# TAYLOR'S CHECKERSPOT (*EUPHYDRYAS EDITHA TAYLORI*) OVIPOSITION HABITAT SELECTION AND LARVAL HOSTPLANT USE IN WASHINGTON STATE

By

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# Abstract

## Taylor's checkerspot (*Euphydryas editha taylori*) oviposition habitat selection and larval hostplant use in Washington State

Taylor's checkerspot (*Euphydryas editha taylori* (W.H. Edwards 1888)), a Federal Endangered Species Act candidate species, is found in remnant colonies between extreme southwestern British Columbia and the southern Willamette Valley in Oregon. This butterfly and its habitat have declined precipitously largely due to anthropogenic impacts. However, this butterfly appears to benefit from some land management activities and some populations are dependent on an exotic hostplant.

Oviposition sites determine what resources are available for larvae after they hatch. Larval survival and growth on three reported hostplants (*Castilleja hispida*, *Plantago lanceolata*, and *P. major*) were measured in captivity to determine the suitability of hostplant species and to develop captive rearing methods. Larvae successfully developed on *C. hispida* and *P. lanceolata.*

Parameters of oviposition sites were measured within occupied habitat at four sites in Western Washington. Sampling occurred at two spatial scales with either complete site censuses or stratified systematic sampling on larger sites. Within the sampled or censused areas, oviposition sites were randomly selected for paired oviposition/adjacent non-oviposition microhabitat measurements.

Taylor's checkerspot oviposited on all reported perennial hosts available. Oviposition was significantly associated with high hostplant density at three of four sites. All but one of 31 oviposition locations selected for microhabitat measurement contained >10,000cm$^3$ hostplant volume within 1m$^2$ surrounding oviposition sites. Other habitat factors examined were suggestive but not statistically significant as influencers of oviposition perhaps because only occupied habitats were measured and thus they were de facto broadly suitable for checkerspot use.

The consistent preference for high density hostplant patches indicates that assessments of habitat and restoration objectives should weight high density hostplant areas more heavily than mean site level host plant abundance or cover and that, within areas of broadly suitable grassland vegetation structure, sites containing high densities of hostplants can be found and used for oviposition.

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# I. Introduction

Taylor's checkerspot (*Euphydryas editha taylori*) is a modestly sized yet charismatically colored butterfly endemic to Northwest grasslands between the Cascade Range and the Pacific Ocean. Historically found from southern Vancouver Island, British Columbia, Canada, to the southern Willamette Valley, Oregon, in the United States, its range has been dramatically fragmented by habitat loss. The few populations that remain are threatened with extinction. This study was designed to support the conservation and recovery of this butterfly by identifying critical aspects of oviposition and larval habitats important for the survival of individual checkerspots.

The survival of Taylor's checkerspot individuals is in large part dependent on its surrounding habitat. The amount of habitat available is restricted by the ability and motivation of the individual to move. In the life cycle of Taylor's checkerspot, mobility increases from zero at the egg stage, to a few centimeters in early instars, up to tens of meters in later instars, and up to thousands of meters in adults though most adults stay within a small area relative to their potential dispersal distance. Because of this relationship between movement ability and habitat, and because previous work has indicated prediapause larval survival is a major population driver, this study focused on habitat selected by adult female butterflies for oviposition and larval survival on two oviposition host plant species. By raising larvae in captivity this study also developed methods for larger scale captive rearing that can generate individuals for population reintroduction or augmentation.

The need for a study of Taylor's checkerspot was driven by its endangered conservation status coupled with a near absence of information about certain aspects of its life cycle and habitat needs. This introduction will clarify this checkerspot's conservation status and outline ways in which the studies presented herein address information gaps in our understanding of this butterfly.

This thesis has taken a number of unusual turns. It began in 2004 with a study of Taylor's checkerspot oviposition at Bald Hill in Thurston County, Washington, and captive rearing to develop methods and determine larval performance on various hostplants. Taylor's checkerspot captive rearing and larval performance is presented in Part II. The Bald Hill population with hundreds of adults spread across numerous small balds was, at the time, considered the most secure population. That population crashed for unknown reasons in 2005 which put thesis work on hold until the population recovered. Unfortunately, Taylor's checkerspot has not been seen in the Bald Hill Area since 2007 despite substantial survey efforts and the population is likely extirpated. In 2010, I restarted work on the thesis by gathering data on Taylor's checkerspot oviposition at four western Washington sites; that work is found in Part III.

### ***Conservation status***

Native grasslands throughout the Willamette Valley-Puget Trough-Georgia Basin Ecoregion, the habitat of Taylor's checkerspot, have declined in extent to about 3% of the area they occupied in 1850 (Crawford and Hall 1997). This decline is primarily due to development for agriculture, urbanization, gravel mining, succession to forest, and non-native invasive species. Many species of prairie dependent vertebrate animals have declined dramatically and some are threatened with extinction or have already been extirpated from prairies (Leonard and Hallock 1997; Rogers, Norman, and Rolph 1997; Rogers 2000; Ryan 1997; Stinson 2005). Plants of the prairie have declined as well, with golden paintbrush (*Castilleja levisecta*), a Taylor's checkerspot host plant, federally listed as threatened under the Endangered Species Act (Caplow 2004). Several other plants are listed as sensitive under the Washington State Natural Heritage Program.

Prairie dependent butterflies show a similar pattern of population declines and extirpations. Four butterflies of the Puget Prairies are listed as “endangered” or “species of concern” by the state of Washington. Thirteen butterflies that use grasslands in the Willamette Valley-Puget Trough-Georgia Basin Ecoregion are listed as endangered, threatened, candidate, or extirpated (or equivalents) by national or state/provincial governments. Taylor’s checkerspot is listed as “endangered” by the State of Washington (Stinson 2005) and considered “critically imperiled” by state and provincial natural heritage programs. The species has been petitioned to the U.S. Fish and Wildlife Service and is a candidate for emergency listing under the Endangered Species Act (Stinson 2005). This means there is likely enough evidence to warrant listing but the US Fish and Wildlife Service has yet to list the species. Agencies in British Columbia have developed draft recovery plans that call for the reestablishment of three robust metapopulations within the area formerly occupied by the butterfly (Guppy, Kondla, and Schaeffer 2003) and investigation of the one remaining Canadian population of Taylor’s checkerspot continues (Page et al. 2009)

Taylor’s checkerspot was abundant in 60 documented, but probably thousands of localities in 1800, but certainly many of these were gone by the early 1900’s by which time much of the habitat had been converted to agriculture. Now however the butterfly is almost gone with one population in Canada, approximately 10 small populations in Washington, and one small population in Oregon. It was known to be abundant in some colonies in fairly recent times in Oregon (Dornfeld 1980) and Washington (Stinson 2005) and, as recently as 1996, several populations in the South Puget Sound numbered in the thousands. By 2000 only a few populations were found and even in the largest of these, few checkerspots were found (Fleckenstein and Potter 1999; Remsberg 2000; Stinson 2005). A likely metapopulation spread across several miles of bald habitat in

Washington went from hundreds (maybe thousands) of adults down to zero between 2003 and 2006.

There are no clear answers as to why Taylor's checkerspot populations have crashed recently even in protected areas. Habitat loss likely played the major part in earlier declines and may be driving recent extirpations as well by disrupting metapopulations and confining butterflies to marginal sites. Many other populations of *E. editha* act as metapopulations and exhibit tremendous variability in abundance and local distribution (Thomas, Singer, and Boughton 1996; Harrison 1989; Boughton 1999; McLaughlin et al. 2002; Ehrlich and Murphy 1987). The fragmentation of prairies and the low vagility of Taylor's checkerspot indicates the isolated populations can no longer act as metapopulations (Char and Boersma 1995). Isolation increases the chances that individual populations will become permanently extirpated by making natural recolonization unlikely. Climate change may also be a cause of population extirpations and this is likely to increase in the future (Hellmann 2004).

Butterflies are often considered to be both good indicator (Black, Shepard, and Allen 2001; Pollard and Yates 1993; Samways 1994) and umbrella (Launer and Murphy 1994; Dunn 1998) taxa. Thus the imperiled nature of prairie endemic butterflies along with massive habitat loss may indicate the prairie system is in crisis.

A number of research and conservation efforts targeting *E.e. taylori* habitat are planned or ongoing. Efforts by the Washington Department of Fish and Wildlife and others are underway to survey historical locations in Washington. So far, all these efforts have failed to find *taylori* populations except for a few small populations in the northeastern Olympic Peninsula lowlands (Potter pers. com.). Invasive plant control has been a focus for several years on many *E.e. taylori* sites including three of the four sites used in this study.

Because Taylor's checkerspot has been extirpated from numerous sites containing suitable habitat and sites that may contain suitable habitat in the future with restoration and enhancement, it is necessary to develop methods for reintroduction. Most of these sites are beyond dispersal distance for butterflies in extant colonies. Captive rearing is an essential part of a successful reintroduction strategy.

Captive rearing is necessary for propagating enough individuals for reintroduction into areas from which this species has been extirpated and for detailed study of biological and ecological attributes impossible to study in the field. The reproductive potential of founder adults can produce at least a 100 fold increase in the captive population for release into the wild once techniques are refined. Reintroduction success is positively correlated with the number of released individuals (Oates and Warren 1990). *E. editha* had been thought to be difficult to rear though stimulation of oviposition in captivity is straightforward (Singer et al. 1992). Protection, restoration and enhancement of habitat is far more important than captive rearing in the long run but captive rearing can help bridge the gaps in fragmented habitats and the gaps in biological knowledge.

### ***Biology and description***

*Euphydryas* butterflies have been extensively investigated as model organisms for metapopulation studies, genetics, and conservation and population biology (Britten et al. 1995; Ehrlich 1992; Ehrlich and Hanski 2004; Ehrlich and Murphy 1987; McLaughlin et al. 2002). Another subspecies, *Euphydryas editha bayensis* is one of the most thoroughly studied non domesticated organisms on earth (Ehrlich and Hanski 2004; New 1997). *Euphydryas editha* is a highly variable species with many subspecies though there are differences of opinion on exactly how many subspecies are valid (Austin and Murphy 1998; Baughman and Murphy 1998; Guppy and Shepard 2001; Hodges et al.

1983; Scott 1986) but the most recent authoritative taxonomic catalog lists 26 (Pelham 2008). The case for subspecific rank in *taylori* is fairly strong—that is to say, it likely designates real and properly delimited (monophyletic) nearly discrete variation within *Euphydryas editha* resting on three attributes: phenotype, ecology, and distribution but molecular evidence is as yet unavailable.

Like other members of its genus, *E.e. taylori* is quite variable in the proportions of colors expressed on wings, but wings are usually spectacularly checkered with white and brick orange-red spots on a deep brown/black background (Figure 1). The variation is striking, from specimens that are dorsally mostly black to those that are quite light with narrower black lines between the orange and white "cells." The ventral side of the hind wing has an "*editha* line" that reliably separates *editha* from other *Euphydryas* (Guppy and Shepard 2001; Pyle 2002; Scott 1986). Antennae are ringed with alternating bands of black and orange on basal segments while the terminal club segments are banded

**Figure 1:** Adult male *Euphydryas editha taylori* perching on unopened *Camassia quamash* adjacent to *Plectritis congesta,* a post diapause host. Adult from the now apparently extirpated Bald Hill (Thurston County, Washington) area population.

in orange. Males are smaller than females overall with a wingspread averaging 3.8cm. Female wingspread averages 4.1cm with a proportionately longer and wider abdomen than the male.

The life cycle of Taylor's checkerspot is similar to most other Lepidoptera with the exception of a long larval diapause between early summer and late winter (Table 1). First instar larvae are tan with small dark spines along the dorsal and lateral surfaces. Third instar larvae are brown with branched spines. The larvae gradually become darker with each instar change so that by the fourth and later instars the larvae have black to dark brown skin splotched with pearlescent lighter spots and a row of orange tubercles topped with complex black spines along the dorsal surface. Larvae feed in groups within a loose silken web during the first through third instars. During the third instar larvae will often leave the web and by the fourth instar larvae begin a mostly solitary existence.

**Table 1:** Life history of *Euphydryas editha taylori* in Washington. Note that there is some variation in the number of instars each larva goes through. Temperature, insolation, and the condition of host plants influence speed of development and larval movement.

| ***Life stage*** | ***Behavior*** |
|---|---|
| **Egg** | Cluster of 15-100 laid on or near the base of the pre-diapause host, *Castilleja hispida* (Figure 2), or *Plantago lanceolata*. Egg laying begins inbetween the beginning of April and ends in late June depending on elevation, latitude and seasonal weather variations. Most laying occurs within three weeks in each population. |
| **1** | Eggs hatch about 7-14 days after laying and larvae begin feeding communally in a web they create on host plant. Instar lasts about one week. |
| **2** | Larvae continue feeding communally in a web on host plant. Instar lasts about one week. |
| **3** | Larvae continue feeding communally in a web on host plant. Larvae start to wander more outside the web but stay near host plants unless searching for a new host. Instar lasts about ten days. |
| **4** | Larvae continue feeding on host plant quickly becoming much more solitary. After feeding for about ten days larvae begin to disperse and diapause. Larvae diapause until late winter. After emergence from diapause, larvae feed on a wider range of host plants. Hosts may be spatially separate from prediapause hosts so they may have to move to find food. |
| **5** | Larvae continue feeding on host plant until shortly before pupation. They stop eating and wander until choosing a location for pupation. |
| **Pupae** | The pupal stage generally lasts about three weeks. Pupae have been found close to the ground hanging from the underside of plants and litter. |
| **Adult** | Adults begin eclosing in mid-March (males eclose first) or later depending on weather conditions. Adults probably live from a few days to about two weeks based on data from other subspecies. |

Pupae have irregular splotches of muted white, reddish yellow and black, similar to those found on the underside of the adult wing but with grey overtones. They have been found suspended from low growing vegetation within a few centimeters of the ground during pupation (D. Ross pers. com.).

**Figure 2:** Taylor's checkerspot eggs on the base of *Castilleja hispida* at 91st Division Prairie. Usually the eggs are on the underside of one of the lowest leaves or on the stem near the base of the plant. Each egg is approximately 1mm in diameter.

*Euphydryas editha* has one of the most complex taxonomic histories of any butterfly in North America. There are at least 26 currently valid (non-synonymous) subspecies (Pelham 2008) with 21 in California alone (Murphy et al. 2004). Edwards originally named *E.e. taylori* in 1888 and subsequently Gunder named both *victoriae* and *barnesi* which are considered synonymous with *taylori*. The phenotype of *E.e. taylori* is, on average, darker than any other *E. editha* subspecies with a higher proportion of dark ground color scalation and it has a distinctively rounded forewing apex and outer margin (Warren 2005). Some of the distinctions are subtle and there is some overlap with other subspecies. Taken as a whole it can be reliably distinguished from other subspecies though the most reliable characteristic is the location the butterfly was found (Figure 3).

*Euphydryas* is derived from the Greek euphys (a goodly shape) and dryas (a dryad or wood nymph). It has been assumed that the specific epithet editha came from a woman named Edith (Guppy and Shepard 2001) though this is not clear in the original description (Boisduval 1852). Subspecies *taylori* is named after the Reverend George W. Taylor, an early British Columbia lepidopterist (Guppy and Shepard 2001). There have been two widely used common names. Taylor’s checkerspot is obviously based on the subspecific designation and probably the most “proper” common name since it was first applied in print by Holland (Holland 1898). It has also been referred to as the Whulge checkerspot (Pyle 1989) after the Salish word for what later became known as Puget Sound.

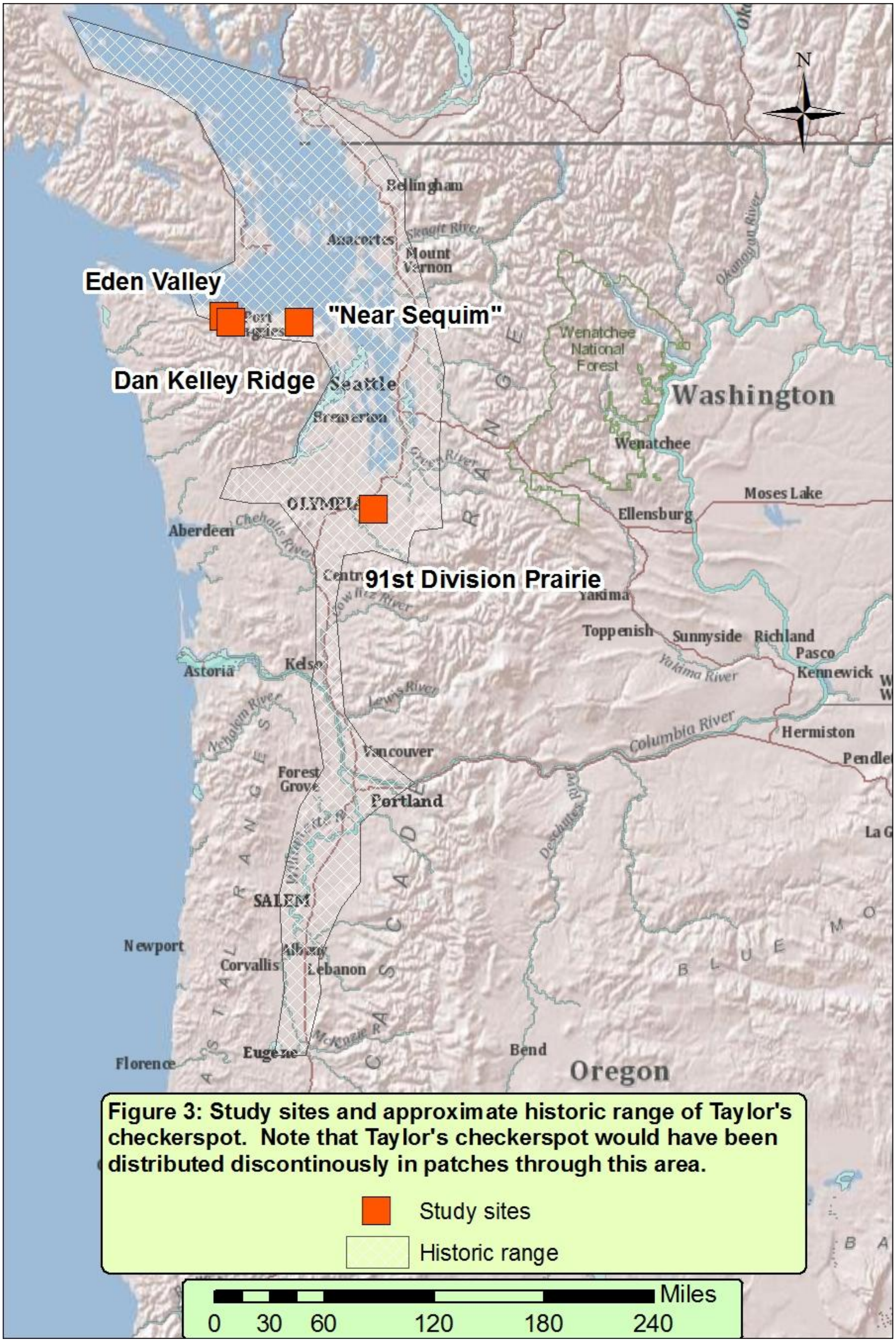

Figure 3: Study sites and approximate historic range of Taylor's checkerspot. Note that Taylor's checkerspot would have been distributed discontinously in patches through this area.

### ***Anthropogenic habitats***

The story of human interactions with the land is not a sideline to the main narrative of ecological processes; rather it is at the core of what created and maintains the unique habitat of Taylor's checkerspot. Fire was a tool used by native people throughout North America and it had tremendous influences on the grassland habitats used by Taylor's checkerspot. Both fire and the harvest of camas (*Camassia quamash*, *C. leichtlinii*) and other foods occurred in what is now Taylor's checkerspot habitat (Carpenter 2002). Native people in what is now western British Columbia, western Washington, and western Oregon developed in synergy with the prairies and the surrounding habitats that together created a rich mosaic of resources for people and wildlife (Boyd 1999b; Leopold and Boyd 1999; Turner 1999). In some areas these native practices and ecological processes created some of the most productive agricultural land in the western United States, but that productivity did not last long under the European model of agriculture (White 1980).

Taylor's checkerspot may have already existed in the ecoregion when the first humans arrived after the end of the Vashon glaciation. The historical distribution of the Taylor's checkerspot is Willamette Valley-Puget Trough-Georgia Straight grasslands and grassy balds from southern Vancouver Island through the Willamette valley in Oregon (Figure 3). It likely co-evolved with the "natural" communities that came into being as a result of Native American cultural burning practices (Stinson 2005).

Many of the cultural practices of the European settlers were profoundly different than those of the Native American inhabitants. Domesticated cattle and sheep, exotic plants from Europe, plowing, disruption of the fire regime, logging, growth of cities, introduction of exotic insects by organic gardeners, suburban development, hobby farming, chemical and concentrated dairy waste fertilizers, military training, and finally anthropogenic climate change have all profoundly altered the available habitat.

The relationship between anthropogenic habitat modifications and Taylor's checkerspot is complex and apparently contradictory because, while human activity has led to the extirpation of populations and climate change may be a range wide threat, human activities both past and present may be essential for the persistence of this butterfly where it currently exists. All remaining populations occupy areas heavily modified by humans including road edges, an artillery impact area, and habitats modified by ecological restoration actions including shrub and tree removal and herbicide use. Exotic invasive plants have reduced habitat quality in most areas but one exotic plant, *Plantago lanceolata* has been adopted as a host with some populations apparently dependent on this exotic host for completion of their life cycle (Severns and Warren 2008). The patterns of vegetation and host plant abundance can be driven in unexpected ways by complex factors including grazing and anthropogenic nitrogen deposition (Weiss 1999).

### ***Taylor's checkerspot ecology***

*Euphydryas editha* is perhaps the best known butterfly species in North America since it has been very intensively studied by a large number of workers (Ehrlich and Hanski 2004), and it is likely that many aspects of that information can be, with caution, applied to *taylori*. Other *Euphydryas* species provide additional pertinent information on study methodologies (Konvicka, Hula, and Fric 2003). The larvae need non-senescent host plants to eat and sun to increase their metabolism (Hellmann 2002; Kuussaari et al. 2004; Weiss, Murphy, and White 1988). Females are picky about where, on which plant species, on which individuals, and under what conditions they oviposit. There are also heritable differences in these oviposition preferences between even nearby populations (Rausher, Mackay, and Singer 1981; Parmesan, Singer, and Harris 1995; Singer, Ng, and Morre 1991; Singer, Ng, and Thomas 1988; Singer 2004). Adult *taylori* usually avoid tall dense non-native vegetation, and all extant colonies are in native grasslands and balds (Hays et al. 2000; Stinson 2005).

Extant *Euphydryas editha taylori* populations in Washington are known to use *Castilleja hispida* and *Plantago lanceolata* as host plants for oviposition and prediapause feeding (Guppy and Shepard 2001; Pyle 2002; Severns and Grosboll 2011). Canadian populations also use *Veronica scutellata* (Page et al. 2009) while Oregon populations appear to be restricted to *P. lanceolata*. Recent work has shown post-diapause feeding on the oviposition hosts as well as *Orthocarpus pusillus, Collinsia parviflora, C. grandiflora*, and *Plectritis congesta* and (apparently rare) use of other species (e.g., *Symphoracarpos alba*, (unpublished data)) and *Veronica scutellata*, *V. serpyllifolia, Plantago major*, and *Centaurium erythraea* (Page et al. 2009) by post-diapause larvae. Other populations of *E. editha* are known to use various Scrophulariaceae and a few Valerianaceae as hosts and some populations require more than one host for development—one or more prediapause and one or more postdiapause (Cushman et al. 1994; Hellmann 2002; Rausher 1982; Singer 2004; Singer, Ng, and Thomas 1988; Singer, Thomas, and Parmesan 1993; White and Singer 1974). The distribution of *Castilleja hispida* in prairies is highly patchy with many existing prairies containing few plants. The non-native *Plantago lanceolata* is widespread but locations where *E.e. taylori* is dependent on this host appear to have not only widespread *P. lanceolata* but high density areas dominated by this plant. The other potential oviosition hosts are either absent or nearly absent from lowland prairie sites in Washington. *Euphydryas editha bayensis* is less able to persist in areas with only *Plantago erecta* (a native annual) hostplants than it is in areas with *Castilleja* (Hellmann 2002), and *taylori* may depend primarily on perennial hosts prediapause and annual hosts post diapauses.

In many *E. editha* populations, most larvae starve before diapause (Kuussaari et al. 2004; White 1974), but this is strongly influenced by weather. This appears to be the case for *E.e. taylori* as well (unpublished observations). All extant populations appear to exclusively use perennial hosts for oviposition which increases larval survival odds because most individuals of their perennial hosts senesce later than their annual hosts, but many hostplants still senesce prior to larval diapause. Unless they can move to a non-senescent host, larvae on those plants lose the race to diapause and do not survive.

Slope and aspect influence many aspects of habitat for many species as soil development, vegetation, temperature, insolation, plant phenology, and rainfall can change dramatically depending on slope and aspect. In some populations *Euphydryas editha* larval survival is largely determined by the slope and aspect where larvae occur (Weiss 1997). Shading influences larval development times and hostplant senescence and known hostplants are all associated with open unshaded habitats.

Nectar plants used by the *E.e. taylori* include *Camassia quamash, Lomatium triternatum* (Hays et al. 2000 and Dunn, unpublished data), *L. utriculatum* (Guppy and Shepard 2001), *Balsamorhiza deltoidea* (Hardwick and Potter pers. com.), *Plectritis congesta, Marah oraganum* (pers. obs.), *Fragaria virginiana*, *Callicortis* spp. and *Malus* spp. (Severns and Warren 2008; Ross 2003). Like most butterflies they can, to some extent, adapt to different nectar sources but in most cases use native plant sources that are particularly abundant and in bloom when they are in flight. Extant populations are found in areas with a high proportion of native grass cover. In areas heavily invaded by non-native vegetation, *E.e. bayensis* is less successful (Weiss 1999) and this is probably the case for the *E.e. taylori* as well (Severns and Warren 2008). Most other western Washington prairie specialist butterflies avoid dense, tall non-native grasses (Hays et al. 2000) and anecdotal accounts suggest that *E.e. taylori* from open prairies often avoid flying into dense vegetation over about 0.75m in height while flight behavior of populations in balds is less predictable (pers. observation). Restricted nectar sources can severely limit egg production in most butterfly species though this may be of slightly less importance in *E. editha* than some other species (Boggs 1997). In any case, nectar distribution likely influences oviposition site choice by increasing the probability that females will be near nectar sources.

Many populations of *E. editha* act as metapopulations and exhibit tremendous variability in abundance and local distribution (Baughman 1999; Baughman, Murphy, and Ehrlich 1988; Ehrlich 1961; Harrison, Murphy, and Ehrlich 1988; McLaughlin et al. 2002; Singer and Ehrlich 1979). The low vagility of *E.e. taylori* combined with habitat fragmentation, makes it likely that most of the now isolated populations can no longer act as metapopulations (Char and Boersma 1995). Recolonization of suitable formerly occupied habitat or colonization of new habitat is unlikely because distances between most suitable habitat sites are beyond the ability of the butterfly to disperse and most matrix habitat between sites is poor quality even for dispersal. In order for populations to survive, habitat occupied by the butterfly has to be suitable for population persistence under all conditions; given the complexities of this species' population biology, and its interactions with anthropogenic habitat and climate alteration, recolonization of otherwise suitable habitat is unlikely.

As articulated above, Taylor's checkerspot requires healthy larval hostplants located in a microclimate and vegetation structure that makes these hostplants available to ovipositing females and hospitable for larval feeding. Thus I contend that populations of Taylor's checkerspot butterfly may be partially limited by the lack of suitable habitat preferred for oviposition. In this thesis I will show the characteristics of preferred oviposition habitat essential for the recovery of this endangered species. These characteristics can be used in combination with information developed about larval survival on different hostplant species, different hostplant populations, oviposition preference in captivity, and hostplant transplant methods to optimize restoration of habitat for this endangered butterfly within the human dominated/degraded Willamette

Valley-Puget Trough–Georgia Basin Ecoregion. The characteristics of the selected oviposition habitats indicate that the long history of anthropogenic habitat modification continues and that this butterfly is likely bound to human alteration of the landscape in unexpected ways.

# II. Captive rearing methods and a comparison of prediapause larval survival using *Castilleja hispida* and *Plantago lanceolata* as hostplants for Taylor's checkerspot (*Euphydryas editha taylori*)[1]

Pre-diapause Taylor's checkerspot larval hostplants are largely synonymous with oviposition hostplants because larvae (particularly in the first two instars) have very limited abilities to move given their minute size, slow locomotion, and limited energy reserves. Because of the larvae's dependence on oviposition hosts it is essential to understand whether larvae survive and grow at different rates on different hostplant species. To investigate their affects on larval survival and growth, two pre-diapause hosts, *Castilleja hispida* and *Plantago lanceolata* were fed to different groups of prediapause larvae.

Butterfly captive rearing for reintroduction is an expanding initiative in North America (Lipman et al. 1999; Anderson et al. 2001) that has a long history in the United Kingdom (Duffy 1977; Oates and Warren 1990; Pullin 1997). The potential for captive rearing to contribute to the conservation of wild populations has been recognized for some time in the United States (Pyle 1988), but only a few species have been reared for reintroduction thus far. Rearing butterflies is an effective method for investigating aspects of their behavior and biology as well.

In contrast to butterflies and other invertebrates, captive rearing of vertebrates is well established as an emergency tool in conservation biology. Vertebrates are more forgiving in captive situations because they tend to have significantly longer reproductive lives than butterflies but, when captive rearing methods are well developed, butterflies have the capacity to produce larger numbers of individuals quickly. In the case of these butterflies there is only one opportunity, about two weeks long, for an individual animal to

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[1] Portions of this work were previously reported in Grosboll (2004).

successfully reproduce. Mistakes at any point in the butterfly life cycle commonly are catastrophic for the captive population and mistakes are likely during initial rearing. Because of their short life cycle, probable negative genetic consequences associated with captive breeding (Brook et al. 2002; Bryant et al. 1999; Nieminen et al. 2001; Saccheri et al. 1999) and substantial long term augmentation of wild populations with captive bred animals (Heath et al. 2003), captive rearing using limited numbers of wild mated females to produce eggs primarily for reintroduction is more conservative and more appropriate for butterflies than transferring of large numbers of individuals from depressed wild populations.

## **Methods**

Captive rearing method development remains an iterative process that proceeds through experiment and experience. A relatively large number of eggs are needed for experimental rearing in order to test various treatments but clearly removal of individuals from the wild should be minimized. In this study, one female Taylor’s checkerspot provided enough (126) eggs for initial testing of three different host plants, different group sizes, and feasibility testing of outdoor rearing.

This rearing project used a combination of methods developed by Gordon Pratt (pers. com.) for Quino checkerspots (*Euphydryas editha quino*), and large scale rearing techniques developed by the Oregon Zoo for Oregon silverspots (*Speyeria zerene hippolyta*) (Anderson et al. 2001). Techniques developed at the Zoo are particularly applicable to large scale rearing. The standard techniques include control of cross contamination by pathogens by keeping larvae segregated, treatment of all surfaces that will come into contact with larvae by autoclave, alcohol, or chlorine disinfection, frequent hand washing by workers, searching host plant material for predators, and host plants unsprayed with either pesticides or biologically active liquids (i.e., compost tea) (Sullivan pers. com.; Anderson pers. com.).

Immediately after hatching larvae were moved to Petri dishes and given appropriate food and space using larger dishes as they grew (Table 2). Larvae were moved by picking them up with a cleaned water moistened size 0 paintbrush during the first instar until midway through the third instar under a stereomicroscope at 8x – 16x magnification. In later instars larvae were moved by scooping them with a small flat spatula. The larvae were segregated into groups as they hatched to minimize the transmission of disease and to allow the testing of different methods. Larvae were never allowed to come in contact with larvae outside their group after hatching. All surfaces that touched the larvae were disinfected to minimize pathogen transmission among groups. Petri dishes were washed and soaked in a 2% chlorine bleach solution between uses. The paintbrushes and spatulas used for handling larvae were washed in 95% ethanol then washed twice in distilled water between handling each group of larvae.

Day length and average temperature were similar to those under outdoor conditions at a lowland South Puget Sound grassland. The rearing area was located on the Thurston County Glacial Heritage Preserve in an unheated room with large windows that were constantly open to the outdoors during the pre-diapause larval feeding season. The amplitude of temperature change throughout the day was likely less than outdoors because of the sheltered location. Diapausing Taylor's checkerspot larvae continued to be segregated and were placed into either an outdoor "humidor" chamber in an insulated cooler that contained 4L of water in the bottom or a "humidor" chamber in a non frost-free refrigerator at 4°C. Larvae were individually checked weekly throughout diapause for mortality and illness. When mold, mildew, or bacterial growth were found in containers or in humidifying water the larvae were moved to new containers and the humidor chamber was cleaned.

**Table 2:** Care of captive laboratory reared Taylor's checkerspot larvae fed *Castilleja hispida* and *Plantago lanceolata*. There was some instar overlap with individuals at varying life stages.

| ***Life Stage*** | ***Action*** |
|---|---|
| **Egg** | Eggs laid on *Castilleja hispida* in captivity. Ninety-two of 94 eggs hatched. |
| **Instar 1** | Placed in 5.5-cm petri dishes with moist, not wet, filter paper in bottom. Two groups of five, two groups of ten were placed on *P. lanceolata*. One group of three, five groups of five and two groups of ten were placed on *C. hispida*. One group of five was provided with both hosts. Moved to new dish/fresh plants every other day. Two medium (2.5 cm) *C. hispida* or one medium (6 cm) *P. lanceolata* leaf per five larvae |
| **Instar 2** | Treatments and dishes as above. One medium *C. hispida* or half a *P. lanceolata* leaf per larvae |
| **Instar 3** | 8.5-cm dish, otherwise as above. Two medium (2.5 cm) *C. hispida* or one medium (6 cm) *P. lanceolata* leaf per larvae |
| **Instar 4** | 13.5-cm dish, otherwise as above. Four medium (2.5 cm) *C. hispida* or one medium (6 cm) *P. lanceolata* leaf per larvae. Began to treat as diapausing when they stopped feeding despite access to fresh host material. |
| **Diapause** | After all larvae began diapause, they were weighed individually on an analytical balance. Petri dishes were maintained in a humidified chamber at outdoor ambient temperatures until October 6 at which point larvae were moved to well plates and Toledo jars. Two groups of larvae were kept in refrigerator at 4°C through early March, one group in Toledo jars initially then transferred back to petri dishes on 12 November; the other group was maintained in well plates. One group was kept in an insulated cooler hydration chamber outdoors. In each case larvae were checked at least weekly. |
| **Instar 4** | Returned to feeding in early March. Dish treatment as in pre-diapause instar 4. Extra fresh plants seemed to stimulate feeding. Experimental feeding groups were abandoned shortly after diapause ended because not enough *C. hispida* plant material was available. Some larvae re-entered diapause. |
| **Instar 5** | Treatments and dishes as above; moved daily to new clean dish with fresh plants daily |
| **Pupae** | Pupae allowed to eclose in cages kept in sheltered area outdoors. |

A single adult female was captured and destined to be a voucher specimen in a newly discovered colony of Taylor's checkerspot on a grassy bald near Port Angeles, Washington. She was kept alive in captivity and laid 126 eggs on a *C. hispida* plant in the one gallon cage in which she was kept. Thirty two of the eggs were given to David Nunnalee, a local volunteer captive rearing expert. Those larvae subsequently died in the second instar after feeding on *Plantago major*, apparently not an acceptable prediapause host though postdiapause feeding has been seen in other populations (Page et al. 2009). Those larvae will not be considered further. The remaining 94 eggs were used for this captive rearing study (Table 1). There were 15 groups overall with nine small groups (3-5 larvae) and five large groups (10 larvae). One group was raised outdoors on a fine mesh enclosed and potted *C. hispida* plant.

The plant material provided to the caterpillars was cut from plants and immediately washed. Washing was accomplished by putting whole stems or leaves of host plant into a salad spinner strainer and washing plant material with a forceful, but not plant bruising, stream of water for at least 30 seconds while moving plant material to ensure thorough coverage. After washing, the plant material was spun in a salad spinner to remove most of the water. The plants were then examined under 5x magnification under a stereomicroscope to remove all other insects and eggs. Whole stems of *C. hispida* were cut from the plants and larvae were provided a piece of stem with attached leaves. When leaves were removed from stems of *C. hispida*, the leaves would dry out quickly and become unpalatable to larvae. Whole leaves of *P. lanceolata* were cut from plants growing in an area that received some water from a nearby lawn sprinkler in an area 200m from a recently extirpated *P. lanceolata* feeding Taylor's checkerspot colony. Only leaves that appeared to be fairly new and tender without significant herbivore damage were selected.

After they began to diapause, all groups were kept in a humidified environment in the same room where they were reared until October 1 except Outdoor Groups 1 that was reared outdoors on a potted plant. On October 1 three groups were placed into a refrigerator at 4°C: Indoor Group 1 was kept in Petri dishes throughout; Indoor Groups 2 was placed into well plates in which the area between the cells was filled with distilled water; Indoor Groups 3 was placed into Toledo jars (modified narrow mouth one pint mason style canning jars) in which caterpillars are suspended above water in a silk organza cell but these Indoor Groups 3 larvae were moved back into Petri dishes (similar to Indoor Group 1) in mid November because of problems with condensation in the jars. The final group (In/Out Group) group was placed into an insulated outdoor enclosure and exposed to outdoor conditions.

Statistical analysis were performed using Microsoft Excel and MINITAB statistical software (MINITAB Statistical Software 2000).

## Results

Larvae were successfully reared through diapause using any of the methods when fed either *Plantago lanceolata* or *Castilleja hispida*. Survival for all hand reared Taylor's checkerspot larvae was 84% from egg through to the end of diapause. The difference between survival on the two hosts *P. lanceolata* and *C. hispida* was not statistically significant (85% for *C. hispida,* 90% for *P. lanceolata*, two proportions test P= 0.517). Weight gain also seemed to be unaffected by host plant (mean weight 2.62mg when fed *C. hispida*; 2.59mg when fed *P. lanceolata*, Student's t-test P = 0.809). One group of five was fed both *P. lanceolata* and *C. hispida* and they fed readily on both hosts generally choosing whichever they encountered. Their weight (2.42mg) was not significantly different than the others though the tiny sample size gives very little power to detect differences with this group.

There was no difference in survival or weight between 5 or 10 larvae per container (five larvae reared mean wt. 2.53mg, ten larvae reared 2.60mg, Student's t-test, P=0.602). There were no significant interactions between group size, host plant, and weight.

Hand raised larval mortality was associated with molting. Often one of the group would fail to molt, become lethargic and die. Larvae died more often in early instars and less often as the larvae entered the fourth instar and diapause. Of the nine larvae placed on a potted *C. hispida* plant, four made it through the winter and began feeding again on warmer days in late February. They moved within the enclosure during the winter, down into a gap between the pot and the potting soil during colder weather, and up onto the wooden supports or leaf surfaces during warmer weather but none of them successfully pupated. The pot was exposed to temperatures as low as -11°C but was slightly protected under the eaves of a building.

Most of the *C. hispida* plants used for rearing survived the regular cutting they received but plants in larger pots that included another plant (either *Festuca roemeri* or *Eriophyllum lanatum*) they could parasitize recovered more quickly than those grown alone. As a facultative hemiparasitic species, *C. hispida* was likely able to replace some of the production lost from its own leaves by that produced by its hosts.

## **Discussion**

This work demonstrated that it is possible to captive rear Taylor's checkerspot on either *Castilleja hispida* or *Plantago lanceolata*. Methods used for Taylor's checkerspot worked remarkably well to get caterpillars from egg through diapauses and some larvae successfully pupated and eclosed to successfully complete their life cycle.

The Petri dish rearing system was effective (Figure 4). The low volume and slow but adequate ventilation, combined with a large bottom for dispersing frass keeps the larvae moist enough while at the same time reducing mold formation. There have not been any escapes and the dishes are easy to handle. At a larger scale, Petri dish racks could be used to stack the containers which would ease handling and minimize the space required.

**Figure 4:** Second instar larvae feeding on Castilleja hispida in captivity. Note medium tip ball point pen on left for scale. The larvae in this photo have only been on the plant for a few minutes but frass is already visible both on the leaves and filter paper substrate.

*P. lanceolata* and *C. hispida,* at least in captivity, appeared to work equally well as host plants in this study and larvae will eat either and readily switch hosts, both in captivity and in the wild (pers. obs.). Subsequent rearing efforts with larger numbers of larvae at the Oregon Zoo indicate that mean weight of caterpillars is less when fed *C. hispida* but survival through diapause was unimpaired (Linders 2007). These somewhat ambiguous results may be the result of factors other than hostplant species such as differences in the nutritional content of hostplant material caused by growing conditions. For example, *C. hispida* is a facultative hemiparisitic plant that is affected by the condition or presence of a host from which it draws nutrients.

The survival of larvae on both hosts is encouraging because, even though there are significant efforts underway to plant *C. hispida* in proposed reintroduction areas, *P. lanceolata* is widespread. Whether there are any tertiary effects, such as reduced levels of compounds protecting caterpillars against predation, is unknown.

The effectiveness of *P. lanceolata*, an exotic species of European origin, as a host creates a conundrum for habitat restoration and enhancement. Generally ecological restoration attempts to create a functional ecological community or ecosystem using native species and ecological processes. Several populations of Taylor's checkerspot appear to be completely dependent on *P. lanceolata* as a host, laboratory measurements indicate that it works well for the butterfly, and other populations of *Euphydryas editha* have exhibited heritable evolutionary shifts away from native hosts to *P. lanceolata* (Singer, Thomas, and Parmesan 1993).

Group size did not affect rearing weight and therefore, because it is less labor intensive to rear larvae in large groups, captive rearing should use larger group sizes. It might be possible to increase the group size even more in hand rearing; this would be more similar to wild larval groups which are usually much larger. Rearing in larger group sizes would need to occur in a larger sized rearing dish to accommodate the additional larvae. The space per larvae available for ten larvae in a 13.5cm petri dish is $13cm^2$, and should probably not be reduced for fourth or fifth instars because of waste generation, unless the petri dish is to be changed once a day or more.

Outdoor enclosure rearing of Taylor's checkerspot was promising. Low temperatures were the likely cause of mortality for five of the nine larvae as there were at least seven alive prior to the cold temperatures. An insulated retreat area would likely reduce this mortality. Not all larvae can be always seen so there is a loss of information on exact numbers of larvae but enough can be seen to indicate whether there has been a catastrophic change. Potentially a raised bed of *C. hispida*, *Collinsia parviflora*, *Plantago lanceolata*, and *Plectritis congesta* could be constructed and netted to raise larvae in an inexpensive and more natural situation. This could both reduce costs and decrease selection for captivity. Excluding predators from such an enclosure is difficult but it should be possible to eliminate many of them.

Potted *C. hispida* plants required regular attention and perhaps in the ground plants would be more effective in a larger rearing operation.  Fifty fairly well established *C. hispida* plants were just barely enough for the fifty caterpillars feeding on this host.  A ratio of two plants for each larva would be better and reduce the stress on plants from excessive cutting.

Scaling up rearing gradually provides opportunities to learn additional information about the species' natural history and minimizes the loss of animals.  During this project, with 92 larvae in captive rearing, a few inferences about different methods could be drawn.  With more larvae in the captive rearing program, investigation of rearing using adaptive management techniques has become more powerful and rigorous.  Current captive rearing at the Oregon Zoo is producing many hundreds of larvae each year for reintroduction (Anderson, pers. com) and this has generated additional biological information.

Because larval mortality is cumulative and higher in early instars, reintroduction can be done with larvae either at the beginning or end of diapause, or as adults. Releasing larvae rather than adults eliminates the unique problems of handling pupae and freshly eclosed adults but it is difficult to know what happens to the cryptic larvae once they are released (Fimbel 2008).  It also exposes larvae to natural conditions which may increase their propensity to exit diapause in the spring.  Current rearing efforts use a mix of larval and adults in experimental reintroduction releases (Linders 2007). Success in getting butterflies through diapause and to pupate and eclose at the same time as their wild counterparts is essential for scaling up for successful adult reintroduction releases into suitable habitat.

Initial captive rearing results were promising but details of winter diapause emergence, pupation and eclosion timing still needed additional effort after this project. After this mid scale exploratory attempt, current attempts have built on the natural history information generated and allow increasingly rigorous comparisons between different rearing protocols.  Subsequent projects proved the effectiveness of these methods (Linders 2007) and refined them further leading to experimental reintroduction attempts at three sites.

# III. Oviposition in the field: Where Taylor's checkerspot chooses to lay eggs[2]

Several aspects of Taylor's checkerspot oviposition were of particular interest in this study. First, the spatial distribution of oviposition at each site has habitat management, as well as ecological, implications. Second, understanding which aspects of habitat are most important to an ovipositing checkerspot is critical for undertaking habitat restoration and selecting suitable sites for reintroduction of checkerspots. The remaining two questions are of primarily scientific rather than management significance: 1) are there physical characteristics of hostplants, hostplant distribution, or nectar plants that drive the choice of oviposition; and 2) are there microhabitat parameters for oviposition that differ from broad scale habitat preferences.

Oviposition habitat was measured in four populations (three in Clallam County, Washington: Dan Kelley Ridge, Eden Valley, and "Near Sequim", and one in Pierce County, Washington: 91st Division Prairie). The exact locations of some of these sites cannot be given in this thesis because of concerns that populations will be damaged by either malicious individuals or unscrupulous butterfly collectors; thus map locations of study sites are deliberately imprecise (Figure 3 map of locations/range). The "Near Sequim" population is on privately owned habitat and access to the site was given with the stipulation that its exact location not be publicized.

Stratified random sampling was used to subsample each site and some smaller areas were censused. Measurements for hostplants not chosen for oviposition (no eggs or larvae) were compared to hostplants chosen for oviposition (with eggs). The variables in Table 3 were measured for each plot. Differences between plants or microsites

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[2] Paul Severns, Ph.D., was jointly responsible with the author for field work and study design; the author was responsible for field work, study design, analysis, and writing.

**Table 3:** Variables measured to determine oviposition site preference. Note that vegetation, number of host plants, and vegetation height was also recorded for search plots.

| ***Measure*** | ***Explanation*** |
|---|---|
| **Aspect/slope** | GPS coordinates on digital elevation model determined aspect and slope of each sample plot. |
| **Larval clusters** | Count of egg clusters/larval masses on plant. |
| **Plant species** | Species on which oviposition occurred. Based on earlier observations and accounts from other subspecies, only likely hosts were searched. |
| **Nearest plant** | Nearest conspecific hostplant. |
| **Plant height** | Maximum height of plant. |
| **Plant # stems** | Number of stems on the plant. |
| **Plant volume** | Plant area as measured by distance between furthest points horizontally on the plant multiplied by the distance across the plant perpendicular to that axis multiplied by the height. |
| **Plant phenology** | Categorical data: flowering, senescent, etc. |
| **Canopy shade** | Shade experienced by the plant using the sunpath method on hemispherical photographs. |
| **Vegetation** | Percent cover for 1m$^2$ quadrat centered on the oviposition site, and adjacent randomly selected non-oviposition plot. |

selected for oviposition should indicate which variables are most important in how butterflies chose where they oviposit. Searching for larval masses (groups of larvae sharing a webbed shelter) was effective, and damaged habitat less than searching for eggs because larval masses are much more visible (Figure 5) than the inconspicuous eggs and required far less trampling and disturbance of hostplants (Figure 2). Moving

**Figure 5:** Larval webbing on *Plantago lanceolata*. This webbing is visible within two to three days after eggs hatch. These larvae are likely at least two weeks old and in the second and third instar. Photo by Paul Severns.

leaves to examine the underside often detaches leaves or dislodges eggs or larvae either of which can lead to the death of eggs or larvae.

## Methods

### *Sampling scheme*

A stratified random scheme to sample for oviposition sites and habitat was used in most areas. Some habitat patches were small enough that a total census was performed by searching the entire area. Randomization, dispersion, minimizing disturbance to habitat, sampling efficiency, and safety (two sites contained steep cliffs and one contained unexploded artillery shells) were considered in developing a sample scheme for each site. In addition, landowners restricted access to two of the sites which limited the time available for sampling. The biology of the butterfly also influenced the available search time because larval groups begin to disperse in search of food as hostplants are denuded or become senescent. Because we could control the sampling scheme but not the other factors we attempted to maximize the available time at each site to generate as many samples as possible.

The sampling was nested in order to determine both broad site preferences and microsite preferences. Search plots were 5m x 5m which was an area that could be searched in a reasonable amount of time and in which the vegetation could be characterized in a straightforward manner. Plots were enclosed in a 5m x 5m rope quadrat prior to search (Figure 6). Within plots, all potential host plants were systematically counted and searched for the presence of larvae. Larval masses were flagged as they were found. Vegetation and other cover within the 5m x 5m plot was characterized using visual estimation of cover by species within two 1m x 2.5m sample quadrats randomly placed on each side of the 5m x 5m quadrat and placed across

**Figure 6:** Search plot at Eden Valley. Note distinct edge of bald habitat behind author and moderately steep south facing slope. The edges of bald habitat were generally clear with a minimum of ecotone habitat between the bald and surrounding forest. Photo by Paul Severns.

(lengthwise) to any environmental variation (e.g., perpendicular to a road, across an ecotone). This was done to measure broad scale habitat preferences within the site. If multiple oviposition sites were found in a search plot, one was randomly selected for microsite measurement. A 1m x 1m quadrat was centered and oriented square to the cardinal directions on the selected larval mass (when present) and vegetation and hostplants were measured at that scale as well as in a randomly selected adjacent and larvae free 1m x 1m quadrat to measure finer scale habitat selection (Figure 7). Categories measured included bare ground, cryptogam, shell, rock, gravel, sand, wood, thatch, and plastic. Ocular estimation was also used to measure cover in the 1m x 1m paired oviposition quadrats. Cover values were recorded to the nearest 1% for species/categories with > 1% cover and to the nearest 0.1% for species/categories with <1% cover. For known and potential host plants (*Castilleja hispida*, *Collinsia parviflora*, *Plantago lanceolata*, *Plectritis congesta*, and *Triphysaria pusilla* were found in plots) we recorded cover to the nearest 0.1%.

In addition, within the paired microplots all host plant heights, lengths, and widths were measured and the number of stems of *Castilleja hispida* were counted. To obtain a coarse estimate of vegetation height within the 5m x 5m oviposition search plots, we measured the height of the tallest vegetation occurring within each of the 25 1-m2 in the search plot. For plants taller than 2m we estimated to the nearest decimeter and to the nearest 0.5m for plants taller than 4m.

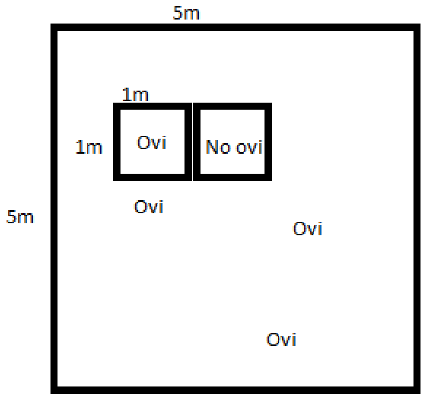

**Figure 7:** Search plot layout showing paired plots. Randomly selected oviposition site (Ovi) paired with adjacent randomly selected non-oviposition site (No ovi).

### *Finding oviposition sites*

To study the interaction between where Taylor's checkerspot lays eggs and its habitat it is essential to find oviposition sites; this is difficult for several reasons. First, butterfly eggs are small, in the case of Taylor's checkerspot, less than 1mm in diameter. Second, the butterfly nearly always lays eggs in a clutch of 6-50 eggs which helps in spotting them but reduces the number of total oviposition sites (compared to most species which lay single eggs) and thus the probability of detection on an individual plant. Third, the eggs are nearly always laid in a hidden location near the base of a host plant, which can be incredibly abundant, either on the stem or under a leaf. Fourth, Taylor's checkerspot is an endangered species living in fragile, rare, and declining habitats so trampling and disturbance of host plants (which sometimes dislodges eggs) needs to be minimized. Finally, the timing of the flight period and thus egg laying generally lasts two to four weeks which means that some eggs are still being laid when others have already hatched.

In order to overcome the challenge of finding oviposition sites we used larval groups as a proxy for oviposition sites, and we only searched known host plants. When larvae hatch from a clutch they immediately begin feeding and forming a larval web starting at the oviposition site that serves to protect them from some predators and parasitoids and perhaps to assist in thermoregulation. Larvae stay within and near the web as long as the host plant remains palatable. Early instar larvae have very limited dispersal range and will likely die when the host they are on is either consumed or senesces if they do not find a new host plant very quickly; early instar larvae will not successfully feed on non-hosts. Feeding webs become visible from several feet away within a few days thus making the larval aggregation visible without disturbing the host plant or larvae (Figure 5). At some point, often in the third or fourth instar, the larvae usually begin to disperse to find other host plants as the original plant has been largely consumed. Because *Euphydryas editha* is known to have high fidelity to its host plants, the assumption was made that *taylori* would follow the same pattern. All observations of *taylori* actually ovipositing have been on known host plants and even if there were oviposition on other plants, larval survival is unlikely and thus feeding webs would never be formed. We assumed that egg laying on non hosts was negligible. Even if some eggs were laid on non-hosts it is unclear that useful information would be generated. Since the purpose of this study was to develop information useful for the conservation of this species, we found no practical value for restoring habitat or recovery of Taylor's checkerspot in the finding that some of the butterflies lay eggs where larvae would invariably starve though finding such an apparently maladaptive behavior would be of evolutionary interest. Based on these factors and the impossibility of searching every plant thoroughly for eggs, short of an inefficient and habitat damaging search using large numbers of people, searching for larval webbing was used as a proxy for oviposition site.

*Euphydryas editha* uses plants in a few families in the order Lamiales and Valerianaceae for oviposition. All members of these families, which included *Castilleja hispida*, *Collinsia parviflora*, *Mimulus* spp., *Plantago lanceolata*, *Plectritis congesta*, and *Triphysaria pusilla*, were searched within the search plots and search areas.

### *Hemispherical photos*

At two sites (Eden Valley and Dan Kelley Ridge, which will be introduced below) hemispherical photographs of the sky above each oviposition site and each search plot were taken using a Nikon Coolpix 4500 coupled with a Nikon FC-E8 0.21x fisheye converter. The camera was placed on a tripod in the center of each plot directly above the vegetation, oriented to point north, and leveled. If an oviposition site was present and greater than 1m from the center of the plot an additional photo was taken and that location. Exposure was adjusted to achieve maximum contrast using methods intended to maximize contrast between sky and everything else (Zhang, Chen, and Miller 2005), and photos were taken prior to sunrise, after sunset, and under cloudy conditions. All hemispherical photographs were taken July 17-23, 2010. No hemispherical photographs were taken at “Near Sequim” or 91st Division Prairie because neither has substantial canopy or terrain that would be captured using this method. The resulting digital hemispherical photographs were manually thresholded (see Appendix A for examples of original and thresholded images) and analyzed using Gap Light Analysis V2.0 software to estimate the amount of solar radiation at each plot.

### ***Analysis methods***

Because this study measured variables at four different sites each with their own characteristics and at different spatial scales within sites, the results were analyzed first within sites and then, when appropriate, across sites. This study generated a large amount of data and sifting through the data to find the information that is important at each site and to then zoom out to consider common aspects of Taylor's checkerspot oviposition across all sites was challenging. For purposes of the oviposition study, approximately random sampling as well as census data at individual sites was treated as blocks for analysis among sites. Paired oviposition-non-oviposition plots were sampled at every site while hemispherical photos were only taken at two sites. In each case the sites used in multi-site analysis are noted. Statistically significant findings that are unlikely to be biologically or ecologically significant are reported and placed in a biological context.

GIS analysis was limited to developing information for other analysis. GPS locations for oviposition search habitats were projected onto a digital elevation model of the area to determine the slope and aspect of each search plot. Those results were used as an input for Gap Light Analysis software and were analyzed statistically to determine their influence on oviposition site selection.

Previous vegetation analysis using Multi-Rank Permutation Procedure (MRPP) (Mielke 1984) and Indicator Species Analysis (ISA) (Dufrene and Legendre 1997) indicated that most vegetation measurements were not predictive of oviposition at the scale of the study at any site (Severns and Grosboll 2011). Perhaps this is because the selected sites were already known as occupied habitat that contained a small subset of the available habitats and vegetation within the range of Taylor's checkerspot. By some measures, species richness for example, the vegetation sampled at every site is dramatically different between sites and between areas within sites. By other measures, such as vegetation structure, the sampled areas were very similar across and within sites. Rather than re-analyze plant community and vegetation data as a whole, analyses of vegetation were focused on selected attributes of vegetation data (particularly host plants) that were associated with oviposition.

Data were transformed to improve normality where appropriate and transformations used followed the recommendations of Zar (1999). Transformation methods are noted where used. Initial data entry and manipulation was done in Microsoft Excel. Statistical analyses were performed in Minitab and Analyze-it where noted.

## Site descriptions and methods

The four sites, Eden Valley, Dan Kelley Ridge, “Near Sequim”, and 91st Division Prairie, are spectacularly different in many ways but all contain primarily low growing herbaceous vegetation with either very thin, excessively well drained, or somewhat excessively well drained soils that dry out during the usual summer drought. Sampling methods were adapted to each site as necessary because of site differences and constraints. Additional site information is available for researchers with approval from the Washington Department of Wildlife.

### Eden Valley

Eden Valley is a grassy bald complex west of Port Angeles topping out at approximately 400m. It contains steep bald and cliff habitats with shallow soil over bedrock in a matrix dominated by second or third growth mixed conifer/deciduous forest. Portions of this site are inaccessible due to the steep terrain but most of the area occupied by host plants is accessible with caution. *Plantago lanceolata*, *Castilleja* *hispida*, *Plectritis congesta, Mimulus* sp. and *Collinsia parviflora* occur on the site. Rough estimates of adult Taylor's checkerspot range between 100 and 300 in years when they are most abundant but monitoring has been sporadic and not designed to give a true population estimate (Ann Potter pers. com.). Our goals at Eden Valley were to sample, detect, and describe the habitat and species used for oviposition in the large bald (thought to contain a large proportion of the population) and to census the smaller balds to determine if they are used for oviposition.

### *Eden Valley sampling methods*

Eden Valley vegetation was sampled June 19-29, 2010 though earlier visits to the site were used to lay out the sampling scheme and provide anecdotal information about the butterfly flight period (in 2010 adults were observed beginning April 21 through June 27, much longer than the average 3-4 week flight period). The vast majority of adult butterflies were gone by the beginning of June, two weeks prior to the initiation of oviposition sampling, but three were spotted during sampling; thus adults, eggs, and larvae were all present at the same time.

The main bald (Figure 8) was defined as our sample universe for this site but unfortunately not all of it could be randomly sampled because of vertical cliffs and some of it needed to be excluded because it was not bald habitat. Even with the use of climbing gear sampling the cliffs would have been impractical and because they were covered in a fragile and poorly anchored layer of cryptogams, would have caused substantial damage for little gain. Arguably these cliffs are not bald habitat but cliff habitat and therefore could be removed from the sampling scheme for that reason as well. Fortunately many cliff areas could be seen from other vantage points and no *Castilleja hispida* was seen. We developed rules for excluding sample sites that included avoiding areas that had poor footing and exposure to life threatening falls. We also excluded forest and large shrub dominated inclusions in the bald (>95% tall shrub cover or tree canopy cover >75% directly over plot) and used these same parameters to identify the bald edges. These areas were excluded because, while they were present within and adjacent to the bald, they are not bald habitat and they clearly were not habitat used by Taylor's checkerspot.

The bald was divided into a 5m x 5m grid and north-south transects every 20 meters, with a random start, were established. For each transect an origin of 0m, 5m, or 10m from the northern edge of the bald was randomly selected using a random number generator. The remainder of the transect was sampled every 15m with the 5m x 5m rope quadrat until the edge of the sample bald was reached either because of tree/shrub cover or encountering cliffs. This created a randomized but well dispersed sampling scheme across the large bald (Figure 8) and yielded 51 search plot locations four of which were excluded as not bald habitat (patches of dense forest) though they were within the boundaries of the bald.

The smaller balds to the north of the main bald were systematically censused for both host plants and larval masses. The searchers walked transects across the balds 2-5m apart (depending on vegetation density) searching for and counting host plants and larval masses. The balds are largely surrounded by continuous forest. A small portion of two of the balds was searched in part with binoculars because of poor footing and exposure to cliffs but this did not likely substantially degrade the search effort in those areas because vegetation was very sparse and individual plants were easy to see and search.

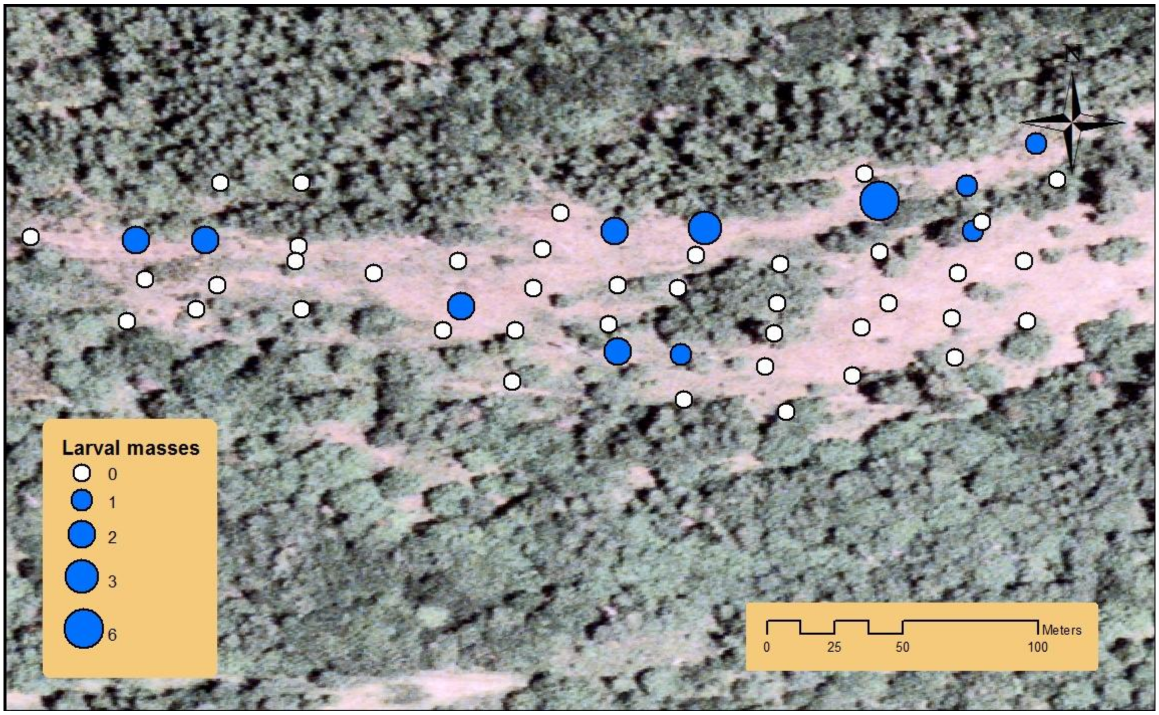

**Figure 8:** Search plot distribution in main bald at Eden Valley. Plots in blue show larval masses. Note the two plots in the upper left area that appear to be in a shrub or tree habitat are actually in an area that was cleared of shrubs after this orthophoto was taken.

### *Eden Valley Results*

Eden Valley yielded 51 5m x 5m search plot samples, 11 of which contained checkerspot larvae with a peak of 6 oviposition sites in one plot (Figure 9). A total of 83 plant species (Appendix B) were recorded in these plots along with the cover of bare soil, gravel, rock, wood, thatch, and qualitative data on previous restoration treatments.

Vegetation as a whole proved to be a poor predictor of oviposition habitat with multivariate examination leading to statistically insignificant results (MRPP: A-statistic = 0.009, p = 0.13) when host plants were excluded from the analysis (Severns and Grosboll 2011). Host plant density, in this case *Castilleja hispida*, is strongly associated with oviposition. Oviposition was also observed in five of the six small censused balds outside the main bald. With the exception of one larval mass found on *Plantago lanceolata* in one of the small balds, all oviposition sites were on *C. hispida*.

**Figure 9:** Search plot at Eden Valley. Blue flags mark larval masses found in this dense patch of Castilleja hispida (flowering orange/red in photo). The density of oviposition appeared to be similar throughout areas with high cover of this plant.

The correlation between *C. hispida* cover and oviposition is strong when all n-51 plots are considered (untransformed data, Pearson's Correlation Coefficient=0.622, p<0.000) but this is biologically trivial because it includes plots that include no host plants and therefore will not contain larvae by definition. Considering only plots that contained *C. hispida* the relationship weakens but is still significant (data transformed Log (x+1), linear regression: adjusted $r^2$=27.4%, p=0.006). The results of binomial regression, to test oviposition versus no oviposition (*C. hispida* cover transformed Log (x+1)), binomial logistic regression p=0.032) versus *C. hispida* cover. The count of *C. hispida* was less powerful as a predictor of oviposition (untransformed data, Pearson's Correlation Coefficient=0.272, p=0.054). The level of *Castilleja hispida* cover is significantly associated with Taylor's checkerspot oviposition compared to *C. hispida* cover plots without oviposition (data transformed Log (x+1), Students t-test, *p* = 0.013).

Results from the paired 1m^2 plots echo the findings at the broader scale 5m x 5m plots. Removing from analysis all paired non-oviposition plots without *Castilleja hispida* leaves n=9 pairs of plots. Using a paired t-test on untransformed measurements of total *C. hispida* volume (Figure 10) oviposition plots contained a mean of 44580 cm^3 *C. hispida* compared to 3621 cm^3 in the paired non-oviposition plots (*p* = 0.006).

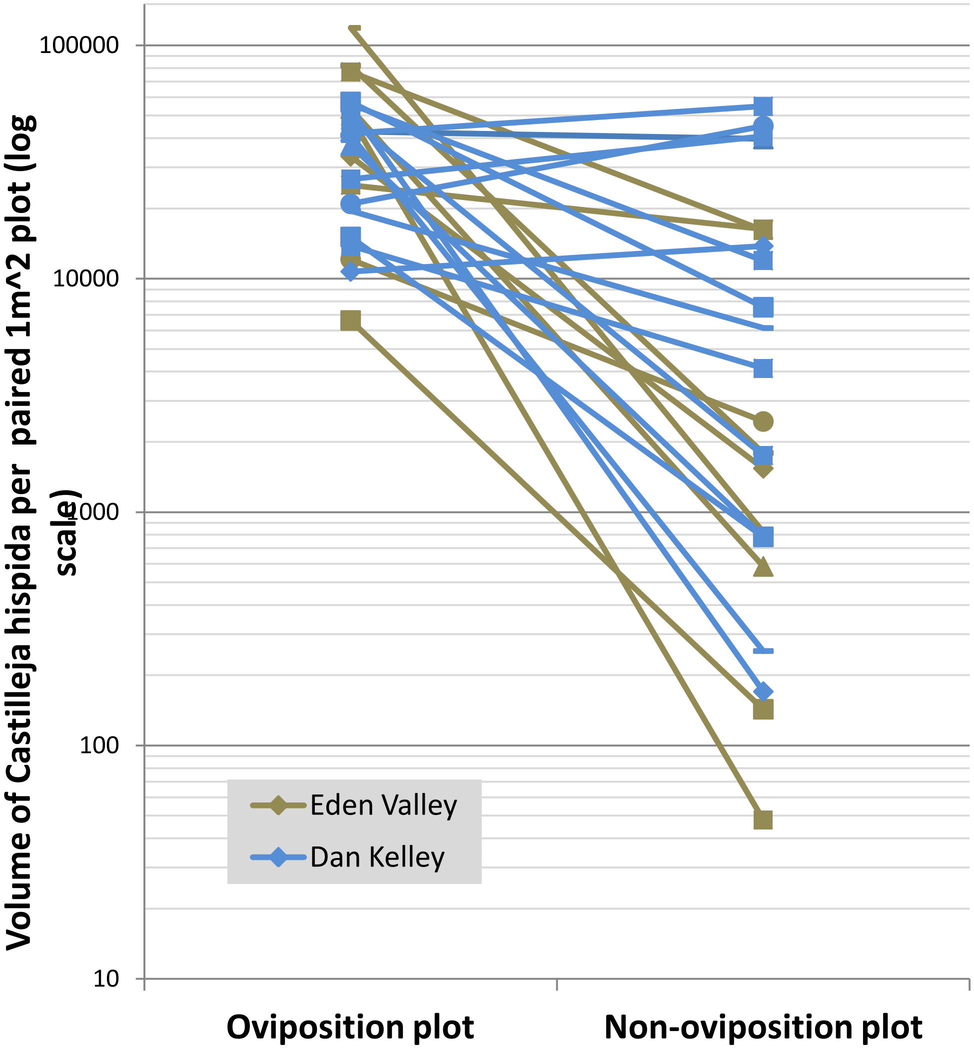

**Figure 10:** Paired plots at Eden Valley and Dan Kelley. Volume of *Castilleja hispida* estimated by measuring the height x length x width of each plant in plots. Non-oviposition plots without *C. hispida* (n=7) were excluded. The oviposition plot was higher in *C. hispida* volume than randomly selected adjacent plot in 18 of 23 pairs (Wilcoxon paired 2-tailed t-test: Eden Valley *p*=0.0039; Dan Kelley Ridge *p* = 0.0353).

Hemispherical photographs of each plot illuminate differences between plots. The range of transmitted light oviposition plots received was not significantly different than those not used for oviposition. Despite this, ovipositon sites received more light than non-ovipostion sites.

## Dan Kelley Ridge

This is another steep grassy bald complex and the highest elevation site studied topping out over 600m in elevation. Much of the site burned in a wildfire in 1987 and was apparently salvage logged thereafter. Many of the balds have been succeeding to shrubs (*Holodiscus discolor* is particularly abundant) and young *Pseudotsuga menziesii*. Anecdotal evidence indicates that the dominance of shrubs has substantially increased in recent years thus likely reducing the abundance and availability of *Castilleja hispida*. Substantial portions of this site are inaccessible because of the steep terrain and much of the habitat is seriously degraded by invasive species and logging activities. Taylor's checkerspots were discovered on the site in 2003 but their numbers and distribution on the site are largely unknown. Anecdotes about oviposition are concentrated on the road edge but this perhaps is more indicative of the distribution of biologists than the distribution of checkerspot oviposition. The goals at Dan Kelley Ridge were to determine what habitats and species were being used for oviposition in particular within previously identified and restored habitats and in general across the accessible portions of the site. *Plantago lanceolata*, *Castilleja hispida*, and *Plectritis congesta* occur on the site.

### *Dan Kelley Ridge sampling methods*

Dan Kelley Ridge vegetation was sampled July 13-20, 2010. Site visits in June and early July indicated that the flight period for Taylor's checkerspot extended to early July and this was the earliest that we could be sure that most eggs had hatched. By mid

July many host plants were stressed by a lack of water and had begun to senesce thus likely leading the starvation of many larvae.

Steep cliffs, large site size, and limited information about the distribution of checkerspots led us to focus our search area on three particular locations: 1) a small natural grassy bald that was outside the burn and recent logging area, 2) an adjacent area, recently treated to control shrubs, that contained substantial *Castilleja hispida,* 3) the road edge, 4) old log landings/pull outs where oviposition had been previously observed, and 5) a search of accessible areas along the crest of the ridge.

The natural grassy bald and the adjacent treated area were each census searched and larvae were flagged. Both balds are steep south facing slopes. In 2009 a former bald area that contained large numbers of suppressed *Castilleja hispida* was treated to control shrubs (Dave Hays pers. com.). The census in the treated area was more haphazard than would be ideal because the terrain is steep, with thousands of short stumps from shrub removal and old logging debris on the treated area and very loose soils throughout most of the site. The resulting census likely undercounted the number of larval masses. We attempted to count as many as possible as systematically as possible using approximate 2m transect spacing without completely destroying either the habitat or ourselves. Butterflies have occasionally been seen in these balds in very low numbers in the past but most have been observed along the ridge top road several hundred meters to the east (Ann Potter pers. com.). Nineteen of the masses were randomly selected for paired microplot vegetation sampling and hemispherical photographs were taken of each.

The road along the ridge was subsampled and the old log landings/pull outs were censused. The road is too wide for the 5m x 5m quadrat to straddle and there are few host plants in the road along much of its length. Therefore the sides of the road were sampled with one edge along the furthest vegetation into the road and the remainder of the quadrat perpendicular to the road. A random start point in the first 50m of the road starting on the eastern end at the Washington Department of Natural Resources property line was used as the origin, and search plots were located every 50m alternating between the north and south sides of the road giving n=22 search plots. Measurements were stopped at the beginning of each of the old log landing/pull-outs and restarted on the other side in order to exclude this separately searched area from the sample. These areas were searched and host plants were counted but vegetation was not quantified. Several hours of earlier haphazard searches in what appeared to be the most likely locations did not turn up any larvae, so characterizing the vegetation was not especially useful. Systematic overlapping transect searches of host plants in the four old log landing/pull-out areas provided a thorough census of larval masses in those areas. The old log landing/pull-outs are anthropogenically flattened wide areas through which the road passes, now used sporadically as parking spaces and illicit ATV spinout areas. They have generally sparse vegetation growing up through the gravel. In addition, we searched and counted all host plants, in this case *Plantago lanceolata*, growing in the center of the road.

### *Dan Kelley Ridge results*

Dan Kelley samples contained 57 plant species (Appendix C). Vegetation as a whole proved to be a poor predictor of oviposition habitat in paired plots with multivariate examination leading to statistically significant but perhaps biologically trivial results (MRPP: A-statistic = 0.045, p = 0.007) (Severns and Grosboll 2011). ISA showed *Castilleja hispida* and bare ground significantly associated with oviposition (Severns and Grosboll 2011). Given the large scale shrub control and associated ground disturbance done to open up habitat specifically in areas with *C. hispida*, bare ground may be an artifact of workers' boots disturbing the soil rather than a true indication of oviposition preference.

The two searched balds yielded 57 oviposition sites of which half were randomly selected for sampling. When the randomly selected sites were less than 2m apart, one of them was randomly selected to avoid overlapping samples resulting in n=19 paired 1m x 1m samples.

The road sampling (n = 22) found no larvae but both *Castilleja hispida* (mean of 5.3 plants per plot) and *Plantago lanceolata* (mean 80.5 plants per plot) were scattered along and in the road (n = 487 *P. lanceolata* in the road were searched). Much of the road edge is shrub dominated and some sections are shaded making the habitat less suitable. Censuses of the old log landings were more productive of oviposition sites sampled and censused respectively. The road edge is used even if rarely (Figure 11).

**Figure 11:** Taylor's checkerspot ovipositing on *Plantago lanceolata* at Dan Kelley Ridge. Her abdomen is curled under the leaf on which she is perched and methodically laying a clutch of eggs. This plant was found in an area with a high cover of *P. lanceolata* in the road verge.

## “Near Sequim”

“Near Sequim” is a privately owned stabilized saltwater shoreline sand dune east of Port Angeles with a maximum elevation of approximately 5m. The landowners allowed access to the site for this study subject to certain restrictions. It contains a typical dune plant community with salt, wind, and summer drought tolerant species. The site is bordered by natural salt marsh which is surrounded by agricultural fields (primarily berries), and an occasionally used two track gravel road runs through the middle of the site. Sampling at this site was stratified to measure the road, the dune, and a mesic ecotone along a brackish slough bordering the site. This Taylor’s checkerspot population is unique in its ocean proximity, stabilized dune habitat, and very early flight period. In the past Taylor’s checkerspot may have been more widespread in this habitat (Pyle 1989). Most of the dune habitat in Washington and Oregon has been severely altered by invasive species and development (Wiedemann, Dennis, and Smith 1999) and other dune and shoreline grassland associated butterflies have declined precipitously on the Northwestern United States coast (e.g., Oregon silverspot (*Speyeria zerene hippolyta*) and sand verbena moth (*Copablepharon fuscum*)) (Hammond and McCorkle 1983).

Taylor’s checkerspot is known to use *Plantago lanceolata* as an oviposition host on this site but there are other potential hosts present including *Triphysaria pusilla* and *Collinsia parviflora*. Post-diapause larvae have been observed feeding on those hosts as well (Ann Potter pers. com.). Taylor’s checkerspots have been monitored at this site for several years but the data have not yet been analyzed. In some years there are at least several hundred individuals in the adult population.

The goals of the study at “Near Sequim” were to determine the distribution of oviposition across three identified potential habitat areas: road, dune, and the mesic ecotone between the dune and a bordering slough.

### *"Near Sequim" sampling methods*

The "Near Sequim" site was sampled May 23-25 and June 1-3, 2010. Site access was limited by the landowner to Tuesday through Thursday 0800-1700. Earlier site visits indicated that this time frame would likely coincide with full larval webbing development for most clutches. Adults regularly fly in early April, though during the study a lone adult female was seen on May 25 but that was clearly an outlier as most larvae were well into their second instar by then.

Oviposition had in the past been observed along the road that bisects the site but it was unknown whether the anecdotally observed oviposition was representative of the distribution of butterflies or the distribution of biologists. The road was sampled by using the 5m x 5m search plot quadrat centered on the road and located randomly within each of 16 segments along the road. Between the dune and the deflation plain wetland behind it is a narrow linear mesic ecotone a bit more than 450m in length. The ecotone vegetation is clearly distinct from both the dune and wetland and it contained a much higher density of *Plantago lanceolata* than the dune. This was sampled (as with the road) by dividing it up into 50m segments and randomly locating the search plot, with one side on what appeared to be the edge of the wetland in each segment (n=9). The dune habitat was sampled by randomly locating a search plot in 50m x 50m grid areas (n=13) across the site. If a randomly selected location fell within road or ecotone habitat we skipped that plot. The road and ecotone were intentionally oversampled relative to the dune habitat because, while the vast majority of the area of the site is dune, the vast majority of the *Plantago lanceolata* is in the road and ecotone areas. *Collinsia parviflora* was common in the dune habitat but it had nearly all senesced making it unusable as a pre-diapause host plant. We decided it was more important to quantify the habitat they were using for oviposition rather than the habitat they were less likely to use. If we had more time on the site, we would have sampled the dune habitat more intensively.

### *“Near Sequim” Results*

“Near Sequim” is much different from the previous two sites with obviously lower levels of biomass and increased bare ground. 48 species were found (Appendix D). Vegetation as a whole proved a poor predictor of oviposition habitat in paired plots.

A total of 48 larval masses were found in the road habitat and the total number of larval masses along the road was estimated to be between 275 and 1504 (95% CI). The number of larvae per mass averaged 38.1 thus giving a total larval population estimate in the road habitat alone of 10500 to 57300 larvae.

There were more larval masses per search plot in the road habitat than in the ecotone and more in the ecotone than in the dune habitat (Figure 12). The road contained areas of dense *Plantago lanceolata* as did some areas of the ecotone. Most of the dune habitat was devoid of *P. lanceolata* but low density (and senescent) *Collinsia parviflora* was scattered through many of the search plots

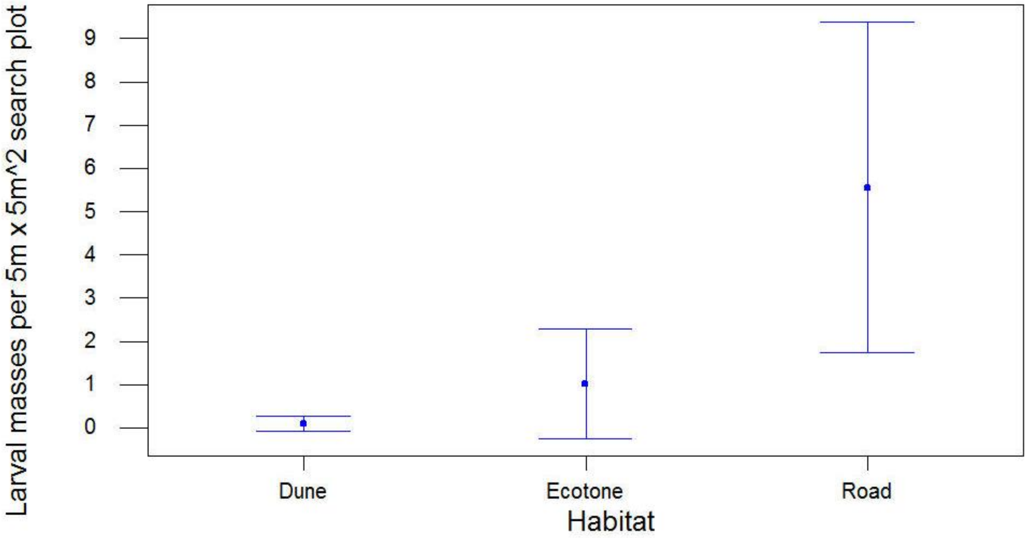

**Figure 12:** Larval masses per search plot at “Near Sequim” (error bars 95% CI). The road habitat was used unevenly with some parts receiving high density oviposition and other areas <50m distant receiving none.

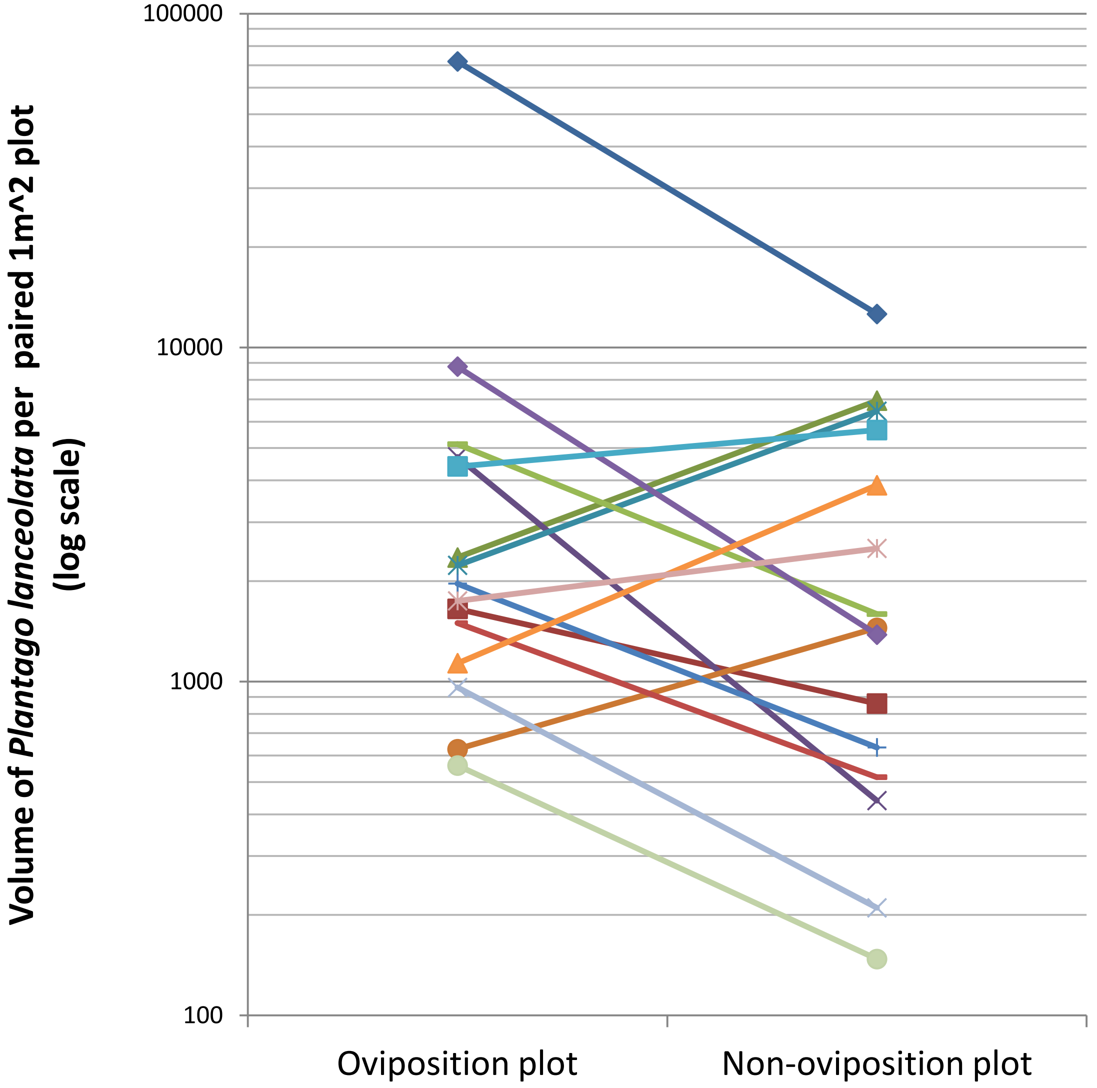

**Figure 13:** Paired plots at “Near Sequim”. Volume of *Plantago lanceolata* estimated by measuring the height x length x width of each plant in plots. Excludes paired plots without host plants in non-oviposition plots. Volume of *P. lanceolata* in the road habitat oviposition plots (mean = 2639 cm$^3$) was not significantly different (n=11, paired t-test, p=0.303) than in paired adjacent non-oviposition plots (mean = 2220 cm$^3$). Small sample sizes containing both larvae and host plants prevented paired analysis in Dune (n=1) and Ecotone (n=3) habitats.

Paired plot results from “Near Sequim” were dramatically different than results from the three other sites. Oviposition occurred in microsites surrounded by less hostplant volume and only one 1m$^2$ oviposition plot contained >10,000cm$^3$ *P. lanceolata*.

There was not a significant difference in hostplant volume between oviposition and non-oviposition paired plots (Figure 13)

At the site scale oviposition was associated with higher counts of *P lanceolata* in 5m x 5m search plots (Figure 14). Within the densest hostplant areas oviposition density was as high as 26 in one plot.

Because larval masses were both abundant and exposed relative to other sites where vegetation was more dense, larvae in the masses were counted. Larval masses (n = 48) along the road were sampled by carefully prodding and unfolding plants and webbing to count larvae. Assuming each mass webbing was a single mass the mean of 38.1 larvae (95% CI 30.1 - 45.5) is on the high end of reported *Euphydryas editha* average clutch size but the data distribution indicated the likelihood that some masses contained two or three clutches.

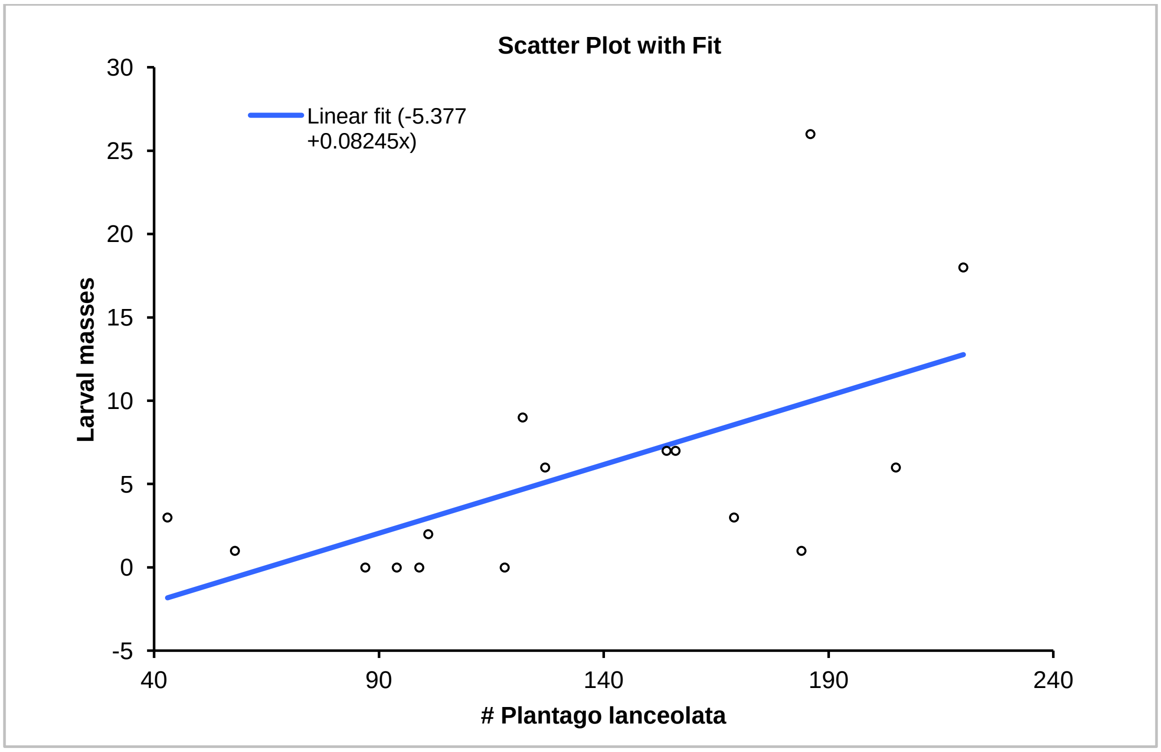

**Figure 14:** Linear regression of the count of larval masses versus number of *Plantago lanceolata* per 5m x 5m search plot within the road habitat at “Near Sequim” ($R^2 = 0.36$; adjusted $R^2 = 0.31$; slope $p = 0.0155$).

### 91st Division Prairie

The Pierce County site, 91st Division Prairie, in part the Artillery Impact Area for Joint Base Lewis-McChord, is a typical South Puget Sound prairie remnant with Spanaway-Nisqually Gravelly Sandy Loam soils and grassland vegetation. This site is used for high intensity training by the United States Army and contains perhaps the highest quality remaining prairie habitat west of the Cascade Range in either Washington or Oregon along with what is likely the largest population of Taylor's checkerspot in the world at Range 76. Portions of the site are concrete fighting positions, gravel vehicle pads, a mix of wide graded gravel roads, and two track roads. Sampling at this site was stratified to sample road edges and open prairie. Access to the site is limited because of military training exercises and, in some areas, the risk of unexploded ordinance. *Castilleja hispida*, *Collinsia grandiflora, C. parviflora*, *Plantago lanceolata*, *Plectritis congesta*, and *Triphysaria pusilla* occur on the site and prior to this study only *Plantago lanceolata* was known as an oviposition host at Range 76 while *Castilleja hispida* had been observed as oviposition hosts at Range 51 6km to the west on the opposite end of 91st Division Prairie.

Taylor's checkerspot may occur elsewhere in 91st Division Prairie but some areas are impossible to access because of high densities of unexploded ordinance. A checkerspot population that occurred at Range 50/51 on the opposite side of this 9,000 acre prairie appears to have been extirpated thus the Range 76 population is the center of the known Taylor's checkerspot distribution and perhaps the only currently occupied portion of 91st Division Prairie.

#### *91st Division Prairie sampling methods*

We sampled in Range 76 in the northeast corner of 91st Division Prairie on June 4 and 7-11, 2010. The ideal sampling period may have been 10-15 days later than the available access. Access to this area is often a challenge because it receives heavy training use by soldiers stationed at Joint Base Lewis-McChord though site availability was above average for 2010 because many units were deployed overseas. Training plans for the site are set months in advance which precludes the possibility of adapting access for research to the weather in a particular year. Checkerspots were still flying during the sampling period (albeit in low numbers) because the flight period was extended by historically cold wet weather and many of the eggs had yet to hatch.

Sampling at Range 76 was stratified by habitat. As at “Near Sequim” and Dan Kelley Ridge, oviposition had been observed in road edges at Range 76. Open prairie, that also contains of hostplants, surrounds the roads. These two habitats were sampled as blocks. There are other sub-types of habitat that could have been sampled with additional time on the range. Of particular interest would be disturbance areas of various types that often are colonized by *Plantago lanceolata.*

The two track road was sampled along 400m of its length by delineating it into 50m segments and randomly locating a search plot within each segment. The road was narrow enough to center search plots on the road and capture both edge and middle strip vegetation.

Given the limited time available we sampled n=32 search plots in open prairie by randomly selecting among the 700m long distance monitoring transects used for Taylor’s checkerspot. We stratified our random selection to choose one transect from each of three blocks in the sample grid. These transects form a regular grid across the known checkerspot occupied area but some of them were truncated by the presence of unexploded ordinance at the time of our sampling. Therefore we had n=14 50m long segments in transect #12, but only n=10 and n=6 in transects #7 and #3. We sampled one randomly located search plot along each segment alternating between placing the search plot north and south of the transect line.

In addition to randomized sampling, all *Castilleja hispida* within Range 76 were searched for both eggs and larvae. This hostplant is highly visible when flowering (Figure 15) and rare within Range 76 though it is common at Range 50/51. Most of the plants are the result of a small direct seeding experiment by the author in 2004.

Results from this site are burdened with the most caveats of any in this study. Because of access restrictions, the sample period was prior to the peak of observable larval masses and portions of the site were unavailable for sampling because of access restrictions caused by unexploded munitions. Together this resulted in fewer samples collected from a non-random subset of the available habitat. For this reason, results from this site must be seen as preliminary at best. Approximately 50% of the eggs on thoroughly searched host plants had not hatched as of the end of the survey period therefore many potential larval masses were not seen during the sample period.

**Figure 15:** *Castilleja hispida* used for oviposition on 91st Division Prairie. This is one of a very few *C. hispida* plants at the site. None occurred in search plots but all *C. hispida* plants at the site were searched and eggs or larvae were found on 9 of 24 plants.

### ***91$^{st}$ Division Results***

Sampling at 91$^{st}$ Division Prairie identified 64 plant species (Appendix E) and 10 categorical cover variables in 38 search plots, 30 in open prairie and 8 along a gravel road. The vast majority of oviposition occurred in road edge habitat (7 of 8 samples) not in the open prairie (considering only plots containing host plants: Road: 7 of n = 8; Prairie: 1 of n = 13, two proportions test p = 0.000). This was likely due in part to the association between *P. lanceolata* and roads at the site (mean # *P. lanceolata* in search plots containing that species: road = 242; open prairie = 63). The single open prairie oviposition microhabitat paired plot contained more host plant volume than any other oviposition site detected, indicating that checkerspots can find high density hostplant sites in open prairie as well. An MRPP test revealed that search plots containing oviposition were different than search plots not containing oviposition sites (A-statistic = 0.072, p = 0.0046) (Severns and Grosboll 2011) but this was a spurious finding based on lumping road samples with open prairie samples; when treated separately the association disappears. MRPP did not indicate a significant difference between paired microhabitat oviposition versus non-oviposition plots.

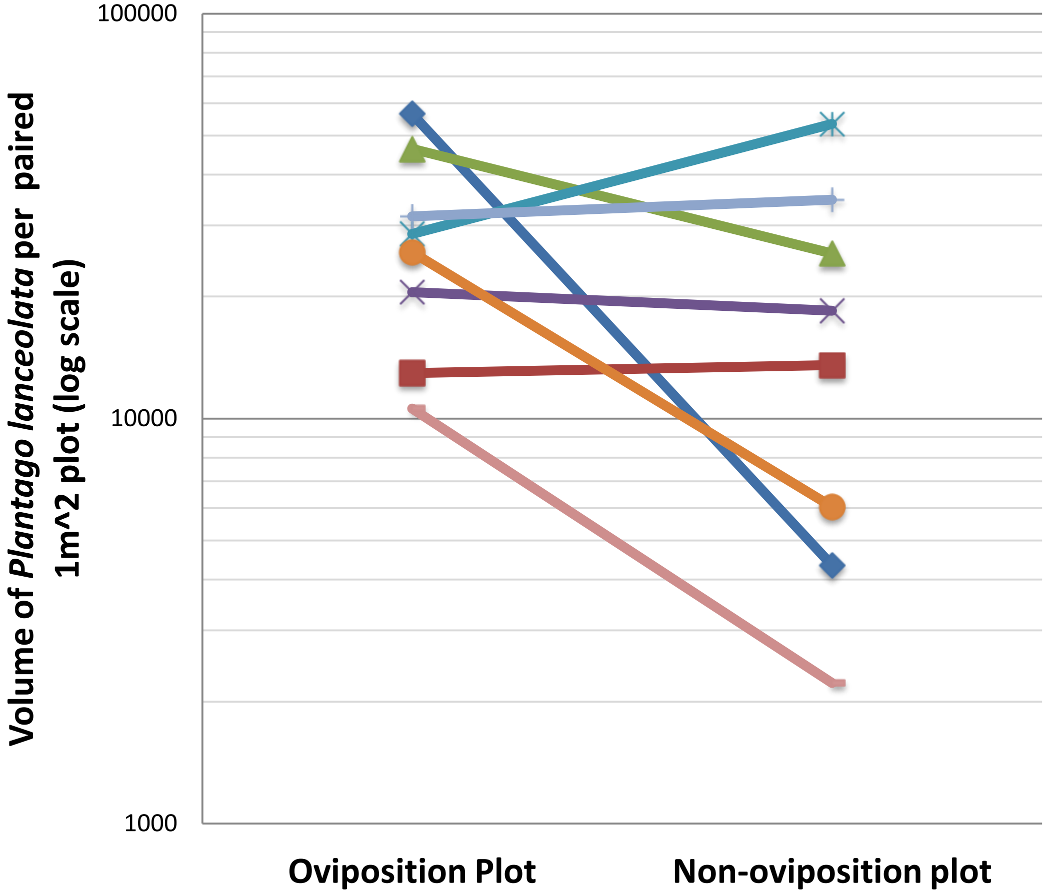

**Figure 16:** Paired plots at 91st Division Prairie. Volume of *Plantago lanceolata* estimated by measuring the height x length x width of each plant in plots. Excluding n=1 paired plot sampled outside the road block the differences between the volume of *P. lanceolata* in the oviposition plots (mean = 25178 cm^3) was not significantly different (n=7, paired t-test, p=0.303) than in paired adjacent non-oviposition plots (mean = 21984). Note that no oviposition sites samples contained less than 10000 $cm^3$ *P. lanceolata* and that when oviposition occurred in plots with less host plant volume, both plots had >10000 $cm^3$ *P. lanceolata*.

Among all road edge search plots that contained *Plantago lanceolata* (n = 8) at 91st Division Prairie, oviposition was very strongly and significantly (Spearman $rs = 0.7$, $p = 0.026$) correlated with the number of *P. lanceolata* plants but not significant at $p < 0.05$ within the smaller paired oviposition plots although the association was positive. The power to detect significant differences was low with n=7 but all oviposition sites contained >10,000$cm^3$ hostplant volume (Figure 16).

The complete census of *C. hispida* plants included a thorough search for eggs as well as a search for larvae. These plants have an unusual distribution because most are growing from seed planted in a line approximately 50m long though a few are scattered over several acres (Figure 15). Of the 24 plants searched, nine had eggs or larvae (37%). In contrast, of the 2757 *P. lanceolata* searched, only 15 had larval masses (0.54%). While this is not conclusive because the search methods used were different, and the distribution of *C. hispida* is unusual, it does strongly suggest the population of Taylor's checkerspot at this site may prefer *C. hispida* to *P. lanceolata*.

# V. Summary, synthesis, and recommendations

Taylor's checkerspots like the rest of *Euphydryas editha* are maddeningly complex to understand. Why is this butterfly Endangered – even though it can depend on a host present on nearly every lawn and roadside in the Northwest, does not require nectar for reproduction, and is part of a species complex that ranges widely across western North America? The findings of this thesis suggest that perhaps oviposition site selection (and thus prediapause larval habitat) is a key, though perhaps a key in a haystack.

Taylor's checkerspot populations at the four sites studied are intimately intertwined with their prediapause hostplants, *Castilleja hispida* and *Plantago lanceolata*. In captivity larvae appear to develop equally well on either host, and in the wild populations studied used both hostplants when they were available. Other factors play a role with respect to where checkerspots oviposit but those factors are overwhelmed by hostplant density. This indicates that the future of this butterfly is utterly dependent on having hostplants in high enough densities within otherwise suitable habitat.

### ***Hostplant density drives oviposition***

Eden Valley and Dan Kelley Ridge are similar habitats and located 1.5 miles apart. Paired plots at the two sites were analyzed together to determine whether the biomass density of *Castilleja hispida* selected for oviposition was similar across the two sites. The patterns and densities at the two sites were remarkably similar (Figure 8). Only one of 23 paired oviposition sites (*C. hispida* plant volume minimum 6624, maximum 118838; 95% CI +/-11538) occurred in a plot with less than 10,000 cm^3 hostplant volume. The distribution of paired non-oviposition plots overlapped substantially with oviposition plots overall (*C. hispida* volume minimum 48, maximum

54825; 95% CI +/-7233), but in only five of 23 paired plots was the volume of *C. hispida* greater in the non-oviposition plot, and in all samples where oviposition occurred in a plot with lower *C. hispida* volume than in the paired non-oviposition plot, both plots had greater than 10,000cm$^3$ volume of hostplant. Once hostplant volume reached 10,000cm$^3$ only half of eight pairs received oviposition in the higher density plot, which is consistent with results from 91st Division Prairie where two of five plots where each member of a pair had >10000cm$^3$ contained oviposition.

Perhaps 10,000 cm$^3$ is a threshold that indicates "enough" hostplant to an ovipositing female. Ovipositing females may not be able to perceive additional density beyond 10,000 cm$^3$ per m$^2$ or, as the preference-performance hypothesis (also known as the "mother knows best" principle) (Jaenike 1978; Gripenberg et al. 2010) would predict, resources beyond that level may be irrelevant for larval survival because they are in excess of those needed for optimal development so females will not seek higher densities. The one site that did not fit this pattern was "Near Sequim" where overall biomass was obviously lower and only one set of paired plots contained plots with greater than 10,000cm$^3$ *C. hispida* volume. With little or no nectar, an exceptionally early flight period contending with cold, wind, and limited sunlight, oviposition search flight capacity may be limited and eggs may of necessity be laid on whatever host plants are encountered. The number of potential oviposition sites containing greater than 10,000 cm$^3$ *C. hispida* volume on the site is likely very small even if butterflies searched extensively.

Taylor's checkerspot and road interactions are strong at "Near Sequim" and 91st Division Prairie. Even at Eden Valley and Dan Kelley, where roads may be less important overall, oviposition on *P. lanceolata* was associated with roads. What this means for the butterfly is more difficult to ascertain. Taylor's checkerspot requires a match between oviposition preferences and larval survival on selected hosts for populations to persist from egg to diapause. Measurements in the field demonstrated that while oviposition may be driven by multiple factors, hostplant density is by far the strongest. Captive rearing proved that larvae can survive from hatching through diapause on both *C. hispida* and *P. lanceolata*. While there is much more involved in recovery of Taylor's checkerspot than this, clarification of these aspects of the butterfly's biology is essential for effective habitat restoration and enhancement.

One overriding factor appears to drive Taylor's checkerspot oviposition at the study sites: density of host plants for oviposition. Because the sample areas were all within extant populations of Taylor's checkerspot, this finding must be interpreted with caution. There may very well be significant drivers of oviposition that were not captured in this study because they do not occur within the currently occupied population areas. Additionally, enough was known about the habitat requirements of this butterfly that areas that were known to be unsuitable were excluded from sampling. While each site is unique and results across sites should be used with caution, some aspects of this study lend themselves to cross site analysis. The nearly consistent lack of statistically or biologically meaningful associations between oviposition and most measured habitat variables was surprising. In part that was due to using study locations that were obviously, due to the presence of Taylor's checkerspot populations, broadly suitable habitat. At all sites additional statistically significant associations might be found with higher sampling intensity but these associations are likely to be somewhat biologically weak. An association between host plant density and oviposition is hardly surprising, but the strength of that association at both the broad site scale and at paired oviposition microsite sampling was unexpected.

One of the most striking findings of this study was that across three sites with a similar biomass of herbaceous vegetation, Eden Valley, Dan Kelley Ridge, and 91st Division Prairie, only one of 31 oviposition events sampled occurred in a plot with less than 10,000 $cm^3$ of host plant volume, but 18 paired non-oviposition plots contained less than 10,000 $cm^3$. This is particularly interesting from a restoration prescription perspective because it may indicate a preferred “threshold level” for oviposition.

### ***Slope, aspect, vegetation, and canopy shading***

In other populations of *Euphydryas editha* slope and aspect appear to be significant drivers of populations but that may not be true for *E. e. taylori*. The most intensely studied populations of *E. editha* occur in central and southern California where, regardless of slope and aspect, the habitat around checkerspot colonies is grassland (Weiss 1997; Weiss et al. 1993; Weiss, Murphy, and White 1988). At the two sites with substantial topographic relief (Dan Kelley Ridge and Eden Valley) all open habitat is flat or south facing. Because the climate in Western Washington even in the rain shadow of the Olympic Mountains, where the Clallam County sites are located, is wetter and cooler than the California sites, slopes that are not south facing invariably become forested and thus do not provide habitat for Taylor’s checkerspot.

Canopy shading would likely be a controlling factor for oviposition in some situations, but at the two sites where there were large numbers of trees and shrubs the results were not statistically significant. The data were suggestive of a positive correlation between sun exposure and oviposition which would fit with a pattern of increased activity when adults are in the sun during the typically marginal flight weather in the Pacific Northwest spring. Because weather varies from year to year, the impact of shade, slope, and aspect could be highly variable among years.

### *Climate change impacts oviposition and larval survival*

Global climate change may have complex effects on *E. e. taylori* populations. Parmesan (Parmesan 1996) indicated that global warming was causing a net northward shift in the distribution of *E. editha*. This seems manifestly untrue for *E. e. taylori*, which has nearly vanished from the northern end of its range, though evidence from other butterfly species supports this general pattern (Warren et al. 2001). There is evidence that increases in climate variability can negatively affect *E. editha* (McLaughlin et al. 2002) and that this is particularly true in homogenous habitats (McLaughlin et al. 2002). Local populations of a species may be less fit throughout its range in some cases rather than showing a positive response to warming at the northern limits and a negative response at the southern limits (Hellmann 2004). Climate change may make extirpations more likely (McLaughlin et al. 2002) and extirpations are even more likely when the population depends on only one host (Hellmann 2002).

Data on regional climate change is coarse relative to the scale of available Taylor's checkerspot habitat. Even fine scale regional climate change models are generally in the 15km grid range (Salathe Jr. et al. 2008). All currently occupied Taylor's checkerspot habitat in British Columbia, Washington, and Oregon adds up to less than the 225km$^2$ in one grid and in Washington alone occurs in three rare specialized habitat "types" (stabilized dune, grassy bald, and Puget Sound grassland) so evaluating the effects of climate change is somewhat subjective and qualitative. Nonetheless some aspects of climate change can be considered for its impacts on oviposition sites. All sites reported in this thesis will be impacted by changes to temperature and precipitation averages and patterns and one site may be inundated by sea level rise.

Changes in mean precipitation and temperature would impact Taylor's checkerspot oviposition and larval development. The CCSM3-WRF and ECHAM5-WRF models indicate relatively little change in mean precipitation between 1970-1999 measurements and 2030-2059 projections with the exception of an agreed increase of a 1-2cm/month decrease in spring precipitation under CCSM3-WRF and an increase in spring precipitation under ECHAM5-WRF (Salathe Jr. et al. 2008). If there is a decrease in spring precipitation as modeled in CCSM3-WRF, and as both models predict, and average temperatures increase throughout the year, then hostplant senescence could arrive earlier in the butterfly life cycle which would decrease survival between egg and diapause. Even though Taylor's checkerspot would likely emerge from diapause, develop, and pupate earlier in response to increased winter temperatures this may or may not be early enough to oviposit with enough time for larvae to develop (Hellmann 2002).

Globally, climate changes are projected to increase variability of both temperature and precipitation; however, within the range of Taylor's checkerspot, models show limited change in variability of either. Models agree on increasing variability in both temperature and precipitation in much of the Pacific Northwest but Taylor's checkerspots are located in the Puget Trough and in the rain shadow of the Olympic Mountains where little change is predicted (Salathe Jr. et al. 2008). This is encouraging for conservation because increases in variability can drive populations to extinction (McLaughlin et al. 2002).

The most recent Assessment Report of the Intergovernmental Panel on Climate Change (IPCC) projected global sea level rise in this century will range between 18cm and 59cm depending on the emissions scenario used, and sea level in Washington is expected to closely follow this trend (Mote et al. 2008). "Apparent sea level change" is the difference between change in land level and change in sea level. Deformation of the earth's crust is causing the area in which "Near Sequim" is located to rise approximately 1.5mm/year (Verdonck 2006) but it is possible that local factors (e.g., subsidence due to sediment compaction or dune movement) could change that on the site scale. At the lowest projected rate of sea level rise under the lowest emissions scenario, local crustal deformation (15cm/100years) nearly equals the rate of sea level rise (18cm/100 years) and the apparent rise would be relatively trivial. Even at the lowest level (26cm) of the high emissions scenario projections, additional erosion may impact this narrow strip of habitat while at the higher end of high emission projections the “Near Sequim” site will likely be periodically overtopped by storm waves with catastrophic consequences for the Taylor’s checkerspots on the site.

### ***Future directions for research and lessons learned***

This study attempted to measure habitat where eggs were laid to understand what parameters were critical for egg laying by females and thus where prediapause larvae would develop. It found that hostplant density was a strong predictor of egg laying but that many other aspects of the habitat were highly variable, at least within occupied checkerspot habitat. A substantial hurdle was finding enough oviposition sites for statistical analysis while using statistically defensible sampling methods. Measuring habitat only within areas containing hostplants would increase the number of observations without losing significant information.

Following females through habitats to observe where individuals lay eggs (along with other spatially explicit behavioral observations) is effective for species that oviposit frequently (Beyera and Schultz 2010) but less effective when following a butterfly like Taylor’s checkerspot that may only lay one clutch of eggs per day and is quite cryptic when laying eggs. Given the impossibility of following butterflies across the steep landscapes where some Taylor’s checkerspots are found, other methods must be used. Sequential hostplant testing is effective for determining preferences that individuals have for particular plants (Singer 2004) but less effective at determining where, across the occupied area, oviposition occurs. Sampling efficiency is critical for detecting enough oviposition sites for analysis.

Captive rearing methods are being applied concurrently with habitat restoration, enhancement, and invasive plant management, which will ensure that there are habitats available for reintroduction. The outlook for restoration is thus promising because, based on this research, it appears that substantial habitat can be restored simply by increasing hostplant density within occupied (or potentially occupied) habitats. Understanding the oviposition preferences, larval survival and performance on different hostplants, and development of methods for enhancing hostplant resources, are essential elements in the recovery of the endangered Taylor's checkerspot butterfly. Further the context of climate change is critical for understanding future habitat availability. Without this knowledge, and application of this knowledge in restoration, the butterfly is likely to go extinct in the near future.

Humans have likely created habitats for this butterfly for thousands of years and that story—both the science and the culture—is an integral part of who we were and who we are. Perhaps surprisingly, that story of human habitat modification is also part of the genome of Taylor's checkerspot: its use of European introduced hostplants and its behavioral affinity for grasslands and balds maintained by Native use of fire. The author hopes the work performed in this thesis will not only increase knowledge about Taylor's checkerspot but that it will also lead directly to better management decisions in its habitat.

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## **Appendix A:** Hemispherical photography.

**Figure 17:** Hemispherical photo of typical steep south facing slope at Eden Valley.  Exposure method designed to be used with cloud cover or pre-sunrise sky makes the sky look nearly white for high levels of contrast with surrounding obstructions.

**Figure 18:** Manually thresholded image of Figure 17. When thresholded this becomes a black and white record of sky obstruction. When the image from this search plot was processed through Gap Light Analysis 60% of the canopy is open but that underestimates the amount of sunlight the plot gets because of its slope, aspect and where trees block the sun. Total direct sunlight reaches this plot is 90.4% of the time. North is at the top of photo.

**Appendix B:** List of plants identified at Eden Valley.

*Acer macrophyllum*
*Achillea millefolium*
*Agrostis sp.*
*Aira caryophyllea*
*Aira praecox*
*Amelanchier alnifolia*
*Apocynum sp.*
*Arabis sp.*
*Arrhenatherum elatius*
*Balsamorhiza deltoidea*
*Berberis aquifolium*
*Berberis nervosa*
*Brodiaea congesta*
*Bromus carinatus*
*Bromus mollis*
*Castilleja hispida*
*Carex inops*
*Cerastium arvense*
*Cerastium glomeratum*
*Clarkia amoena*
*Claytonia perfoliata*
*Corallorhiza maculata*
*Cytisus scoparius*
*Dactylis glomerata*
*Danthonia californica*
*Daucus carota*
*Delphinium menziesii*
*Elymus glaucus*
*Epilobium sp.*
*Eriophyllum lanatum*
*Festuca occidentalis?*
*Festuca roemeri*
*Festuca rubra*
*Festuca subuliflora*
*Fragaria virginiana*
*Fritillaria affinis*
*Galium aparine*
*Habenaria sp.*
*Heuchera spp*
*Holcus lanatum*
*Holodiscus discolor*
*Hypericum perforatum*
*Hypochaeris radicata*
*Koehleria macrantha*
*Lathyrus sp.*
*Lomatium utriculatum*
*Lonicera ciliosa*
*Lotus micranthus*
*Luzula campestris*
*Madia sp.*
*Microseris laciniata*
*Microsteris gracilis*
*Moehringia macrophylla*
*Montia parviflora*
*Myosotis discolor*
*Nemophila parviflora*
*Osmorhiza purpurea*
*Pentagramma triangularis*
*Plectritis congesta*
*Poa bulbosa*
*Poa spp.*
*Polystichum imbricans*
*Polystichum munitum*
*Prunella vulgaris*
*Prunus avium*
*Pseudotsuga menziesii*
*Ranunculus uncinatus*
*Rosa nutkana*
*Rubus ursinus*
*Rumex acetosella*
*Satureja douglasii*
*Sedum spathulifolium*
*Stellaria media*
*Symphoricarpos albus*
*Trifolium spp.*
*Trifolium repens*
*Trifolium wormskjoldii*
*Veronica spp.*
*Vicia hirsuta*
*Vicia sativa*
*Vulpia spp*
*Whipplea modesta*
*Zigadenus venenosus*

# **Appendix C:** List of plants identified at Dan Kelley Ridge.

*Acer macrophyllum*
*Aira caryophyllea*
*Aira praecox*
*Anaphalis magaritacea*
*Arbutus menziesii*
*Arrhenatherum elatius*
*Berberis aquifolium*
*Berberis nervosa*
*Bromus carinatus*
*Bromus mollis*
*Bromus sp.*
*Castilleja hispida*
*Carex inops*
*Cerastium arvense*
*Cerastium glomeratum*
*Cirsium edule*
*Clarkia amoena*
*Daucus carota*
*Elymus glaucus*
*Epilobium sp.*
*Erodium cicutarium*
*Festuca rubra*
*Fragaria virginiana*
*Fritillaria affinis*
*Galium aparine*
*Gaultheria shallon*
*Geranium spp.*
*Heuchera spp*
*Hieracium cynoglossoides*
*Hieracium sp*
*Holodiscus discolor*
*Hypochaeris radicata*
*Lathyrus sp.*
*Leucanthemum vulgare*
*Luzula campestris*
*Microseris laciniata*
*Myosotis discolor*
*Osmorhiza purpurea*
*Penstemon sp.*
*Philadelphus lewisii*
*Plectritis congesta*
*Polystichum munitum*
*Prunella vulgaris*
*Prunus emarginata*
*Pseudotsuga menziesii*
*Ranunculus uncinatus*
*Ribes sanguineum*
*Rosa nutkana*
*Rubus discolor*
*Rubus ursinus*
*Satureja douglasii*
*Symphoricarpos albus*
*Trifolium spp.*
*Vicia hirsuta*
*Vulpia spp*
*Whipplea modesta*
*Zigadenus venenosus*

## **Appendix D:** List of vascular plants identified at “Near Sequim”.

*Abronia latifolia*
*Achillea millefolium*
*Agrostis spp*
*Aira caryophyllea*
*Aira praecox*
*Ambrosia chamissonis*
*Artemesia campestris*
*Aster spp.*
*Beach grass*
*Bromus mollis*
*Bromus rigidus*
*Bromus tectorum*
*Cerastium glomeratum*
*Cerastium nutans*
*Cirsium arvense*
*Cirsium vulgare*
*Collinsia parviflora*
*Crataegus sp.*
*Dactylis glomerata*
*Epilobium sp*
*Erodium cicutarium*
*Festuca rubra*
*Festuca/Lolium pratensis*
*Galium aparine*
*Grindelia*
*Holcus lanatum*
*Hordeum spp.*
*Hypochaeris radicata*
*Juncus spp.*
*Lathyrus nevadensis*
*Lotus spp.*
*Matricaria chamomilla*
*Orthocarpus pusillis*
*Plantago lanceolata*
*Poa bulbosa*
*Poa spp.*
*Polygonum paronychia*
*Potentilla anserina pacifica*
*Rosa nutkana*
*Rumex acetosella*
*Sagina maxima*
*Sonchus arvensis*
*Taraxacum officinale*
*Trifolium dubium*
*Trifolium oliganthum*
*Vicia hirsuta*
*Vicia sativa*
*Vulpia spp*

## **Appendix E:** List of vascular plants identified at 91st Division Prairie.

*Achillea millefolium*
*Agrostis diegoensis*
*Aira caryophyllea*
*Aira praecox*
*Anthoxanthum aristatum*
*Anthoxanthum odoratum*
*Apocynum sp.*
*Arrhenatherum elatius*
*Balsamorhiza deltoidea*
*Bromus carinatus*
*Bromus mollis*
*Camassia quamash*
*Campanula rotundifolia*
*Carex inops*
*Cerastium spp*
*Cytisus scoparius*
*Danthonia spicata*
*Agropyron canina*
*Erigeron speciosus*
*Eriophyllum lanatum*
*Erodium cicutarum*
*Festuca roemeri*
*Filago arvensis*
*Fragaria virginiana*
*Fritillaria camschatcensis*
*Galium aparine*
*Holcus lanatum*
*Hieracium cynoglossoides*
*Hypochaeris radicata*
*Hypericum perforatum*
*Koehleria macrantha*
*Leucanthemum vulgare*
*Lomatium triternatum*
*Lomatium utriculatum*
*Lotus micranthus*
*Lupinus albicaulis*
*Lupinus bicolor*
*Lupinus lepidus*
*Luzula campestris*
*Microseris laciniata*
*Microsteris gracilis*
*Orthocarpus pusillis*
*Panicum spp.*
*Parentucellia viscosa*
*Plantago lanceolata*
*Poa spp.*
*Potentilla gracilis*
*Potentilla recta*
*Pteridium aquilinum*
*Ranunuculus occidentalis*
*Rubus sp.*
*Rumex acetosella*
*Saxifraga integrifolia*
*Solidago spp.*
*Spergularia spp.*
*Spiranthes romanzoffiana*
*Teesdalia nudicaulis*
*Tragopogon dubius*
*Trifolium arvense*
*Trifolium dubium*
*Vicia hirsuta*
*Vicia sativa*
*Vulpia spp*
*Zigadenus venenosus*