Taylor's checkerspot (*Euphydryas editha taylori*)
Captive Rearing and Translocation:
South Puget Sound, Washington, 2018-2019

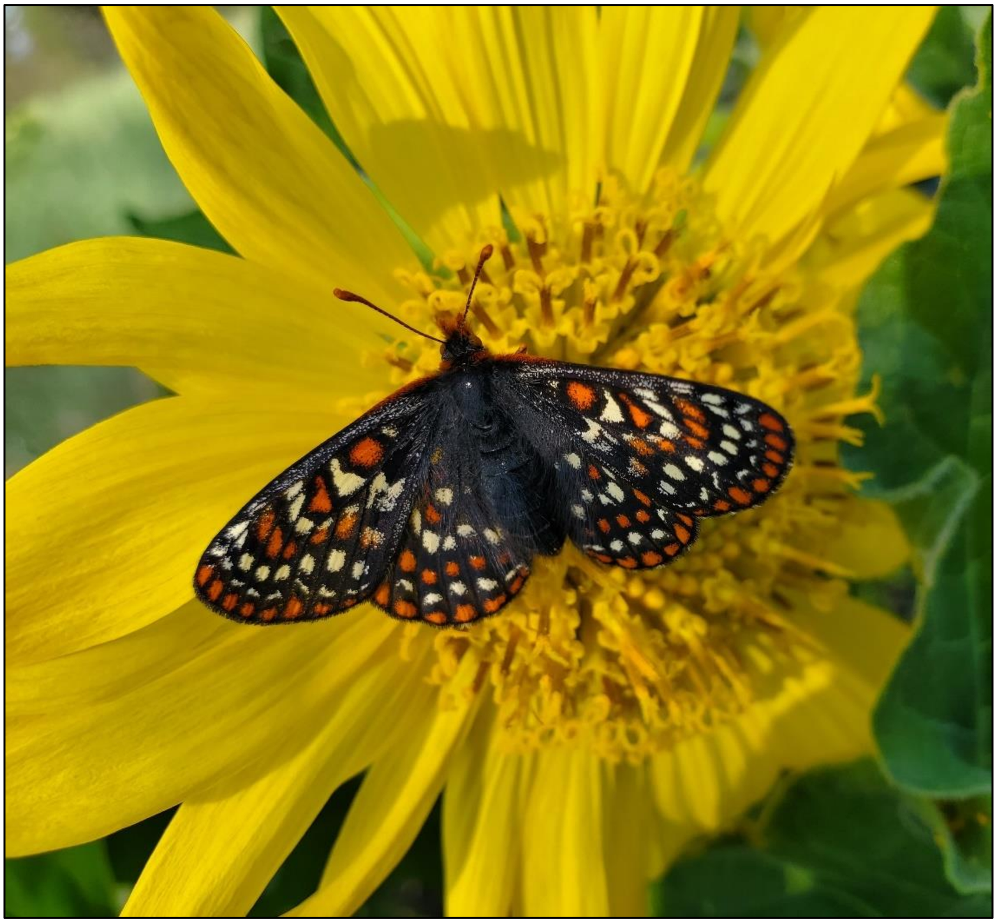

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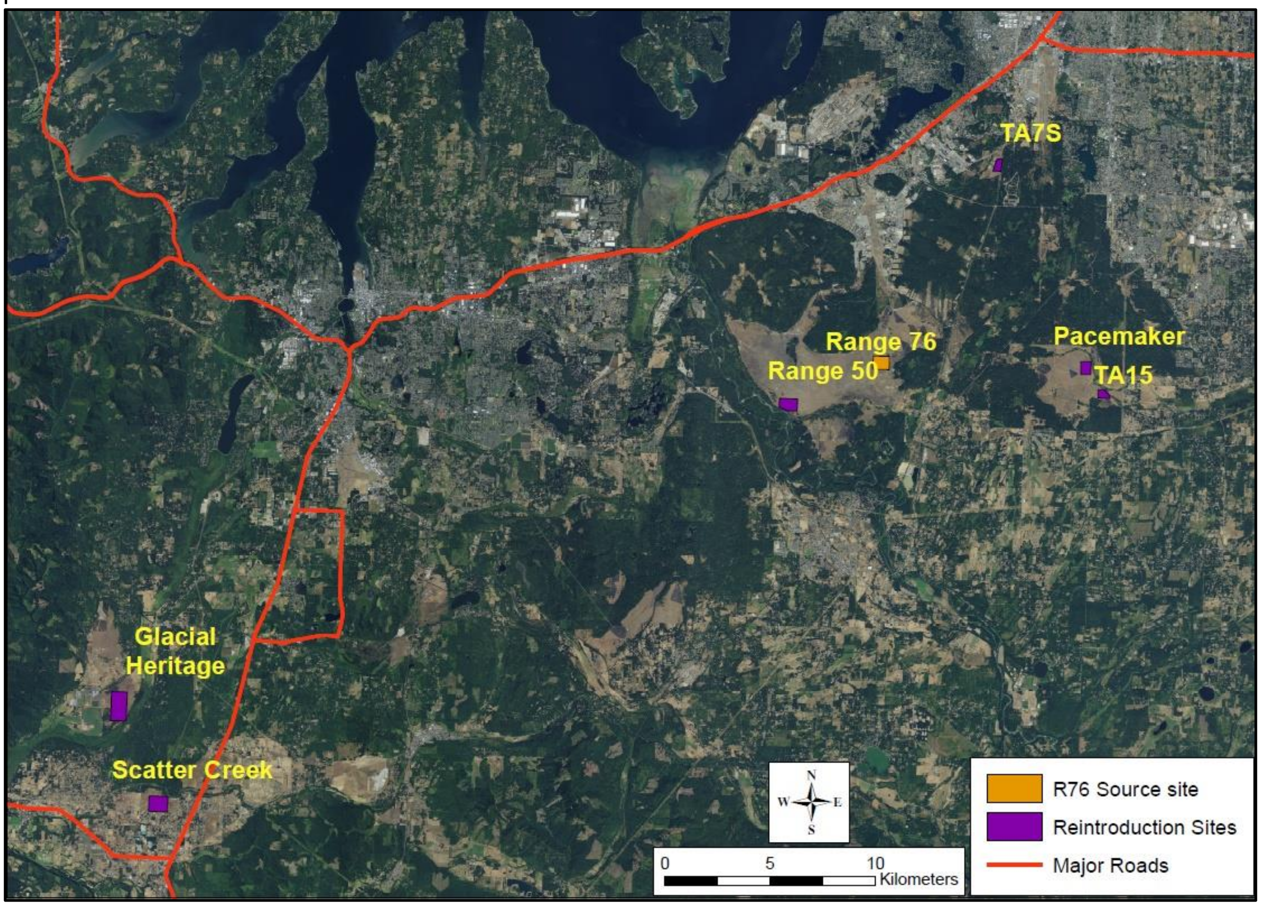
Table of Contents

List of Tables

List of Figures

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 July 2018-December 2019. The captive rearing section borrows heavily from annual reports produced by the Oregon Zoo (Lewis et al. 2019) and Mission Creek (Curry et al. 2019) programs. At least 9,200 eggs from wild females and 8,000 eggs from captive-bred females were harvested at captive rearing facilities at the Oregon Zoo and Mission Creek Corrections Center for Women in 2018. Due to high oviposition rates at Mission Creek and low productivity among captive females at the Zoo, 989 wild and 2,049 captive eggs were transferred to the Zoo to balance captive populations. In all, 15,371 prediapause larvae (8,546 wild and 6,825 captive) were produced, 5,175 were released prior to diapause (Linders et al. 2019) and 9,563 (7,458 wild, 2,105 captive) surviving larvae entered diapause in captivity in June 2018. Mortality increased at both facilities in the weeks following wake up. In both cases, poor food quality appeared to play a role in sickening larvae after heavy snow and persistent cold prevented harvest of *Plantago* from outdoor raised beds for several weeks. It is believed the same pattern was playing out in the field over the course of the 6-wk cold snap in late spring. Mortality at the Oregon Zoo from diapause to release was similar to levels in 2018 and remains significantly higher (49 percent) than normal (1-2 percent).

Unseasonably cold conditions that persisted through February and March 2019 significantly impacted host plant availability through top kill so much so that new release plots had to be established on three consecutive occasions prior to release. As a result, we were also unable to establish and monitor the plots typically used to estimate postdiapause larval survival following release. A total of 3,714 postdiapause larvae were released at TA7S on 10 and 15 Mar 2019, including 1,510 from Oregon Zoo and 2,204 larvae from Mission Creek. In addition, 2,847 larvae were released at TA15 (1,199 from Oregon Zoo and 1,648 from Mission Creek) on 17 Mar 2019.

At TA7S, 198 adults (85 females, 113 males) from the Oregon Zoo were released on 7 May 2019 with 194 adults (101 females, 93 males) from Mission Creek released at TA15 the same day. Seventeen pupae were also received from the Oregon Zoo on 7 May, most of which were reared to pupation prior to release. Of these, six females were released at TA7S on 9 May 2019, and six pupae and four adults (2 females, 1 male and on unknown) were released at TA15 on 11 May 2019; one pupa died. Nineteen females, including 8 wild females were released from the Zoo at TA15 on 17 May 2019 along with 3 males and 55 females from Mission Creek. Another relatively warm and dry spring resulted in good breeding conditions in captivity and good production from wild females, which led to release of 3,250 prediapause larvae that were in excess of what was needed in captivity were released at TA15 on 11 Jun 2019.

In 2019 we monitored the population source site (R76) to justify take, and to support military training at JBLM, and at Range 50 (R50; initiated in 2009), in case the site was needed for collection and to comply with JBLM's federal reporting requirements. Monitoring for establishment continued Scatter Creek South Unit 1 (SCS1; initiated in 2008), with a second unit (SCS2) monitored for evidence of colonization. We also monitored two active reintroduction (TA7S and TA15) sites for release success and population development. On all sites we used distance sampling to quantify daily population density, daily population size, and to illustrate the distribution of adults. A total of 16,464 checkerspot observations were recorded during distance surveys, with the greatest number observed at R50. Flight season initiation was intermediate in its timing relative to past years, with peak counts occurring on most sites 29 Apr-1 May. The peak count at SCS2 was one week later (8 May), suggesting it is influenced by dispersal from SCS1. The peak count at TA7S on 10 May followed a large release of adults 3 days prior, mimicking dispersal at SCS2. The peak day encounter rate at R50 (0.20 checkerspots /m) was about 30 percent lower than in 2018 and was comparable to SCS1 (0.22/m). At R76 (0.22/m for transects 1-16; 0.25/m for transects 1-12) encounter rates were slightly higher than in 2018. Peak day encounter rates

at TA15 (0.037 /m), SCS2 (0.014/m) and TA7S (0.004/m) also increased in 2019. 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 effectively occupy the entire 24.8-ha monitoring area at R50 for a second straight year, having increased their spread annually since 2014. However, the peak single day abundance estimate for 2019 (5,851; 95% CI: 3,927-8,716) was 34 percent lower than in 2018. This together with the widespread dispersal observed in surrounding areas, suggests this population may be at carrying capacity. Habitat monitoring using RHA should be resumed to evaluate current condition and ensure habitat threats are continuing to be addressed. In contrast, the peak single day abundance estimate for R76 (19,435; 95% CI: 14,596-25,877), was 36 percent higher than in 2018 and the population occupied nearly the entire monitoring area. Checkerspots at SCS1 returned a peak single day abundance estimate of 5,484 (95% CI: 4,214-7,137), up 13 percent from 2018 and well above the level required to meet establishment criteria for the 3rd year running and remains on track to meet all establishment criteria by 2021. Checkerspots at SCS1 occupied 93.0 percent of the 20-ha monitoring unit in 2019, with restoration continuing across the site. Similar to R50, checkerspots at SCS 1 are dispersing into surrounding habitat where colonization potential is high (e.g., SCS2). One adult checkerspot was observed at Glacial Heritage in 2019, although no formal surveys were conducted. Releases were discontinued at GHP in 2018 and will not resume without a better understanding of the factors affecting success, which may include the condition of food plants, availability of microsites, pesticide residues or other unidentified factors.

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 2018-2019 activities but includes some longer-term summaries as well. Objectives for 2019 were to:

1) Identify areas for release of larvae in collaboration with land managers,
2) Maintain 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) Release excess adults and prediapause larvae as appropriate.
5) 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, and the Washington Department of Corrections. For clarity and cohesion, this report covers all 2018-2019 captive propagation and translocation activities regardless of funding source.

This 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 sq m (0.37 ac) of habitat (or 1 sq m per larva) with abundant host plants including at least 20,000 sq m (about 5 ac) 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.

Within release plots, we flagged locations for larval release where *Plantago* or *Castilleja* plants were expected to be at least 1000 cm^3 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 (2014-2019), and TA15 (2018-2019) 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.

Changeable conditions in winter 2019 significantly impacted host plant availability. Warm conditions, which prevailed in January, caused many *Plantago* plants to break dormancy and set the stage for a very early season. By early February, however, unseasonably cold conditions set in, followed in mid-February by 21 inches of snow that lingered for over a week. Even after the snow melted, additional periods of freezing temperatures persisted well into March which repeatedly top-killed emerging host plants (Fig. 1). To encourage *Plantago* to break dormancy and spur growth, small shelters made from inverted berry baskets with clear lids were secured over release plants to serve as miniature greenhouses. Grass clumps were also wrapped around the north side of the plants as a backstop to increase microsite temperature (Fig. 1). Ultimately, release plots were moved three times prior to release to ensure release plants were producing new green growth. As a result of this effort, we were unable to establish and monitor the plots typically used to estimate postdiapause larval survival following release.

Figure 1. Host plant (*Plantago lanceolata*) top-killed by cold weather (left); inverted berry basket with lid used to spur growth and development of host plants on release sites for Taylor's checkerspot larvae (center), and host plant benefitting from a warm microsite created by a pile of dry grass on its north side, Puget Lowlands, Washington, March 2019.

In 2019 postdiapause releases occurred at TA15 (2nd year) and TA7S (6th year), consistent with our existing strategy (Linders et al. 2015b). We did not prioritize a prediapause release at TA7S due to restoration constraints, preferring instead to maximize the number of larvae at the newer release site, TA15. We established four postdiapause and two prediapause release plots at TA15 (Appendix C, Fig. 4), and one postdiapause release plot at TA7S (Appendix C, Fig. 3). 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. 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. Both TA 7S and 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 0.50 sq m/larvae of habitat at TA15.

Table 1. Size and area of release 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 2019.

Site	Plot ID	Sq meters	Acres
TA 7S - postdiapause	D	**4039.8**	**0.998**
TA 15 - postdiapause	A	1651.6	0.408
	C	489.2	0.12
	F	1152	0.284
	G	306.8	0.075
	H	275.3	0.068
Postdiapause total		**3874.9**	**0.955**
TA15 - prediapause	I	483.1	0.119
	J	1169.1	0.288
Prediapause total		**1652.2**	**0.407**

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. 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 using the guidelines that follow. 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 general guidance from the Population Management Center (Schad 2008). Wild females are collected with the aim that they will supply about half of the 10,000 eggs (5,000 per facility) needed, with the remaining half 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 24 gravid wild females were collected from R76 on JBLM with same day delivery to Mission Creek (10 on 8 May; 14 on 14 May 2018, respectively) following methods reported previously (Linders and Lewis 2013). In addition, 19 females were collected on 8 May 2018 from R76 in addition to one misidentified male; all were delivered to the Oregon Zoo the same day in good condition.

Captive mating

Methods

In all, 193 adults from 8 maternal lines originating as eggs in 2017 (17FL and 17SC) were available for inclusion in the captive breeding colony at the Oregon Zoo in 2018. Similarly, 220 adults from 20 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 whereby the first matriline enters the chain when 40 percent of postdiapause larvae pupated (Lewis et al. 2015). Remaining matrilines were added once females eclosed, which ensures that males, which eclose first, are 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).

Results and Discussion

The Oregon Zoo conducted breeding introductions between 25 April and 8 May 2018, with copulations taking place between 25 April and 5 May (Table 3). Twenty-eight females did not copulate despite seven of those having multiple opportunities. At Mission Creek, copulations occurred between 2 and 9 May, from introductions spanning 30 April to 9 May 2018 a total of 12 MC females did not copulate (Table 3). Copulation rates were markedly different between facilities. Those at Mission Creek showed a sharp

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, spring 2018.

Matriline	Adults	Females	Males
Oregon Zoo			
17FL705	59	24	35
17FL710	30	15	15
17SC735	30	20	10
E17FL742	7	3	4
E17FL761	4	2	2
E17FL764	25	18	7
E17FL768	16	13	3
E17FL769	22	16	6
OZ Total	**193**	**111**	**82**
Mission Creek			
15FL551	2	1	1
15FL557	3	1	2
16FL613	3	2	1
16FL614	7	2	5
16FL622	2	2	-
16FL625	3	-	3
16FL630	5	3	2
16FL643	12	7	5
16FL649	5	3	2
16FL655	4	3	1
17FL701	21	8	13
17FL703	24	10	14
17FL705	21	8	13
17FL708	5	1	4
17FL709	18	10	8
17FL712	16	5	11
17FL716	14	4	10
17FL719	20	8	12
17FL720	26	11	15
17FL723	9	5	4
MC Total	**220**	**94**	**126**

improvement over 2017, presumably as a result of re-aligning breeding methods with those developed previously (Lewis et al. 2013), especially retaining more larvae per matriline, aging males for 3 days prior to introducing females and using all available males (Linders et al. 2019). In addition, they identified that the ceiling of the greenhouse was a prime location for breeding success. In contrast, breeding success at the Zoo was again suppressed, perhaps due to adults being in poor condition as a result of larval development issues.

Captive rearing: oviposition to adult

Methods

Taylor's checkerspots 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 10,000 eggs in 2018. 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).

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.

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 no longer needed for breeding were released.

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 2018.

Female ID	Male ID	Females	Intros	Copulations	Productive females
Oregon Zoo					
17FL705	17FL710	10	11	5	2
17FL710	17FL705	9	16	1	0
17SC735	17FL710,	10	13	2	0
E17FL742	17FL705	3	4	2	2
E17FL761	17FL710	1	4	0	- - -
E17FL764	17FL705	5	5	2	0
E17FL768	17FL710	2	2	2	0
E17FL769	17SC735	3	3	1	1
OZ Total	**NA**	**43**	**58**	**15**	**5**
Mission Creek					
15FL551	16FL630	1	1	1	1
15FL557	16FL643	1	1	1	1
16FL613	16FL643	2	2	1	1
16FL614	16FL649	1	1	1	1
16FL622	16FL614	2	2	2	2
16FL630	15FL557	1	1	1	1
16FL643	16FL614	2	2	2	2
16FL649	16FL614	1	1	1	1
16FL655	15FL551	1	1	1	1
17FL701	17FL703	7	10	5	3
17FL703	17FL723	2	6	1	1
17FL705	17FL701	7	9	4	4
17FL708	17FL712	1	1	1	1
17FL709	17FL705	8	10	6	4
17FL712	17FL720	2	2	2	2
17FL716	17FL708	3	3	3	3
17FL719	17FL716	4	4	3	2
17FL720	17FL719	2	2	2	2
17FL723	17FL709	6	8	4	3

MC Total	NA	54	67	42	36

Results and Discussion

At Mission Creek, 20 of 24 (83 percent) wild females laid eggs that hatched (i.e., were productive), with 17 of 19 (89 percent) wild females productive at the Oregon Zoo in 2018 (Table 4). At Mission Creek, 86 percent of captive-mated females were productive in contrast to 33 percent at the Oregon Zoo (Tables 3 and 5). At least 9,199 eggs from wild females and 8,005 eggs from captive-bred females were harvested at the two facilities. Eight captive-bred females at the Zoo laid 988 eggs that did not hatch, so 989 eggs from wild females and 2,049 eggs from captive females were transferred from Mission Creek to the Zoo for rearing (Tables 4 & 5). Numerous egg to hatch ratios >1.0 (Table 5) at Mission Creek indicates persistent undercounting despite efforts to standardize techniques, which invalidates efforts to track egg to hatch ratios. Survival from egg to hatch is the most variable life stage both in captivity and in the wild and is influenced by several factors outside of our control including the amount of sunlight during oviposition and hatching. For this reason, we are re-evaluating how egg data are collected and whether they will be tracked, since the hatch count is ultimately more reliable.

Table 4. Oviposition outcomes to diapause by female for wild-caught Taylor's checkerspots reared at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2018. Only productive females (i.e., laid eggs that hatched) are included; number hatched taken at 3rd instar.

Female ID	Eggs laid	Eggs Retained	Hatched	Prediapause Release	Diapause In
Oregon Zoo					
P18FL801	361		317	0	313
P18FL803	297		252	0	246
P18FL806	152		125	0	125
P18FL807	210		187	0	183
P18FL808	325		276	0	273
P18FL812	336		295	0	290
P18FL813	163		148	0	146
P18FL815	532		489	0	475
P18FL816	220		215	0	202
P18FL817	311		305	0	304
P18FL818	276		276	0	271
P18FL820	190		177	0	175
P18FL823	407		399	0	397
P18FL824	405		371	0	363
P18FL826	357		346	0	337
P18FL827	250		255	0	253
P18FL829	221		172	0	169
Subtotal	*5266*		*4605*	*NA*	*4522*
Ave	292.6		255.8	NA	266.0
St Dev	98.0		113.4	NA	94.6
Eggs transferred from Mission Creek to Oregon Zoo					
P18FL809	179		171	170	0
P18FL811	154		148	148	0
P18FL814	160		153	152	0
P18FL831	174		166	96	68[1]
P18FL833	78		70	0	70
P18FL838	129		121	0	103
P18FL844	115		113	0	109[1]
Subtotal	***989***		***942***	***566***	***350***

Total	**6255**		**5547**	**566**	**4872**
Mission Creek					
P18FL802	61	61	60	0	62
P18FL804	161	161	166	0	163
P18FL809	333	154	162	0	159
P18FL811	316	162	211	0	208
P18FL814	315	155	167	0	165
P18FL819	240	240	235	0	230
P18FL822	221	221	223	0	219
P18FL825	217	217	236	0	235
P18FL828	311	311	328	0	307[2]
P18FL831	225	51	51	0	49
P18FL832	127	127	132	0	130
P18FL833	180	102	87	0	86
P18FL834	169	169	155	0	155
P18FL835	156	156	156	0	157
P18FL837	183	183	165	0	165
P18FL838	307	178	174	0	128
P18FL840	48	48	48	0	46
P18FL842	67	67	72	0	60
P18FL843	97	97	87	0	85
P18FL844	199	84	84	0	84
Total	**3933**	**2944**	**2999**	**0**	**2586**
Ave	**196.7**	**147.2**	**150.0**	**0.0**	**136.1**
St Dev	**89.5**	**69.2**	**74.0**	**0.0**	**62.0**

[1] One larva did not diapause and developed directly to the adult stage in 2019.
[2] Nine larvae did not diapause and developed directly to the adult stage in 2019.

In all, 15,371 prediapause larvae (8,546 wild and 6,825 captive) were produced, with 5,175 larvae released prior to diapause (Linders et al. 2019) and 9,563 larvae (7,458 wild, 2,105 captive) entering diapause in captivity (Tables 4 and 5). Diapausing larvae were moved outdoors for cold diapause on 30 September 2018 at the Oregon Zoo, and 12 September 2018 at Mission Creek. Once in diapause most larvae are inactive, although some movement is normal, especially in late diapause (Fig. 2). In contrast to 2018, larval movement in 2019 was high across facilities in late January during relatively warm conditions (low 50s F.) but was most pronounced at Mission Creek. Larvae settled in response to much cooler temperatures in early February (30s) before increasing activity again later in the month (Fig. 2).

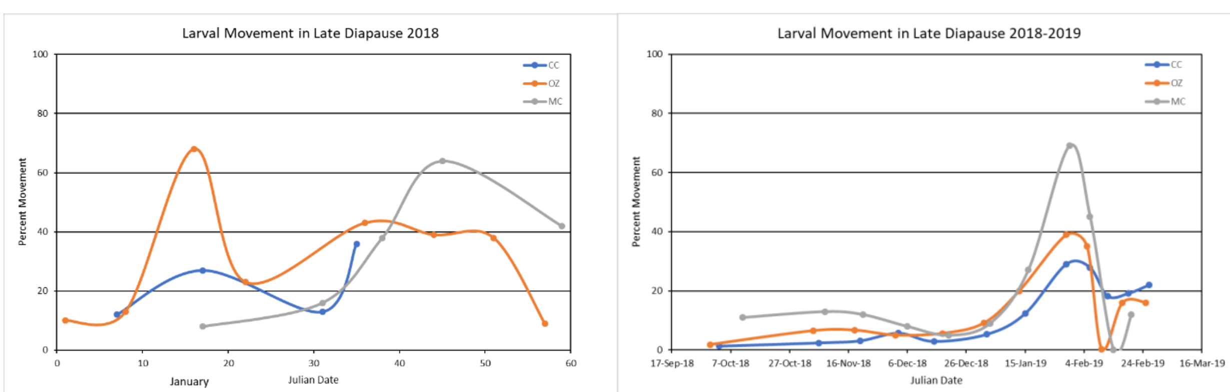


Figure 2. Percent of diapausing Taylor's checkerspot larvae by rearing institution that moved their location within diapause cups between checks, winter 2018 (left) and 2018-2019 (right). CC = Coffee Creek Corrections Center, OZ = Oregon Zoo, Portland, Oregon; MC = Mission Creek Corrections Center, Belfair, Washington.

Monitoring diapause movement across facilities in different geographic areas can improve our understanding of whether and how movement may relate to mortality at the Oregon Zoo and Coffee Creek facilities (Oregon population) in Portland. Movement at Coffee Creek followed movement patterns similar to the Zoo's in 2019 and to a lesser degree in 2018 (Fig. 2). Movement at the Zoo in 2018 tracked both the record warming in January (Mass 14 Jan 2018) and the record cold in mid-February (Mass 19 Feb 2018). Tracking diapause movement in different parts of their range may also help us understand how checkerspots respond to their environment in terms of behavior and survival in the face of climatic perturbations. As in 2018, larvae from both facilities were pulled from diapause in three batches between 28 February and 5 March, 7-10 days later than in years past. This was done to manage food resources, workloads, and weather-related uncertainty.

To support a captive breeding, Mission Creek retained 668 larvae from 19 matrilines including 24 second diapause (17FL) larvae (Table 6). At the Zoo, 310 larvae from ten 2018 matrilines were retained for breeding (Table 7). Only offspring of wild adults were retained for breeding. All offspring of captive-mated adults and those from wild females in excess of those needed for captive breeding were released.

Table 5. Oviposition outcomes for captive-mated Taylor's checkerspots by female at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2018. Only productive females (i.e., laid eggs that hatched) are included; number hatched is taken at 3rd instar.

Female ID	Eggs	Eggs Retained	Hatched	Prediapause Release	Diapause In
Oregon Zoo					
17FL705-14P	345		315	NA	310
17FL705-24F	98		1	NA	1
E17FL742-4P	87		42	NA	41
E17FL742-5P	355		4	NA	4
E17FL769-22F	106		13	NA	13
Total	**991**		**375**	**0**	**369**
Ave	**198.2**		**75.0**	**NA**	**73.8**
St Dev	**138.8**		**135.1**	**NA**	**133.0**
Eggs transferred from Mission Creek to Oregon Zoo					
15FL51-02	83		6	6	0
15FL57-02	23		23	23	0
16FL13-03	134		116	113	3
16FL14-06	141		138	138	0
16FL22-01	171		114	112	2
16FL22-02	194		98	94	3
16FL30-06	143		134	134	0
16FL43-04	174		144	119	6
16FL43-09	136		130	130	0
16FL55-02	82		119	119	0
17FL712-15	71		63	61	2
17FL712-18	80		83	83	0
17FL719-09	40		46	46	0
17FL719-12	154		151	151	0
17FL720-08	163		55	51	0
17FL720-26	130		129	119	4*

17FL703-23	130		129	129	0
Total	**2049**		**1678**	**1628**	**20**
Ave	**120.5**		**98.7**	**NA**	**3.3**
St Dev	**48.9**		**44.6**	**NA**	**1.5**
Mission Creek					
15FL551-02	213	110	102	0	102
15FL557-02	85	50	69	0	70
16FL613-03	231	97	96	96	0
16FL614-06	304	163	169	89	77
16FL622-01	474	336	254	181	71
16FL622-02	194	0	0	0	0
16FL630-06	197	54	60	0	61
16FL643-04	256	82	69	0	54
16FL643-09	272	136	105	0	105
16FL649-03	151	151	138	0	135
16FL655-02	140	58	70	0	69
17FL701-17	168	168	138	32	103
17FL701-18	180	180	195	44	149
17FL701-20	185	185	190	62	122
17FL703-23	271	133	100	95	5
17FL705-14	200	200	208	159	46
17FL705-17	117	117	65	0	55
17FL705-18	162	162	154	117	37
17FL705-19	188	188	203	117	85
17FL708-05	31	31	27	27	0
17FL709-09	143	143	157	110	47
17FL709-14	106	106	140	142	0
17FL709-18	26	26	26	26	0
17FL709-19	185	185	183	183	0
17FL712-15	237	166	174	163	11
17FL712-18	274	194	184	47	136
17FL716-12	100	100	102	102	0
17FL716-14	126	126	137	137	0
17FL716-15	124	124	129	129	0
17FL719-09	216	176	152	152	0
17FL719-12	250	96	85	85	0
17FL720-08	300	137	83	28	54
17FL720-26	302	172	171	46	122
17FL723-05	241	241	264	264	0
17FL723-07	116	116	120	120	0
17FL723-08	256	256	253	253	0
Total	**7021**	**4965**	**4772**	**3006**	**1716**
Ave	**195.0**	**137.9**	**132.6**	**NA**	**47.7**
St Dev	**86.7**	**67.7**	**64.9**	**NA**	**49.3**

Increased mortality was observed at both facilities in the weeks following wake up. At Mission Creek larvae took over a week to begin feeding rather than a few days, and appetites remained low. This was more severe than similar observations in 2018, which also suffered a mid-February cold snap (Linders et al. 2019). Some larvae had diarrhea and then died; others recovered. Symptoms were not evident until after the second field release (15 March 2019) and appeared more common in captive-bred larvae than those from wild females. Multi-diapause larvae were the most affected group (Curry et al. 2019). At the Zoo, as in recent years (e.g., Linders et al. 2018, Linders et al. 2019), there was an initial spike in mortality (~ 19 percent) following wake-up in 2019 (Lewis et al. 2019). However, one week later, larvae at the Zoo also had diarrhea and were lethargic, resulting in another mortality (~ 45 percent) spike.

In both cases, poor food quality appeared to play a role in sickening larvae, after heavy snow and persistent cold prevented harvest of *Plantago* from outdoor raised beds for several weeks. Instead all available potted plants were heavily harvested, including plugs destined for out-planting. Feeding from such plants has raised concerns in the past due to questions about fertilizer and plant chemistry (Linders et al. 2019). At release sites, host plants also exhibited retarded growth and larvae were noted missing from locations where previous feeding had been observed, suggesting the long cold spell may have also affected wild populations by increasing the rate of return to diapause and/or mortality. Persistent patterns of diapause mortality at the Oregon Zoo over the past several years (Linders et al. 2015, 2016, 2018, 2019) point to host plant quality as a factor (Lewis et al. 2018). But while improvements in food quality have increased larval weight, which is correlated with survival, diapause survival has not yet improved significantly, suggesting other factors are also at play.

Table 6. Numbers of Taylor's checkerspot larvae by matriline that successfully emerged from diapause, retained for captive breeding at Mission Creek Corrections Center for Women, Belfair, Washington, or were released, March 2019. Quality Control (QC) larvae were separated from their siblings at some point in the rearing process.

Matriline	Diapause out	Retained	Released @TA7S	Released @TA15
16FL622	0	0	0	0
16FL625	0	0	0	0
16FL630	0	0	0	0
16FL643	0	0	0	0
16FL655	4	0	0	4
17FL701	0	0	0	0
17FL703	4	0	0	4
17FL705	1	0	0	1
17FL708	15	15	0	0
17FL709	5	5	0	0
17FL716	2	2	0	0
17FL719	6	0	0	6
17FL720	0	0	0	0
17FL723	2	2	0	0
Subtotal	*39*	*24*	*0*	*15*
18FL802	61	0	31	30
18FL804	162	44	118	0
18FL809	155	27	128	0
18FL811	208	44	163	0
18FL814	160	40	60	60
18FL819	231	45	0	186
18FL822	218	45	143	30
18FL825	233	45	0	188
18FL828	302	43	244	15
18FL831	49	0	0	49
18FL832	130	45	0	85
18FL833	86	44	11	30
18FL834	155	46	16	93

18FL835	157	45	0	110
18FL837	161	41	0	120
18FL838	127	45	0	82
18FL840	46	0	0	46
18FL842	59	0	0	59
18FL843	85	45	0	40
18FL844	81	0	0	81
Subtotal	*2866*	*644*	*914*	*1304*
18MC551-02	102	0	87	15
18MC557-02	70	0	70	0
18MC614-06	77	0	0	77
18MC622-01	71	0	41	30
18MC630-06	61	0	61	0
18MC643-04	53	0	53	0
18MC643-09	105	0	105	0
18MC649-03	134	0	0	134
18MC655-02	69	0	69	0
18MC701-17	103	0	103	0
18MC701-18	149	0	134	15
18MC701-20	122	0	91	31
18MC703-23	5	0	0	5
18MC705-14	46	0	46	0
18MC705-17	0	0	0	0
18MC705-18	37	0	37	0
18MC705-19	85	0	85	0
18MC709-09	47	0	47	0
18MC712-15	11	0	0	11
18MC712-18	136	0	121	15
18MC720-08	45	0	45	0
18MC720-26	122	0	107	15
Subtotal	*1650*	*0*	*1302*	*348*
QC-1 &2	80	0	0	80
QC-D2	3	0	0	3
Subtotal	*83*	*0*	*0*	*82*
Total	**4638**	**668**	**2216**	**1749**

Outcomes of postdiapause rearing, including progression to the adult stage, are presented in Table 8. Rates of return to diapause appear to fluctuate somewhat between facilities (Linders et al. 2018, Linders et al. 2019), similar in some years and more variable in others. Return rates at Mission Creek were twice as high (26 percent) as they were at the Zoo (12 percent) in 2019 (Table 9), which were on the low end. Multi-diapause larvae are retained and paired with other multi-diapause adults whenever possible. This trait is believed to be important for long-term population persistence especially in the face of climate change, and in the wild, likely results in genetic mixing across years. In all, 483 adult checkerspots (297 males and 303 females) made up the 2019 captive breeding colony (Table 8).

Table 7. Numbers of Taylor's checkerspot larvae by matriline that emerged from diapause, were retained for captive breeding at the Oregon Zoo, Portland, Oregon, or were released, 2019. Quality Control (QC) larvae were separated from their siblings at some point in the rearing process.

Matriline	Diapause Out	Retained	TA7S Release	TA15 Release
E17FL742	0	0	0	0
E17FL755	7	0	0	7
E17FL761	9	0	0	9
E17FL764	0	0	0	0
E17FL768	0	0	0	0
E17FL769	0	0	0	0
Subtotal	*16*	*0*	*0*	*16*
17FL705	0	0	0	0
17FL710	2	0	0	2
17SC735	0	0	0	0
LCC	0	0	0	0
Subtotal	*2*	*0*	*0*	*2*
18FL801	132	0	132	0
18FL803	128	30	0	128
18FL806	41	0	41	0
18FL807	19	36	0	19
18FL808	108	30	0	108
18FL812	22	0	0	22
18FL813	81	0	81	0
18FL815	296	28	0	296
18FL816	154	30	154	0
18FL817	156	0	0	156
18FL818	225	0	225	0
18FL820	76	30	76	0
18FL823	103	36	103	0
18FL824	126	30	120	6
18FL826	174	30	174	0
18FL827	192	0	190	2
18FL829	85	30	85	0
18FL831	65	0	0	65
18FL833	39	0	39	0
18FL838	92	0	76	16
18FL844	109	0	0	109
Subtotal	*2423*	*310*	*1496*	*927*
18OZ705	207	0	0	207
18OZ742	25	0	0	25
18OZ769	13	0	0	13
Subtotal	*245*	*0*	*0*	*245*
18MC613	0	0	0	0
18MC622	3	0	0	3
18MC643	1	0	0	1
18MC712	1	0	0	1
18MC720	4	0	0	4

Subtotal	*9*	*0*	*0*	*9*
QC	14	0	14	0
Total	**2709**	**310**	**1510**	**1199**

Rearing conditions

Methods

Our rearing strategy attempts to mimic ambient conditions to the greatest degree possible, because instituting environmental controls assumes detailed knowledge exists on which conditions maximize survival. In fall 2015 based on existing data, expert opinion and field observation, we refined our environmental targets (Table 9) to exclude potentially detrimental ambient outdoor conditions (e.g., freezing) and increase consistency and comparability between rearing facilities.

Table 8.   Numbers of Taylor's checkerspots by life stage and matriline reared for breeding at the Oregon Zoo, Portland, Oregon and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2019. Improperly eclosed (IE) adults are a subset of all adults.

Matriline	Larvae	2nd diapause	Pupae	IE	Males	Females	Males released	Females released
Oregon Zoo								
18FL803	30	6	24	6	10	10	10	10[1]
18FL807	36	2	32	4	15	13	13	14
18FL808	30	3	27	1	13	14	13	14
18FL815	28	2	26	3	18	7	14	9
18FL816	30	0	28	0	13	12	12	12[1]
18FL820	30	1	23	6	14	7	12	2[1]
18FL823	36	5	31	5	17	13	13	12
18FL824	30	13	14	1	6	8	5	6
18FL826	30	4	25	1	13	11	13	10
18FL829	30	1	26	1	10	14	9	16[1]
Total	**310**	**37**	**256**	**28**	**129**	**109**	**114**	**105**
Mission Creek								
17FL708	15	0	1	0	1	0	1	0
17FL709	5	1	3	1	1	2	0	1
17FL716	2	0	1	0	1	0	1	0
17FL723	2	0	2	1[1]	0	0	0	0
Subtotal	***24***	***1***	***7***	***2***	***3***	***2***	***2***	***1***
18FL804	44	26	13	0	4	7	2	2
18FL809	27	13	12	0	5	7	3	7
18FL811	44	4	32	0	16	14	13	15
18FL814	40	5	31	1	12	17	11	15
18FL819	45	5	37	1	15	20	5	22
18FL822	45	6	32	0	20	7	14	7
18FL825	45	9	29	1	16	11	3	10
18FL828	43	1	37	0	17	12	15	12
18FL832	45	15	21	1	10	8	3	6
18FL833	44	14	27	0	14	12	5	10
18FL834	46	21	9	0	2	7	1	7
18FL835	45	7	34	2	16	15	15	9
18FL837	41	12	26	0	12	12	3	12

18FL838	45	29	14	2	6	4	4	2
18FL843	45	0	44	1	21	19	21	19
Subtotal	***644***	***167***	***398***	***9***	***186***	***172***	***118***	***155***
Total	**668**	**168**	**405**	**11**	**189**	**174**	**120**	**156**

[1] In additional releases include: 1 18FL803 pupa and 1 unsexed adult; 1 18FL829 pupa; 2 18FL816 pupa; 2 18FL820 pupa.

Differences in climate and facility design require that supplemental light and heat be used at the Oregon Zoo to achieve target conditions, whereas shade cloth and cooling are needed to achieve target conditions in the Mission Creek greenhouse (Table 9). In addition, SPP received a grant from USFWS in 2017 to construct a second greenhouse and expand rearing capacity. Construction was completed by February 2019 (Curry et al. 2019). The new greenhouse is similar to the original facility in concept but has significant design changes. The new greenhouse is 28x12 ft in size with an 18x12-ft main room and a 10x12-ft small room, adding 96-ft^2 of additional space. It has wall-mounted heaters like the original greenhouse that maintain a minimum temperature and UV-transmitting glass was still used for the side walls. However, the new greenhouse has a polycarbonate roof and end walls designed to reduce heat build-up. For cooling it also features a combination of three 24-inch exhaust fans (one in the small room, two in the main room) wired to a thermostat, along with continuous side- and roof-vents that open manually.

Following a series of environmental tests, rearing was initiated in the new greenhouse with postdiapause larvae in 2019 (Curry et al. 2019). Conditions were carefully monitored throughout the season and compared with the original structure to ensure targets were being met. To track rearing conditions at all 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 9. 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, 2018-2019.

Life stage	**Mission Creek**	**Oregon Zoo**	**Target**		**Mission Creek**		**Oregon Zoo**	
	Location	**Location**	**Temp (°F)**	**Ave min RH (%)**	**Heat/Cooling**	**Light**	**Heat**	**Light**
Males	GH Main Room (Floor)	TCB room	≤85 78-85 for 2-6 h/day	≥50	Ice packs; wet towels	Full shade	40-watt bulb 8-9 AM & 1-1:30 PM	None
Females and Oviposition	GH Small Room	TCB room	50-90 78-90 for 4-8 h/day	≥50	40-watt bulb 10 AM-2 PM or outside	Partial shade	40-watt bulb 10 AM-2 PM or outside	None
Egg & Prediapause	GH Main Room	TCB room	50-90; ave min ≤65	≤65	Ice packs; wet towels	None	None	7 AM - 7 PM
Warm diapause	GH Main Room	TCB room	50-90	≥40	Ice packs; wet towels	None	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 ≥60 daytime	≥50	None	None	None	7 AM - 7 PM
Pupation	GH Main Room	OSB room	≥50 night ≥65 daytime	≥65	Misting/ humidifier	None	7 AM-7 PM	7 AM - 7 PM

Results and Discussion

Temperature (°F) and relative humidity (percent RH) metrics recorded at the Oregon Zoo and Mission Creek in 2018-2019 are in listed in Table 10. Rearing conditions at both facilities followed very similar patterns across the rearing year. In most cases temperatures met target levels, with the most notable exceptions being the number of days that targets were met during the adult life stage, when broad ambient temperature swings make conditions more difficult to control. Nighttime temperatures during postdiapause rearing at Mission Creek were intentionally increased to stimulate larvae to feed, which was greatly slowed as a result of the very cool conditions that prevailed during that period (Curry et al. 2019). Humidity levels were more variable, tending to be lower than target levels in several instances. Not surprisingly, average humidity levels were low during the adult stage when temperatures also reach their maximum. In fact, there are heat and moisture gradients within adult enclosures such that suitable conditions can be found, as evidenced by good activity levels and longevity (Lewis et al. 2019). None of the metrics that were outside target ranges are thought to have affected outcomes (see Survival of captive animals below). Rearing conditions in the two greenhouses at Mission Creek were similar, but the new greenhouse had higher highs and lower lows than the original structure (Curry et al. 2019). A lack of shade cloth initially and improper installation (e.g., draping over the roof vents) explain the greatest differences in performance of the new greenhouse. It also takes time to understand the relationship between weather and climate controls in any new rearing structure.

Table 10. Actual average (ave) temperature (°F) and relative humidity (% RH) conditions and absolute range (rng) 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, 2018-2019. 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	27 Apr – 11 May 2018	24 Apr - 11 May 2018	mean max (rng)	81 (49-89)	87 (63 - 91)	mean	**38**	**39**
			#days w/i rng	**11 of 15**	**3 of 11**	(rng)	(**26**-57)	(**31**-69)
Females and Oviposition	2 - 22 May 2018	25 Apr -22 May 2018	mean (rng)	67 (49-95)	63 (46-103)	mean	**38**	**23**
			#days w/i rng	**14 of 21**	**9 of 22**	(rng)	(**22**-57)	(**6**-74)
Egg & Prediapause	3 May – 12 Jul 2018	27 Apr - 13 Jul 2018	mean (rng); ave min	67 (47-96); 54	71 (61 - 84); 65	mean (rng)	42 (19-62)	**71** (47-**94**)
Warm diapause	12 Jul – 12 Sep 2018	14 Jul - 29 Sep 2018	mean (rng)	70 (51-97)	67 (50 - 84)	mean (rng)	41 (**21**-66)	65 (38-93)
Cold diapause	12 Sep 2018 – 5 Mar 2019	30 Sep 2018 - 4 Mar 2019	#days min ≤35	70 of 175	**50** of 157	mean (rng); ave max	83 (**37**-99); 91	?? (**28**-100); 85
Postdiapause	28 Feb – 30 Apr 2019	4 Mar - 1 May 2019	#nts ≤45	**0 of 53**	59 of 59	mean	53	54
			#days ≥60	52 of 53	59 of 59	(rng)	**14**-92	(**18**-94)
Pupation	1 Apr – 8 May 2019	7 Apr - 6 May 2019	#nts ≥50	38 of 38	29 of 29	mean	60	76
			#days ≥65	38 of 38	29 of 29	(rng)	**27**-92	(**45**-100)

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 include any larvae known to perish prior to that point.

Results and Discussion

Stage specific survival rates by life stage for both captive rearing institutions appear in Table 11. Mortality rates from egg to hatch (3rd instar) are often the highest of any life stage, but also the most variable (Linders 2010, 2011, 2012, Linders and Lewis 2013, Linders et al. 2014-2019). At Mission Creek, 50 percent of hatch counts exceed the associated egg counts, adding to questions about the usefulness of these data. What is evident is that hatch rates for wild-sourced eggs at the Zoo were high relative to those from captive-mated females, however at Mission Creek, hatch rates for both captive (18MC) and wild-sourced (18FL) eggs was very high in 2019 (Table 11). Hatch to diapause survival at both facilities was high for all cohorts except one (Captive 18MC at the Zoo) with a small sample size. As in recent years (Linders et al. 2014-2019), survival from diapause to release at the Zoo was poor compared to Mission Creek, which has consistently maintained high survival for larvae entering first year diapause (i.e., 2018 cohorts). For larvae emerging from 2nd or 3rd year diapause (17FL/SC; E17FL; and 17/16-1FL)

Table 11. Number and survival (Kaplan-Meier 1958) by life stage and cohort for Taylor's checkerspot butterflies at the Oregon Zoo (OZ), Portland, Oregon and Mission Creek Corrections Center for Women (MC), Belfair, Washington, 2018-2019. Cohort 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 egg year (e.g., 16=2016). Includes only females with eggs that hatched; hatched larvae recorded at 3rd instar; those of unknown origin (QC) are excluded.

Oregon Zoo										
Cohort	**Wild 18FL**		**Captive 18OZ**		**Captive 18MC**		**Wild 17FL/SC**		**Wild E17FL**	
Life stage	**#**	**Survival**	**#**	**Survival**	**#**	**Survival**		**Survival**	**#**	**Survival**
Eggs	6255		991		2049					
Egg to hatch	5547	0.89	375	0.38	1678	0.82				
Prediapause release	*566*				*1628*					
Hatch to diapause[1]	4872	0.98	369	0.98	20	0.40	71		83	
Diapause to release	2423	0.50	245	0.66	9	0.45	2	0.03	16	0.19
Postdiapause	310									
Return to diapause[2]	38	0.12								
Pupation	255	0.94								
Eclosion	237	0.93								

Mission Creek						
Cohort	**Wild 18FL**		**Captive 18MC**		**Wild 17/16-1FL**	
Life stage	**#**	**Survival**	**#**	**Survival**	**#**	**Survival**
Eggs	2946		7021			
Eggs to OZ	*989*		*2049*			
Egg to hatch	2999	1.02	4772	0.96		
Prediapause release			*3006*			
Hatch to diapause[1]	2893	0.96	1716	0.97	105	

Diapause to release	2866	0.99	1650	0.96	39	0.37
Postdiapause	644				24	
Return to diapause[2]	167	0.26			1	0.04
Pupation	398	0.83			7	0.30
Eclosion	358	0.90			6	0.86

[1] Hatch rates not adjusted for undercount of eggs.

[2] Proportion of postdiapause larvae that returned to diapause.

survival was considerably lower at both facilities. However, as there is little data on which to base expectations it is difficult to judge success except to say that survival at Mission Creek was lower in 2019 than in 2018, when about half of the multi-diapause larvae pupated, and nearly all eclosed. That was the largest group (n = 108) ever to make it through multi-diapause to eclosion (n = 47). 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. 2014-2019), well above those expected in the wild (Moore 1989).

Mortality at the Oregon Zoo from diapause to release (Table 11) was similar to levels in 2018 and remains significantly higher than normal (Linders et al. 2019). Warm overwinter temperatures and disease have been ruled out as causal factors. Other concerns included providing old food (cut several days prior to being fed) and feeding from plants with varying levels of fertilizer including no supplementation. Unfortunately, larval feeding trials on the effects of fertilizer did not provide a clear solution, although larvae fed from either fertilized potted plants or from plants grown in the ground generally fared better than those fed from unfertilized plants grown in pots in terms of both weight gain going into diapause and survival thereafter (Lewis, unpub. data). One other notable pattern was that larvae fed from fertilized plants were less likely to return to diapause (16 percent) and with higher survival to pupation (91 percent) compared to those fed from unfertilized plants (50 and 77 percent, respectively). Larvae fed from plants grown in the ground had a mixed response (52 and 92 percent, respectively). Once larvae pupated all had equally high rates of eclosion (96, 94, and 96 percent, respectively. At the Zoo, Lewis (unpub. data) also found that larvae that are heavier going into diapause are more likely to survive.

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

On average, wild females taken to Mission Creek were lighter than those taken to the Oregon Zoo in 2018 (p <0.05), which is likely because Mission Creek received more than half of their females six days after the first delivery to both facilities (8 May), and 16 days past the flight season peak. This tendency for females to be lighter when collected later in the season has been consistent across years (Curry 2019). As in 2017 (Linders et al. 2019), there was no difference in wing size between wild females by facility (p =0.066). Not surprisingly, wild females were also lighter than captive females at both facilities (p > 0.0001 in both cases). However, both male (p <0.001) and female (p = 0.0037) pupae and adults were heavier at Mission Creek than at the Oregon Zoo (Table 12), which has a trend (Linders et al. 2018, 2019). However, there was no significant difference in wing size between captive females from either facility or with those collected from the wild ($p > 0.070$ in all cases), which is promising, since differences have been observed at times in the past. However male wing area did differ between facilities, with those at the Zoo significantly larger than those at Mission Creek ($p > 0.01$), which is opposite results for 2017, and despite differences in weight. It is not readily apparent what would cause this pattern reversal.

Table 12. 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, 2018; for adults whose wings eclosed improperly, only their weights are included.

Unit of Analysis	Statistic	Pupal Weight (g)	Adult Weight (g)	Wing Area (cm2)
Oregon Zoo				
	N	83	83	34
Captive males	mean	0.1607	0.0826	1.0441
	var	0.0004	0.0003	0.0156
	N	111	109	58
Captive females	mean	0.1999	0.1291	1.3213
	var	0.0006	0.0004	0.0251
	N	NA	19	19
Wild females	mean	NA	0.1530	1.4463
	var	NA	0.0005	0.0313
	N	NA	1	1
Wild males	mean	NA	0.0800	1.4160
	var	NA	NA	NA
Mission Creek				
	N	125	125	72
Captive males	mean	0.2004	0.0924	0.9631
	var	0.0004	0.0003	0.0105
	N	94	94	86
Captive females	mean	0.2528	0.1583	1.2631
	var	0.0010	0.0004	0.0191
	N	NA	24	21
Wild females	mean	NA	0.1241	1.2948
	var	NA	0.0012	0.0440

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.

A total of 3,714 postdiapause larvae were released at TA7S on 10 and 15 Mar 2019, including 1,510 from Oregon Zoo and 2,204 larvae from Mission Creek. In addition, 2,847 larvae were released at TA15 (1,199 from Oregon Zoo and 1,648 from Mission Creek) on 17 Mar 2019 (Fig. 3). Eighteen larvae from Mission Creek and 125 from the Oregon Zoo were dead on arrival and were not released. Of the 3,714 larvae released at TA7S, 2,400 were offspring of wild females, 1300 were offspring of captive-mated females, and 14 were of unknown (QC) origin as they were separated from their sibling groups at some point in the rearing process. Of the 2,847 larvae released at TA15, 2,190 were offspring of wild females, 575 were offspring of captive-mated females, and 82 were of unknown (QC) origin. Numbers reported may not match those in the captive rearing section exactly, but discrepancies are small. Weather on release days was mixed with temperatures 5.0-20.2°C, average wind speed 1.3-4.7 mph, and clear to partly cloudy skies; weather in the days following both releases was generally dry, warm and mostly sunny. At least 19 people assisted with releases, working from about 1000-1700 h each day to complete the task.

Adult release

Once their roles in captive mating and/or oviposition were complete, 198 adults (85 females, 113 males) from the Oregon Zoo were released at TA7S on 7 May 2019. The same day, 194 adults (101 females, 93 males) from Mission Creek were released at TA15; 24 males were dead on arrival. Seventeen pupae were also received from the Oregon Zoo on 7 May and most of which were reared to pupation. Of these, six females were released at TA7S on 9 May 2019, and six pupae and four adults (2 females, 1 male and on unknown) were released at TA15 on 11 May 2019; one pupa died. Nineteen females, including 8 wild females were released from the Zoo at TA15 on 17 May 2019 along with 3 males and 55 females from Mission Creek; four males and seven females from Mission Creek died in route. As in previous years, males and females were co-housed in net enclosures to encourage mating in transit. Once on site they were released directly into the environment from open cages or placed on nectar plants as needed. Release conditions were warm (17.1-21.5 °C), with light winds (4.8-6.8 mph average), and clear or overcast skies (Fig. 3).

Prediapause larval release

The timing of the 2019 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. As a result, 3,250 prediapause larvae were released at TA15 on 11 Jun 2019. Of these, 2,514 prediapause larvae were from Mission Creek and 736 were from the Oregon Zoo. Two larvae from Mission Creek were dead on arrival. Of larvae released at TA15, 3,223 were offspring of captive-mated females, and 27 were of unknown origin (QC).

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 checkerspots are surviving and reproducing on site. To confirm site occupancy, quantify minimum survival, and identify issues that may be cause for concern, we typically conduct surveys for postdiapause larvae in 4x4-m survival plots on two occasions in the weeks following release. However, due to the persistence of cold conditions and its effect on host plant availability in 2019, no survival plots were established (see Section I).

Winter and spring 2019 contained an undulating mix of warm and cold conditions in western Washington. Warm very mid-winter conditions in January changed to persistent cold and snow in mid-February that lasted well into March (see Section 1 for impacts to host plants) when conditions changed again, becoming unseasonably hot and dry. In spite of over 20 inches of snow that fell in a single event in February, precipitation in March was well below normal, resulting in slow growth of host and nectar plants. Asa result, this was the first time that we observed host plants grazed in an even manner across the top, which we concluded was the result of larvae consuming them as they emerged from the ground (Fig 3). Such foraging evidence was widespread across most sites.

Figure 3. Release of prediapause larvae (left), heavy larval forage sign (center) and release of adult (right) Taylor's checkerspots at Training Areas 15 and 7S (right), respectively, Joint Base Lewis-McChord, Washington, spring 2019.

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. Four translocation sites (SCS1, R50, TA7S, and TA15), one colonization site (SCS2), and one extant site (R76) were surveyed for adult checkerspots during the 2019 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 2 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 13. The closer transect spacing at translocation sites ensures 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.

<u>Data analyses.</u> Analyses were conducted using Program Distance, Version 7.3 (Thomas et al. 2010) with density estimates computed by 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) modules. Summary statistics, including observation frequency tables calculated by observer date, and transect line, were calculated first in SAS. We also generated tables of encounter rates (observations per unit line length surveyed) by date and observer. For the MCDS analyses, we also computed univariate statistics and

plots of distance data for potential covariates, such as observer, butterfly behavior, survey protocol, and weather, as recommended by Marques et al. (2007).

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.

Table 13. Number and size (m) of distance sampling units, standard survey length and survey area for density estimation by site for extant (R76) and reintroduced Taylor's checkerspot populations, South Puget Sound, Washington, 2010-2019. Range 76 (R76), Scatter Creek South Units 1 (SCS1) and 2 (SCS2), Range 50 (R50), Training Areas 7 South (TA7S) and 15 (TA15), Glacial Heritage Units 2 (GHP2) and Unit 2 with *Castilleja levisecta* (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	5400	42.0
R76	2016-19	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	2017-19	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	2014-19	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	2016-19	19	25	7-14	25	175-350	5125	12.8
SCS2	2017-19	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
TA15	2019	21	25	4-24	25	100-425	6350	15.9

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

In 2019, there were 4 different observers conducting surveys. All observers had experience with distance sampling for Taylor's checkerspots, although the amount of experience 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 four translocation sites (SCS, R50, TA7S and TA15), one colonization site (SCS2) and for R76 are reported here for 2019.

Results and Discussion

From six to seven complete distance surveys were conducted at each of six occupied Taylor's checkerspot sites in 2019 (Table 14); partial surveys were also conducted at R76, R50 and SCS1. A total of 68.7 km were surveyed at R50, 59.0 km at SCS1, 32.4 km at SCS2, 35.9 km at TA7S, 44.5 km at TA15, and 77.2 km at R76.

After a dry March, conditions were again cool with wetter than normal patterns in April, which persisted throughout the month (Mass 2019). Dry conditions again prevailed again in May, with overcast skies becoming common after the first week of the month, making for overall suitable weather conditions for surveys throughout much of the flight season. Notably, all of the back and forth changes in the weather, led to some unusual observations in 2019, including the simultaneous presence of larvae, pupae, adults and eggs during the latter half of April (Fig. 4). What was particularly unusual was that larvae were last observed on 2 April, then a whole cohort of larvae suddenly reappeared on the 19 April following the start of the flight season. Presumably these larvae had initially decided to go into second diapause, but then changed course, re-emerging two weeks later to resume feeding. Hundreds of larvae and extensive feeding damage was observed, particularly across Range 76, where larvae continued to feed until the early May. While some larvae were confirmed to have been parasitized, this was definitely not true for all, which led to speculation that a group of late-emerging adults might appear near the end of the flight season. If true, this would be evidence for a mechanism with the potential to increase flight season duration and overall population size. And in fact, the flight season duration was 6.5 weeks long, a full week longer than we have ever documented in the Puget Lowlands. Furthermore, while weather and access prevented us from confirming a population increase at R76 late in the flight season, a sample of 19 adults that were located on 22 May included 6 males, 10 females and 3 of unknown sex. In addition,

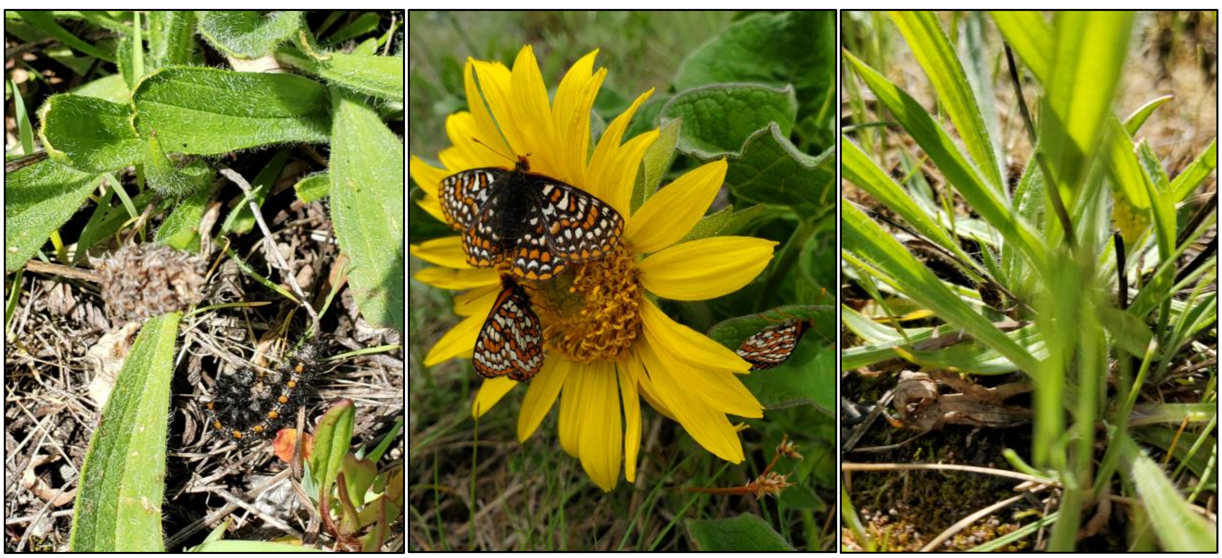


Figure 4. The simultaneous presence of Taylor's checkerspots as postdiapause larvae (left preparing to pupate), pupae (not pictured), adults (center) and eggs (right) has never before been documented in Washington state, Range 76, Joint Base Lewis-McChord, Washington, spring 2019.

on a wing wear scale of 1.0 to 3.5, 16 adults had wing wear scores of 1.0 (fresh or very fresh), with two others showing mild wear (1.5), and only one showing moderate wear (2.0). While the sample is small, both the sex ratio and the number of fresh adults suggests the majority of these individuals were part of the cohort of late-feeding larvae which contributed to the extension of the flight season and illustrated a mechanism with the potential to greatly expand population size when conditions are suitable. While somewhat anecdotal, these findings have huge implications for how this species and others might have the potential to respond and adapt in the face of a changing climate, so long as habitat resilience is also secured through host and nectar plant diversification.

Access to R76 and R50 was primarily limited to a few Mondays and weekend days in April, with only 3 dates where weather corresponded with access between 15 and 30 April; some dates restricted the footprint at R76 so as to make surveys unfeasible. In May access and weather lined up more favorably with the Saturday-Monday access schedule, except in late May, when we were attempting to document the flight season extension resulting from late-feeding larvae (see above). Ultimately, we achieved the minimum of five qualifying surveys at high priority AIA sites and others (Table 14), although weather conditions in 2019 made that challenging at times.

Table 14. Number of Taylor's checkerspots counted by site and date during distance sampling surveys at Scatter Creek South Units 1 (SCS1) and 2 (SCS2), Range 50 (R50), Range 76 (R76 Transects 1-16) and Training Areas 7 (TA7S) and 15 (TA15), Puget Lowlands, Washington, spring 2019. Surveys modified by adult release are indicated.

Date	R76	R50	SCS1	SCS2	TA7S	TA15	Comments
15-Apr	23	2					R50: abort due weather
20-Apr	313	393					R76: omit T 1, 2, 7, & 12 due to access time
21-Apr					0	21	
24-Apr			654	8			
26-Apr			48		6	176	SCS1: abort due to weather
27-Apr	388	1670					R76: abort due to weather, R50: ~2/3 in protocol
29-Apr	2395						
30-Apr			1786	21	9		
1-May		1938				238	
3-May					12		
4-May			1311	51			
6-May	1435	1293				208	
7-May					11		Release: TA15 - 194 adults; TA7S - 199 adults postsurvey
8-May			696	76			
10-May					19		
11-May	418	376				54	Release: TA15 - 10 adults, 6 pupae; TA7S - 6 adults
13-May			200	49			
18-May	53	62				5	Release: TA15 - 77 adults
22-May					0		TA7S: Marginal temps
23-May			26	14		5	
29-May			1				
30-May	0						R76: 1 checkerspot observed off survey
Total	**5025**	**5734**	**4722**	**219**	**57**	**707**	

Density and abundance estimates 2019

Distance data were analyzed for six sites (R76, R50, SCS1 SCS2, TA7S and TA15) in 2019; results are presented here (Table 15) and in Appendix B. A total of 16,464 checkerspot observations were recorded during distance surveys, with the greatest number observed at R50 (Table 14). Group size ranged from 1 to 12, with 119 groups comprised of four or more adults. Flight season initiation was intermediate in its timing relative to past years, with peak counts occurring on most sites 29 Apr-1 May. The peak count at SCS2 was one week later (8 May), suggesting it is influenced by dispersal from SCS1. The peak count at TA7S on 10 May followed a large release of adults 3 days prior, mimicking dispersal at SCS2. The peak day encounter rate at R50 (0.20 checkerspots /m) was about 30 percent lower than in 2018 and was comparable to SCS1 (0.22/m). At R76 (0.22/m for transects 1-16; 0.25/m for transects 1-12) encounter rates were slightly higher than in 2018. Peak day encounter rates at TA15 (0.037 /m), SCS2 (0.014/m) and TA7S (0.004/m) also increased in 2019. One checkerspot was observed at GHP, although no transect surveys were conducted. Staff from Joint Base Lewis-McChord worked with partners from CNLM and WDFW to monitor parts of TAs 6, 7S, 14, 15 and the perimeter of 91st Division Prairie (the Artillery Impact Area-AIA) using transects with a 100-m spacing; those data are reported by JBLM staff, but data are included on maps displayed in Appendices C and D.

SCS1. Eight surveys were conducted at SCS1 in 2019 between 24 April and 29 May; butterflies were observed on all dates. All were qualified surveys (Linders and Olson 2014) except on 26 April when the survey was aborted due to poor weather (data omitted from analyses). The number of observers per day varied from 2 to 4, with effort and encounter rates similar for three of the observers; the fourth observer had a higher effort and lower encounter rate. Observer-specific detection functions were modeled for all dates except 23 May; data were truncated at 8m to eliminate outliers. Of 3,678 observations used in the analyses, the greatest number (1,376) were recorded on 1 May. Daily density and abundance estimates with 95% CIs are presented in Table 15.

SCS2. Six surveys were conducted at SCS2 in 2019 between 24 April and 23 May; butterflies were observed on all dates. All were qualified surveys (Linders and Olson 2014). The number of observers per day varied from 2 to 4, with effort distributed equally within dates. Due to smaller numbers of observations on some dates (24 & 30 April, 23 May), these data were pooled across dates to estimate detection functions. On the remaining dates, detection functions were modeled separately by date; all data were truncated at 8m to eliminate outliers. Of 210 observations used in the analyses, the greatest number (71) were recorded on 8 May. Daily density and abundance estimates with 95% CIs are presented in Table 15.

R50. Seven surveys were conducted between 15 April and 18 May by 2-4 observers in 2019; butterflies were recorded on all dates. All were complete surveys except on 15 April when the survey was aborted due to non-protocol conditions. In addition, all analytical models run on data from 27 April produced a very poor fit and were omitted from further analyses; presumably this was due to the high winds that drove observers to abort the survey at R76 the same day. A total of five qualifying surveys (Linders and Olson 2014) were completed in 2019. The number of observers varied from 2 to 4 per day with survey effort fairly evenly distributed among observers, although encounter rates varied by observer and date. Sufficient observations were recorded on 20 April and 1 May to fit detection functions by observer; estimates for 6, 11 May utilized detection functions with observers pooled by date. Data were generally truncated at 8 m to reduce unnecessary adjustment terms. In all, 3,255 adult observations were used in the analyses, with the greatest number (1,556) on 1 May. Density estimates with 95% CIs (CI) are presented in Table 15 along with daily abundance estimates and 95% CI for the entire survey area.

TA7S. Seven surveys were conducted at TA7S in 2019 between 21 April and 22 May; no butterflies were observed on the first and last dates. All other surveys were qualified (Linders and Olson 2014). The number of observers per day varied from 2 to 4. There were only enough detections to run models pooled over all observers and dates; data were not truncated. Of 53 observations used in the analyses, the greatest number (19) were recorded on 10 May. Daily density and abundance estimates with 95% CIs are presented in Table 15.

TA15. Seven surveys were conducted at TA15 in 2019 between 21 April and 23 May by 2-4 observers; butterflies were observed on all dates. All were qualifying surveys (Linders and Olson 2014). Because line length varied from 100-425 m, distribution of effort among observers within dates was more variable than at other sites. Data for 21 April, 18, and 23 May were pooled over all dates and observers, and data grouped into 6 intervals. All other estimates were date-specific generated by observer or with observer covariates. Data were truncated at 8 m. Of 1,116 observations used in the analyses, the greatest number (193) were recorded on 1 May. Daily density and abundance estimates with 95% CIs are presented in Table 15.

Table 15. Number of observations (#Obs) used to estimate density (#/ha) and adult abundance (#/site by date), and 95% Confidence Intervals (CI) for Taylor's checkerspots at Scatter Creek South Units 1 (SCS1) and 2 (SCS2), Range 50 (R50), Range 76 (R76) and Training Areas 7 (TA7S) and 15 (TA15), Puget Lowlands, Washington, 2019.

		Survey	Density			Abundance		
Date	**#Obs**	**area (ha)**	**#/ha**	**Lower CI**	**Upper CI**	**#/site**	**Lower CI**	**Upper CI**
SCS1								
24-Apr	491	20.00	123.1	90.0	168.3	2462	1801	3366
30-Apr	1376	20.00	274.2	210.7	356.8	5484	4214	7137
4-May	1038	20.00	172.7	136.8	218.1	3455	2736	4362
8-May	574	20.00	118.3	91.2	153.3	2365	1825	3066
13-May	177	20.00	33.2	24.9	44.4	664	497	888
23-May	22	20.00	4.3	2.4	7.8	86	48	155
SCS2								
24-Apr	40[b]	13.5	3.0	0.9	9.6	41	13	130
30-Apr	40[b]	13.5	7.5	3.6	15.9	101	48	215
4-May	42	13.5	10.5	6.3	17.6	142	85	237
8-May	71	13.5	38.1	25.4	57.2	514	342	772
13-May	47	13.5	21.9	12.9	37.0	295	174	500
23-May	40[b]	13.5	4.5	1.7	11.7	61	23	157
R50								
20-Apr	342	24.75	41.5	19.0	90.9	1028	470	2249
1-May	1556	24.75	236.4	158.7	352.2	5851	3927	8716
6-May	978	24.75	155.5	132.8	182.1	3849	3287	4507
11-May	322	24.75	31.2	25.7	38.0	772	635	940
18-May	57	24.75	10.7	6.1	18.7	264	150	463
TA7S								
26-Apr	53[a]	12.8	2.1	0.9	5.3	27	12	68
30-Apr	53[a]	12.8	3.0	1.5	6.0	38	19	76
3-May	53[a]	12.8	4.4	1.4	14.0	57	18	179
7-May	53[a]	12.8	3.7	1.6	8.4	47	21	108
10-May[c]	53[a]	12.8	6.3	2.8	14.3	80	35	183
TA15								
21-Apr	572[b]	15.9	4.2	2.5	7.0	66	39	112
26-Apr	139	15.9	35.3	25.6	48.8	561	406	775
1-May	193	15.9	43.2	34.8	53.1	685	553	842
6-May	161	15.9	34.8	24.8	49.0	553	394	777
11-May	51	15.9	8.0	5.6	11.4	126	88	181
18-May[d]	572[b]	15.9	1.2	0.4	3.3	18	6	53
23-May	572[b]	15.9	1.2	0.5	2.5	18	8	40
R76								
15-Apr	21	54.50	3.3	1.4	7.5	180	78	411
20-Apr	245	54.50	51.5	28.1	94.5	2806	1529	5150
29-Apr	1861	54.50	356.6	267.8	474.8	19435	14596	25877
6-May	1177	54.50	206.6	174.6	244.5	11261	9517	13325
11-May	327	54.50	48.5	34.3	68.4	2641	1871	3729

18-May	50	54.50	6.6	3.7	11.8	361	203	643

[a] Due to small sample size, one detection function, derived from combined data for all dates, was applied to each date.
[b] Detection function was fit to data pooled over these dates for this site; sample size given is the pooled value.
[c] 198 adults released following the survey on 7 May2019.
[d] 58 adults released 17 May 2019.

R76. Eight surveys were conducted by 2-4 observers from 15 April to 30 May 2019. No butterflies were observed on 30 May, for a total of 7 qualifying surveys (Linders and Olson 2014). One survey (27 April) was aborted due to weather, and a second survey eliminated 4 lines, but these were scattered across the site and did not significantly impact results. Four observers were present on most survey dates, with effort well-distributed among 3 observers and dates, and a fourth observer surveying 900 m of additional line length. Sufficient observations were recorded on some dates to fit detection functions by observer (29 April) or with observer covariates (20 April, 6 May). Alternatively, estimates for 15 April, 11,18 May utilized detection functions pooled over all observers within each date. Data were truncated at either 8 m. A total of 3,681 adult observations were used in the analyses, with the greatest number (1,861) observed on 29 April. Daily density and abundance estimates with 95% CIs are presented by date in Table 15.

Other observations

Nectar

Nectar-feeding observations have been recorded opportunistically during Taylor's checkerspot distance surveys since 2011 (Fig. 5), totaling 8,007 observations on 32 species across seven survey areas. Three species: *Balsamorhiza deltoidea*, *Lomatium triternatum* and *Saxifraga integrifolia (=Micranthes integrifolia)* accounted for 84.4 percent of all nectar observations (n= 7,845). The importance of these species across sites and years (Linders et al. 2019) appears two-fold: 1) consistent (high quality?) nectar production, and 2) provision of basking and/or roosting sites (Linders, pers. obs.). Both functions can increase longevity and fitness in adults (Murphy et al. 1983, Dover et al. 1997, Dennis 2010). Adults seek shelter at night and during inclement weather (hot, cold or wet extremes).

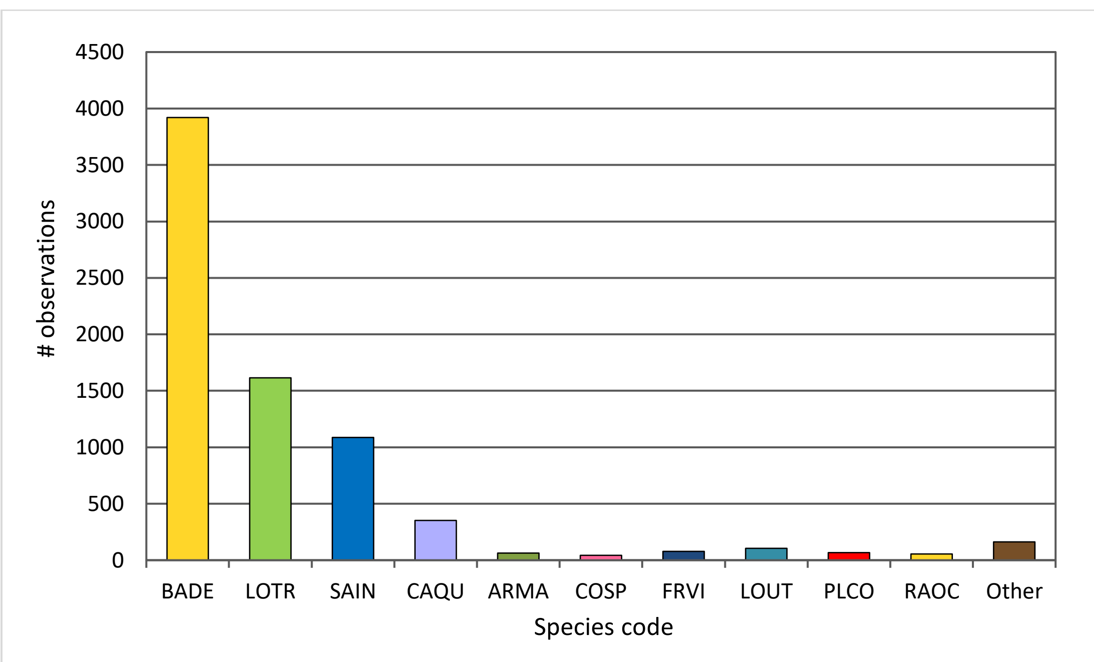


Figure 5. Number of nectar observations (n = 7,845) by species for Taylor's checkerspots observed during surveys in the Puget Lowlands, Washington, 2011-2019. BADE - *Balsamorhiza deltoidea*; LOTR- *Lomatium triternatum*; SAIN

- *Saxifraga integrifolia*; CAQI -*Camassia quamash*; ARMA – *Armeria maritima*; COSP – *Collinsia species*; FRVI – *Fragaria virginiana*; LOUT-*Lomatium utriculatum*; PLCO- *Plectritis congesta*; RAOC - *Ranunculus occidentalis*; Other = 22 other native and non-native species accounting for a total of 2 percent of all observations.

Mardon skipper

As in years past, state endangered mardon skipper (*Polites mardon*) butterfly sightings were systematically recorded during 2019 distance sampling surveys for Taylor's checkerspot at the three sites where they co-occur. In total, 231 mardon skippers were observed on surveys, with 12 additional observations off transect; most were observed at R50 (Table 16). More than twice as many skippers were observed at sites in 2019 compared to 2018, even though fewer surveys were conducted overall. This is the second consecutive year that sufficient observations were collected at a single site (R50) to make calculation of a density estimate possible. Most observations were of lone skippers, but 12 groups of two skippers (1 at R76, 11 at R50) and one group of three skippers (SCS1) were also recorded.

Table 16. Number of mardon skipper butterflies observed by date and site during distance surveys for Taylor's checkerspot in spring 2019 at four sites [Range 76 (R76); Range 50 (R50); Scatter Creek South Units 1 (SCS1) and 2 (SCS2), South Puget Sound, Washington. "Zero" surveys included nearest dates when skippers were observed.

	Distance surveys				Off transect			
Date	**R76**	**R50**	**SCS1**	**SCS2**	**R76**	**R50**	**SCS1**	**SCS2**
29-Apr	0							
1-May		0						
4-May			0	0				
6-May	4	44			2			
8-May			4	1				
11-May	24	106				4		
13-May			1	1			4	1
18-May	4	41				1		
23-May			0	1				
30-May	0							
Total	32	191	5	3	2	5	4	1

Adult distribution

Methods

To illustrate the distribution of adults within survey areas at each site, all Taylor's checkerspot observations collected during 2019 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 checkerspot distribution 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 in some cases cell size changes by site and year as a result of segment length and transect spacing (Table 13). 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 occupancy surveys across all of SCS in 2019 using transects spaced 50 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 17. Checkerspots occupied the majority of survey areas at R50 and SCS. Occupancy at TA7S (Appendix C, Fig. 3) and GHP (1 checkerspot observed, no surveys) remains limited despite repeated releases (Appendix A). At R76, occupancy declined from 2014-2016 and rebounded in 2017-2019. Occupancy at SCS2 rose sharply in 2019, 56.9 percent (123 of 216) of segments surveyed (Appendix C, Fig. 2), up from just 10.6 percent in 2018. This steep increase was clearly supported by dispersal from SCS1, as evidenced by the delay in peak counts between these two monitoring areas (Table 14). Increasing occupancy at TA15 is on a strong positive track and mirrors patterns observed at R50 in its first two years (2010-2011) and at SCS1 in 2015-2016 (Table 17), the point at which those populations increased significantly (Appendix B). At R76, checkerspots occupied 96.1 percent (323 of 336 segments) of the total monitoring area (transects 1-16) in 2019, the largest area recorded to date (Table 20).

Table 17. 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 – 2019. 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	2019
SCS1			0.2	7.1	4.3	4.3	4.3	3.9	5.4	10.6	12.2	15.4	18.6
SCS2											2.9	1.4	7.7
R50				6.5	13.6	18.8	17.5	11.2	15.3	18.4	22.4	24.8	24.3
PCM						3.0	0.0	0.0	0.0	-	-	-	
GHP						0.4	6.3	3.3	3.4	3.7	0.1	0.1	0.0
TA7S								2.1	3.4	0.3	0.1	0.0	2.2
TA15												5.3	9.8
R76 T1-12	39.3	17.0	26.0	38.0	41.8	42.0	39.5	39.4	38.0	21.3	34.5	41.1	40.4
R76 T13-16								11.0	11.0	8.1	7.3	9.1	12.0
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	52.4

A map of scattered sightings and 100-m occupancy survey results for the Artillery Impact Area (AIA) on JBLM shows a significant increase in the number and distribution of dispersing checkerspots in 2019, with concentrations of adults in several disparate locations around the periphery of this 3,075-ha prairie (Appendix D). Surveyors visited transects on all but the south edge of the AIA in 2019. Four adults were observed in TA14 west of PCM in 2019 during 100-m occupancy surveys by JBLM staff; they are presumed to be dispersers from TA15 to the east. Occupancy surveys and scattered sightings at SCS indicate continued spread across the site (Appendix C, Fig. 2), and is confirmed by an increase in encounter rate on occupancy surveys from 0.0029 checkerspots/m in 2018 to 0.0034 in 2019.

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 the Puget Lowlands. 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 has been 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. Data from this naturally occurring population has formed 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. As more data are now available, these criteria are being reviewed and some slight modifications proposed as part of the Reintroduction Plan being developed in cooperation with project partners. 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 at a given site are complete, the distance sampling protocol (Linders and Olson 2014) is implemented for an additional five years to monitor population establishment. Results are evaluated annually against the above criteria using daily abundance estimates, distribution maps and field observations to verify signs of reproduction.

Results and Discussion

Based solely on natural reproduction, adult checkerspots effectively occupy the entire 24.8-ha monitoring area at R50 (Appendix C, Fig. 1) for a second straight year, having increased their spread annually since 2014. However, the peak single day abundance estimate for 2019 (5,851; 95% CI: 3,927-8,716; Table 15) was 34 percent lower than in 2018 (Linders et al. 2019). This together with the widespread dispersal observed in surrounding areas (Appendix D), suggests this population may be at carrying capacity. Habitat monitoring using RHA should be resumed to evaluate current condition and ensure habitat threats are continuing to be addressed. In contrast, the peak single day abundance estimate for R76 (19,435; 95% CI: 14,596-25,877; Table 15), was 36 percent higher than in 2018 (Linders et al. 2019) and the population occupied nearly the entire monitoring area (Table 17). 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. Monitoring continues in order to add to our understanding of checkerspot population dynamics and to support military training at JBLM.

Checkerspots at SCS1 have met criteria 1-3 each year since 2017, with a peak single day abundance estimate of 5,484 (95% CI: 4,214-7,137; Table 15), up 13 percent from 2018 (Linders et al. 2019) and continuing a positive trajectory since 2014 (Appendix B). Checkerspots at SCS1 occupied 93.0 percent of the 20-ha monitoring unit in 2019, with restoration continuing across the site. This site far exceeded its Year 3 goal and remains on track to meet all establishment criteria by 2021. Similar to R50, checkerspots at SCS 1 are also dispersing into surrounding habitat (Appendix C, Fig. 2), where colonization potential is high (e.g., SCS2).

One adult checkerspot was observed at GHP in 2019, although no formal surveys were conducted. Releases were discontinued at there in 2018 and will not resume until factors affecting our success are better understood. These may include the condition of food plants, availability of suitable microsites, pesticide residues or other unidentified factors. See next section for a discussion of research needs related to larval host plant availability and microsite.

Future Plans and Recommendations

Captive Propagation

Mortality rates from egg to hatch (3rd instar) are often the highest of any life stage, but also the most variable both in the lab and in the field, which has raised questions about the usefulness of these data for tracking program outcomes. In addition, hatch rates for wild-sourced eggs are generally as high or higher than those from captive females. Consequently, there is an opportunity to re-evaluate what data we collect. Furthermore, considering on-the-ground success, we question the efficacy of continuing the captive mating component of the project vs. simply collecting the number of wild females needed to meet all of our oviposition needs. At the time of this writing the COVID-19 pandemic has upended the direction of almost everything, including captive rearing efforts for this project. As a result, both issues are being evaluated and have the potential to lead to significant program changes and provide some cost-saving as well.

Mortality at the Oregon Zoo from diapause to release (Table 11) was similar to 2018 and remains significantly higher than normal. The good news is that once the wave of postdiapause mortality passes, survival does not differ between facilities. Unfortunately concerns about providing old food (cut several days prior to being fed) were not addressed as completely as we thought, so may have continued to be factor in 2019. In addition, a standard protocol for fertilizing plants has been developed and implemented in response to the larval feeding trials conducted in 2018-2019. Finally, a thorough review of new vs. old methods also revealed that use of UV bulbs was inadvertently dropped when transitioning from the old to the new greenhouse in 2013. As UV light kills bacteria and viruses, it may be important for developing larvae. Although little information on the costs and benefits of UV light on caterpillar development is readily available in the literature, we were advised by invertebrate specialists at other zoos to use UV emitting glass in greenhouse construction. Additional work will be required to determine whether UV light plays a critical role in the patterns of mortality observed at the Oregon Zoo.

Taylor's checkerspot translocation in the Puget lowlands

The significant increase in the number of adults at R76 in 2019 relative to R50 and SCS1, and the rise to an all-time high, was stunning and underscores the ongoing importance of this site to Taylor's checkerspot recovery and long-term persistence on JBLM and in the Puget Lowlands as a whole. This site continues to provide critical new information that speaks to the dynamic potential of Taylor's checkerspot populations to rebound and to the plasticity of the species to respond to environmental change where opportunity exists. This is evidenced both by large increases in numbers (Appendix B), and by the one-week flight season extension that resulted from hundreds of larvae returning to the surface to feed two weeks after initiation of the adult life stage (see Adult presence and relative abundance).

Similarly, numbers at SCS1 and SCS2 rose markedly in 2019 with significant dispersal to surrounding areas. The potential for this site to fuel colonization of surrounding areas is great so long as habitat restoration and maintenance keep pace with threats from invasive weeds. This site differs from many other sites being restored for reintroduction because it is already home to numerous rare plant and animal species that must be protected in the course of habitat restoration. As a result, weed control requires a more delicate approach that reduces the effectiveness of some treatments, and requires they be implemented over a longer time frame.

In contrast to 2018, numbers at R50 in 2019 declined markedly, which may be a natural response to the very high numbers reported in 2018 and the dispersal of adults throughout the AIA (Appendix D). Although the site appears to support fewer host plant than in the past, no RHA work has been done since the original assessment in 2013 that would allow for a direct comparison. In general, R50 receives relatively little military training use, although use in the surrounding area has increased.

Monitoring results at TA15 in 2019 were very promising for a second-year reintroduction site and the expectation is that 2020 will be the final release year. Habitat conditions are excellent with an abundance and diversity of host plants across the site, which occur in high density patches on a range of microsites from cool north-facing ridges along the creek to warm south-facing slopes at the site's interior. Nectar plants are also varied and widespread, with many new plantings taking hold. The most promising of these is Puget balsamroot, a key nectar plant which also provides critical roost sites for adults, one of the few details missing in some areas of the site. Occupancy is also expanding across at TA15 and overall the site mirrors patterns observed at R50 in its first two years (2010-2011) and at SCS1 in 2015-2016, periods when those populations increased significantly (Appendix B).

Collectively, the potential of these large high-density sites to "reseed" surrounding areas also speaks to their importance for recovery at the landscape scale. Given the dynamic nature of checkerspot populations and their propensity to take advantage when conditions are right, a strategic approach to recovery should include development of multiple high-density populations that can function as sources of dispersing adults. When surrounded by satellite sites harboring high quality habitat, colonization events would have a high likelihood of success (Thomas et al. 2011), leading to a network of occupied sites that function as a mega- or metapopulation. Evidence that these conditions are developing (SCS; Appendix C, Fig. 2) or already occur in some areas (AIA; Appendix D) is strong, suggesting that if we can provide suitable habitat in a permeable landscape and initial populations of sufficient size and diversity, the species has what it takes to do the rest. Occupancy surveys are doing a good job of delineating checkerspot distribution and identifying concentration areas but understanding the size and persistence of these population segments and their role in recovery will require a more strategic approach to determine whether habitat conditions are likely to lead to long-term persistence. If there is one message that can be drawn increasingly from this work, it's that habitat quality, especially host plant patches, really matters. Where that is low (e.g., GHP), populations fail to maintain the critical densities needed for persistence, but where quality is high, habitat provides the mechanism needed to spur rapid population growth and sustained persistence. 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 consistent with the Rapid Habitat Assessment standards, including restoration of satellite sites within dispersal distance of source locations.
2) Define and then increase the number and distribution of population source sites on the landscape, and
3) Increase the number and size of populations, especially through colonization, but also by continuing active translocation to suitable sites.

Current translocation-related plans

This is the fourteenth year of the Taylor's checkerspot captive rearing and translocation project. The continued robust response of the checkerspot population at SCS and its broad distribution across the 121-ha site affirms the recommendation that additional releases are unnecessary, and the site is on track to meet or exceed the establishment target by 2021 (Tables 15 & 17 and Appendix C, Fig. 2). Occupancy surveys using a 50-m transect spacing were quick and easy to implement and should be continued to help inform restoration and site management. The restoration response to post-wildfire treatments has generally been positive, however full restoration and mitigation of fire-related impacts will require many more years. Unfortunately, combating the effects of the fire resulted in a reduction in restoration effort in core occupied parts of the site and at other WDFW sites being restored for checkerspot reintroduction. The collective setbacks of the 2017 wildfire, the loss of JBLM-ACUB funds, loss of access to inmate crews assisting with restoration and now the constraints of SARS-Cov2 have reversed the trajectory from maintenance to restoration. Habitat restoration at these sites must be a top priority to insure checkerspot recovery off JBLM is not delayed further.

If the doubling of the population at R50 in 2018 was considered a surprise, that may also be true for the drop observed in 2019 and is a reminder that our understanding of Taylor's checkerspot population dynamics is still limited. This is confounded by the significant amount of adult dispersal and apparent colonization of remote parts of the AIA. While the R50 population met establishment criteria in 2016, given the need to 1) secure existing populations for recovery, 2) establish multiple source populations, and 3) fully understand habitat potential at the site, we recommend monitoring at R50 continue.

Training Area 15 on JBLM has responded quickly and positively to reintroduction and was the highest priority site for release in 2020, both because of abundant "reintroduction-ready" habitat (Waters 2016) and a desire to reinforce the upward population trajectory (Table 15 and Appendix B). Given the number of larvae that have already been released on this site and the positive population response, the hope is that releases will be complete by spring 2020. This site promises dividends like those observed at R50 and SCS1 and would be an excellent location for further research on larval habitat quality that could highlight parameters critical to repeated translocation success (Thomas et al. 2011).

In contrast, TA7S continues to underperform for reasons that are not entirely clear despite having three 5-ac "reintroduction-ready" units. Reasons for this may include habitat configuration, poor microsite conditions, ongoing intensive restoration, bird predation, herbicide residue and other unidentified factors. In addition, while the site is rich with checkerspot host and nectar resources as well as adult cover, it is also the smallest reintroduction site and has a long narrow shape surrounded by open areas and suitable habitat that is not protected from concentrated pedestrian and vehicular training impacts. Unlike most sites, the restoration challenge at TA7S appears to be one of too much vegetation, even if much of it is native. The challenge of restoring this site and the generally poor response to reintroduction is another reminder that waiting to initiate releases until habitat is fully restored is a more effective strategy (e.g., Thomas 1989, Oates and Warren 1990, Stamps and Swaisgood 2007, Thomas et al. 2011) that should be employed whenever possible. Our reintroduction strategy on TA7S is to maintain occupancy while restoration continues and to identify and address potential threats as they arise.

In light of the broad distribution of checkerspots on JBLM and the fact that Johnson Prairie is not yet ready for reintroduction, turning attention to releases off base is of rising importance. Tenalquot Prairie and Bald Hill are two sites that have topped the list of priorities in Thurston County. Because habitat patches at Bald Hill are small and dispersed, a successful strategy there will require a large input of larvae at several locations simultaneously. Given the current funding structure and the need for a standardized, broad-scale habitat assessment at Bald Hill, that site is a lower priority for immediate release. Alternatively, restoration at Tenalquot Prairie, a small site adjacent to the Rainier Training Area, appears promising and is a good location to initiate releases in 2020 despite the lack of a recent habitat assessment. While this is not the preferred order of operations, it is at times the reality.

Continuing broad-scale searches outside of standard monitoring grids to look for adults and identify potential colonization areas remains a high priority to understand and document the scale of recovery. Staff at JBLM have done a great job conducting surveys on base that delineate occupancy across a broad landscape. While it is important for this work to continue in some areas, in areas where occupancy has been well-established in recent years new approaches should be employed to increase our knowledge of population size and persistence, to better understand the contribution of these sites to regional recovery. Documenting new breeding populations is a key element of recovery that will improve our understanding of checkerspot demography, ultimately reducing the cost of captive propagation and translocation by providing a supplemental path to increasing the size and number of populations.

Funding for both captive rearing and translocation is in increasingly short supply, with current monies projected to last only until September 2020 unless new funds are secured from JBLM and/or the USFWS. Funds have been requested from USFWS for 2020 which includes money for habitat assessments at Both Tenalquot Prairie and Bald Hill. Unfortunately, the funding outlook at JBLM looks very poor for 2020. Funding from additional sources is badly needed to stabilize the revenue stream, including monies to support widespread restoration off base. The loss of JBLM-ACUB funds has had profound effects on the pace and success of off-base restoration and subsequently, reintroduction efforts for Taylor's checkerspot. Continued success of habitat restoration and population growth at Scatter Creek is contingent upon sufficient funding to maintain adequate habitat in a suitable condition, which is also true of our ability to successfully establish population on other off-base sites.

A translocation plan for the Puget lowlands is nearly complete and lays out a long-term project approach including additional site selection criteria. The plan will be finalized in collaboration with conservation partners in 2020. Our objectives will be to continue with large multi-stage releases over several years, maintain more than one release site whenever possible, and insure that habitat is in a suitable condition prior to initiating releases. Sites are also 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, somewhat unpredictable and is a source of ongoing concern. The fundamental dependence of the project on those occupied sites and the vast amount of potential habitat on base continue to underscore the importance of JBLM to species recovery and long-term persistence in the Puget Lowlands. Access to R76 and R50 was primarily limited to a few Mondays and weekend days in April, with only 3 dates where weather corresponded with access between 15 and 30 April; similar issues arose near the end of the flight season in late May. Some dates also restricted the access footprint at R76 so as to make surveys unfeasible. 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 minimize access requests and accommodate uncertainty, even at a cost to monitoring on other sites. A more predictable management agreement would reduce the time and effort required to meet the survey requirements as contracted and create a more predictable training environment.

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 lifetables 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).

3) Can modeling using existing Puget Lowland data on population growth and spread be used to identify milestones and pathways for Taylor's checkerspot recovery?

Data generated by distance sampling, occupancy transects, scattered sightings and habitat assessment efforts indicate a tremendous expansion in checkerspot occupancy in the Puget Lowlands, with several strong population centers connected by a network of scattered individuals. These data span a period from 2007, when the species was reduced to a single local population, to 2019 when checkerspots exhibited strong populations on at least 3 sites and several satellite locations, in addition to hundreds of other sightings spread across thousands of acres. While individual movement data are not available, the history of scattered sightings data indicates these adults ultimately originated from one of the known populations, which could shed light on the species potential for landscape scale recovery and population level structure. Recovery planning objectives will ultimately require metrics for population size, persistence, dispersal, and landscape permeability. More advanced modeling using these data would provide a significant boost to our understanding and confidence in setting recovery objectives and developing structured processes for the decisions required to down-list and de-list the species and could also inform which strategy, reintroduction vs. passive colonization, will be a more effective solution for a given locale.

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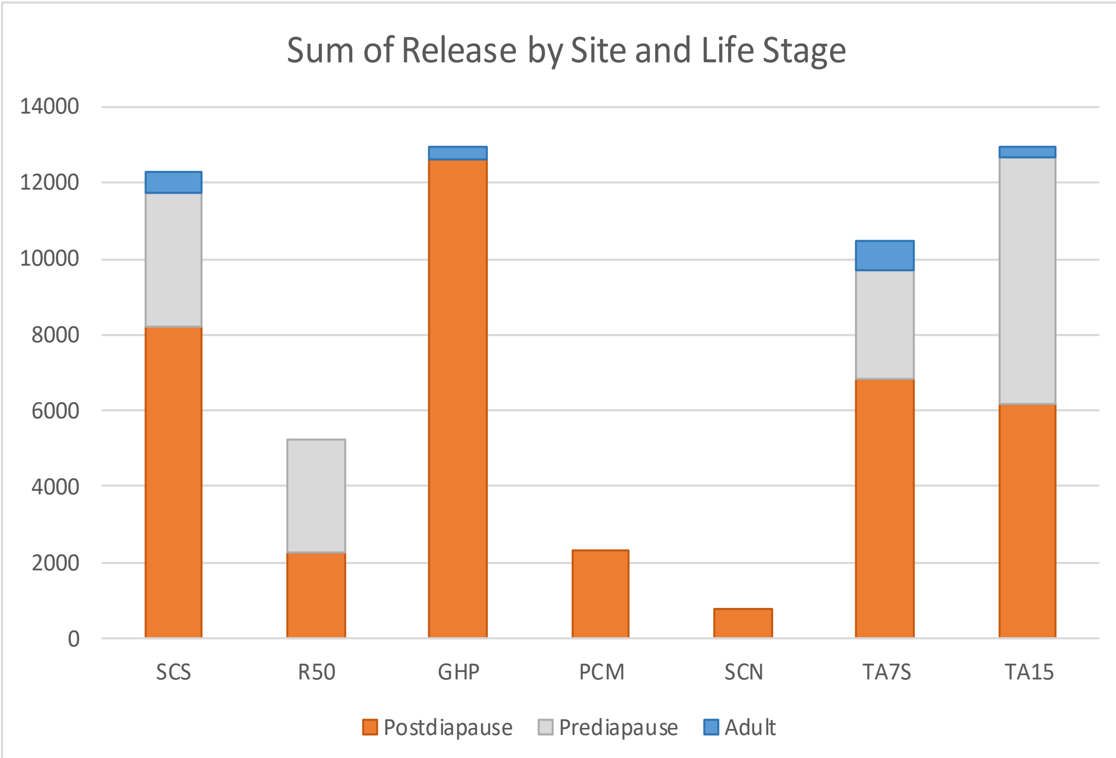
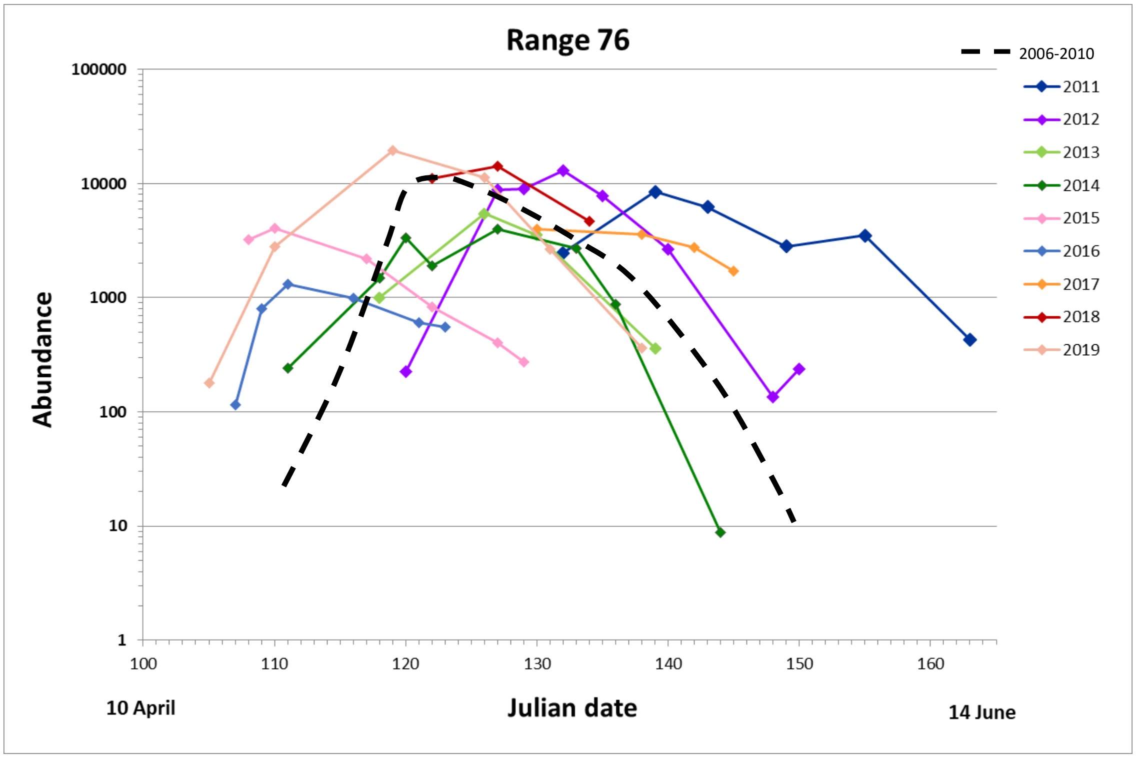
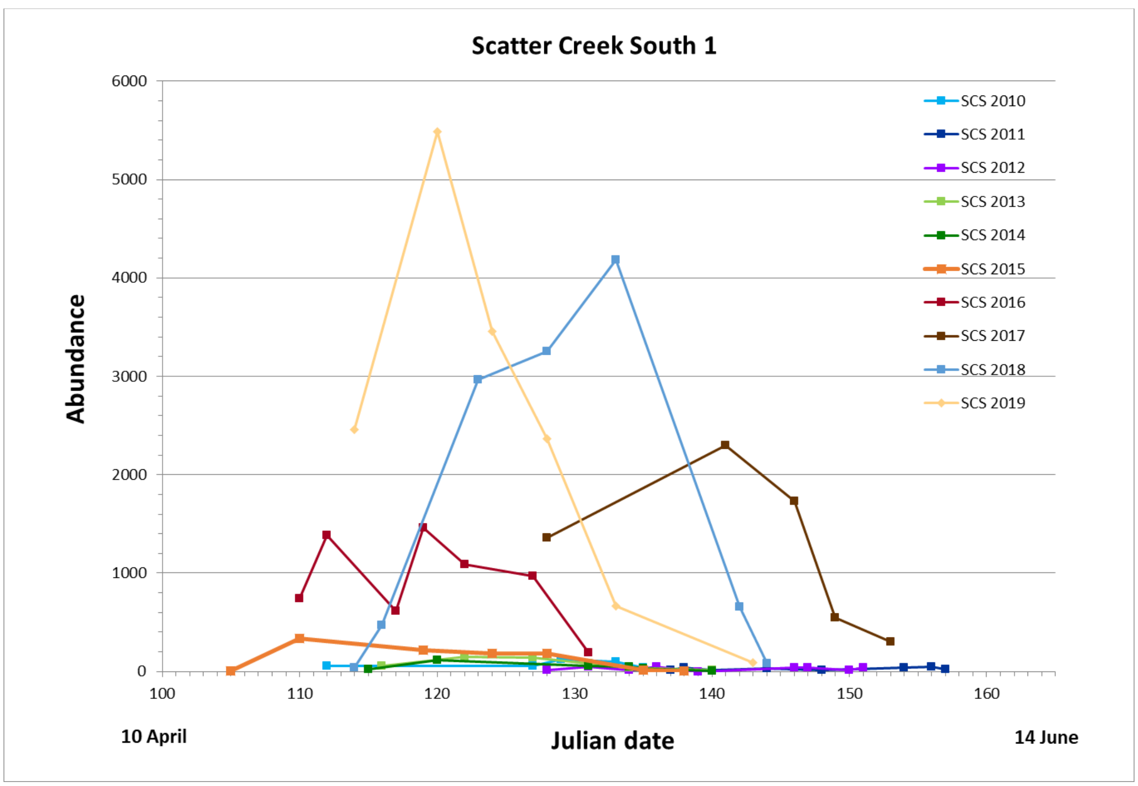
Appendix A. Approximate number of Taylor's checkerspot butterflies released by year, site and life stage (Po = postdiapause larvae; Ad = adult; Pr = prediapause larvae) and site (SCS = Scatter Creek South; R50 = Range 50; GHP = Glacial Heritage Preserve; PCM = Pacemaker Airstrip; SCN= Scatter Creek North; TA7S = Training Area 7 South; TA15 = Training Area 15) in South Puget Sound, Washington, 2007-2019. See also the graph by life stage and site on next page.

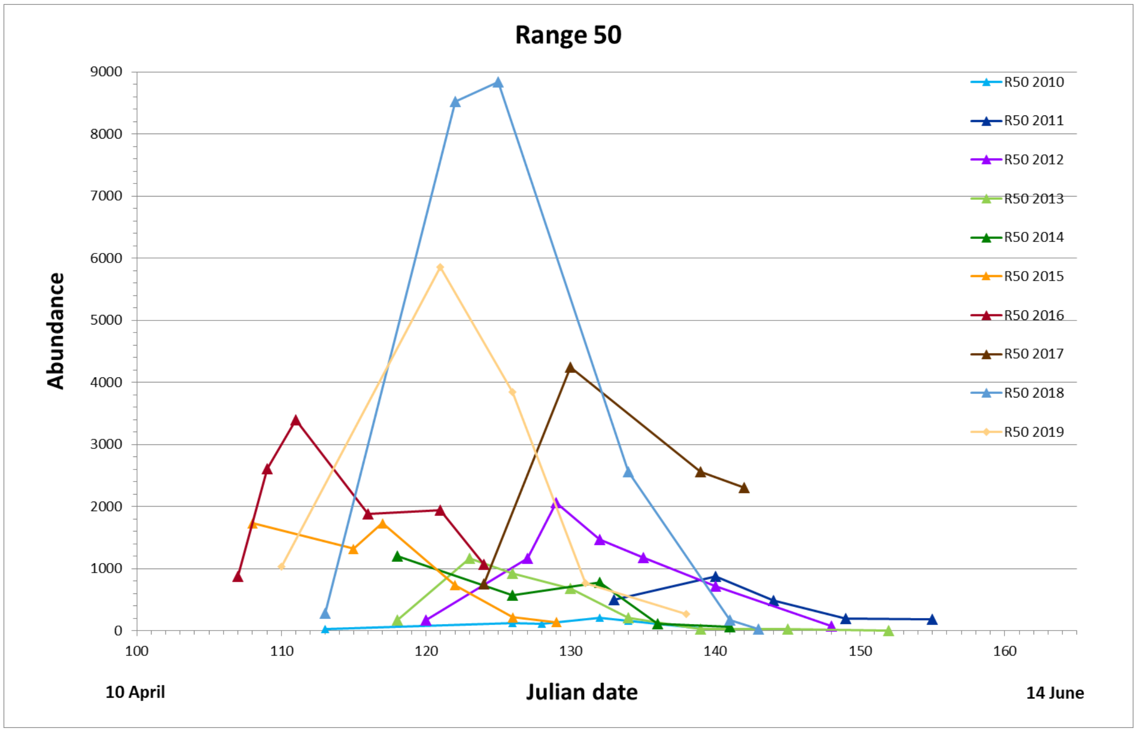
Year/Site	Stage	SCS	R50	GHP	PCM	SCN	TA7S	TA15
2007	Po	199						
2008	Po	340						
2009	Po	747			741	759		
	Pr	2487	2956					
2010	Po	891	1145					
	Ad	202						
2011	Po	1109	1141					
	Ad	167						
	Pr	1036						
2012	Po			975	1565			
	Ad	133						
2013	Po	3250		3372				
	Ad			107				
2014	Po			1522			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
2019	Po						3714	2847
	Ad						205	93
	Pr							3250

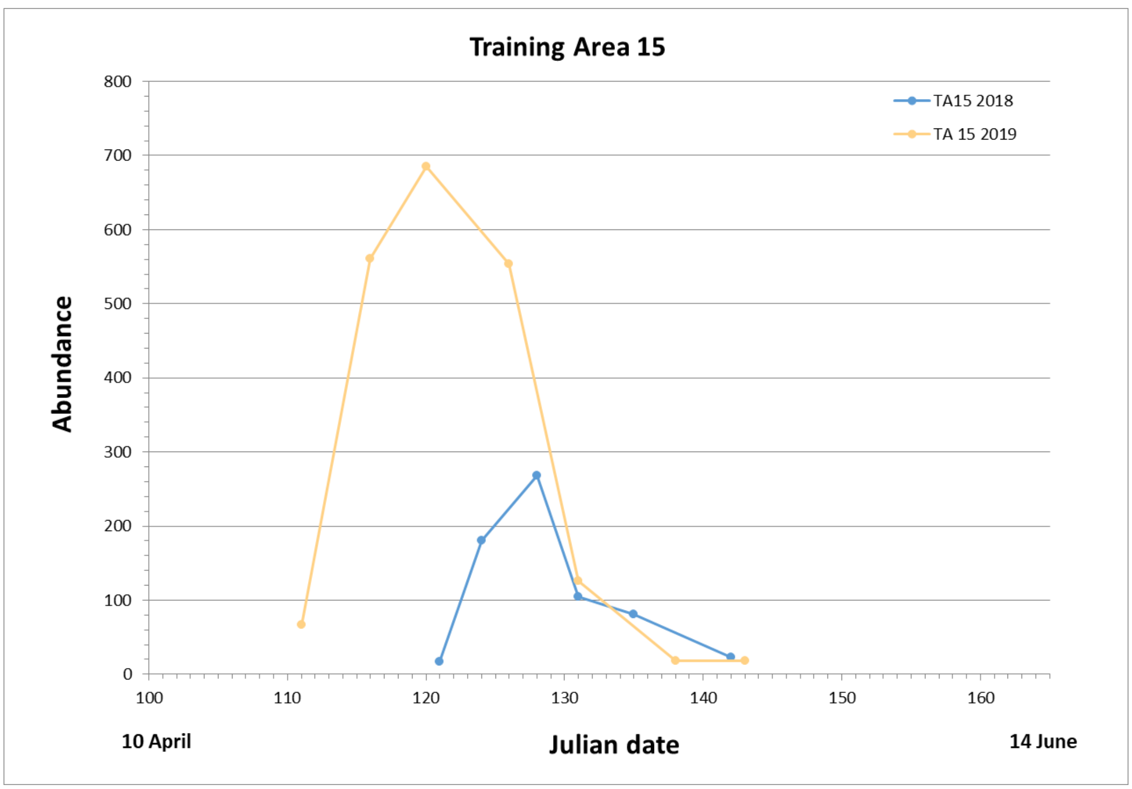

Sum of Release by Site and Life Stage
14000
12000
10000
8000
6000
4000
2000
0
SCS
R50
GHP
PCM
SCN
TA7S
TA15
Postdiapause
Prediapause
Adult

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 inclusive (▬), and in 2011-2019; 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 1 bottom) 2010-2019, Range 50 2010-2019 (page 2 top), and Training Area 15 2018-2019 (page 2 top). Area surveyed varies by site and year; see Table 13 and text for details.






Range 50
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Abundance
100
110
120
130
140
150
160
10 April
Julian date
14 June
R50 2010
R50 2011
R50 2012
R50 2013
R50 2014
R50 2015
R50 2016
R50 2017
R50 2018
R50 2019


Training Area 15
800
700
600
500
400
300
200
100
0
Abundance
100
110
120
130
140
150
160
10 April
Julian date
14 June
TA15 2018
TA 15 2019

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 2019.

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 2019, South Puget Sound, Washington.

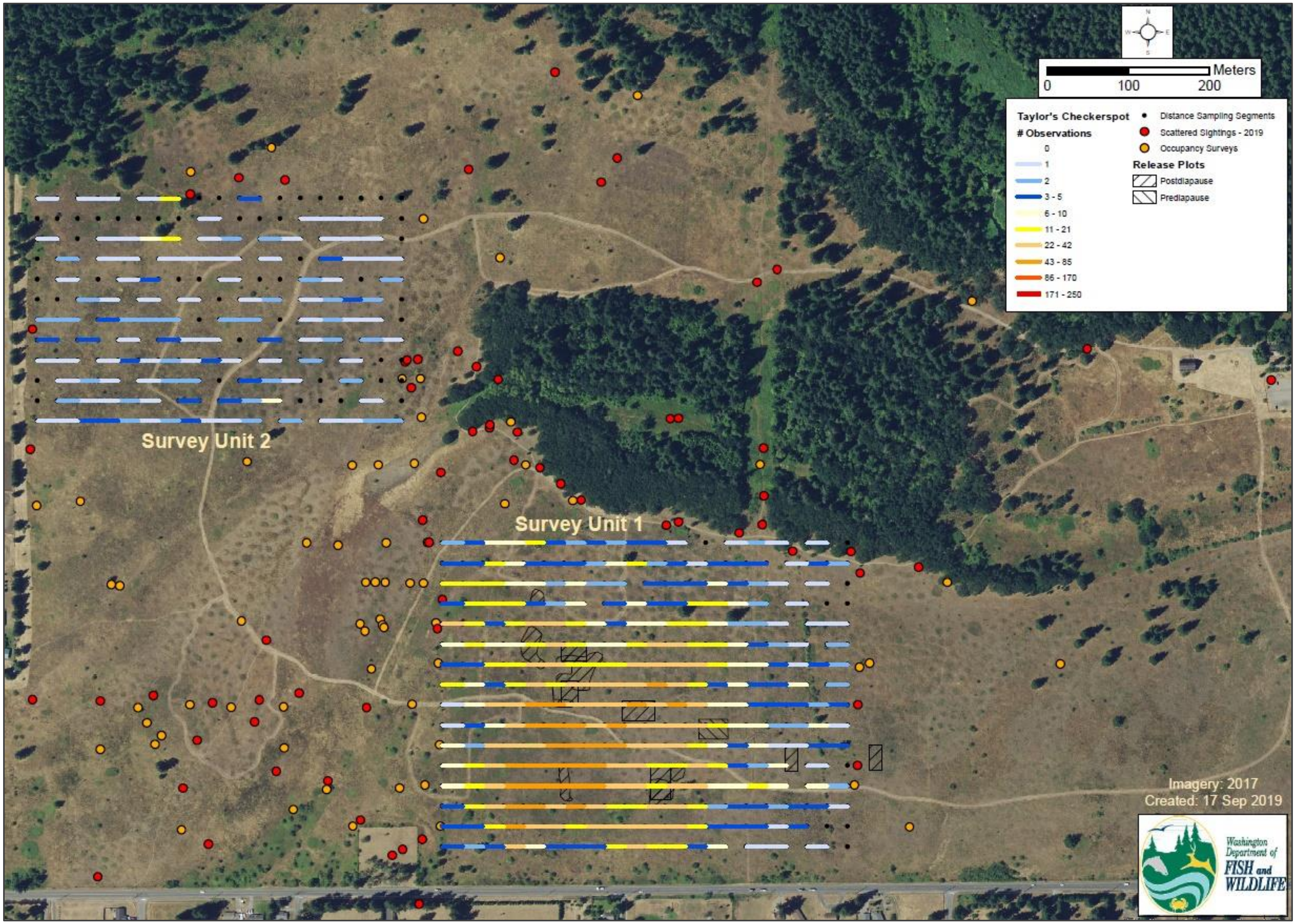


Figure 2. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Scatter Creek South in survey areas 1 and 2, combined across all survey dates in spring 2019, South Puget Sound, Washington. Also shown are scattered sightings and results of 50-m occupancy surveys.

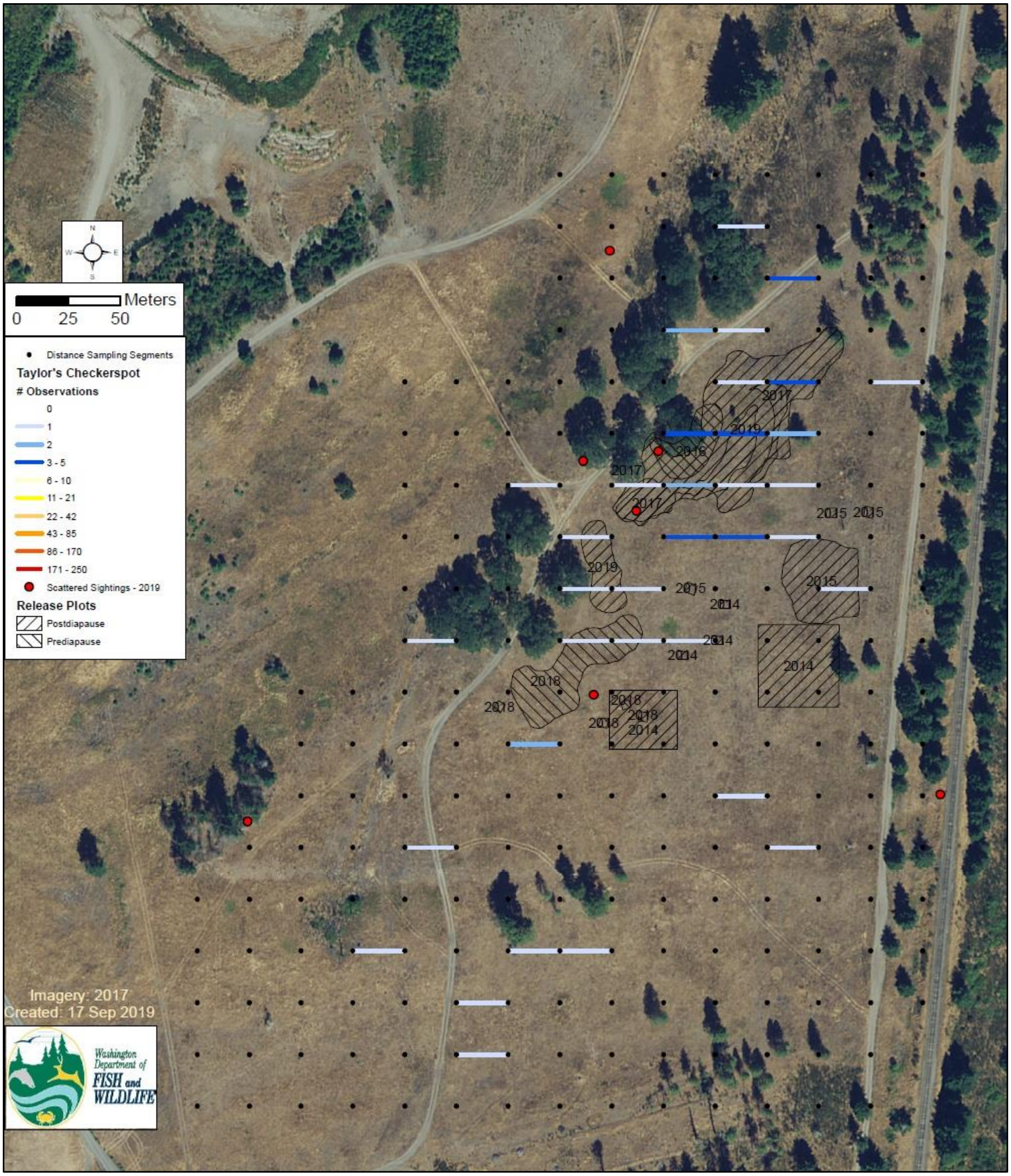


Figure 3. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Training Area 7S, combined across all survey dates in spring 2019, South Puget Sound, Washington.

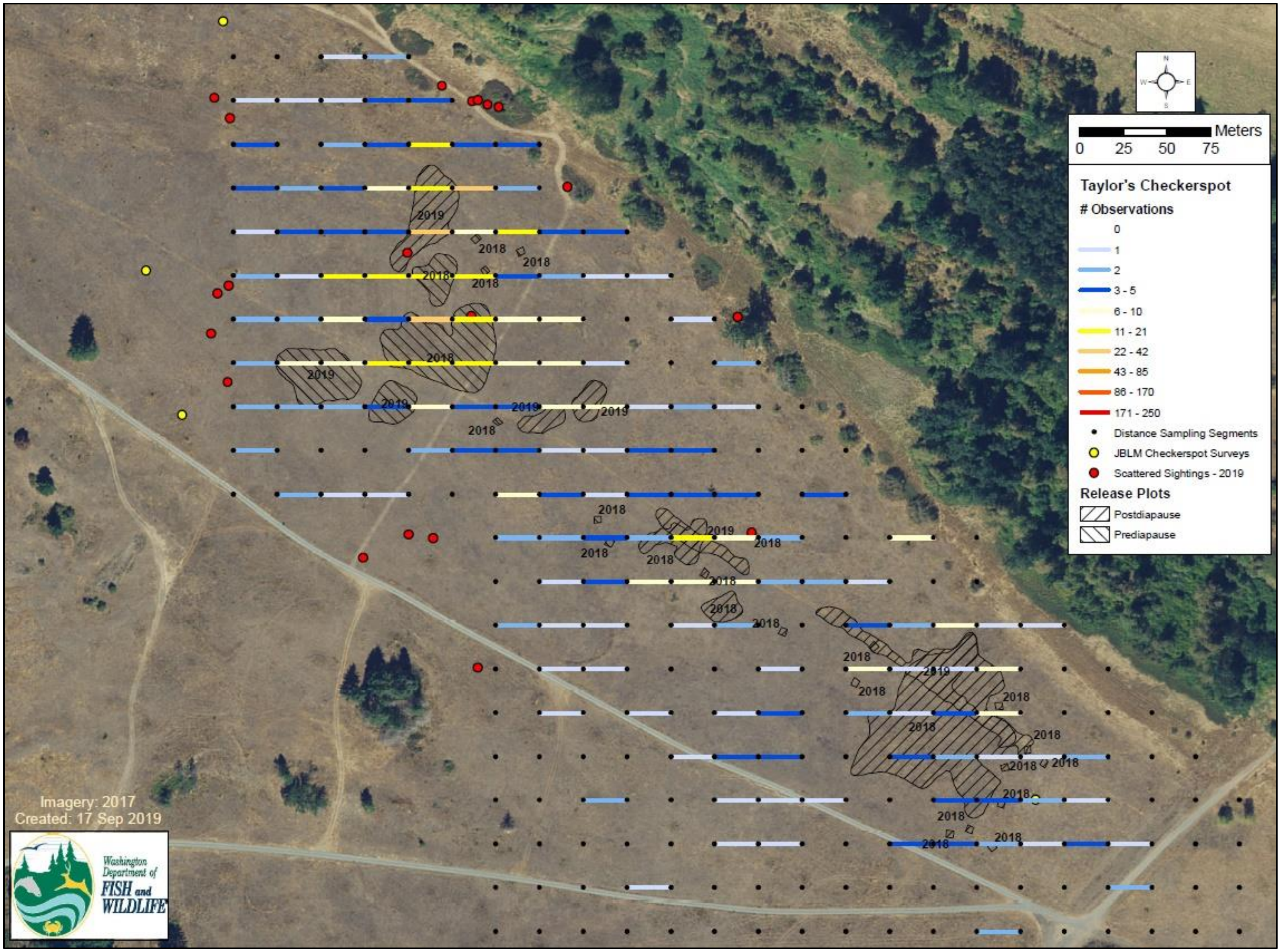


Figure 4. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Training Area 15, combined across all survey dates in spring 2019, South Puget Sound, Washington.

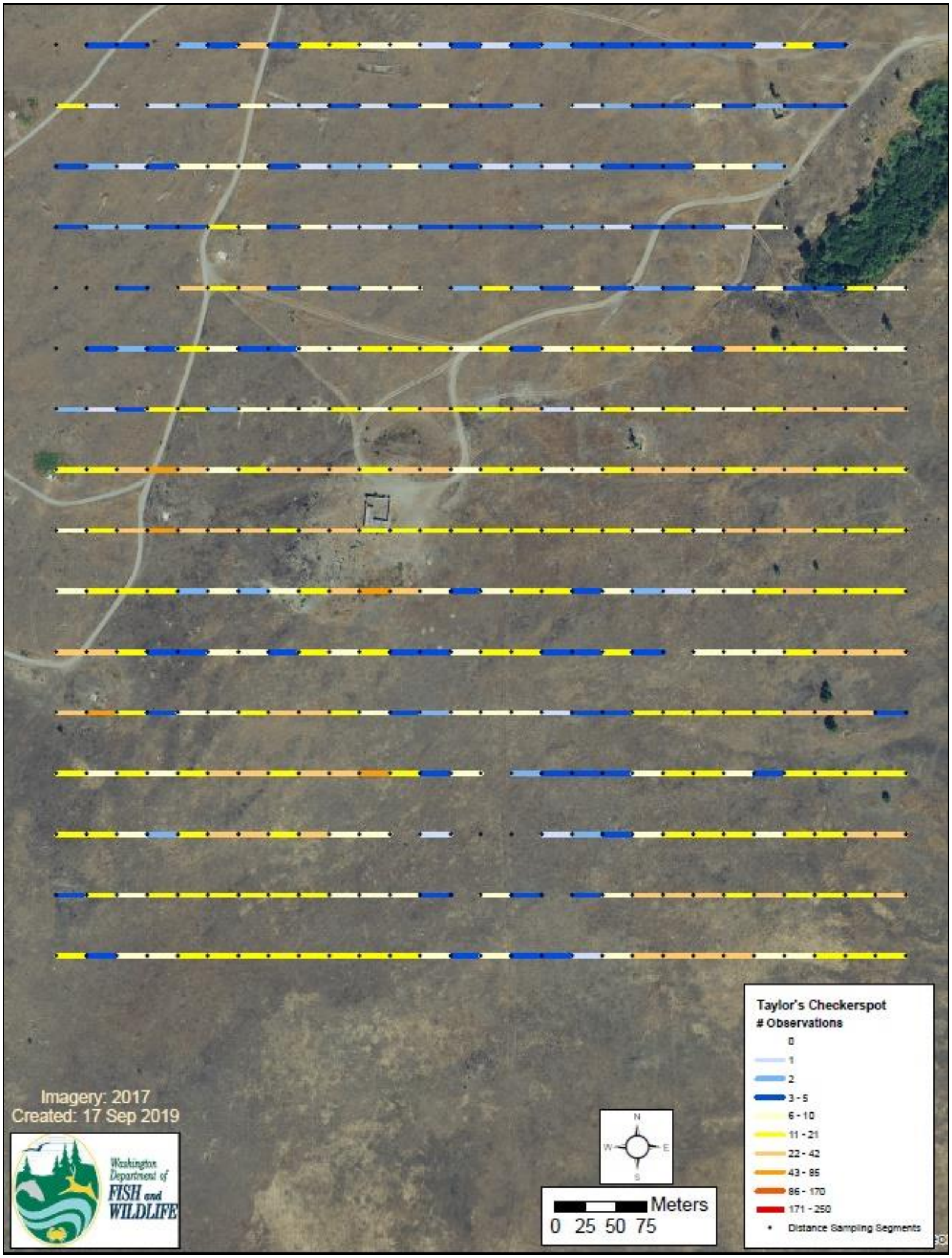


Figure 5. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Range 76, combined across all survey dates in spring 2019, South Puget Sound, Washington.

Appendix D. Adult Taylor's checkerspot observations (scattered sightings and occupancy survey transects) on the Artillery Impact Area (AIA) relative to standard monitoring areas (light green shade), Joint Base Lewis-McChord, Washington, 2019. North-south transect surveys with a 100-m spacing around the periphery of the AIA were implemented by JBLM staff and partners.