Taylor's checkerspot (*Euphydryas editha taylori*) Captive Rearing and Translocation: South Puget Sound, Washington, 2019-2020

2020 Final Annual Report associated with:
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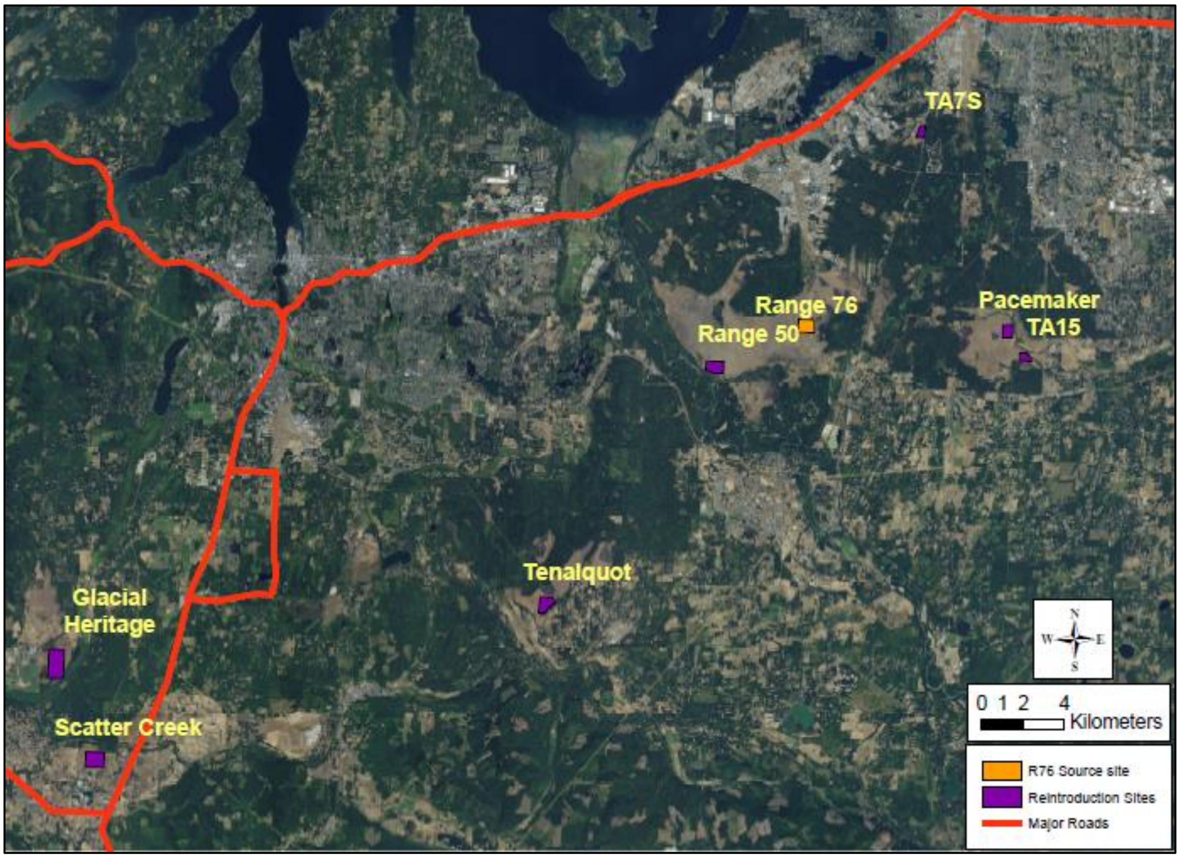
Project Sites

Captive rearing facilities are located at the Oregon Zoo, Portland, OR, and Mission Creek Corrections Center for Women in Belfair, WA. Translocation and monitoring sites for 2020 included Scatter Creek Wildlife Area – South Unit, (SCS1; releases in 2007-14, 2016); Training Area 15, JBLM, (TA15; releases in 2018-2020), Range 50, Joint Base Lewis-McChord (JBLM) (R50; releases in 2009-2011); and Range 76, JBLM (R76; population source for captive propagation). A second monitoring area at Scatter Creek South (SCS2) was established to document colonization. Past reintroduction sites that were not monitored in 2020 include Pacemaker Airstrip, JBLM (PCM; release in 2012); Glacial Heritage Preserve (GHP; releases in 2012-2017); and Training Area 7 South, JBLM (TA7S; releases in 2014-2018).



Project Partners

This project, initiated by the Washington Department of Fish and Wildlife (WDFW) in 2004, has grown to include a diverse set of public and private partners. Funding and support to restore habitat for Taylor's checkerspot throughout the Puget lowlands has been provided by US Fish and Wildlife Service (USFWS), Joint Base Lewis-McChord (JBLM) Army Compatible Use Buffer (ACUB) and Fish and Wildlife programs, WDFW, Washington Department of Natural Resources (WDNR), Washington State Recreation and Conservation Office, the Natural Resources Conservation Service and others. The Oregon Zoo, The Evergreen State College and Mission Creek Corrections Center for Women (Washington Department of Corrections - WDOC) are primary partners involved in captive propagation. The USFWS and JBLM-ACUB and Fish and Wildlife programs have been primary contributors of project funding. In-kind support has been provided by WDFW, JBLM Fish and Wildlife, The Oregon Zoo, WDOC, and the Center for Natural Lands Management (CNLM). Ultimately, JBLM, WDFW, WDNR, CNLM, Thurston County, Wolf Haven International and the University of Washington work cooperatively with USFWS to protect and enhance habitat for Taylor's checkerspot and other at-risk prairie species on 13 priority Puget Lowland sites.

Executive Summary

Taylor's checkerspot butterfly (*Euphydryas editha taylori*), is listed as federally endangered under the Endangered Species Act (ESA) and is State-endangered in Washington. It was extirpated from eight sites in the South Puget Lowlands between 1998 and 2006. Natural recolonization was precluded by isolation from the last naturally occurring Puget Lowland population on JBLM's Range 76 coupled with habitat loss and degradation. Captive-rearing followed by reintroduction to historic sites is being used to reverse these trends and to limit the conservation burden on key military training lands at JBLM. Associated conservation actions include but are not limited to habitat restoration, population monitoring, species research, and landowner agreements. Collectively these conservation measures are guided by the Taylor's checkerspot Action Plan developed by WDFW, USFWS and their partners and have been generously funded by USFWS, DoD, JBLM, WDFW and other conservation partners. The strength and flexibility of this coalition has garnered national attention as a model for public/private conservation partnership through joint acquisition, shared access, cooperative restoration, collaborative science and creative funding solutions.

This report summarizes work on Taylor's checkerspot captive rearing and translocation conducted July 2019-December 2020. The captive rearing section borrows heavily from annual reports produced by the Oregon Zoo and Mission Creek programs. At least 8,039 eggs from wild females and 8,923 eggs from captive-bred females were harvested at rearing facilities at the Oregon Zoo and Mission Creek Corrections Center for Women in 2019. In all, 12,924 prediapause larvae (7,212 wild and 5,712 captive) were produced, with 3,317 larvae released prior to diapause in 2019 and 9,506 larvae (7,034 wild, 2,472 captive) entering diapause in captivity in June 2019. Mortality at the Oregon Zoo from diapause to release remains higher than normal.

In a substantial departure from our strategy to date, all offspring of both captive-mated and wild females from both rearing facilities were released into the field in March 2020. This decision was the result of several factors including 1) ongoing high mortality following wake up at the Oregon Zoo, 2) declining funding projections and lost revenue sources, 3) the cost of the captive mating process relative to production, and 4) concerns over our ability to operate during the impending COVID-19 pandemic in early March 2020. In addition, a key objective of the captive mating program was to retain a captive colony to protect against loss of the wild source population. As there are now three strong populations in the South Puget Lowlands, maintaining this objective is less urgent than it was in 2008 when work began. For these reasons we don't anticipate re-initiating captive breeding in the near future, although sufficient documentation exists to make it a viable strategy should it be needed.

Another casualty of the COVID-19 pandemic was the loss of the captive rearing program at the Oregon Zoo. This is highly unfortunate as the Zoo has been fundamental to the inception and development of captive rearing for Taylor's checkerspot, and has been a valuable partner to Mission Creek, TESC and WDFW. We are grateful for their service! The loss of this program and associated funding declines will slow efforts to recover the species in the South Puget Lowlands.

A total of 4,935 postdiapause larvae were released at TA15 on 28 Feb, 4 and 17 Mar 2020, including 654 from Oregon Zoo and 4,281 larvae from Mission Creek. In addition, 2,183 larvae were released at TNQ (550 from Oregon Zoo and 1,633 from Mission Creek) on 9 Mar 2020. No adults were released in 2020. Neither were prediapause larvae available for release due in part to the safety restrictions employed in response to the coronavirus pandemic. The rainy and overcast conditions that prevailed through the majority of the flight season also negatively impacted the production of eggs and larvae.

Flight season initiation (13 April) was technically one day later than the earliest recorded date because 2020 was a leap year. However, COVID-19 restrictions prohibited WDFW staff from conducting most

field work until after 1 May, when the flight season was about half over. 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 11,305 checkerspot observations were recorded during distance surveys, with the greatest number again recorded at R50.

In 2020 we monitored the population source site (R76) to justify take and at Range 50 (R50 initiated in 2009), in case the site was needed for collection. Both sites are also monitored to support military training and to comply with JBLM's federal reporting requirements. Due to restrictions associated with the COVID-19 pandemic, site access and inclement weather we were unable to conduct enough surveys to meet the regional monitoring standards at these sites in 2020. Monitoring for establishment continued Scatter Creek South Unit 1 (SCS1; initiated in 2008) and at a second unit (SCS2) monitored for evidence of colonization. We also monitored two active reintroduction (TA15 and TNQ) sites for release success and population development. Tenalquot (TNQ) is a new site that was initiated in 2020. No distance sampling was conducted at TA7S due to time restrictions and poor results in recent years. However, one adult checkerspot was observed during 100-m occupancy surveys conducted by JBLM staff. No releases occurred there in 2020 and they are not expected to resume until factors affecting success are better understood.

Peak counts were recorded on 1-10 May, but this may not reflect the true flight season peak due to poor access and weather conditions. Based on year's past, the lag between flight season start and peak count suggests 29 Apr-1 May would have been peak flight, so the peak was likely missed on most sites. Peak day encounter rate data were also highest near that time. Peak day encounter rates at TA15 (0.129 /m) and SCS2 (0.064/m) were notable for being significantly higher than in 2019, whereas most sites they were lower. In contrast to year's past, counts at SCS2 followed the same timing and pattern of decrease as at SCS1, suggesting that site is now functioning as a population segment rather than just a pool of dispersing adults from SCS1.

Long-term monitoring and population goals developed in fall 2012 were used to assess progress at R50 and SCS1. Based solely on natural reproduction, adult checkerspots effectively occupy the entire 24.8-ha monitoring area at R50 for a third straight year. The peak single day abundance estimate for 2020 (5,633; 95% CI: 4,858-6,532), was recorded on 4 May and was similar to 2019 despite the fact that it was based on a smaller survey footprint. This together with the widespread dispersal observed in surrounding areas supports the view that this population may be at carrying capacity. Habitat monitoring using RHA has not been conducted since 2013, but anecdotal observation suggests host plant is being lost, which was also the conclusion of work conducted by CNLM using a different methodology (Kronland 2020). Habitat monitoring using RHA should be resumed to evaluate current condition and ensure it is being maintained; habitat loss and degradation is a primary threat to Taylor's checkerspot persistence. The peak single day abundance estimate recorded for R76 (5,725; 95% CI: 4,634-7,072) was collected on 9 May and is thought to be an inaccurate reflection of the true peak due to the later survey date. The R50 population was deemed to have met the project definition for establishment in 2016. The peak single day abundance estimate for checkerspots at SCS1 was 3,741 (95% CI: 2,997-4,669; Table 16) in 2020, well above the target of 250 adults. While this estimate is lower than that in 2019, the 2020 estimate may have been collected after the true peak; overall numbers remain strong. Checkerspots at SCS1 occupied 83.0 percent of the 20-ha monitoring unit in 2020 and are increasing in number at SCS2. Restoration is ongoing across the site but impacts from the 2017 wildfire cannot be negated. This site remains on track to meet all establishment criteria by 2021. Like R50, checkerspots at SCS1 are dispersing into surrounding habitat, where colonization potential is high (e.g., SCS2).

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 2019-2020 activities but includes some longer-term summaries as well. Objectives for 2020 were to:

1) Identify areas for release of larvae in collaboration with land managers,
2) Maintain captive propagation facilities and produce at least 4,500 postdiapause Taylor's checkerspot larvae for release,
3) Conduct postdiapause larval releases at two translocation sites (Training Area 15 and Tenalquot Prairie),
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 Area 15 and Tenalquot Prairie), and
    b. Evaluate translocation success based on presence, distribution and relative abundance of adults at three past translocation sites (Scatter Creek South Unit 1, Training Area 14 and Range 50) and one potential colonization site (Scatter Creek South Unit 2).

We implemented this year's work with joint funding from JBLM's Fish and Wildlife program, and the USFWS Recovery Implementation Program, with in-kind support from JBLM, the Oregon Zoo, and the Washington Department of Corrections. For clarity and cohesion, this report covers all 2019-2020 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 2015). 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 USFWS, WDFW and JBLM personnel and other state and local conservation partners. 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 2015). 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 2015). 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

Eight 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). Five sites, SCS (2007-2014, 2016), R50 (2009-2011), GHP (2012-2017), TA7S (2014-2019), and TA15 (2018-2020) have received multiple sequential releases (Appendix A). Two sites (SCN and PCM) were also included in a research-related release in 2009 which failed to produce more than a few adults. These were subsequently dropped, although one site (PCM) received a single release three years later (2012), after which releases were restricted by JBLM. A new site, TNQ, received its first release in 2020.

Overall warm conditions followed plenty of rain in January and February, which helped spur growth of *Plantago*. Host plants were abundant and easy to find at TA15, whereas heavy thatch obscured host plants in places at TNQ and raking was needed to expose plants to the sunlight and spur growth. No prediapause releases occurred in 2020 and no sites were prepped.

In 2020 postdiapause releases occurred at TA15 (3rd year) and TNQ 1st year), consistent with our existing strategy (Linders et al. 2015b). We did not prioritize a postdiapause release at TA7S due to restoration constraints, preferring instead to maximize the number of larvae at the newer release sites, TA15 and TNQ. We established thirteen postdiapause release plots at TA15, 9 of which were 4x4-m plots for monitoring survival (Appendix C, Fig. 4), and eleven postdiapause release plots at TNQ, 7 of which were survival monitoring plots (Appendix C, Fig. 3). Both TA15 and TNQ 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. At both TA15 and TNQ the number of larvae released

Table 1. Size and area of release plots for Taylor's checkerspot larvae by life stage and translocation site: Training Areas 15 (TA15) and Tenalquot (TNQ), South Puget Sound, Washington, spring 2020.

Site	Plot ID	Sq meters	Acres
TA 15 - postdiapause	K	675.3	0.167
	L	1457.1	0.36
	M	993.3	0.245
	N	504.1	0.124
Survival plots = 16m²	S20 – S28	144	0.035
Postdiapause total		**3773.8**	**0.931**
TNQ - postdiapause	A	555.7	0.137
	B	365.4	0.09
	C	458.4	0.113
	D	277.4	0.068
Survival plots = 16m²	S1 – S7	112	0.027
Postdiapause total		**1768.9**	**0.435**

exceeded the minimum habitat requirement, providing less than 1 square meter of habitat per released larva (See Postdiapause larval release). However, many host plants were very large and dense in places, so that total amount of forage was believed to be adequate.

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. In 2019, a second rearing structure was completed at Mission Creek, increasing rearing capacity by about 30 percent.

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 1500 adults at R76 is sufficient to supply the minimum of 30 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 1500 adults (Appendix B). Should something happen to the wild population, 10 females per rearing structure 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 15,000 eggs (5,000 per rearing structure) 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 40 gravid wild females were collected from R76 on 3 May 2019 JBLM following methods reported previously (Linders and Lewis 2013). Half of these were delivered to Mission Creek and half to the Oregon Zoo on the same day as collection; all were in good condition.

Captive mating

Methods

In all, 237 adults from 10 maternal lines originating as eggs in 2018 (18FL) were available for inclusion in the captive breeding colony at the Oregon Zoo in 2019. Similarly, 361 adults from 18 maternal lines were included in Mission Creek's breeding colony (Table 2). Following eclosion, adults were processed and housed according to established procedures (Barclay et al. 2009, Lewis et al. 2013). As in the past, a daisy chain was used to assign pairing of males and females by matriline (Schad 2008; Lewis et al. 2013). Daisy chains were created using a female driven, late binding strategy (Lewis et al. 2015).

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 2019.

Matriline	Adults	Females	Males
Oregon Zoo			
18FL803	20	10	10
18FL807	28	13	15
18FL808	26	13	13
18FL815	25	7	18
18FL816	25	12	13
18FL820	21	7	14
18FL823	30	13	17
18FL824	14	8	6
18FL826	24	11	13
18FL829	24	14	10
OZ Total	**237**	**108**	**129**
Mission Creek			
17FL708	1	0	1
17FL709	3	2	1
17FL716	1	0	1
18FL809	12	7	5
18FL811	30	14	16
18FL814	29	17	12
18FL819	35	20	15
18FL825	27	11	16
18FL828	29	12	17
18FL833	26	12	14
18FL835	30	14	16
18FL837	24	12	12
18FL843	40	19	21
18FL804	11	7	4
18FL822	27	7	20
18FL832	17	7	10
18FL834	9	7	2
18FL838	10	4	6
MC Total	**361**	**172**	**189**

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 could copulate only once to increase the genetic contribution from each line. We moved females to oviposition chambers when copulation was complete.

Results and Discussion

The copulation rate at the Oregon Zoo was higher in 2019 (0.43 copulations per introduction, Table 3) than in 2018 (0.26; Linders et al. 2020). However, at Mission Creek, a decline from 0.86 copulations per introduction in 2018 to 0.22 in 2019 (Table 3) reversed gains made in 2018, which were attributed to more exacting procedures. It's unclear what contributed to the reduction in copulation success in 2019, because there were no difference between the new and old greenhouses at Mission Creek (Table 3).

Table 3. Taylor's checkerspot captive breeding by matriline and dyad (ID), including numbers of introductions, unique females, copulations and productive females (i.e., those with eggs that hatched) conducted at the Oregon Zoo, Portland, Oregon and Mission Creek Corrections Center for Women, Belfair, Washington, spring 2019.

Female ID	Male ID	Introductions	Females	Copulations	Productive females
Oregon Zoo					
18FL803	18FL816	5	5	2	2
18FL807	18FL815	2	2	2	1
18FL808	18FL803	4	4	2	2
18FL815	18FL808	11	8	1	0
18FL816	18FL807	5	5	2	1
18FL820	18FL829	7	4	2	0
18FL823	18FL820	5	5	2	0
18FL824	18FL826	3	3	2	2
18FL826	18FL824	5	5	2	0
18FL829	18FL826	3	3	2	0
OZ Total		**50**	**44**	**19**	**8**
Mission Creek	Raven – old greenhouse				
17FL709	17FL708	2	2	1	1
17FL709	18FL837	2	1	0	0
17FL723	18FL837	1	1	1	0
17FL723	18FL843	1	1	0	0
18FL809	18FL811	22	7	3	3
18FL811	18FL835	9	8	2	1
18FL814	18FL825	30	16	3	1
18FL819	18FL809	11	7	3	3
18FL825	18FL837	9	6	2	1
18FL828	18FL814	6	4	2	1
18FL833	18FL819	13	11	5	2
18FL835	18FL833	25	13	4	0
18FL837	18FL843	4	4	3	3
18FL843	18FL828	10	10	3	2
	Turtle – new greenhouse				
18FL804	18FL822	17	6	4	2
18FL822	18FL834	10	7	1	1
18FL832	18FL804	7	4	2	1
18FL834	18FL838	7	7	2	2
18FL838	18FL832	11	4	2	1
MC Total		**197**	**119**	**43**	**25**

Captive rearing: oviposition to postdiapause

Methods

Taylor's checkerspots readily oviposit in captivity, enabling collection of about 250 eggs from each gravid wild and captive female to meet a target of 15,000 eggs for 2019. 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. Larvae are reared exclusively on freshly cut *Plantago lanceolata* leaves rather than native host plants because it is 1) easy to grow and handle in the lab, 2) less prone to mold and desiccation, and 3) results in high survival (Linders 2007). Female choice of oviposition host species is a genetically derived trait and is unrelated to the host they fed upon 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 (19 and 24 Feb 2020 at Mission Creek; 21 and 25 Feb 2020 at Oregon Zoo), 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 larvae were ready for release.

Results and Discussion

At Mission Creek, all 20 wild females laid eggs that hatched (i.e., were productive), with 17 of 20 (85 percent) wild females productive at the Oregon Zoo in 2019 (Table 4). At Mission Creek, 58 percent of captive-mated females were productive (Tables 3 and 6) in contrast to 42 percent at the Oregon Zoo (Tables 3 and 5). At least 8,039 eggs from wild females and 8,923 eggs from captive-bred females were harvested at the two facilities. However, at the Oregon Zoo, three wild females (207 eggs) and 10 captive females laid eggs that did not hatch. At Mission Creek, 16 captive females laid 1,929 eggs that failed to hatch. Egg to hatch ratios >1.0 (Tables 5 &6) indicate inaccurate counts, a persistent challenge despite standardized techniques that makes hatch ratios hard to compare. Egg to hatch survival is the most variable life stage 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. Consequently, we are re-evaluating whether and how egg count data are collected as 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 2019. Only productive females (i.e., those with eggs that hatched) are included; number hatched taken at 3rd instar.

Female ID	Eggs	Hatched	Diapause In
Oregon Zoo			
P19FL901	385	407	394
P19FL903	283	246	243
P19FL905	210	173	171
P19FL907	299	279	223
P19FL909	280	276	266
P19FL911	346	315	306
P19FL913	280	262	259
P19FL915	311	322	319
P19FL917	185	174	171
P19FL921	166	140	139
P19FL923	219	227	224
P19FL925	193	180	179
P19FL927	297	256	215
P19FL929	113	91	90
P19FL933	161	119	119

P19FL935	218	199	198
P19FL937	107	100	100
OZ Total	**4243**	**3766**	**3616**
Ave	**213.0**	**188.3**	**180.8**
St Dev	**101.8**	**113.3**	**107.9**
Mission Creek			
19FL902	216	134	149
19FL904	211	192	186
19FL906	217	166	167
19FL908	200	209	203
19FL910	76	79	78
19FL912	121	121	121
19FL914	91	101	100
19FL916	86	93	95
19FL918	202	202	209
19FL920	292	270	252
19FL922	63	47	46
19FL924	282	256	254
19FL926	122	116	116
19FL928	165	171	169
19FL930	198	203	204
19FL932	177	183	183
19FL934	224	117	112
19FL936	169	172	172
19FL938	348	300	293
19FL940	319	314	309
MC Total	**3779**	**3446**	**3418**
Ave	**189.0**	**172.3**	**170.9**
St Dev	**80.8**	**73.5**	**71.0**

In all, 12,924 prediapause larvae (7,212 wild and 5,712 captive) were produced, with 3,317 larvae released prior to diapause in 2019 (Tables 5 & 6) and 9,506 larvae (7,034 wild, 2,472 captive) entering diapause in captivity (Tables 4-6). One larva (matriline P19FL905) at the Oregon Zoo did not diapause and continued development to pupation. Diapausing larvae were moved outdoors for cold diapause on 20 October 2019 at the Oregon Zoo, and 11 September 2019 at Mission Creek. Once in diapause most larvae are inactive, although some movement is normal, especially in late diapause (Fig. 1).

Table 5. Oviposition outcomes for captive-mated Taylor's checkerspots by female at the Oregon Zoo, Portland, Oregon, spring 2019. Only productive females (i.e., laid eggs that hatched) are included; number hatched is at 3rd instar. See Linders et al. (2020) for information on prediapause release in 2019.

Female ID	Eggs	Hatched	Prediapause	Diapause In
18FL803-14	214	190	125	60
18FL803-17	174	156	149	4
18FL807-22	212	56	45	6
18FL808-17	203	157	103	53
18FL808-21	157	111	98	7
18FL816-16	207	144	141	1
18FL824-03	191	95	72	0
18FL824-05	34	1	1	0

Total	**1392**	**910**	**741[1]**	**131**
Ave	**174.0**	**113.8**	**NA**	**16.4**
St Dev	**59.9**	**61.9**	**NA**	**25.0**

[1] Includes 7 quality control (QC) larvae were released from the Oregon Zoo.

Table 6. Oviposition outcomes for captive-mated Taylor's checkerspots by female at Mission Creek Corrections Center for Women, Belfair, Washington, spring 2019. Only productive females (i.e., laid eggs that hatched) are included; number hatched is at 3rd instar. See Linders et al. (2020) for information on prediapause release 2019.

Female ID	Eggs	Hatched	Prediapause Release	Diapause In
19MC709-01	168	126	73	49
19MC804-05	191	66	15	51
19MC804-11	223	240	135	106
19MC809-06	253	281	96	189
19MC809-09	253	260	202	54
19MC809-12	251	214	162	48
19MC811-24	273	244	188	47
19MC814-28	237	220	169	51
19MC819-09	207	71	5	63
19MC819-11	276	213	69	138
19MC819-20	213	171	122	49
19MC822-21	230	295	188	105
19MC825-22	248	231	181	49
19MC828-07	272	210	95	110
19MC828-09	164	41	0	41
19MC832-08	268	167	62	104
19MC833-12	334	278	81	196
19MC833-24	48	56	0	55
19MC834-06	247	259	118	138
19MC834-08	253	247	73	172
19MC837-05	247	207	75	117
19MC837-08	187	132	72	52
19MC837-15	206	98	50	48
19MC838-01	247	218	0	216
19MC843-29	190	216	105	69
19MC843-39	229	199	147	51
QC	18	22	21	14
Total	**5933**	**4982**	**2504**	**2382**
Ave	**219.7**	**184.5**		**88.2**
St Dev	**65.4**	**79.7**		**54.9**

A comparison of diapause movement across three rearing facilities in early February from 2018-2020 indicates almost twice as many larvae at Mission Creek were active compared to those at the Oregon Zoo (Washington population) and Coffee Creek (Oregon population; Fig. 1). This was also true in 2017. Reduced movement by Zoo larvae suggests they were in trouble prior to wake up, rather than signaling a response to some other difference or change (e.g., weather or early wake up).

Larval mortality during and after wake-up has been an issue at both the Oregon Zoo and Coffee Creek and does not appear to be related to overwinter temperatures (Linders et al. 2018). A more likely explanation is that some aspect of prediapause larval rearing (e.g., food or lighting) is affecting their condition prior to entering diapause. At the Zoo, larvae housed in solo cups in 2020 were more likely to survive to release (61 percent) than those in deli cups (25 percent; *t* (96) =7.37, p<0.001; Lewis et al. 2020), suggesting the smaller solo cups with less ventilation may provide a more suitable microclimate. However, all Mission Creek larvae overwinter in deli cups.

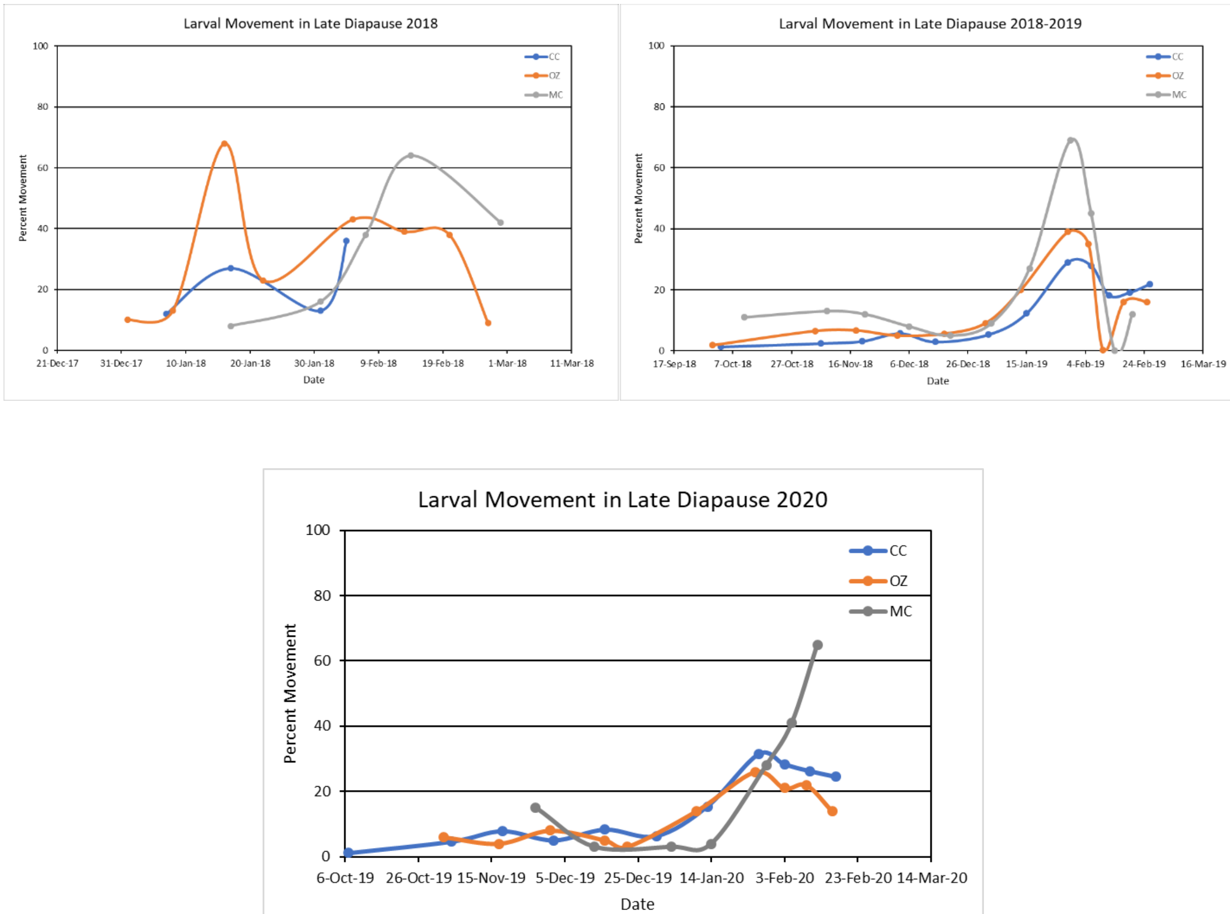


Figure 1. Percent of diapausing Taylor's checkerspot larvae by rearing institution that moved between checks while in diapause, winter 2018 (upper left), 2018-2019 (upper right) and 2019-2020 (lower). CC: Coffee Creek Corrections Center, OZ: Oregon Zoo, Portland, Oregon; MC: Mission Creek Corrections Center, Belfair, Washington.

In a substantial departure from our strategy to date, all offspring of both captive-mated and wild females from both rearing facilities were released into the field in March 2020 (Tables 7 & 8). This decision was the result of several factors including 1) ongoing high mortality following wake up at the Oregon Zoo, 2) declining funding projections and lost revenue sources, 3) the cost of the captive mating process relative to production, and 4) concerns over the impending COVID-19 pandemic in early March 2020. In addition, a key objective of the captive mating program was to retain a captive colony to protect against loss of the wild source population. As there are now three strong populations in the wild in the South Puget Lowlands, the need to maintain this objective is less urgent than it was in 2008 when captive mating began. For these reasons we don't anticipate re-initiating the captive breeding portion of the project in the near future, although sufficient documentation exists that it remains an available strategy should the time come when it is needed once again.

Another casualty of the COVID-19 pandemic was the loss of the captive rearing program at the Oregon Zoo. This is highly unfortunate as the Zoo has been fundamental to the inception and development of captive rearing for Taylor's checkerspot, and has been a valuable partner to Mission Creek, TESC and

WDFW. We are grateful for their service! The loss of this program and associated funding declines will significantly slow efforts to recover the species in the South Puget Lowlands.

Table 7. Numbers of Taylor's checkerspot larvae by matriline that emerged from diapause at Mission Creek Corrections Center for Women, Belfair, Washington and were released by site, March 2020. Quality Control (QC) larvae were separated from their siblings at some point in the rearing process.

Matriline	Diapause out	Released @TA15	Released @TNQ
17FL709	1	1	0
18FL804	25	24	0
18FL809	13	12	0
18FL811	4	1	0
18FL814	5	5	0
18FL819	5	3	0
18FL822	6	6	0
18FL825	8	6	0
18FL828	1	0	0
18FL832	14	0	12
18FL833	13	5	0
18FL834	21	21	0
18FL835	7	6	0
18FL837	12	11	0
18FL838	27	12	14
Subtotal	***162***	***113***	***26***
19FL902	149	149	0
19FL904	186	186	0
19FL906	166	165	0
19FL908	203	197	0
19FL910	78	78	0
19FL912	121	121	0
19FL914	99	95	0
19FL916	97	97	0
19FL918	207	134	72
19FL920	251	30	221
19FL922	46	0	48
19FL924	253	252	0
19FL926	116	115	0
19FL928	169	169	0
19FL930	202	145	58
19FL932	183	183	0
19FL934	112	101	0
19FL936	172	168	0
19FL938	290	299	0
19FL940	308	254	52
Subtotal	***3408***	***2938***	***451***
19MC709-01	48	0	49
19MC804-05	49	0	51
19MC804-11	105	41	45
19MC809-06	188	100	90
19MC809-09	54	39	0
19MC809-12	48	47	0

19MC811-24	47	0	47
19MC814-28	51	51	0
19MC819-09	63	0	60
19MC819-11	135	46	89
19MC819-20	49	0	49
19MC822-21	101	52	50
19MC825-22	49	48	0
19MC828-07	110	97	12
19MC828-09	41	15	26
19MC832-08	104	52	52
19MC833-12	196	100	96
19MC833-24	55	54	0
19MC834-06	138	50	89
19MC834-08	172	113	58
19MC837-05	117	50	67
19MC837-08	52	0	51
19MC837-15	47	46	0
19MC838-01	217	124	105
19MC843-29	69	50	19
19MC843-39	51	0	51
Subtotal	***2356***	***1175***	***1156***
QC	16	17	0
Total	**5926**	**4243**	**1633**

Rearing conditions

Methods

Our rearing strategy attempts to mimic ambient conditions to the greatest degree possible, because instituting environmental controls assumes the existence of detailed knowledge 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 extreme heat) and increase consistency and comparability between rearing facilities. 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 greenhouses (Table 9). Construction of a second greenhouse was completed in February 2019 (Curry et al. 2019), which is similar to the original facility in concept but larger (28x12 ft), and with UV-transmitting glass on the side walls and a polycarbonate roof and end walls designed to reduce heat build-up. It also features three 24-inch exhaust wired to a thermostat, and continuous side- and roof-vents that open manually. The only change noted by the Oregon Zoo was a shift from 40-watt to 160-watt bulbs for adult basking. Conditions were carefully monitored throughout the season and compared with the original structure to verify that 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 and monitor conditions during all rearing stages.

Results and Discussion

Temperature (°F) and relative humidity (percent RH) metrics recorded at the Oregon Zoo and Mission Creek in 2019-2020 are in listed in Table 10. Rearing conditions at all facilities followed similar patterns across the rearing year. In most cases temperatures met target levels, although they appear to have been cooler than desired during the adult stage, which may have affected copulation and egg-laying (Tables 3 & 4). Humidity levels during the adult life stage also tended to be lower than desired, even dropping below 25 percent at times at Mission Creek. Low humidity could affect both energy level and longevity in males. All other metrics reported were generally within range. Numerous staffing changes and other operational challenges at Mission Creek resulted in a loss of environmental during several life stages. However, in addition to data loggers, min/max units are used by technicians to monitor and adjust daily conditions, so do not we expect that conditions were problematic in spite of the data gap. Inadequate shade cloth on the new greenhouse and improper installation (e.g., draping over the roof vents) continued to affect performance in the new greenhouse, but should no longer be a concern in 2021.

Table 8. Numbers of Taylor's checkerspot larvae by matriline that emerged from diapause at the Oregon Zoo, Portland, Oregon and were released by site, March 2020. Quality Control (QC) larvae were separated from their siblings at some point in the rearing process.

Matriline	Diapause out	Released @ TA15	Released @ TNQ
18FL803	5	3	
18FL807	1	0	
18FL808	2	0	
18FL815	1	0	
18FL820	1	0	
18FL823	3	0	
18FL824	5	0	
18FL826	2	0	
Subtotal	*20*	*3*	*0*
P19FL901	328	89	37
P19FL903	194	0	34
P19FL905	152	28	48
P19FL907	195	85	19
P19FL909	210	78	47
P19FL911	214	20	46
P19FL913	153	21	7
P19FL915	42	6	2
P19FL917	151	34	39
P19FL921	86	11	13
P19FL923	184	27	50
P19FL925	156	68	19
P19FL927	206	62	57
P19FL929	90	36	53
P19FL933	110	0	49
P19FL935	81	12	0
P19FL937	80	15	10
Subtotal	*2632*	*592*	*530*
19OZ803	62	35	12
19OZ807	5	5	0
19OZ808	54	16	8
Subtotal	*121*	*56*	*20*
QC	7	3	
Total	**2780**	**657**	**550**

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, 2019-2020.

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 (floor)	TCB room	≤85 78-85 for 2-6 h/day	≥50	Ice packs; wet towels	Full shade	160-watt bulb 8-9 AM & 1-1:30 PM	None
Females and Oviposition	GH Small	TCB room	50-90 78-90 for 4-8 h/day	≥50	40-watt bulb 10 AM-2 PM or outside	Partial shade	160-watt bulb 10 AM-2 PM or outside	None
Egg & Prediapause	GH Main	TCB room	50-90; ave min ≤65	≤65	Ice packs; wet towels	None	None	7 AM - 7 PM
Warm diapause	GH Main	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	TCB room & porch	≤45 night ≥60 daytime	≥50	None	None	None	7 AM - 7 PM

Table 10. Actual average (ave) temperature (°F) and relative humidity (% RH) conditions and absolute range (rng) by life stage and date range during captive rearing of Taylor's checkerspot at the Oregon Zoo, Portland, Oregon, and Mission Creek Corrections Center for Women, Belfair, Washington, 2019-2020. 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 Old	Mission Creek New	Oregon Zoo	Terms	Mission Creek Old	Mission Creek New	Oregon Zoo
Males	23 Apr – 27 May 2019	28 Apr - 6 May 2019	mean max	78 (54-93)	81 (49-95)	**90** (57 - 98)	mean (rng)	**38** (19-70)	**38** (**12**-96)	**37** (32-83)
			#days w/i rng	**16 of 35**	**26 of 35**	**2 of 9**				
Females and Oviposition	8 - 22 May 2019	3 - 6 May 2019	mean (rng)	67 (57-97)	66 (51-90)	69 (55-96)	mean (rng)	48 (21-91)	**40** (**23**-94)	**42** (27-91)
			#days w/i rng	**4 of 15**	**11 of 15**	**4 of 14**				
Egg & Prediapause	8 May – 31 Jul 2019	4 May - 14 Jul 2019	mean (rng); ave min	68 (52-102); 60	66 (48-100); 56	68 (52 - 91); 60	mean (rng)	55 (26-100)	58 (22-97)	**42** (25-98)
Warm diapause	31 Jul – 12 Sep 2019	15 Jul - 19 Oct 2019	mean (rng)	71 (55-115)	NA	66 (54 - 78)	mean (rng)	55 (28-95)	NA	56 (32-99)
Cold diapause	12 Sep 2019 – 19 Feb 2020	20 Oct 2019 - 25 Feb 2020	#days min ≤35	NA	NA	**41** of 129	mean (rng); ave max	NA	NA	?? (32-100); 88
Postdiapause	19 Feb – 17 Mar 2020	22 Feb - 8 Mar 2020	#nts ≤45	NA	NA	10 of 16	mean (rng)	NA	NA	55 (49-95)
			#days ≥60	NA	NA	16 of 16				

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 typically 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, about 36 percent of hatch counts again exceeded associated egg counts (Tables 4 & 6), suggesting we should no longer be using these data as a measure of survival. However, either counts or estimates are still needed to manage egg collection and assess progress in meeting egg targets. In addition, hatch rates for wild-sourced eggs at the Zoo (19FL) and Mission Creek (19FL) were similar and were higher than those for captive-mated females at either facility (19OZ & 19MC) in 2020 (Table 11). Hatch to diapause survival was also higher at the Zoo for wild (19FL) vs. captive (19OZ) larvae, with very high survival reported for both groups at Mission Creek. Survival from diapause to release at the Zoo continues to be poor (Linders et al. 2014-2020) relative to previous results (e.g., Linders 2010-2012) and compared to Mission Creek, which has consistently maintained high survival for larvae entering first year diapause (19FL cohorts). The high rate of survival through diapause for 2nd and 3rd year larvae (18FL/17FL) at Mission Creek is also encouraging and follows progress in bringing this group through diapause to the adult life stage in 2019 (Linders et al. 2020). Checkerspots capable of multi-year diapause are assumed to be important for long-term adaptation to climatic changes. Even with the challenges we've faced at the Zoo, survival rates in captivity (Linders 2012, Linders and Lewis 2013, Linders et al. 2014-2020) continue to meet or exceed those that expected in the wild (Moore 1989), validating the ongoing success of the program.

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.

Table 11. Number and survival (Kaplan and 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, 2019-2020. 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 19FL**		**Captive 19OZ**		**Wild 18FL[2]**	
Life stage	**#**	**Survival**	**#**	**Survival**	**#**	**Survival**
Eggs	4243	---	1392	---	---	---
Egg to hatch	3766	0.89	910	0.65	---	---
Prediapause release	0	0.00	734	0.81	---	---
Hatch to diapause[1]	3616	0.96	131	0.74	36	---
Diapause to release	1124	0.31	77	0.59	3	0.08
Mission Creek						
Cohort	**Wild 19FL**		**Captive 19MC**		**Wild 18/17-1FL[2]**	
Life stage	**#**	**Survival**	**#**	**Survival**	**#**	**Survival**
Eggs	3779	-	5933	-	-	-
Egg to hatch	3446	0.91	4982	0.84	-	-
Prediapause release	-	-	2504	-	-	-
Hatch to diapause[1]	3418	0.99	2382	0.96	162	-
Diapause to release	3389	0.99	2331	0.98	139	0.85

[1] Hatch rates not adjusted for undercount of eggs.
[2] Multi-diapause larvae.

Results and Discussion

Wild female weights (Table 12) did not differ between Mission Creek and the Oregon Zoo in 2019 (p = 0.872), which is to be expected as they were all collected and delivered on the same day, and randomly assigned to the facilities. Wild females are always lighter than captive females at both facilities (p > 0.0001 in both cases) because while we collect fresh wild females, they are of an unknown age, whereas captive females are newly eclosed. However, as in years past (Linders et al. 2018, 2019), both male (p <0.0001) and female (p <0.0001) pupae were heavier at Mission Creek than at the Oregon Zoo (Table 12), but curiously, no difference was found in adult weights (male p = 0.969; female p = 0.953). Captive females from the Oregon Zoo were smaller than those collected from the wild in 2019 (p > 0.001), however this is not a consistent trend (Linders et al. 2020). Wing size data were not analyzed at Mission Creek due to high staff turnover.

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 4935 postdiapause larvae were released at TA15 on 28 Feb, 4 and 17 Mar 2020, including 654 from Oregon Zoo and 4,281 larvae from Mission Creek; 225 larvae were placed in nine survival plots. In addition, 2,183 larvae were released at TNQ (550 from Oregon Zoo and 1,633 from Mission Creek) on 9 Mar 2020 (Fig. 2), including 175 larvae placed in seven survival plots. Of the larvae released at TA15, 3,676 were offspring of wild females, 1,248 were offspring of captive-mated females, and 11 were of

Table 12. Descriptive statistics for pupal and adult weights, and wing area measurements for either wild and/or captive Taylor's checkerspot butterflies at the Oregon Zoo, Portland, OR, and Mission Creek Corrections Center for Women, Belfair, WA, 2018; only weights are included for adults whose wings eclosed improperly.

Unit of Analysis	Statistic	Pupal Weight (g)	Adult Weight (g)	Wing Area (cm2)
Oregon Zoo				
Captive males	N	127	122	95
	mean	0.1873	0.1018	1.0046
	var	0.0005	0.0005	0.0388
Captive females	N	110	99	86
	mean	0.2419	0.1637	1.2609
	var	0.0016	0.0004	0.0328
Wild females	N	NA	20	19
	mean	NA	0.1211	1.4699
	var	NA	0.0010	0.0588
Mission Creek				
Captive males	N	189	189	
	mean	0.2104	0.1034	
	var	0.0005	0.0046	
Captive females	N	174	172	
	mean	0.2726	0.1646	
	var	0.0009	0.0006	
Wild females	N	NA	20	
	mean	NA	0.1165	
	var	NA	0.0005	

unknown (QC) origin as they were separated from their sibling groups at some point in the rearing process. Larvae released at TNQ included 1,007 offspring of wild females, 1,176 offspring of captive-mated females; none 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 10.0-12.9°C, average wind speed 0.6-3.0 mph, and clear to overcast skies; weather in the days following release were dry for at least part of a day, with periods of sun interspersed with rain. At least 21 people assisted with releases, working from about 1000-1600 h each day to complete the task.

Figure 2. Release of postdiapause Taylor's checkerspot larvae (right) by volunteers (left) at Tenalquot Prairie, Thurston County, Washington, spring 2020.

Adult release

No adults were released in 2020. Wild adult females brought into captivity were permitted to lay eggs at will and held in captivity until they perished.

Prediapause larval release

No prediapause larvae were available for release in 2020 due in part to a conservative approach employed in response to the coronavirus pandemic. The rainy and overcast conditions that prevailed through the majority of the flight season also negatively impacted the production of eggs and larvae.

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 typical 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 at release sites.

Methods

To confirm site occupancy, quantify minimum post-release survival, and identify issues that may be cause for concern, we conducted surveys for postdiapause larvae in sixteen 4x4-m survival plots at two reintroduction sites on two occasions in the weeks following release. Plots were placed at least 10 m apart for independence. Nine survival plots were established at TA15 and seven at TNQ (see Postdiapause release) based on the relative number of larvae released at each site.

We used both single and double observer strategies to maximize coverage and measure detectability, respectively. A dependent double observer approach was used for a subset of plots on each survey date. Two observers cannot survey the same square meter simultaneously without interference, so the primary observer, going first, placed a pin flag with the group ID and number observed at each larval group. The secondary observer, following in close proximity, verified the presence of flagged larvae, then flagged and recorded any additional larvae observed. Larvae within 10 cm of one another were considered part of the same group. On occasion, larvae move away from their flagged location prior to confirmation by the secondary observer, so we assumed larvae within 15 cm of a flagged location were already counted unless they were in addition to those already counted. We thoroughly searched each 4x4-m plot area, also noting any larvae within one meter of the plot perimeter. For each group we recorded number of larvae, instar, behavior and substrate, and mapped each location on a plot diagram to reconcile differences between observers. We recorded larval instar because as noted on previously (e.g., Linders and Lewis 2013, Linders et al. 2014, 2015; Table 14), larvae may hide and therefore are unavailable to be counted during molt from 5th to 6th instar (when they are more vulnerable to predation), but re-emerge after reaching 6th instar. Observers switched roles between plots to account for differences in detectability between observers (Cook and Jacobson 1979) and to permit estimation of observer-specific detection probabilities (Nichols et al. 2000). Single observer plots were surveyed and marked in the same manner as double observer plots. All surveys were completed by 20 March to minimize the likelihood that larvae had pupated. The ratio of Single to Double observer plots varied by survey date to evaluate trade-offs between sample size and measurement of detectability (Table 13) during available weather windows.

To estimate abundance, we generated detection probabilities by observer within site (TA15 and TNQ) and date using double observer plot data (Cook and Jacobson 1979). Individual detection rates were estimated by date from double observer plot data (Nichols 2000) and applied to the respective data from single observer plots to estimate abundance (N-hat) for those plots. Similarly, the combined detection rate (p-hat) for both observers was used to estimate N-hat on double observer plots by date. All N-hats were then combined to estimate survival by site (Table 14). Survival estimates were calculated by dividing pooled abundance estimates (N-hat) for each survey date by the total number of larvae released into that set of plots. Problems arose when it came to estimating the variance, however. While Nichols et al. (2000) provide the variance equation for double observer plots, we have not yet succeeded in identifying the appropriate variance equation for single observer plots. To evaluate whether released larvae were surviving at similar rates across sites, we planned to calculate daily survival rates (Powell 2007) by site then compared them by calculating 95 percent CIs and checking for overlap.

Results and Discussion

Results of post-release surveys conducted for postdiapause Taylor's checkerspot larvae at TA15 and TNQ in 2020 appear in Table 13. We had enough good weather to complete two rounds of surveys at each site. In 2020, the effect of 5th to 6th instar ratios on detectability during initial surveys is confounded by the later release date at TNQ (9 Mar) compared to TA15 (4 Mar). However, ratios were similar by the second (final) survey, when nearly all larvae were in 6th instar, increasing confidence that those detection rates likely reflect relative survival between sites.

Table 13. Survey results pooled by site and date from sixteen 4x4-m survival plots, each of which received 25 postdiapause Taylor's checkerspot larvae at two reintroduction sites (Training Area 15 = TA15: n = 9 plots; Tenalquot Prairie = TNQ: n = 7 plots), South Puget Sound, Washington, spring 2020. Survey date, number of days post-release, ratio of 5th to 6th instar larvae observed, ratio of Single:Double observer plots, detection rates (p-hat), #larvae counted (Count), abundance estimate (N-hat), and proportion of larvae surviving (s-hat) are reported.

Date	Days	5:6	S:D	p-hat[1]	p-hat[2]	p-hat[3]	Count	N-hat	s-hat
TA15									
10-Mar	6	0.47	3.00	1.000	0.786	1.000	113	126	0.630
18-Mar	14	0.06	1.25	0.605	0.834	0.935	90	110	0.488
TNQ									
16-Mar	7	0.17	1.00	0.648	0.819	0.937	34	46	0.309
19-Mar	10	0.02	0.75	0.795	0.614	0.921	72	102	0.584

p-hat [1] - Individual detection rate for KC by site/date.
p-hat[2] - Individual detection rate for MJL.
p-hat[3] - Combined detection rate from double observer plots.

Detection rates (p-hat) were relatively high but varied somewhat by observer and date (Table 13). Observer 1 (KC) was new, whereas Observer 2 was experienced. Daily post-release survival was similar and high in both cases (TA15 = 0.955, TNQ = 0.948,), although we couldn't make a direct comparison between sites without estimating 95% Confidence Intervals. This was confounded by lack of a suitable equation for the variance of p-hat; we are continuing to search the literature to fill this gap. As in 2018, larval survival at the end of the survey period appears similar to the weighted average survival (0.476) calculated from Moore (1989) for *E. editha* in California across three years and two subpopulations. We assume most post-release mortality occurs within the first few days of release, as survival in the lab during this period is high (Table 11).

Winter and spring contained an undulating mix of very dry conditions in fall 2019 followed by ample amounts of rain in Jan and Feb 2020. The first half of March was fairly typical with periods of rain and sun interspersed. However, this shifted to very dry, sunny and warm conditions later in the month, pushing larvae to pupate 18-21 March and setting the stage for the flight season to start as early as 1 April. However, conditions returned to a more typical pattern at that time, with far fewer sun breaks, temperatures dropping into the 30s at night and rarely exceeding about 53 F during the day. Several large hailstorms and frequent rain prevailed through the first week of April before better weather prevailed, allowing initiation of the flight season.

Warm conditions during the post-release feeding period and the fact that a large portion of larvae were already in 6th instar at the time of the first survey (Table 13), suggest some larvae may have pupated by the time the second round of surveys had been conducted, which would have the effect of reducing the true survival estimate. In most years, most larvae appear to pupate simultaneously, especially when weather conditions are relatively warm and dry. The opposite may be true in cool and wet years, which tend to 1) emphasize differences in initial body size of larvae emerging from diapause, 2) draw out the postdiapause larval feeding period, and 3) affect the rate of return to diapause. Larvae that emerge smaller must feed longer prior to pupating to make up for limits in the amount of daily forage time available, and some larvae may significantly delay the decision of whether to return to second diapause.

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, TA15 and TNQ), one colonization site (SCS2), and one extant site (R76) were surveyed for adult checkerspots during the 2020 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 surveyed routinely. Transect length and spacing by site and year is shown in Table 14. 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 14. Number and size (m) of distance sampling units, survey length and area for density estimation by site and year on extant (R76) and reintroduced Taylor's checkerspot sites, South Puget Sound, Washington, 2010-2020. 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), and Tenalquot (TNQ).

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	2016-20	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	2017-20	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	2014-20	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	2017-20	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	4-17	25	100-425	6350	15.9
TA15	2020	23	25	7-17	25	175-425	8525	21.3
TNQ	2020	11	25	13	25	350	3850	9.6

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

Results and Discussion

Although fall 2019 was notably dry, rain was abundant in Jan and Feb 2020 with fairly warm conditions prevailing through the spring. In the first 12 days of March rain interspersed with sun made for typical conditions. Following postdiapause release, however, 10 days of warm, very dry and sunny weather led larvae to pupate between 18-21 March with a predicted first flight of April 1. However, in late March and early April, fewer sun breaks along with temperatures in the 30s (F) at night and low 50s during the day slowed development. And on 31 March an intense storm with BB size hail lasted more than 15 minutes, with a second shorter hailstorm on 2 April; it rained most days the first week of April.

Flight season initiation (13 April) was technically one day later than the earliest date recorded because 2020 was a leap year. However, COVID-19 restrictions prohibited WDFW staff from conducting most field work until after 1 May, when the flight season was about half over. Ongoing issues with poor weather and limited staffing meant few complete distance surveys were conducted in 2020 (Table 15).

Access to R76 and R50 was unpredictable because JBLM shifted to a weekly scheduling format in mid-April, although Monday access remained a in place. In addition, some days we lacked PPE and could not enter the duded area while on others the duded area was closed. JBLM prohibited pin flag placement in the Artillery Impact Area (AIA), which left us without a way to mark survey transects. In addition, the location of lark nest protection circles excluded access to some segments. These and other access issues challenge our ability to implement a standardized monitoring protocol. Due to the combination of access, weather, COVID-19 restrictions, limited staffing and other challenges, we were unable to achieve the minimum of five qualifying surveys at any of our sites in 2020 (Table 15).

Table 15. 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), Training Area 15 (TA15), and Tenalquot (TNQ), South Puget Sound, Washington, spring 2020.

Date	R76	R50	SCS1	SCS2	TA15	TNQ	Comments (PS = Partial Survey, NP = Non-protocol weather)
13-Apr	1						First flight
15-Apr			1				First flight
20-Apr	498						PS: T 5-10, NP
24-Apr					4		NP abort
26-Apr	770					1	R76: PS (T5-16), NP count only; TNQ: PS (T5-11), NP abort
29-Apr					220	16	TA15: PS (T11-26), lost weather and access
30-Apr					381		TA15: PS (T4-10), continuation of 29 Apr survey
1-May					1073	7	TA15: Omit segments 31+; TNQ: NP
4-May		1972					R50: PS (T5-16), lost weather and access
5-May			930	344			SCS1: PS (omit T6-7)
7-May					423	12	TA15: Omit segments 31+
8-May			846	297			
9-May	1004						
10-May		2080					
11-May						3	
13-May					29		PS (omit T6, 9, 12, 15, 18, 21, 24; omit segments 31+)
15-May			176	183			
18-May	25						PS (omit T1, 4, 7, 10, 13, 16)
22-May		6					Observed; NP
27 May			2	3			Site check
28-May					0	0	One observer; odd transects only.
1-Jun		0					One observer; odd transects only.
Total	**2297**	**4058**	**1954**	**827**	**2130**	**39**	

Density and abundance estimates 2020

Distance data were analyzed for five sites (R76, R50, SCS1 SCS2, and TA15) in 2020; results are presented in Table 16 and Appendix B. A total of 11,305 checkerspot observations were recorded during distance surveys (Table 15). Peak counts were recorded on 1-10 May, but we lack confidence in the accuracy of that assessment due to poor access and weather conditions. Based on year's past, the lag between flight season start and peak count suggests peak flight would have been 29 Apr-1 May, so we likely missed the peak on most sites. Peak day encounter rate data were also highest near that time (*unpub. data*). Peak day encounter rates at TA15 (0.129 /m) and SCS2 (0.064/m) were notable for being significantly higher than in 2019 (Linders et al. 2019), whereas most sites were lower. In contrast to year's past, counts at SCS2 followed the same timing and pattern of decrease as at SCS1 more closely, suggesting that site is beginning to function on its own rather than just a collection of dispersing adults from SCS1.

At least one checkerspot was observed at TA7 by JBLM staff, although no transect surveys were conducted due to time restrictions on WDFW staff. 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 on by JBLM staff, but data are included on maps displayed in Appendix D.

In 2020, two different observers conducted surveys; both had experience with distance sampling for Taylor's checkerspots. The same two observers were present on all survey dates and effort was evenly balanced between them. Encounter rates within days were generally similar between observers, but one observer had a consistently higher encounter rate than the other. Data for all dates were truncated at 8 m except for TA15 on May 13 and date-specific estimates were generated for all survey dates with sufficient data. Data and density estimates for three translocation sites (SCS, R50, TA15), one colonization site (SCS2) and for R76 (population source) are reported here for 2020.

SCS1. Three surveys were conducted at SCS1 in 2020 (Table 15); butterflies were observed on all dates. All were qualified surveys (Linders and Olson 2014). Best-fit models varied with observer-specific detection functions better on some dates and combined detections better on others. Resulting daily density and abundance estimates with 95% CIs are presented in Table 16.

SCS2. Three surveys were conducted at SCS2 in 2020; butterflies were observed on all dates. All were qualified surveys (Linders and Olson 2014). Best-fit models varied with observer-specific detection functions better on some dates and combined detections better on others. Daily density and abundance estimates with 95% CIs are presented in Table 16.

Table 16. Number of adult observations (#Obs) used to estimate density (#/ha) and 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), Training Areas 15 (TA15) and Range 76 (R76), Puget Lowlands, Washington, 2020.

		Survey	Density			Abundance		
Date	**#Obs**	**area (ha)**	**#/ha**	**Lower CI**	**Upper CI**	**#/site**	**Lower CI**	**Upper CI**
SCS1								
5-May	759	20.0	187.0	149.9	233.5	3741	2997	4669
8-May	652	20.0	112.0	98.4	127.5	2240	1968	2550
15-May	159	20.0	29.5	23.1	37.8	590	461	756
SCS2								
5-May	275	13.5	69.11	55.4	86.2	933	748	1164
8-May	255	13.5	81.56	56.6	117.5	1101	764	1586
15-May	175	13.5	41.27	31.7	53.8	557	427	726
R50								
4-May [a]	1636	19.5	288.9	249.1	335.0	5633	4858	6532
10-May	1742	24.8	187.71	161.26	218.49	4646	3991	5408
TA15								
1-May	862	21.3	134.6	102.6	176.6	2867	2185	3762
7-May	359	21.3	55.5	42.3	72.9	1182	901	1552
13-May	26	21.3	4.3	2.6	7.3	92	55	155
R76								
9-May	830	54.5	105.04	85.03	129.76	5725	4634	7072

[a] Omitted T. 1-4 due to unexpected weather and access restrictions; density applied to smaller area underestimates abundance.

TA15. Three full surveys and three partial surveys were conducted at TA15 in 2020; butterflies were observed on all dates except 28 May. Qualifying surveys (Linders and Olson 2014) occurred on 1, 7 and 13 May. The complete survey on 1 May was analyzed in lieu of partial surveys on 29 and 30 (Table 15) April. Daily density and abundance estimates with 95% CIs are presented in Table 16.

R76. One full survey and three partial surveys were conducted in 202020; butterflies were observed on all dates. Only one qualifying survey (Linders and Olson 2014) was conducted due poor access and limited ability to interpret transect lines where flags were missing. Two partial surveys (20, 26 April) were conducted to validate take but could not be completed due to weather; the second of these was simply a count and did not attempt to estimate distance. A third survey eliminated 6 lines scattered across the site to minimize impacts on data; this was an unbiased survey but had too few observations for analysis. Daily density and abundance estimates with 95% CIs are presented by date in Table 16.

Other observations

Nectar

Nectaring observations have been recorded opportunistically during Taylor's checkerspot distance surveys since 2011 (Fig. 3). A total of 9,148 observations were made on 32 species across eight survey areas, most of those at R50, R76 and SCS1. Three species: *Balsamorhiza deltoidea*, *Lomatium triternatum* and *Saxifraga integrifolia (=Micranthes integrifolia)* accounted for 83.6 percent of all observations. This was the second year in a row where *Lepidium campestre*, a non-native weedy mustard, accounted for a significant number of observations in the "Other" category (Fig. 5). The prevalent use of *B. deltoidea* 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 adult longevity and fitness (Murphy et al. 1983, Dover et al. 1997, Dennis 2010). Adults seek shelter at night and during inclement weather (hot, cold or wet extremes).

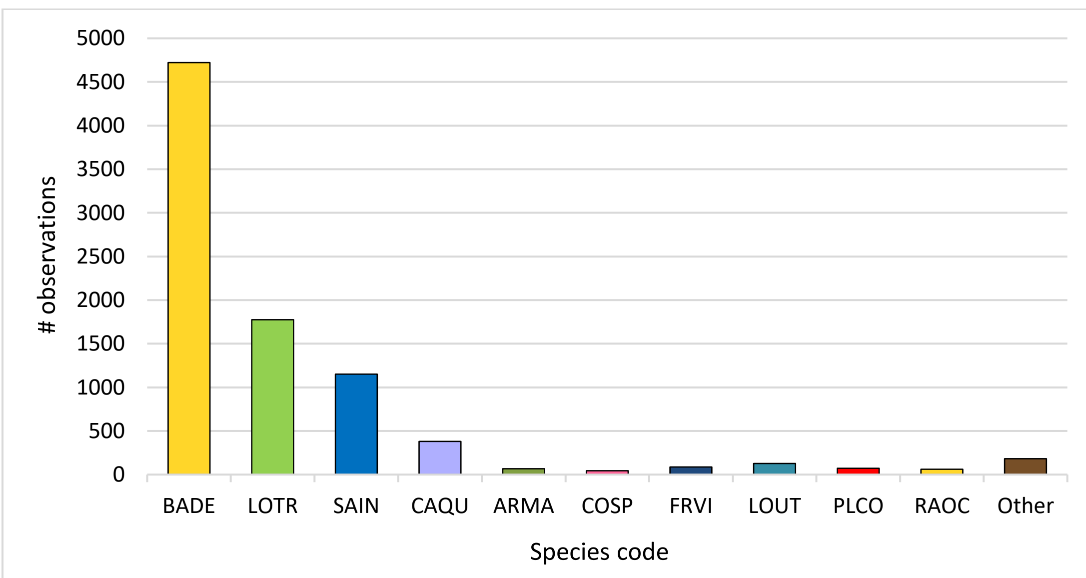


Figure 3. Number of nectar observations (n = 9,148) by species for Taylor's checkerspots observed during surveys in the Puget Lowlands, Washington, 2011-2020. BADE - *Balsamorhiza deltoidea*; LOTR- *Lomatium triternatum*; SAIN - *Saxifraga integrifolia*; CAQI -*Camassia quamash*; ARMA – *Armeria maritima*; 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 2020 distance sampling surveys for Taylor's checkerspot at the three sites where they co-occur. In total, 256 mardon skippers were observed on surveys, with 43 additional observations off transect; most were observed at R50 (Table 17). Approximately 50 more skippers were observed in 2020 compared to 2019, despite fewer surveys overall. This is the third 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, although 10 groups of two skippers (2 at R76, 7 at R50, 1 at SCS1) were also recorded; no larger groups were observed this year.

Table 17. Number of mardon skipper butterflies observed by date and site during distance surveys for Taylor's checkerspot in spring 2020 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**
26-Apr	**0**				**0**			
4-May		42				4		
5-May			1	0				0
8-May			4	1				
9-May	25				5			
10-May		143				6		
15-May			5	7			2	
18-May	24				9			
22-May		4				0		
1-Jun		0						
Total	49	189	10	8	14	10	2	0

Adult distribution

Methods

To illustrate the distribution of adults within survey areas at each site, all Taylor's checkerspot observations collected during 2020 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 2019 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 14). 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. Due to unanticipated staffing reductions, we did not perform occupancy surveys across SCS this season. 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 18. Checkerspots occupied the majority of survey areas at R50, R76, SCS1 and SCS2, and TA15. JBLM Occupancy surveys at TA7S resulted in a single checkerspot observation (no Distance surveys). At R76 occupancy declined in 2020, although this may be the result of fewer surveys and partial surveys, resulting in 71 percent of the total monitoring area (transects 1-16) occupied (312 of 436 segments). Occupancy at SCS2 rose sharply in 2020, to 83.8 percent (181 of 216) of segments surveyed (Appendix C, Fig. 2), compared to 56.9 percent (123 of 216 segments) in 2019. This appears to be in part the result of reproduction as both monitoring areas peaked simultaneously, although dispersal from SCS1 may have kept numbers higher at SCS2 later in the season (Table 15). Increasing occupancy at TA15 is on a strong positive trend and mirrors patterns observed at R50 in its first three years (2010-2012) (Table 18), that population was growing significantly. In addition, 3 observations of checkerspots at TA14 were also reported including one on 16 Apr, just three days after first flight was observed on the AIA. Due to the large survey area at TA15 and fewer observers, 2020 surveys did not extend east of segment 30 except on one partial survey. The first checkerspot release at TNQ in 2020 resulted in 16.2 percent occupancy (25 of 154 segments).

A map of scattered sightings and 100-m occupancy survey results for the Artillery Impact Area (AIA) on JBLM shows adults checkerspots continuing to occupy the periphery of this 3,075-ha prairie, although surveys did not extend into the center in 2020 (Appendix D). Due to staffing issues and complications resulting from the COVOD-19 pandemic, occupancy survey efforts in 2020 were considerably reduced compared to previous efforts. Occupancy transects included Scout's Out prairie for the first time, which lies north of Nisqually Lake in the northcentral part of the AIA. Checkerspots were widely observed there, representing a northward expansion of the occupied area. Eight adults were observed in TA14 near PCM in 2020, half during 100-m occupancy surveys and half as scattered sightings by JBLM staff; they are presumed to be dispersers from TA15 to the east.

Table 18. 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 – 2020. 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, Tenalquot = TNQ, and Range 76 = R76.

Site	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
SCS1			0.2	7.1	4.3	4.3	4.3	3.9	5.4	10.6	12.2	15.4	18.6	16.6
SCS2											2.9	1.4	7.7	11.3
R50				6.5	13.6	18.8	17.5	11.2	15.3	18.4	20.9	24.6	24.3	23.4
PCM						3.0	0.0	0.0	0.0	-	-	-	-	-
GHP						0.4	6.3	3.3	3.4	3.7	0.1	0.1	-	-
TA7S								2.1	2.3	0.3	0.1	0.1	2.2	-
TA15												5.3	9.8	17.3
TNQ														1.6
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	33.9
R76 T13-16								11.0	11.0	8.1	7.3	9.8	12.0	5.1
R76 Total	39.3	17.0	26.0	38.0	41.8	42.0	39.5	50.4	49.0	29.4	41.8	50.9	52.4	39.0

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 third straight year. The peak single day abundance estimate for 2020 (5,633; 95% CI: 4,858-6,532; Table 16), was recorded on 4 May and was similar to 2019 (Linders et al. 2020) despite the fact that it was based on a smaller survey footprint. This together with the widespread dispersal observed in surrounding areas supports the view that this population may be at carrying capacity. Habitat monitoring using RHA should be resumed to evaluate current condition and ensure habitat is being maintained, especially since anecdotal observation suggests host plant is being lost; this was also the conclusion of work conducted by CNLM (Kronland 2020). The population occupied less than the entire monitoring area (Table 18), but the limited survey effort likely contributed to this result. Peak single day abundance estimates at R50 have remained well above the minimum threshold of 250 adults every year since releases concluded in 2011. This population has met or exceeded criteria 1-4 and is officially established based on the project definition. In addition, this site shows many promising signs that it is functioning as a population source, providing dispersing adults to surrounding areas. Monitoring continues in order to add to our understanding of checkerspot population dynamics and in support of military training at JBLM.

Checkerspots at SCS1 have met criteria 1-3 each year since 2017, with a peak single day abundance estimate of 3,741 (95% CI: 2,997-4,669; Table 16) in 2020, well above the target. While this estimate is lower than that in 2019 (Linders et al. 2019), the 2020 estimate may have been collected after the true peak (Appendix B); overall numbers remain strong. Checkerspots at SCS1 occupied 83.0 percent of the 20-ha monitoring unit in 2020 and are increasing in number at SCS2 (Appendix B). Restoration is ongoing across the site but impacts from the 2017 wildfire cannot be negated. This site remains on track to meet all establishment criteria by 2021. Like R50, checkerspots at SCS1 are dispersing into surrounding habitat (Appendix C, Fig. 2), where colonization potential is high (e.g., SCS2).

One adult checkerspot was observed at TA7S in 2020, although only 100-m occupancy surveys were conducted. Releases did not occur there in 2020 and are not expected to resume until factors affecting 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

This is the fifteenth year of the Taylor's checkerspot captive rearing and translocation project. At this point our short-term project objective to establish 3 new populations in the Puget Lowlands by 2022 is close to being met. A new population at R50 was established in 2016, a second population at SCS will be deemed "established" in 2021 (barring a catastrophic event), and TA15 is expected to meet Year 2 of the establishment objectives in 2022, with no further releases planned. Building on that success, a translocation plan for the South 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 2021. Our strategy includes 1) continuing large multi-stage releases at new sites over several years, 2) working on more than one release site whenever possible, and 3) ensuring habitat is in a suitable condition prior to initiating releases. The last of these tasks remains the most challenging and poses the greatest risk to reintroduction success and recovery overall.

Captive Propagation

Several major changes to the captive rearing program occurred in 2020 as a result of staffing and budget complications related to the COVID-19 pandemic coupled with funding losses from JBLM/DoD and other sources. In an effort to reduce costs, streamline the program, and mitigate risk during the pandemic the captive mating portion of the program was eliminated at both rearing facilities in 2020. In addition, the captive rearing program at the Oregon Zoo was closed and lack of funding precludes reopening the program in 2021 or longer. This will affect the rate at which reintroductions can be implemented and extend the time to recovery. The pattern of diapause mortality observed at the Oregon Zoo has also occurred at Coffee Creek Corrections Center (Oregon population), which continues to evaluate factors that may be affecting survival including food quality and UV lighting. Captive rearing at Mission Creek has continued albeit at reduced capacity (3,805 larvae in diapause) to address COVID-19 safety concerns. The recent addition of a second greenhouse provides optimism that the program will continue to produce enough larvae for at least one translocation annually, and more if COVID-19 safety requirements can be maintained simultaneously.

Translocation

The combination of weather, access and staffing restrictions that limited survey opportunities in 2020 results in a fuzzy picture and contributes to uncertainty about what to expect in 2021. The lack of funding to monitor two key sites, R76 and R50, in 2021 adds further complications. Funds in hand only cover take requirements for this project. No funds are available for regulatory compliance or population monitoring, which has been critical in building our knowledge of population dynamics for the species as a whole. Population monitoring in 2021 is particularly important because it could elucidate the latent effects of the wet conditions prevalent in spring 2020.

<u>R76</u>: The peak single day abundance estimate for R76 (5,725; 95% CI: 4,634-7,072) collected on 9 May, may not be an accurate reflection of the true peak due to the late survey date and that estimate is far lower than peak estimates in recent years. In addition to other complications, a major challenge to collecting better estimates in 2020 was the new restriction on pin flag placement in the AIA, which was announced late in the process. Distance estimation requires that surveyors can simultaneously identify the transect line in both the foreground and at a distance; this was easily accomplished with pin flags. Devising an alternative marking system that both visible yet not fixed to the ground will be difficult if not impossible to achieve given 436 points are needed at R76 alone.

<u>R50</u>: Peak estimates for R50 appear similar to 2019 and remained relatively strong. Habitat monitoring using RHA should be resumed to evaluate current condition and identify needed improvements. Habitat loss and degradation is a primary threat to Taylor's checkerspot persistence and ongoing maintenance will be needed to avoid backsliding. An RHA assessment has not been conducted since 2013; work conducted by CNLM suggests host plant is being lost but uses a different methodology and footprint (Kronland 2020). Larval host plants are the key consumable resource for butterflies (Dennis 2010). 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.

<u>SCS</u>: Peak estimates for SCS1 appeared lower than in 2019, but also remained relatively strong, especially given the uptick in numbers at SCS2, the second monitoring unit in the northwest corner of this site. Maintenance of the checkerspot population at SCS and its dispersal across the site affirms 1) that additional releases are unnecessary, and 2) the site is on track to meet or exceed the establishment target by 2021. The potential for this site to fuel colonization on other parts of the site (e.g., SCN) is great so long as habitat restoration and maintenance keep pace with the threat of invasive weeds. However, this has become particularly challenging since the 2017 wildfire, as intensive work has been required across a much larger area than was managed previously. Collectively, setbacks from the 2017 wildfire, loss of JBLM-ACUB funds, loss of inmate crews to assist with restoration and COVID-19 restrictions have reversed the trajectory from one of maintenance to one again requiring restoration. Habitat restoration at sites off of JBLM must be a top priority to ensure checkerspot recovery across the South Puget Lowlands.

<u>TA15</u>: The number and distribution of checkerspots appeared very promising at this third-year reintroduction site and no further releases are planned at this time. 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. Population growth mirrors patterns observed at R50 and SCS as those populations took hold. If this trend at TA15 continues this population can be expected to meet establishment criteria by 2025. 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).

<u>TNQ</u>: Releases at TNQ commenced in 2020 and while flight season numbers were fairly low, adults were observed repeatedly at some locations. The site has some very promising host plant patches, although some are set in a matrix of thick grass, which can cause microsite cooling. A predominant wind out of the southwest was also notable during flight season surveys, which could influence how butterflies use the site. For example, two locations where adults were routinely found were on the east sides of nearby confers. At SCS adults are often found on the south side of trees.

<u>TA7S</u>: As noted in previous reports, TA7S continues to underperform for reasons that are not entirely clear Reasons for this may include habitat configuration (patches are linear and sprawling), poor microsite conditions, ongoing intensive restoration, bird predation, herbicide residue and/or other unidentified factors. In addition, while the site is rich with checkerspot host and nectar resources as well as adult cover, it is the smallest reintroduction site and has a long narrow shape and adjacent suitable habitat that receives concentrated pedestrian and vehicular training impacts. The restoration challenge at TA7S tends toward one of too much vegetation and limited amounts of open ground needed for basking. The ongoing challenge of restoring this site and its poor response to reintroduction is another reminder that initiating releases after habitat is restored is the most effective strategy (e.g., Thomas 1989, Oates and Warren 1990, Stamps and Swaisgood 2007, Thomas et al. 2011). Our reintroduction strategy on TA7S is to maintain occupancy while restoration continues and to identify and address potential threats as they arise.

<u>Occupancy monitoring</u>: Efforts to monitor adult distribution using a 100-m transect spacing in the AIA and surrounding areas were considerably lower in 2020 due to staffing shortages, with the most notable result being the discovery of adults throughout much of the Scouts Out Road area, which extends north from the central part of that site (Appendix D). Similarly, occupancy surveys were not implemented at SCS in 2020 but are scheduled for both SCS and SCN in 2021.

Future Plans

Collectively, the potential for large high-density sites to “reseed” surrounding areas speaks to their importance for recovery at the landscape scale. Given the dynamic nature of checkerspot populations and their propensity to take advantage of good conditions, a strategic approach to recovery should include development of multiple high-density populations that can function as sources for dispersing adults. When surrounded by satellite sites harboring high quality habitat, colonization events would have a high likelihood of success (Thomas et al. 2011) and lead to networks of occupied sites with a greater chance of long-term persistence. Evidence for this approach is strong and suggests that providing suitable habitat in a permeable landscape with high-density reservoir populations will lead the species to do the rest.

Occupancy surveys do a good job of delineating checkerspot distribution and locating concentration areas but understanding the size and persistence of these subpopulation and their role in recovery will require a more strategic approach. If there is one message that can be drawn increasingly from this work, it’s that habitat quality, especially host plant patches, really matter (Thomas et al. 2011). Where they are lacking (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 growth and sustain populations.

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 translocation to suitable sites.

Given the broad distribution of checkerspots on JBLM releases off base are rising in importance with TNQ and BHP topping the priority list in Thurston County. Postdiapause release efforts will be focused at TNQ in 2021 because there are too few larvae in captivity to add a second site at this time. 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 reduced funding and loss of the Oregon Zoo program, it is unlikely that releases would commence prior to 2022 unless prediapause larvae are super abundant in 2021. Both sites are scheduled for habitat assessments (RHA) which will shed important light on their overall readiness and identify localized areas of high-quality habitat that would provide good release targets.

Funding for both captive rearing and translocation is in increasingly short supply, which will reduce the project scope in 2021 by about 30 percent and up to 45 percent in 2022 if no new funding is obtained. We continue to seek funding for both the Oregon Zoo and Mission Creek captive rearing programs and associated translocation activities through a variety of channels, however, at present the funding stream relies solely on USFWS, which has generously provided for 2021-2022, includes money for habitat assessments at Tenalquot Prairie and Bald Hill. Funding from additional sources is badly needed to stabilize the revenue stream, including monies to support widespread restoration off JBLM. Continued success of habitat maintenance and reintroduction activities is contingent upon funding to restore and maintain habitat across occupied sites (e.g., SCS) and prepare new sites for reintroduction.

Continuing broad-scale searches outside of standard monitoring grids to look for adults remains a priority in order to understand dispersal capability and document colonization. Both factors have the ability to greatly reduce the overall cost of recovery. Staff at JBLM have done a great job conducting occupancy surveys across large landscapes. While it is important for this work to continue, in some areas where occupancy is now well-established, new approaches should be employed to assess population size and persistence, which will improve our understanding of how these sites contribute to regional recovery. Documenting new breeding populations will improve our understanding of checkerspot demography and offers a supplemental path to increasing the size and number of populations.

Access to JBLM sites, especially those on the AIA, remains dynamic and unpredictable and is a source of ongoing concern primarily because the scheduling approach frequently changes. The project has gone to great lengths to minimize access requests and accommodate uncertainty and continues to do so even at a cost to monitoring on other sites. A more predictable management agreement would reduce the time and effort required to meet survey requirements and create a more predictable training environment. The fundamental dependence of this project on access to those sites cannot be overstated. In addition, the vast amount of potential habitat on base underscores the important role JBLM plays in Taylor's checkerspot recovery and its long-term persistence in the Puget Lowlands.

Questions for Further Research

1) Does the availability of preferred larval habitat drive population density and population growth of Taylor's checkerspot?

This remains a high priority. Without high quality larval habitat reintroductions do not take. 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:
A) Within site quality of larval habitat: Preferred larval microsites are closely correlated with enhanced larval fitness in the wild.

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

C) 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.

C) 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? How might they influence translocation success as well as long-term population persistence?

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) How do population size, dispersal capability, habitat quality and landscape permeability interact to affect short and long-term population persistence?

Much of our knowledge about Taylor's checkerspot populations has been gleaned in the last 20 years, which saw a period of steep decline (1997-2006) up to listing, followed by discovery of a few new populations and re-establishment of others through reintroduction (2004-2020). In addition, extensive work to restore Taylor's checkerspot habitat and rebuild populations has occurred in the absence of a recovery plan. Taken together, our knowledge of population function, which is critical to setting appropriate recovery criteria, is limited by time, inconsistent monitoring approaches and differences in landscape setting. The most intensive and widespread monitoring has occurred in the South Puget Lowlands as a result of this project, which has revealed population growth and dispersal potential in recent years that has not been observed previously. Data generated by distance sampling, occupancy transects, scattered sightings and habitat assessment efforts indicate several strong population centers connected by a network of satellite locations and hundreds of scattered individuals spread across thousands of acres. These data shed light on the species potential for landscape scale recovery through an understanding of population-level structure.

However, it is not clear that this new insight, which is being proposed as the foundation for setting recovery criteria, represents a sustainable or appropriate model for conservation either locally or range wide. The risks include 1) setting population size/density targets that are too high/low; 2) setting habitat targets that are too high/low; 3) uncertainty about the role of reintroduction vs. colonization for recovery; 4) delineating recovery in inappropriate landscapes (e.g., size and arrangement of patches AND landscape permeability); 5) uncertainty about whether assumptions for one region (e.g., South Puget Lowlands) are appropriate for other regions (e.g., North Olympic Peninsula, Oregon). Potential costs of untested/inappropriate assumptions include 1) lack of political will, 2) inability to obtain necessary funding, 3) reduced/inability to measure confidence in recovery outcomes, and 4) failure to recovery the species. These issues point to the need for a spatially explicit population model to explore the relationship between population size, dispersal capability, habitat quality and landscape permeability and measure their effect on short and long-term population persistence. … would have the ability to assign and measure confidence that proposed actions would be likely to lead to long-term persistence (e.g., 90% confidence that the population/species will persist for 75 years), which is being promoted as the new standard currency for recovery criteria.

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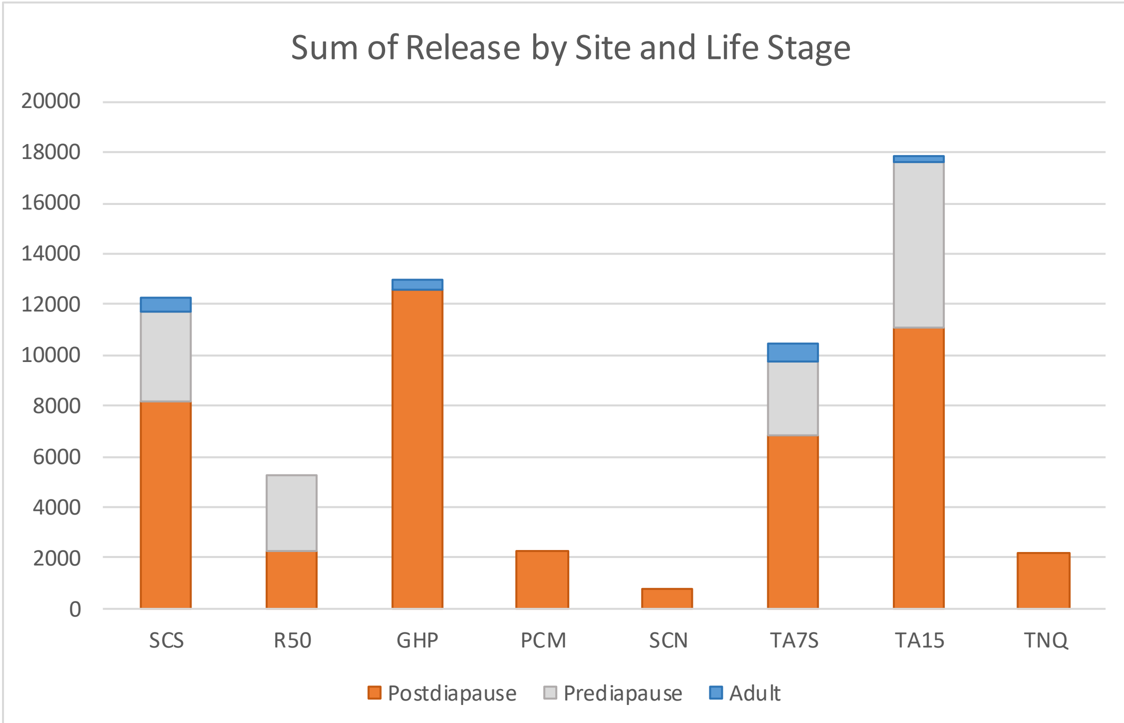
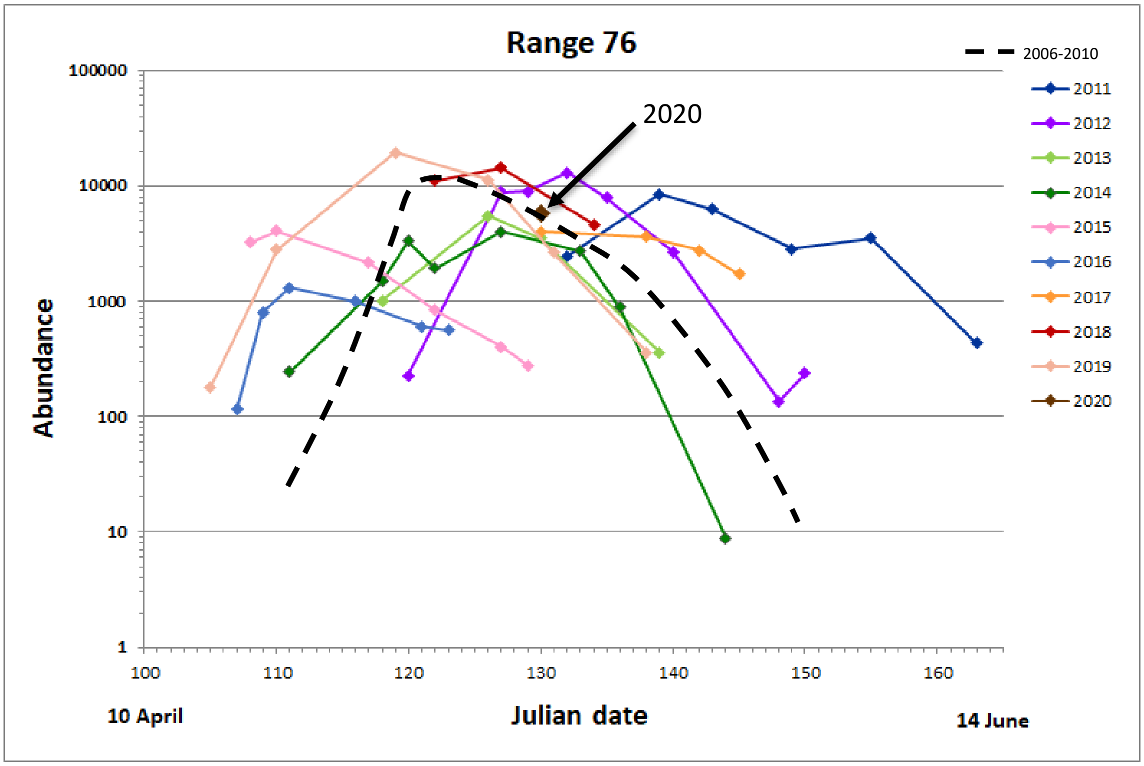
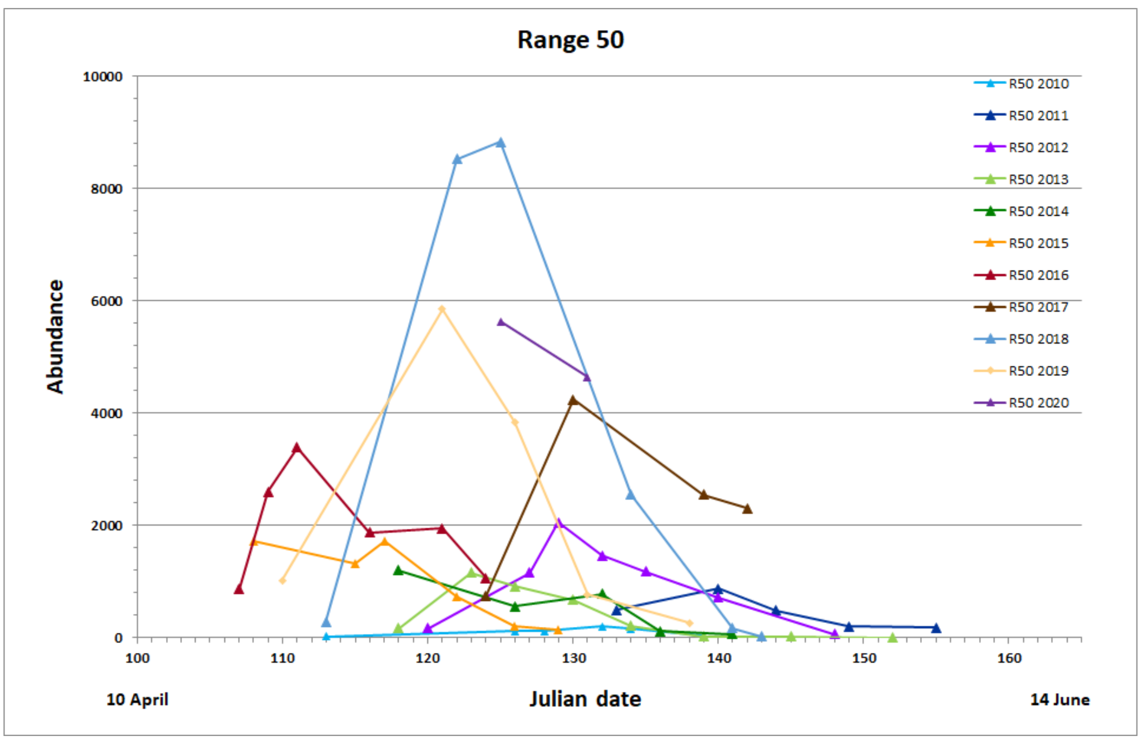
Appendix A. Approximate number of Taylor's checkerspot butterflies released by year, site and life stage (Po = postdiapause larvae; Ad = adult; Pr = prediapause larvae) and site (SCS = Scatter Creek South; R50 = Range 50; GHP = Glacial Heritage Preserve; PCM = Pacemaker Airstrip; SCN= Scatter Creek North; TA7S = Training Area 7 South; TA15 = Training Area 15; TNQ = Tenalquot) in South Puget Sound, Washington, 2007-2020. See also the graph by life stage and site on next page.

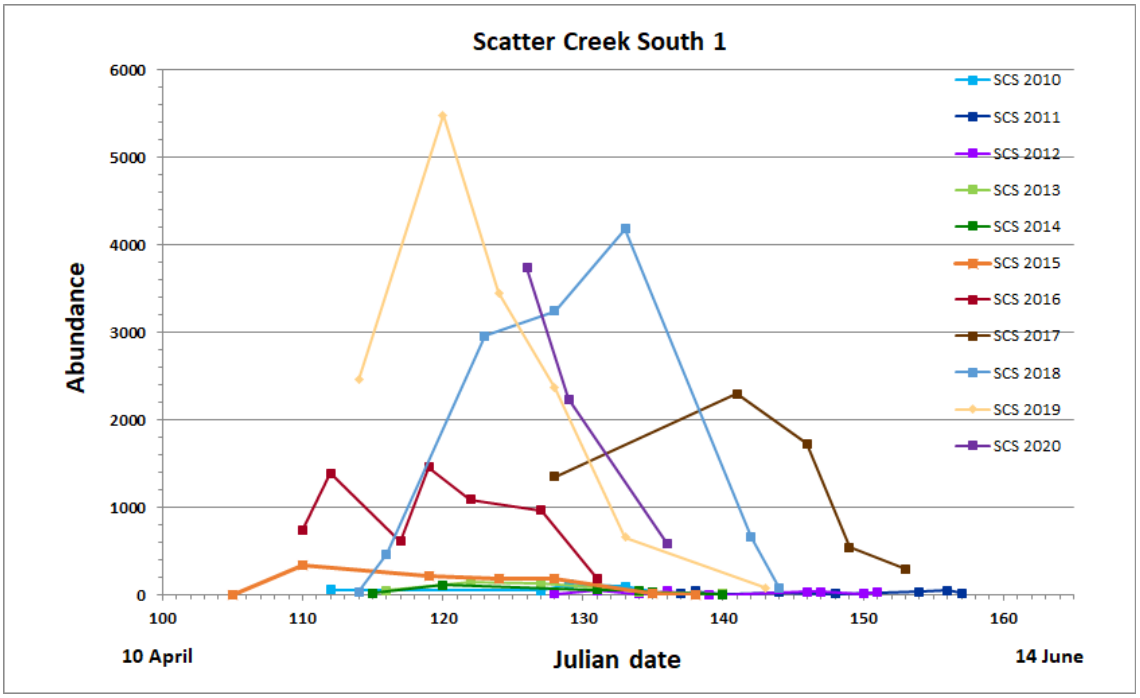
Year/Site	Stage	SCS	R50	GHP	PCM	SCN	TA7S	TA15	TNQ
2007	Po	199							
2008	Po	340							
2009	Po	747			741	759			
	Pr	2487	2956						
2010	Po	891	1145						
	Ad	202							
2011	Po	1109	1141						
	Ad	167							
	Pr	1036							
2012	Po			975	1565				
	Ad	133							
2013	Po	3250		3372					
	Ad			107					
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	
2020	Po							4935	2183
	Ad								
	Pr								

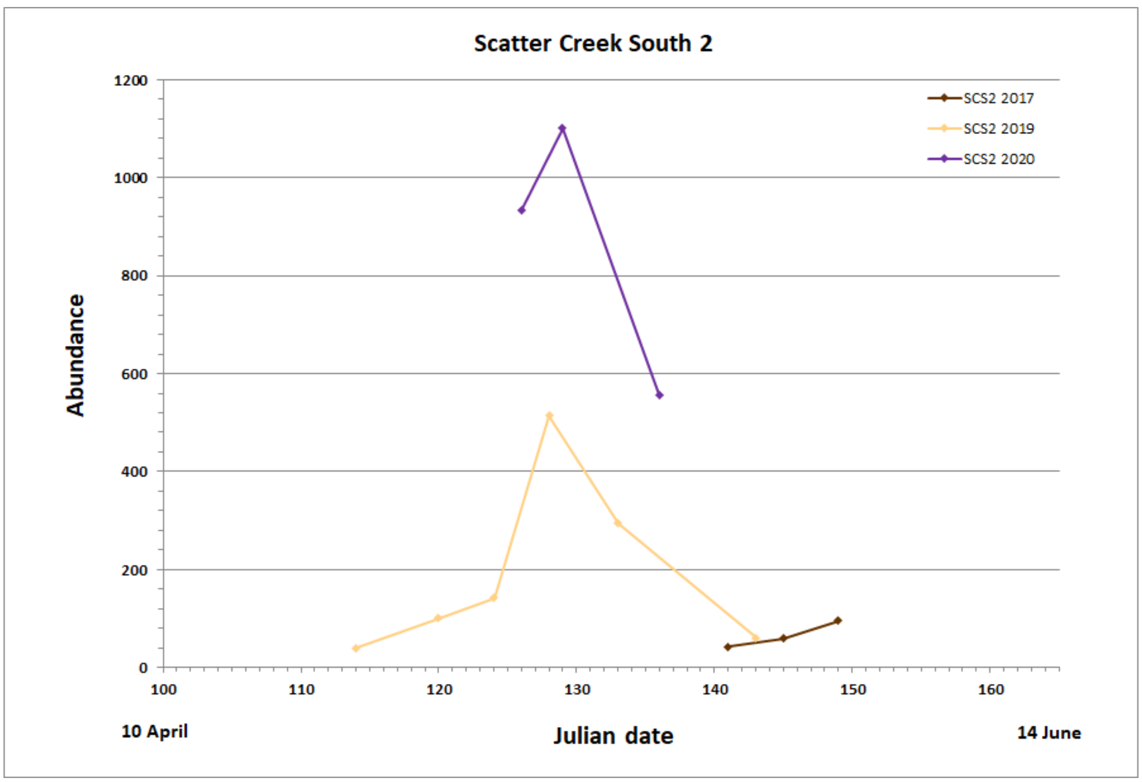

Sum of Release by Site and Life Stage
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
SCS
R50
GHP
PCM
SCN
TA7S
TA15
TNQ
Postdiapause
Prediapause
Adult

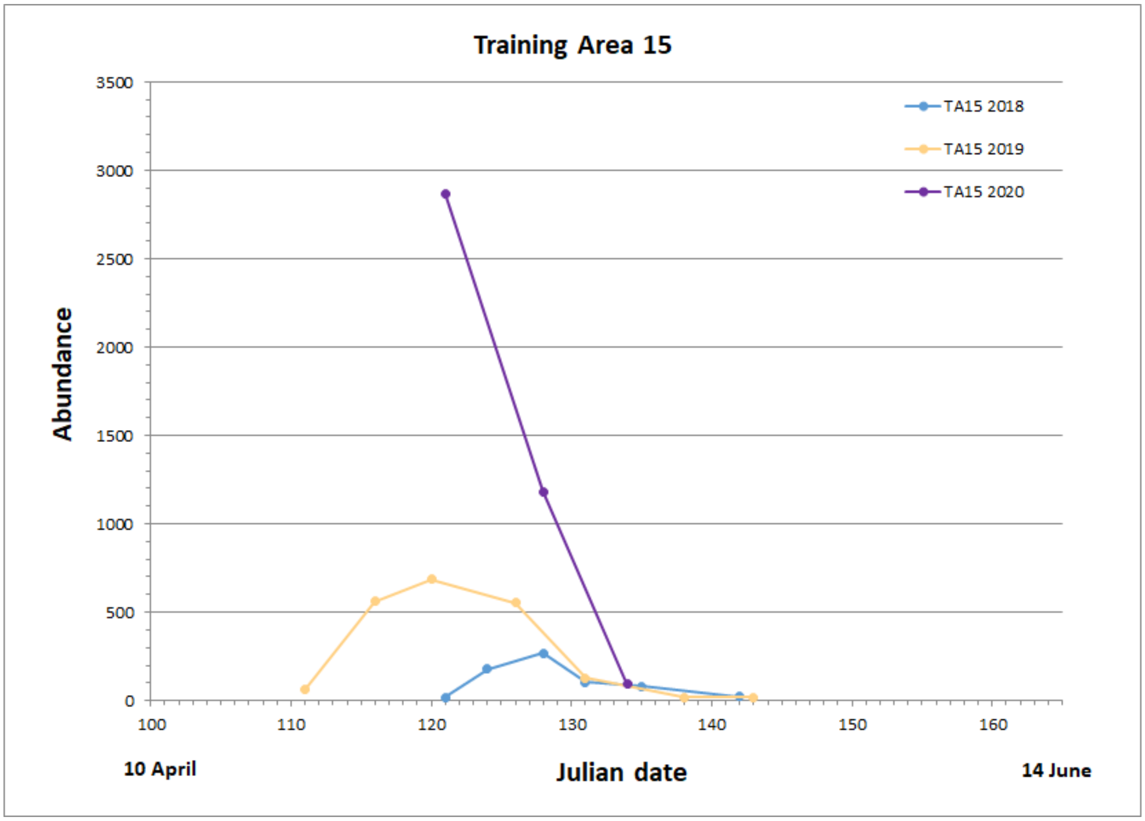
Appendix B. Estimated daily abundance of Taylor's checkerspots based on distance estimation and timing of the flight season at Range 76 (source site) in 2006-2010 inclusive (▬), and 2011-2020; 2020 is a single point on Day 130; note log scale on the y-axis. Three translocation sites also shown with linear scales on y-axis: Range 50 2010-2020 (bottom); Scatter Creek South Unit 1 2010-2020 (page 2 top), Scatter Creek South Unit 2 2017, 2019-2020 (page 2 bottom), and Training Area 15 2018-2020 (page 3 top), South Puget Sound, Washington. Area surveyed varies by site and year; see Table 14 and text for details.






Scatter Creek South 1
6000
5000
4000
3000
2000
1000
0
Abundance
100
110
120
130
140
150
160
10 April
Julian date
14 June
SCS 2010
SCS 2011
SCS 2012
SCS 2013
SCS 2014
SCS 2015
SCS 2016
SCS 2017
SCS 2018
SCS 2019
SCS 2020


Scatter Creek South 2
1200
1000
800
600
400
200
0
Abundance
100
110
120
130
140
150
160
10 April
Julian date
14 June
SCS2 2017
SCS2 2019
SCS2 2020



Training Area 15
Abundance
3500
3000
2500
2000
1500
1000
500
0
TA15 2018
TA15 2019
TA15 2020
100
110
120
130
140
150
160
10 April
Julian date
14 June

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 2020.

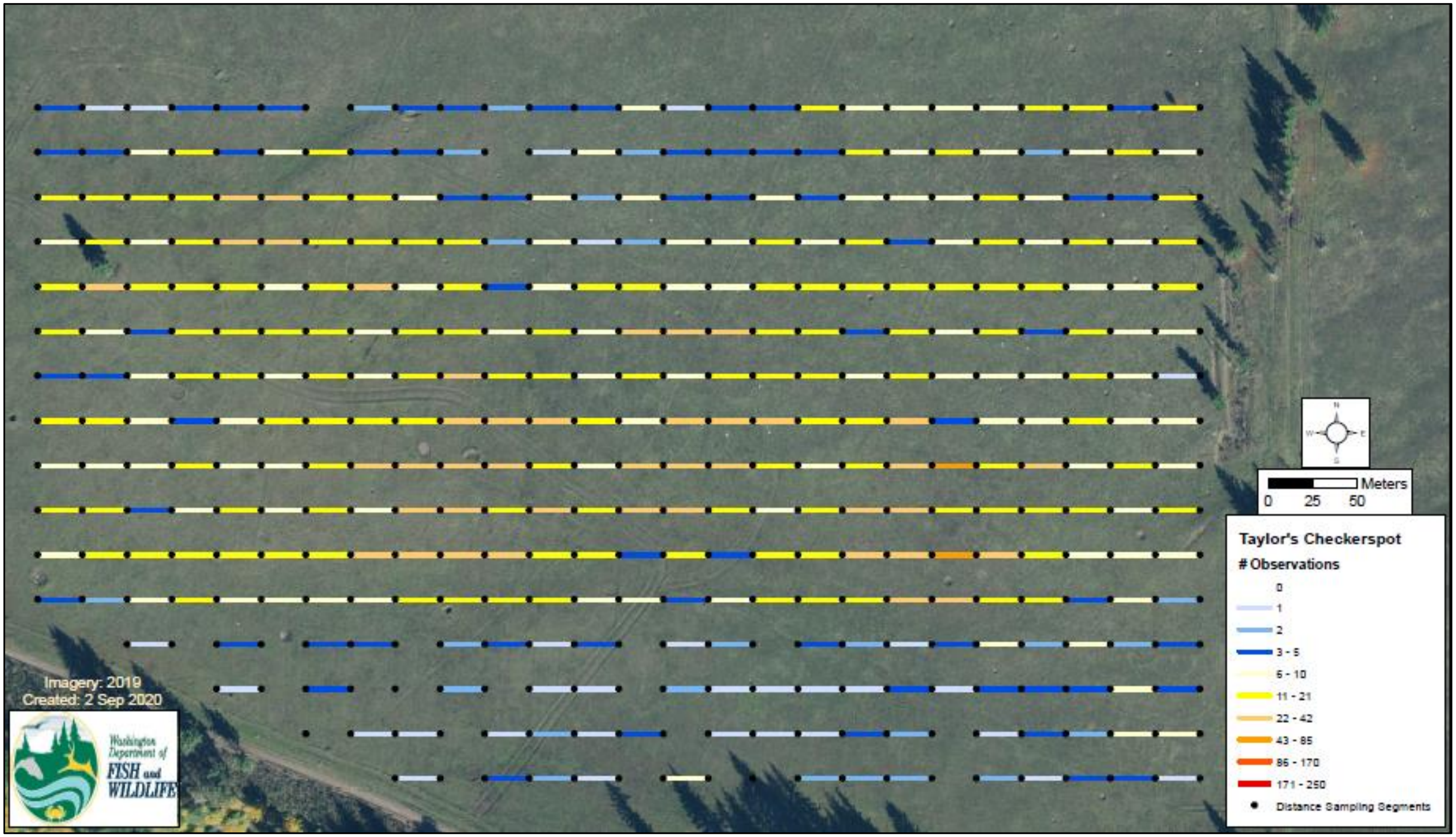


Figure 1. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Range 50 combined across all survey dates in spring 2020, South Puget Sound, Washington.

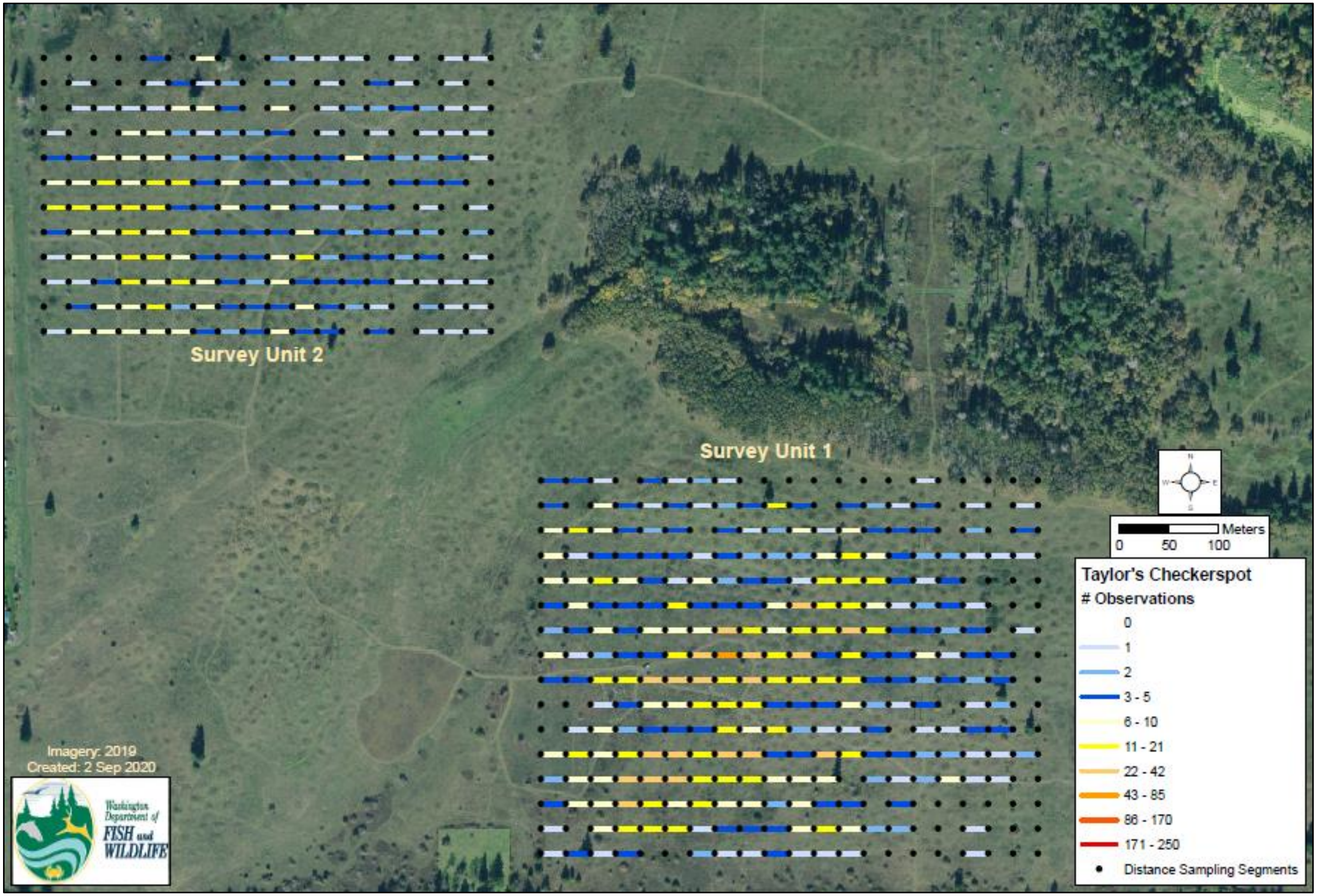


Figure 2. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Scatter Creek South in survey areas 1 and 2, combined across all survey dates in spring 2020, South Puget Sound, Washington.

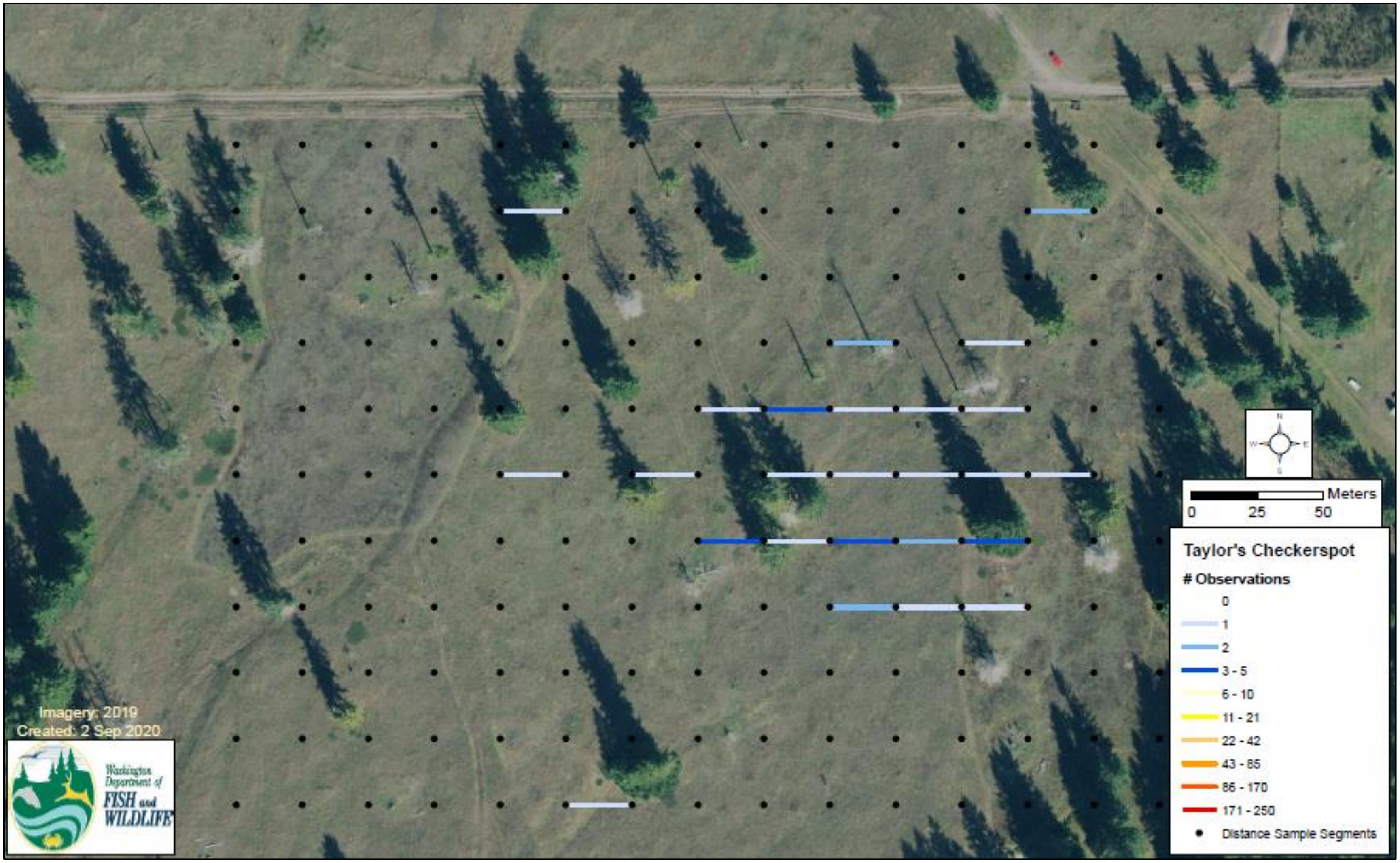


Figure 3. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Tenalquot, combined across all survey dates in spring 2020, South Puget Sound, Washington.

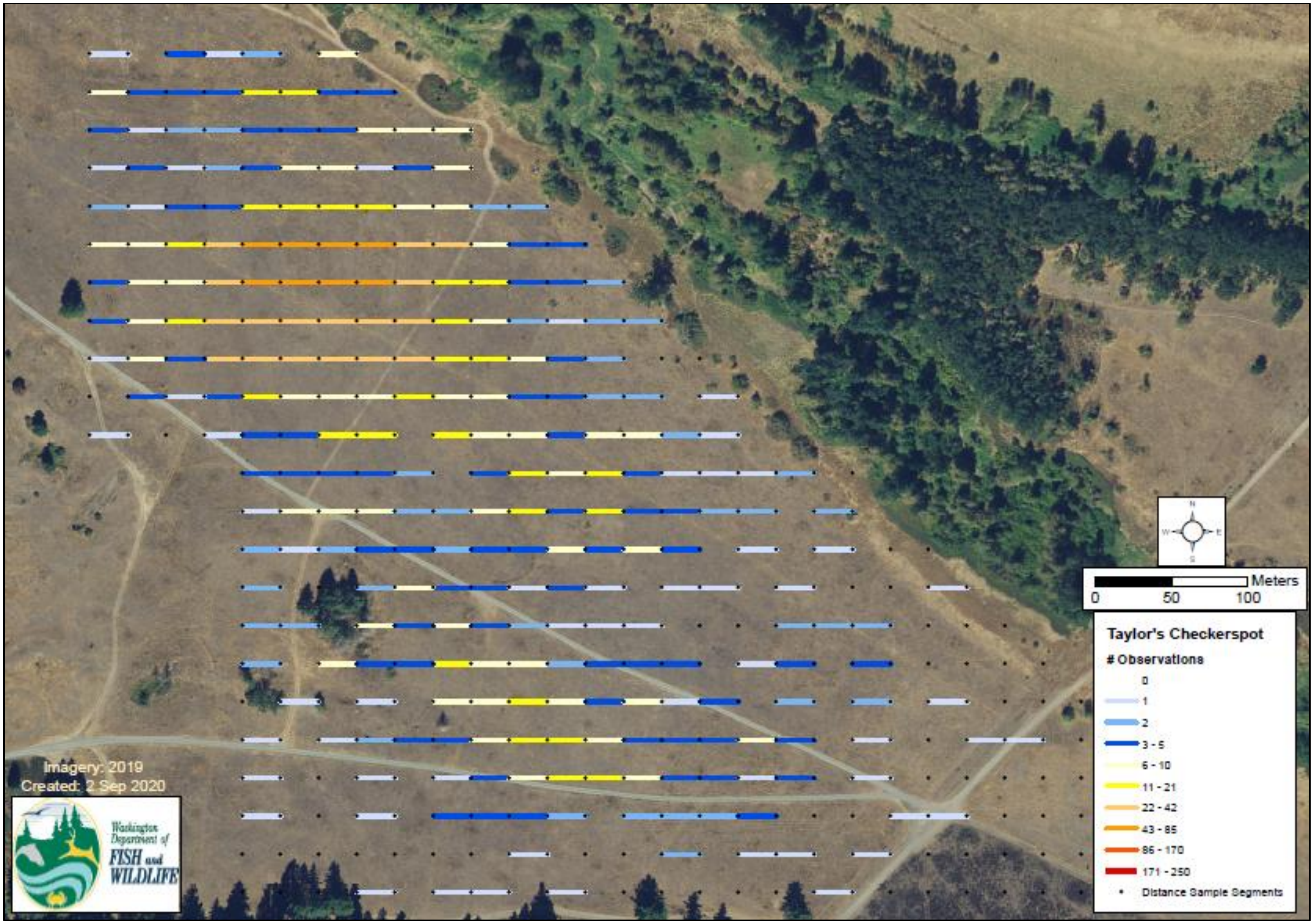


Figure 4. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Training Area 15, combined across all survey dates in spring 2020, South Puget Sound, Washington.

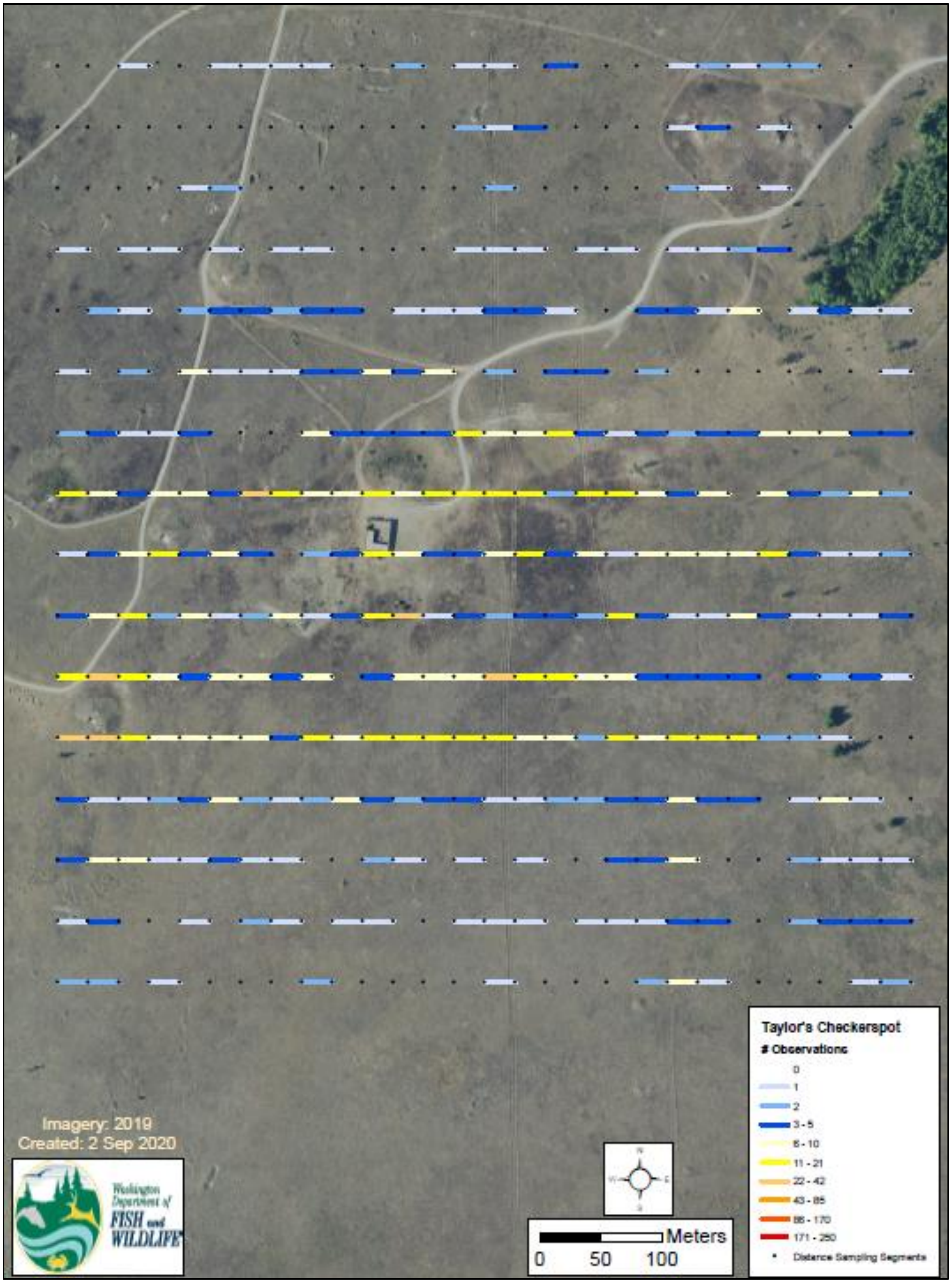


Figure 5. Distribution and number of adult Taylor's checkerspots observed during distance sampling surveys at Range 76, combined across all survey dates in spring 2020, South Puget Sound, Washington.

Appendix D. Adult Taylor's checkerspot observations (scattered sightings and a subset of 100-m occupancy survey transects) by JBLM staff and partners on the Artillery Impact Area (AIA) relative to standard monitoring areas (light green shade), Joint Base Lewis-McChord, Washington, 2020.