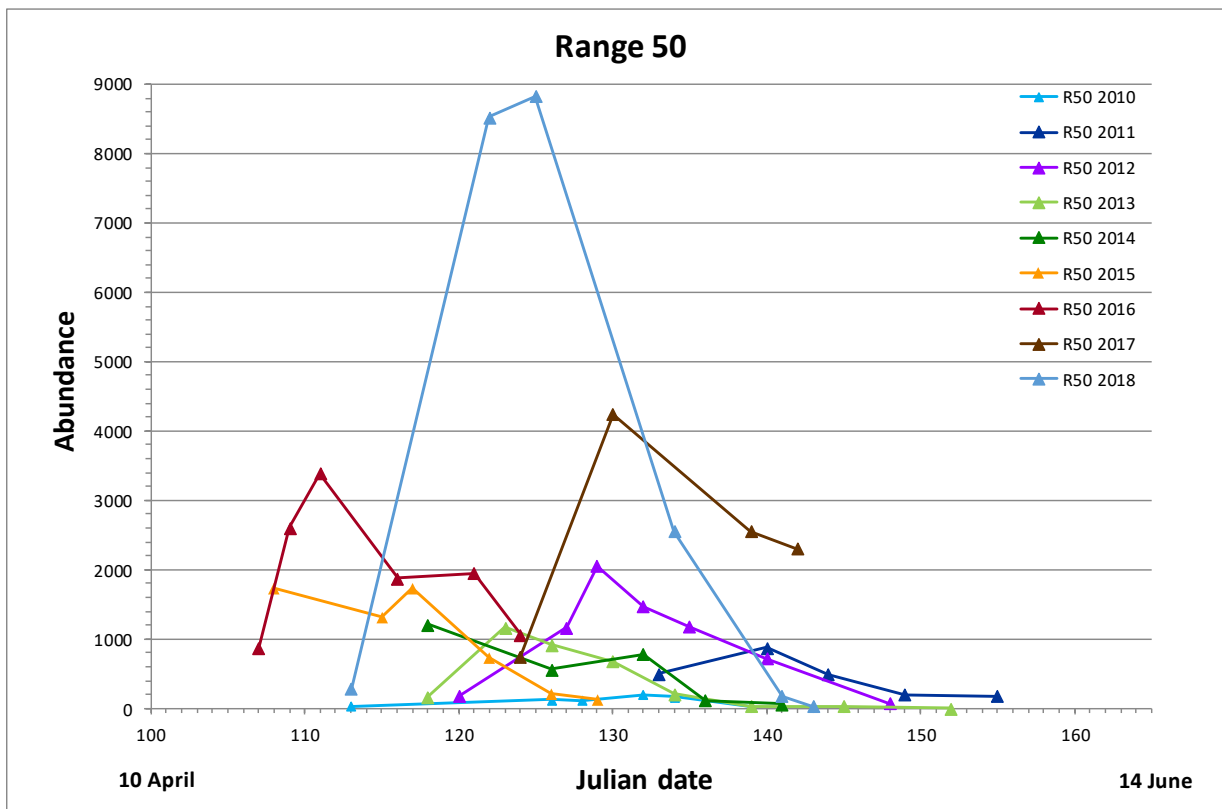
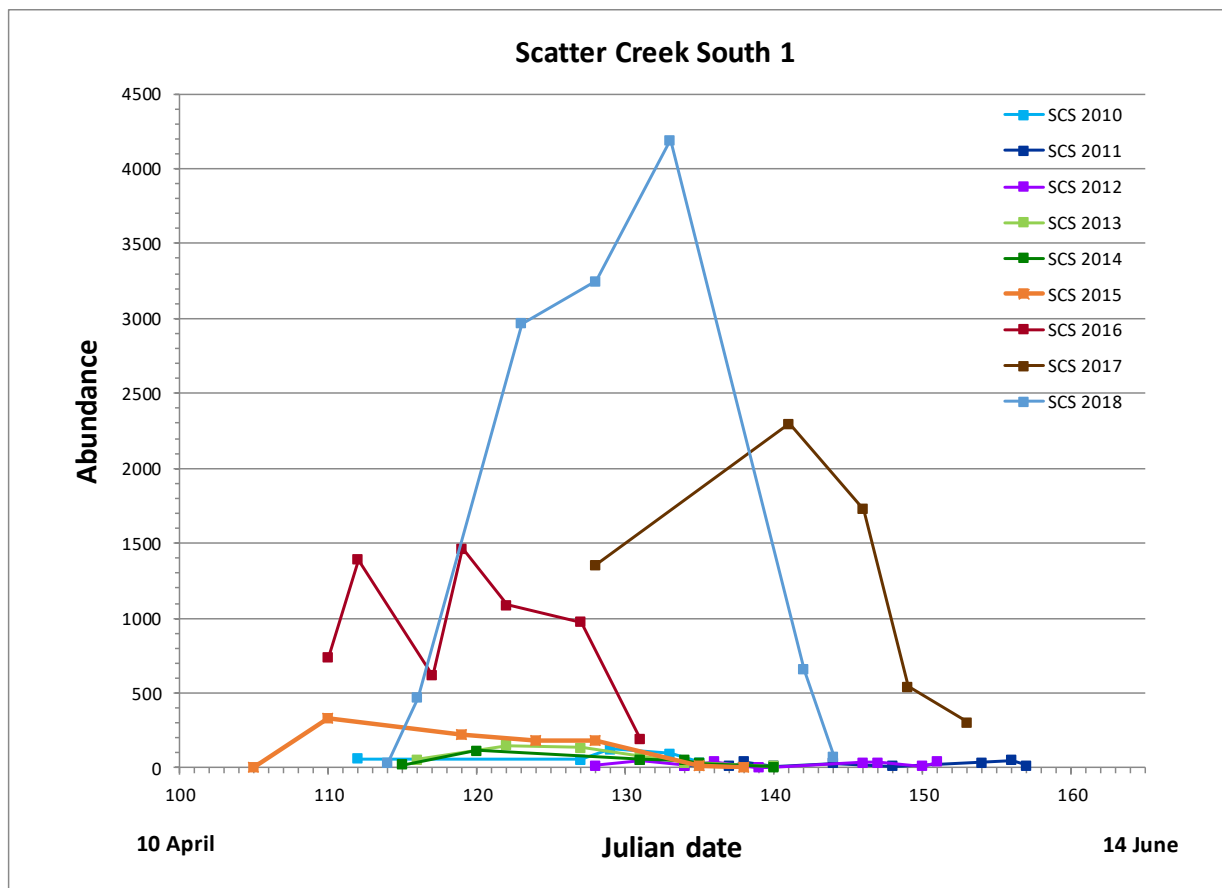
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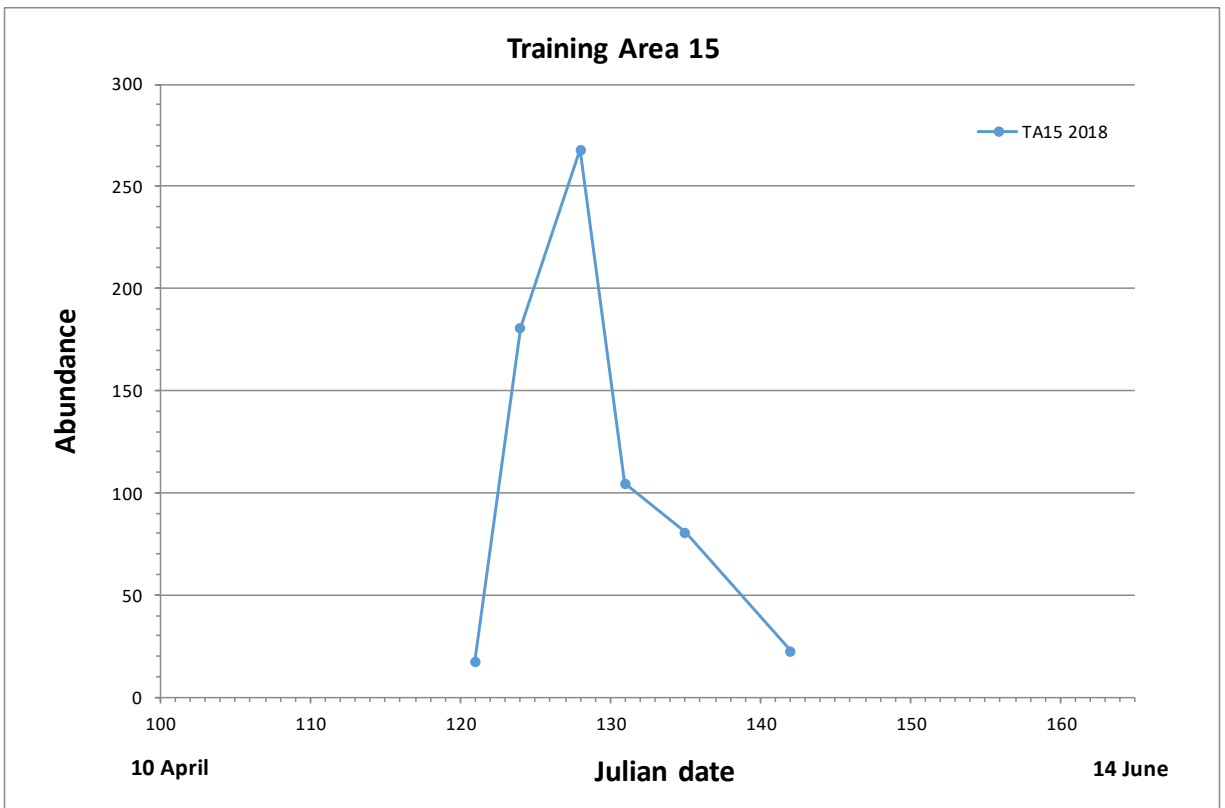
Appendix A. Approximate number of Taylor’s checkerspot butterflies released by life stage (Po = postdiapause larvae; Ad = adult; Pr = prediapause larvae) and site (SCS = Scatter Creek South; R50 = Range 50; GHP = Glacial Heritage Preserve; PCM = Pacemaker Airstrip; SCN= Scatter Creek North; TA7S = Training Area 7 South; TA15 = Training Area 15) in South Puget Sound, Washington, 2007-2018.

Year/Site	Stage	SCS	R50	GHP	PCM	SCN	TA7S	TA15
2007	Po	199						
2008	Po	340						
2009	Po	747			741	759		
	Pr	2487	2956					
2010	Po	891	1145					
	Ad	202						
2011	Po	1109	1141					
	Ad	167						
	Pr	1036						
2012	Po			975	1565			
	Ad	133						
2013	Po	3250		3372				
	Ad			107				
2014	Po			1522			1086	
	Ad	56						
2015	Po			1693			1102	
	Ad			226				
2016	Po	1658		2029				
	Ad						231	
	Pr						1003	
2017	Po			3024			909	
	Ad						145	
2018	Po							3336
	Ad						152	158
	Pr						1913	3262

Appendix B. Estimated daily abundance of Taylor’s checkerspot based on distance estimation, and timing of the flight season at: Range 76 (source site) in 2006-2010 (top), and in 2011-2018 (bottom); note log scale on the y-axis. Data for three translocation sites in South Puget Sound, Washington, are also shown with linear scales on the y-axis: Scatter Creek South Unit 1 (page 2 top) 2010-2018, Range 50 2010-2018 (page 2 bottom), and Training Area 15 2018 (page 3). Area surveyed varies by site and year; see Table 15 and text for details.







Appendix C. Distribution and number of adult Taylor's checkerspot observed during distance sampling surveys at extant site (R76) and translocation sites in South Puget Sound, Washington, spring 2018.

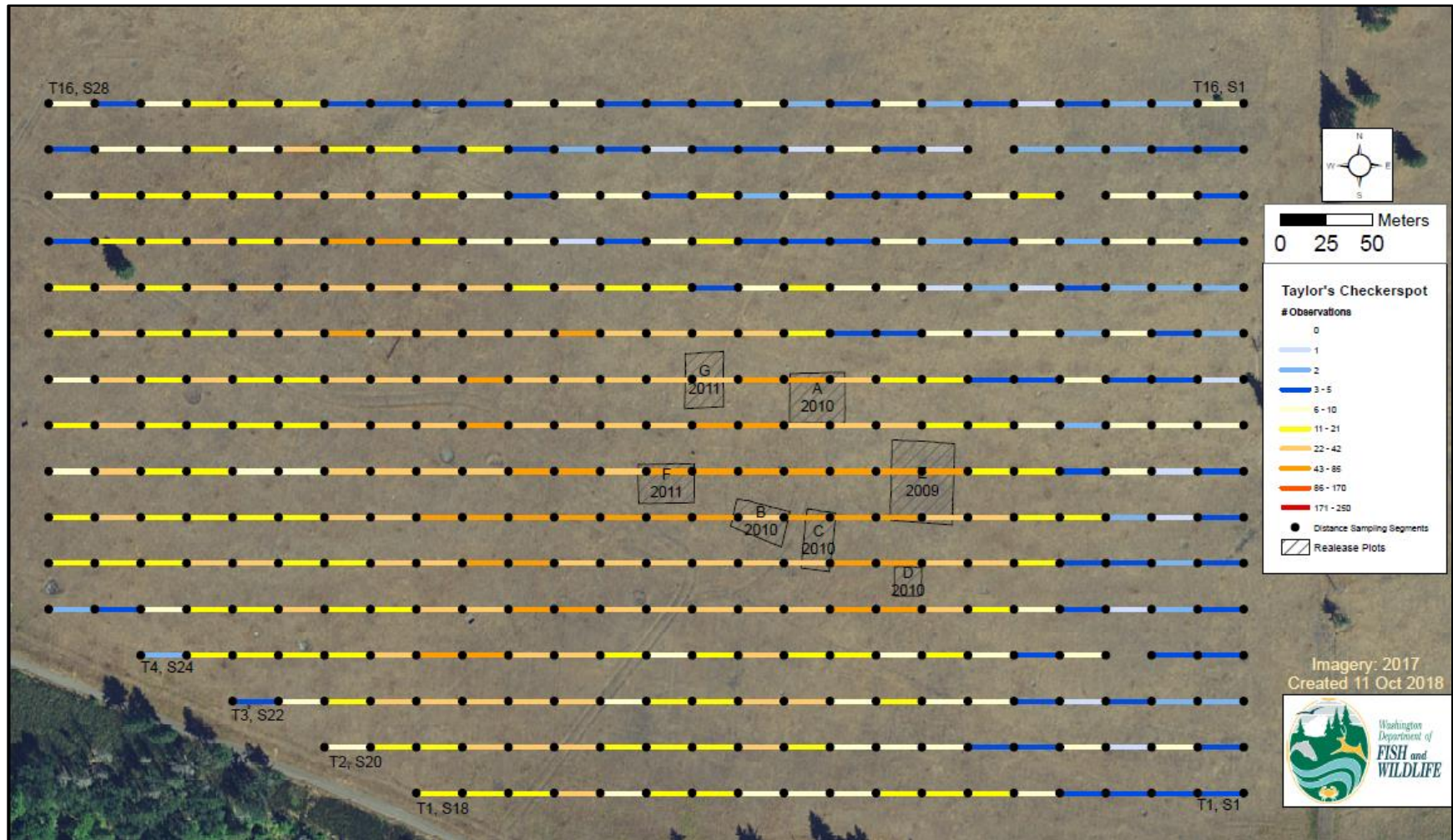


Figure 1. Distribution and number of adult Taylor's checkerspot observed during distance sampling surveys at Range 50 combined across all survey dates in spring 2018, South Puget Sound, Washington.

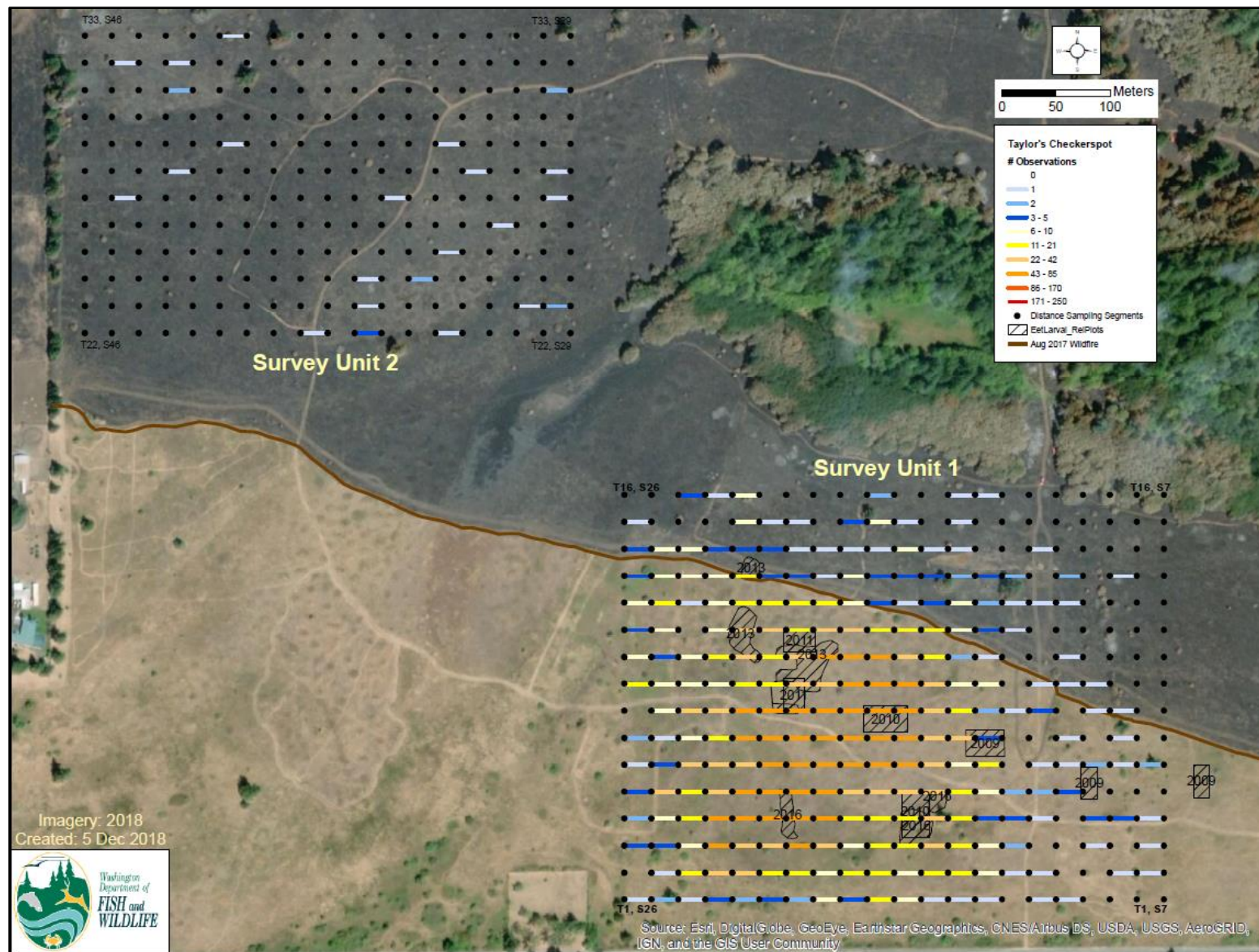


Figure 2. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Scatter Creek South in survey areas 1 and 2, combined across all survey dates in spring 2018, South Puget Sound, Washington.



Figure 3. Two distance sampling areas surveyed at Glacial Heritage Preserve in 2018 based on the prevalence of *Castilleja hispida* (GHP2, right) and *C. levisecta* (GHP2L, left); only two adult Taylor's checkerspot were observed at the sites in spring 2018, South Puget Sound, Washington.



Figure 4. No adult Taylor's checkerspots were observed during distance sampling surveys at Training Area 7S prior to release of adults, data combined across all survey dates in spring 2018, South Puget Sound, Washington.

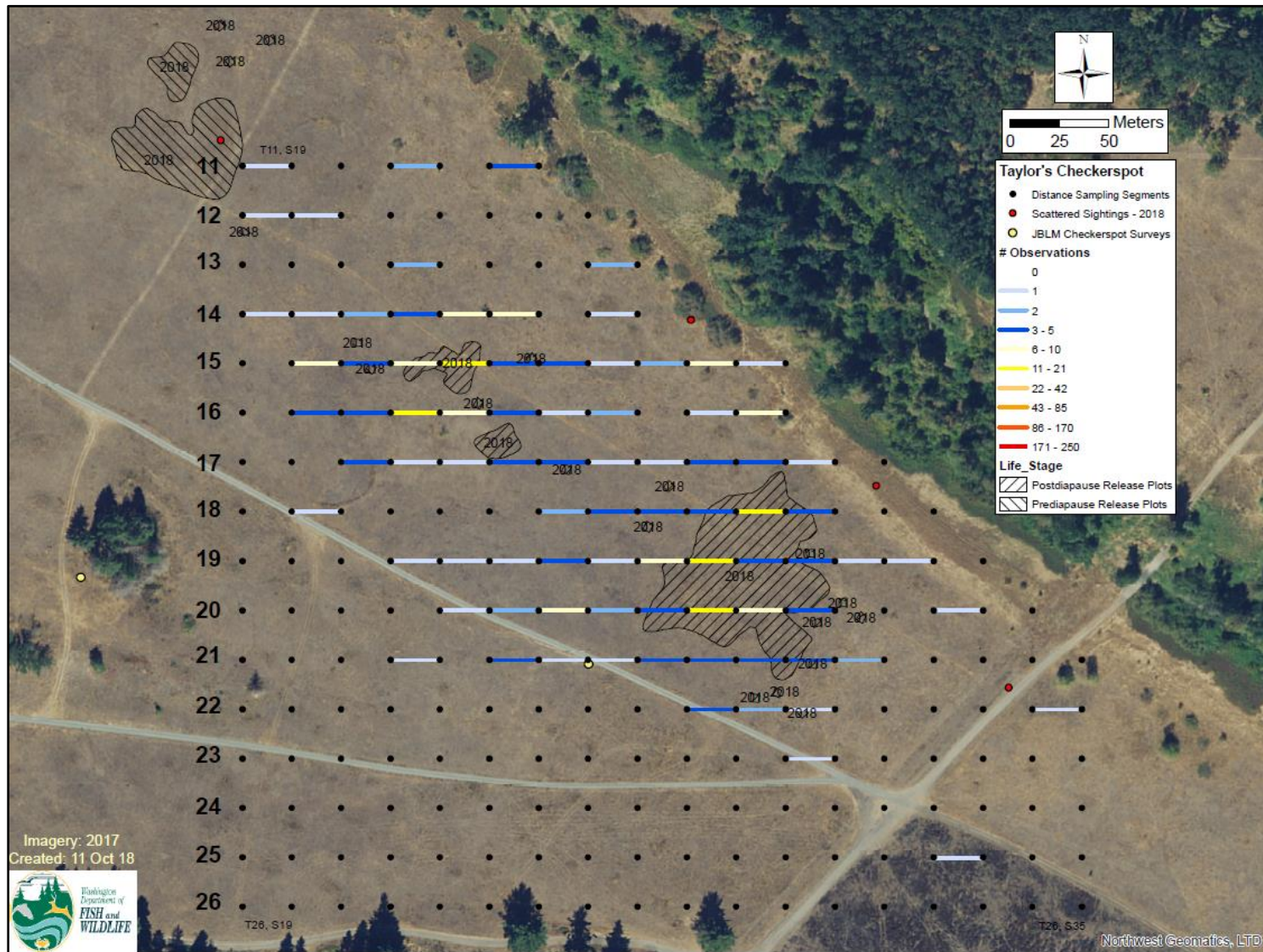


Figure 5. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Training Area 15, combined across all survey dates in spring 2018, South Puget Sound, Washington.

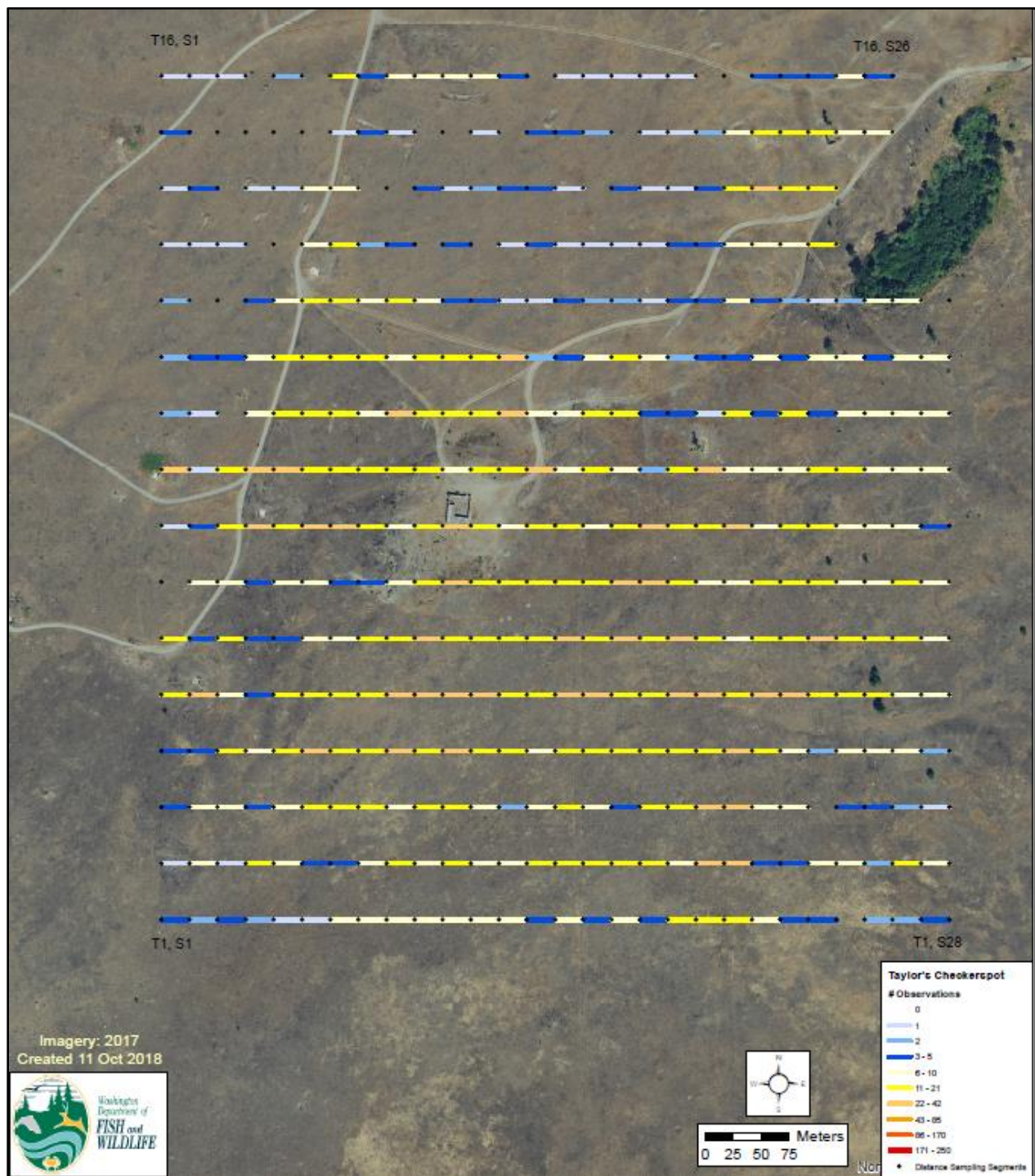


Figure 6. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Range 76, combined across all survey dates in spring 2018, South Puget Sound, Washington.

Appendix D. Scattered sightings of Taylor's checkerspot and 100-m transect survey results.

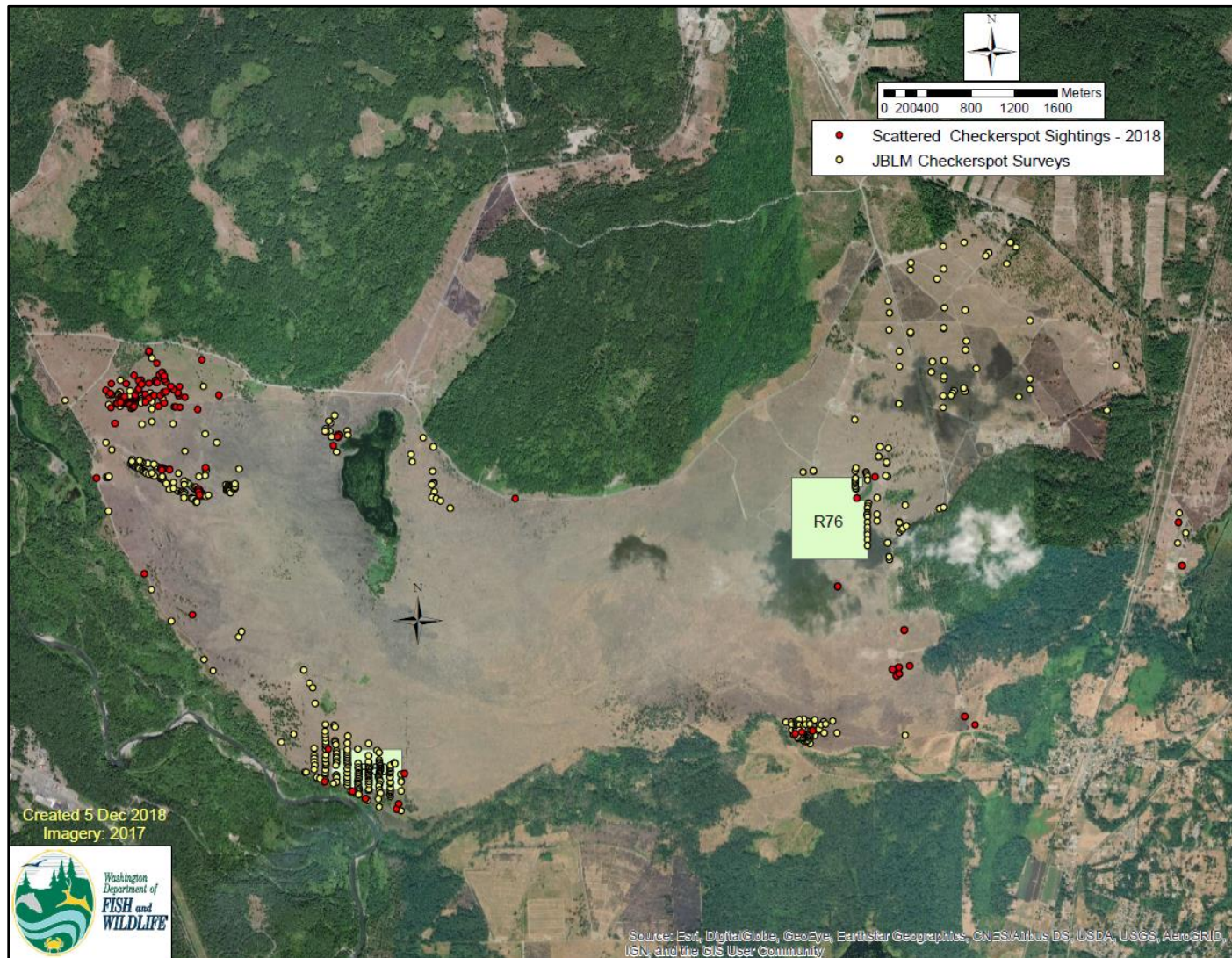


Figure 1. Adult Taylor's checkerspot observations (scattered sightings and JBLM's transect survey results) on the Artillery Impact Area (AIA) relative to standard monitoring areas (light green shade), Joint Base Lewis-McChord, Washington, 2018. North-south transect surveys with a 100-m spacing around the periphery of the AIA were implemented by JBLM staff and partners, with 25-m east-west transects employed at MP13 (lower right).

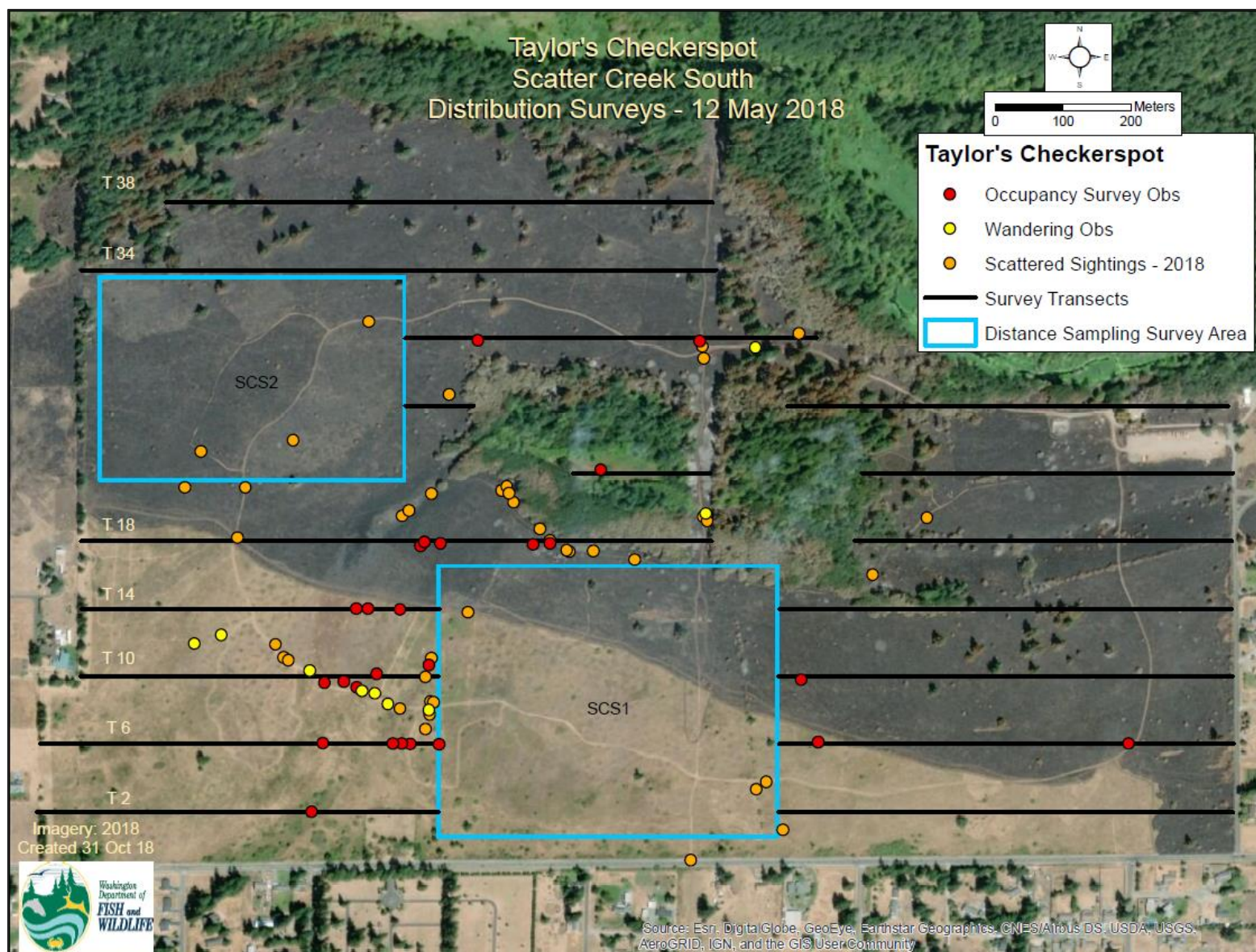


Figure 2. Adult Taylor's checkerspot observations (scattered sightings and 100-m transect survey results) at Scatter Creek South relative to standard monitoring areas (blue boundaries). Wandering observations were recorded in route to start and end points.