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Development of Captive Rearing and Translocation Methods for Taylor's Checkerspot in south Puget Sound, Washington: 2006-07 Annual Report

by Mary J. Linders

Washington Department of
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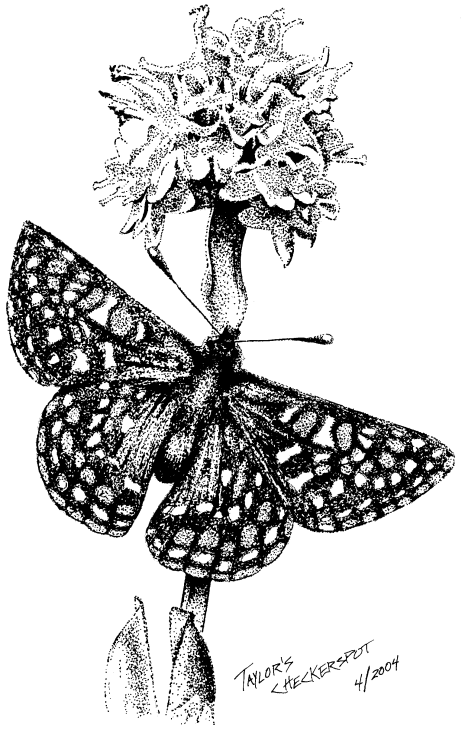
2006-2007 Annual Report



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Wildlife Program – Region 6
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EXECUTIVE SUMMARY

Taylor's checkerspot, a federal candidate and state-endangered species, is known from only two areas in south Puget Sound. The primary goal of this project is to reduce the probability of extinction of *Euphydryas editha taylori* in south Puget Sound. To reduce the likelihood of near-term extinction we are developing captive rearing and translocation techniques, and using experimental releases to learn about potential limiting factors.

Captive rearing

The 2006-2007 rearing season was a banner year with regard to the health and survival of Taylor's checkerspots at the Oregon Zoo. Of 930 larvae hatched, 726 or 78.1 percent were released into the wild (527 prediapause and 199 postdiapause larvae). In addition, 21 larvae pupated in the lab with 17 eclosing as adults and up to 11 or possibly more adults eclosed in one of the field trials. Increased survival during the prediapause phase in 2006 was attributed to good moisture balance in the containers housing larvae and elimination of molt-related complications. Husbandry of pupa and adults was also promising. Mean number of days from diapause to pupation was 38.8 $\pm$ 7.8 (SD) (range 32 – 64) with the first pupae forming on 25 March and the last on 15 May. Pupae eclosed after an average of 20.1 $\pm$ 3.7 (SD) days (range 11 – 27); eclosion dates ranged from 15 April to 26 May. Adult survival ranged from 7 to 36 days, averaging 24.6 $\pm$ 9.3 (SD) days. The first adult butterfly to die did so on 28 April; the last died 2 June 2007. To apply these methods to recovery throughout the state, the program will need to move from a captive rearing program to a captive breeding program.

We also examined the effect of host plant (*Castilleja* and *Plantago*) and container type (yogurt vs. deli) on growth and survival of larvae during diapause. Larvae reared on *Castilleja* weighed significantly less than those reared on *Plantago* at initiation of diapause, but there was no statistical difference in survival during diapause between larvae raised on *Castilleja* and those on *Plantago*. We controlled for observed differences in prediapause larval weights between host plants and found that larvae housed in yogurt containers lost slightly more weight than those in deli containers but the difference was not significant. Diapause survival rates were high for both host plant groups and did not differ between container types. Differences in larval behavior were observed between container types; larvae in deli containers moved more than those in yogurt cups and were less likely to form webbing. Webbing may reduce risk of predation and provide protection from inclement weather during diapause.

Experiment #1: Translocation of eggs via wild females

Eight adult female Taylor's checkerspots were captured at the source site in early May 2006 and placed in net pens on the release site containing either potted or wild host plants; potted plants with eggs were moved to the Oregon Zoo for rearing. We established that females 1) recognize both *Castilleja* and *Plantago* as host species, although only *Plantago* is available at the source site; 2) will lay eggs in a controlled setting; 3) can live several to many days after they stopped producing eggs; 4) have an average life span was 12.9 days (range = 5-19 days; n = 8); 5) produced a minimum average of 142 eggs per female. Survival from egg to diapause in the wild was very low (0.8 percent; 5 larvae from 639 eggs) presumably the result of inclement weather; survival in the lab it was very high (91.4 percent; 412 larvae from 451 eggs), indicating that rearing to warm diapause in the lab is a beneficial strategy.

Experiment #2: Release of wild and captive late prediapause larvae
On 27 June 2006, a total of 608 prediapause larvae were distributed between four 50-m^2 plots on each two Ft. Lewis release sites [Site FL1 (301) and Site FL2 (307)] with similar habitat characteristics. Eighty-one were collected as prediapause larvae from the source site and 527 came from the captive population. Both *Castilleja* and *Plantago* were available on all plots. Plots were monitored during the postdiapause phase and 6 larvae were observed among 3 of the 8 release plots (5 on Site FL1; 1 on Site FL2). The poor release results are thought to be due to extremely dry conditions at the time of release coupled with the strategy of releasing larvae individually rather than in groups.

Management Action #2: Release captive-reared post-diapause larvae
The release of 199 late postdiapause larvae resulted in 15 and 22 larvae observed on Plots A (release 6 Mar 2006) and B (release 16 Mar 2006), respectively, during post-release surveys on 18 March at the State release site. Surveys on 27 March 2007 revealed 5 and 4 larvae on Plots A and B, respectively. Eleven observations of adult checkerspots (6 at Plot A and 5 at Plot B) occurred during the flight season. Nectaring was observed one time each on *Balsamorhiza deltoidea*, *Lomatium triternatum* and possibly on *Ranunculus occidentalus*. This project affirmed our ability to foster Taylor's checkerspot from egg to adult, as we did so in both lab and field. Survival rates relative to the number of larvae released will require further exploration. Rapid dispersal of adults at the release site raises additional questions that will need to be addressed in future release trials.

BACKGROUND

Taylor's checkerspot, a federal candidate and state-endangered species, is known from only two areas in south Puget Sound: the Artillery Impact Area on Fort Lewis and the Bald Hills in Thurston County, a complex of small mid-elevation grassy balds on state and private land. Several Taylor's checkerspot populations have been lost in the past decade due to such factors as habitat degradation, fire, climatic fluctuations and demographic stochasticity. Restoration is proceeding on many of these sites but recolonization may be prohibited by 1) a limited number of source populations, 2) large distances between source populations and potential reintroduction sites, and 3) small source populations. Few wild populations of Taylor's checkerspot in Washington have more than 100 individuals. Captive rearing to reduce larval mortality is thought to be the best way to provide animals for reintroduction while minimizing impacts to source populations (Grosboll 2004). A captive rearing program for Taylor's checkerspot was initiated in 2003 and was transferred to the Oregon Zoo in 2004. This research follows the plan written by the Washington Department of Fish and Wildlife to develop methods for captive rearing, translocation, and release of Taylor's checkerspot with the ultimate goal of increasing the number and size of checkerspot populations in south Puget Sound (Linders 2006).

The goal of this project is to reduce the probability of extinction of *Euphydryas editha taylori* in south Puget Sound. Secondary objectives are to 1) develop translocation and captive rearing techniques that can be used to reduce the probability of extinction, 2) use experimental releases to learn about important life history traits such as rates and sources of mortality, habitat use and suitability, and other potential limiting factors which are difficult to observe at source locations due to site sensitivity and access, and 3) provide recommendations for a translocation plan that will identify how and where to increase the number and/or size of Taylor's checkerspot populations in south Puget Sound. The information gained will contribute significantly to our knowledge of factors limiting checkerspots in south Puget Sound and the potential role of captive rearing in population reestablishment. This report covers all aspects of the project, which has received funding from several sources. The project is still in progress and methods development, translocation monitoring, and translocations are likely to continue for several more years until captive rearing and release methodologies and population establishment have been confirmed. At that point these methods will be incorporated into a translocation plan that will identify how and where population reestablishment will occur throughout the species' Washington range.

The goal of the captive rearing program is to use human intervention to increase the total number of Taylor's checkerspots available for translocation. Objectives are to 1) determine at which life stage(s) survival in captivity exceeds that in the wild, 2) test different rearing methods (e.g., container type, location, host plant, etc.) to reduce mortality rates in captivity, and 3) determine which method maximizes the number of animals available for translocation while minimizing risk to source populations. Objectives for the 2006-2007 rearing season included 1) comparing survival of egg and prediapause larvae in captivity with those in the wild, 2) comparing growth and survival of larvae fed *Penstemon gloxinoides* against those fed *Plantago lanceolata* and *Castilleja hispida*, and 3) building on results of a 2005 experiment in which larvae diapaused in outdoor chambers. Additional objectives for the 2007-2008 rearing season are 1) extend captive care to pupal and adult stages, and 2) test hand-mating techniques for adults. The captive rearing protocol for 2007-2008 is in Appendix A.

In addition to the general project objectives, specific objectives for each translocation experiment or management action were as follows. The objectives of Experiment #1 were to 1) determine whether females in enclosures would lay eggs at the release site, and 2) compare hatch rate and survival of eggs and larvae through the 3rd instar between captive and wild populations. Objectives of Experiment #2 were to determine whether wild and captive-reared larvae differ in 1) overwinter survival, and 2) survival to adulthood. Captive-reared late prediapause larvae from Experiment #1 and a sample of similar-sized wild larvae from the Ft. Lewis source population were translocated to new sites. Management Action #2 sought to determine how well captive-reared postdiapause larvae, which we have protected from inclement weather and presumably parasites, will survive to adulthood following release into the wild. This is a partial repeat of a similar release conducted in 2006 but with a larger sample.

METHODS

Captive rearing

Eggs: Potted host plants (*Castilleja* and *Plantago*) with eggs were moved to the Oregon Zoo 16 May 2006 and eggs were allowed to hatch and larvae develop *in situ* for 3-7 days, at which point they were moved to small yogurt containers and reared as larval groups (Pratt 2005). It was believed that this method would improve captive survival in the early stages because it eliminates several sources of concern associated with Petri-dish rearing: 1) desiccation of the oviposition leaf, 2) break up of larval webs (some larvae are more active spinners), 3) injury of 1st instar larvae due to handling, and 4) inundation by frass. *Plantago* leaves containing egg clusters were also brought to the lab from the source site in Washington due to a lower than expected number of eggs produced on potted host plants (*see Experiment #1*). Egg clusters and larvae were moved into 8 oz. Dannon yogurt containers lined with paper toweling (full sheet folded in quarters) on the bottom (Fig. 1). Clear lids permitted light and a dimpled lip allowed air movement, which reduced mold. Moisture balance was adjusted by addition of host plants, eliminating the need to mist them with water. Egg clusters were left on the leaves until hatching.

Larvae readily feed on a variety of host plants that contain compounds known as iridoid glycosides, including the nonnative host *Plantago lanceolata*. Larval groups were randomly assigned to either a prediapause (P1) or postdiapause (P2) release group. The P1 group was fed leaves of *Plantago* or *Castilleja* consistent with their original host. Those in the P2 group were to be assigned to 1 of 3 feeding treatments: *Plantago*, *Castilleja*, or *Penstemon*. However *Penstemon gloxinoides* was not available for sale in Northwest nurseries at the point it was needed and this plant was abandoned as part of the feeding trials. Oviposition preference is genetically coded and altering larval diet has not been found to affect oviposition preference in adults (Singer 2004).

Figure 1. Prediapause larvae of Taylor's checkerspot in captivity at the Oregon Zoo, Portland, Oregon.

Prediapause larvae: Eggs and larvae were checked daily

and the following data collected every other day: total number of eggs, date of hatching, total # of larvae, # of larvae by instar, and other data as appropriate. Containers were cleaned of frass every 3 to 4 days. Larvae were allowed to molt to the 3rd or 4th instar (just prior to diapause), then weighed using an electronic balance (Appendix B). Larvae from the Captive pens were weighed on 29 June and 3, 5 July and Source site larvae on 4 July 2006 (Andersen et al. 2007). Larvae in the P1 treatment were released into the field on 27 June 2006 (see Experiment #2) about 2 weeks prior to estimated initiation of diapause based on the relative phenologies of field and lab reared larvae. Larvae in the P2 treatment were split according to the diapause treatments outlined below.

Diapause: Larval groups in the P2 treatment were split about equally, with half of each feeding group going to each diapause treatment (deli vs. yogurt container). Deli containers were lined with sphagnum moss; yogurt containers with a paper towel. Occupied yogurt containers were cleaned as thoroughly as possible without disturbing larval diapause shelters. After all larvae entered diapause, yogurt and deli containers were put inside inverted clay pots; pots were placed outside beneath a building overhang on 16 August 2006. Larvae were checked weekly for mortality and moisture adjusted as needed, generally by wetting the clay saucers; occasionally, the sphagnum moss was also wetted, but no water was ever put inside of yogurt containers.

Postdiapause: Larvae were removed from diapause chambers following increased activity levels and corresponding emergence of wild progeny. Larvae feeding on *Plantago* were removed on 21 February 2007, whereas those feeding on *Castilleja* were removed on 12 March 2007 because host plants were not ready. We abandoned the remainder of the feeding trial at this point and moved forward with other objectives of the project; *Plantago* was used as a host instead. Larvae were weighed again at the end of the diapause (Appendix B); *Plantago*-eaters on 21 February 2007 and *Castilleja*-eaters on 12 March 2007 (Andersen et al. 2007).

Once larvae began feeding they were moved into clear plastic Sterilite shoebox bins (12" long x 7.5" wide x 4.5" deep) lined with blue paper shop towels. Bins were covered with netting (tulle) and secured with a rubber band to maximize direct light and airflow. The temperature of the lab was maintained at 65 ºF or lower, with a relative humidity of about 55%. Lids were placed askew over the bins (90% of bin covered) overnight and removed the next morning to prevent dehydration. Fresh food was placed on one end and a mounded paper towel ("mima mound") on the other end to act as a basking structure. A moisture gradient was maintained across the paper towel. Bins were placed on wire racks with full spectrum fluorescent bulbs on a 10-hour light/14-hour dark cycle maintained by timers. One or two 75-watt tungsten lamps provided 4 hours/day of extra light and heat. Room lights were on during the day to increase light intensity.

Data analysis: Statistical tests were calculated using SPSS 12.0 (Andersen et al. 2007). Tests were two-tailed and were significant at alpha $\leq$ 0.05. Student's t-test was used to compare the effect of host plant (*Castilleja* or *Plantago*) on prediapause weight and to compare container effect (deli or yogurt) on weight loss during diapause. A three-way analysis of variance test was used to assess the effect of larval source, host plant and diapause container on diapause survival. Diapause survival was based on mean cohort survival from pre- to postdiapause. Statistical tests used mean larval weight by cohort; a summary of the data included in these analyses can be found in Appendix B.

Experiment #1: Translocation of eggs via wild females
Following estimate of abundance surveys at the source site slightly worn wild females were captured using soft nets and placed in containers inside a cooler to prevent overheating during transport to the State release site. Take was limited to no more than one percent of the minimum number of individuals counted. Females were randomly assigned to one of two treatments: eggs reared in the wild or eggs reared in captivity, and placed inside the appropriate net pen (1 m long x 0.5 m wide x 0.5 m tall) on the day of capture. Four net pens were staked where wild *Plantago* was abundant; four net pens contained potted *Plantago* and *Castilleja*. Supplemental nectar plants were provided in both cases (Fig. 2). The edges of the nets were staked to prevent escape. Females were moved between treatments every two days until all females had died in an effort to maximize genetic diversity within treatments. Wild net pens were placed wherever host plants were the densest and were moved every 2 days to increase the dispersion of eggs across the site. This also permitted a partial count of the number of eggs produced by each female. Net pens with potted plants housed the same two females sequentially. Potted plants containing egg clusters were moved to the Oregon Zoo for rearing.

After removing the nets, wild pens were searched for egg clusters, which were marked with low profile flags; each plot was searched until no new eggs or larvae were found. Each cluster of eggs or larvae was examined every other day until they reached the 3rd instar (Moore 1989) or were gone. Moore (1989) scored larvae as dead from starvation if they ran out of food before reaching the 3rd instar and no other food was available within a few centimeters. In contrast to Moore's observations *taylori* larvae in south Puget Sound appear to diapause in the 4th instar or later (M. Linders, M. Andersen, pers. obs.), so we scored larvae as dead from starvation if they ran out of food before reaching late 3rd or early 4th instar and no other food was available within 30 centimeters. Changes from Moore's method are based on 1) the larger size of *taylori* larvae prior to diapause, 2) observed movements of prediapause *taylori* larvae in the field, and 3) the resilience of perennial as opposed to annual host plants. Data collected were # of eggs/cluster, color of eggs, host plant, date of hatching, total # of larvae, # of larvae by instar, presence of other eggs on or near host plant, presence of insects on or near host plant, condition of host plant, amount of larval foraging sign, and size of larval web.

Figure 2. Wild net pen staked out at a release site for female Taylor's checkerspots in south Puget Sound, Washington in 2006.

Release areas were also monitored for 30-minute periods several times during the 2007 flight season (20 April-18 May), when suitable weather conditions (e.g., ≥10°C, wind <10 mph, bright) permitted. No adult Taylor's checkerspots were observed associated with these release plots.

Experiment #2: Release of wild and captive late prediapause larvae
A total of 608 prediapause larvae were released on 27 June 2006. Eighty-one were collected that morning from the source site; 527 came from the Oregon Zoo. Most larvae came from the captive-reared population because survival of prediapause larvae was higher than expected in the lab, and the mortality rate of eggs and larvae at State site, seemingly related to inclement weather, underscored the need for caution in estimating impacts to source populations. Larvae were split between four 50-m^2 plots on each of two release sites on Ft. Lewis [Site FL1 (301) and Site FL2 (307)] with similar habitat characteristics. Release plots were separated by at least 50 m to maintain independence. Larvae appear unlikely to move this distance and can therefore be distinguished by site and tracked through adult emergence (Moore 1989). About 75 larvae were released per plot; they were placed individually in about equal numbers on one of two host plants, *Castilleja* or *Plantago*. Both host species were available on all plots. Upon release, larvae began to move about, forage and bask.

To control for variation in habitat quality between sites, 50-m^2 plots were selected based on the presence of *Castilleja* and ocular estimates of high-density areas. *Plantago* is a secondary host and did not drive plot selection. Eleven 1-m^2 releve plots were systematically located in each plot (every 5th meter on back and forth transects through the plot) to obtain constancy and percent cover of *Plantago* and constancy and stem count of *Castilleja*. Percent cover was recorded to the nearest one percent; stems of *Castilleja* were counted if ≥ 2.5 cm (Table 1).

Table 1. Constancy and percent cover of *Plantago* and constancy and stem count of *Castilleja* on two release sites for prediapause Taylor's checkerspot larvae in south Puget Sound, Washington, June 2006.

	Plantago		***Castilleja***		***Plantago***		***Castilleja***	
	Mean % cover	Range % cover	Mean cover stems	Range stems	Mean constancy	Range constancy	Mean constancy	Range constancy
Site FL1	1.9	0.4 to 3.0	13	2.7 to 23.6	81.8	54.5 to 100	68.2	45.4 to 90.9
Site FL2	2.2	0.6 to 2.8	8.0	6.5 to 9.5	86.4	63.6 to 100	52.3	45.4 to 72.7

Release plots were monitored during the postdiapause phase using belt transects spaced 3.5 meters apart to cover the plot plus 1 meter on either side. The transects were walked by a single observer at a pace of about 1 meter per minute; about 20 minutes were spent searching each plot. Searches were conducted once each week by the same observer from 26 February to 26 March 2007 when temperatures were ≥10°C, wind ≤ 10 mph, and no more than very light precipitation. Detectability of larvae appeared to drop off considerably outside of one meter. Our original method followed the plot edge to reduce trampling risk and used a wider transect belt.

Release plots were also monitored to see if adults eclosed successfully. Each plot was monitored for a total of 30 minutes in 10-minute intervals several times during the 2007 flight season (20 April-18 May) when suitable weather conditions (e.g., ≥10°C, wind <10 mph, bright sunlight) permitted. No adult Taylor's checkerspots were observed. Release sites will be monitored again during the 2008 flight period because larvae have the capacity to re-enter diapause.

Management Action #2: Release of captive-reared postdiapause larvae
Two shipments of captive-reared postdiapause larvae were brought from the Oregon Zoo in Portland to the State release site. They remained at ambient temperature (unheated vehicle or room) until release. Larvae were placed singly at the base of the densest or largest host plants on one of two 225-m^2 plots (Harrison 1989). The weather during both releases was sunny and seasonal (15-20 °C). On 6 March 2007, 99 postdiapause larvae were released into Plot A. Larvae appeared healthy and active, acclimating quickly to their surroundings. An additional 100 postdiapause larvae were released on 16 March 2007 into Plot B, with similar results (Fig. 3). Larvae had changed dramatically in the intervening 10 days. Those released into Plot B were of 3 distinct sizes: 1) small ($\leq$1 cm) and relatively inactive, 2) medium (1.5 cm) and active, and 3) large (2.5 cm) and active. Small larvae were thought to be returning to diapause, whereas medium and large larvae were believed to be males and females, respectively. If these assumptions are correct, Plot B received 34 females, 16 males and 49 larvae preparing to re-enter diapause. The large number of larvae appearing to re-enter diapause may be explained in part by the fact that larvae fed *Castilleja* (39 of 49 small larvae) were not removed from diapause until 12 March because plants were too small to be cut in spite of "pushing" them in a greenhouse. Also, in the time between releases, some larvae at the Zoo were found to host parasitic wasps in their larval form. These came from 3 cohorts (FL-5, FL-6, FL-15) that had been collected as 1st instar larvae rather than as eggs. Parasites have occasionally been observed in *taylori* larvae brought from the source site on previous occasions. To date, these have only been found in cohorts that were collected as larvae. As a precaution, larvae from these cohorts were excluded from the latter release, although 10 larvae from the FL-6 cohort had already been released 6 Mar.

Plots were monitored post-release on 18 March and 27 March 2007, using belt transects spaced 3.0 meters apart to include the plot plus 1 meter on either side. All plots were monitored by a single observer walking at a pace of about 1 meter per minute, with about 60 minutes spent searching each plot. Searches were conducted once each week from 26 February to 26 March 2007, when temperatures were $\geq$10°C, wind $\leq$ 10 mph, and no more than very light precipitation. Detectability of larvae seemed to drop sharply outside of one meter. This was a modification of the original method, which followed the plot edge and attempted to view a wider area. Release sites were monitored for a total of 30-minutes in 10-minute intervals, several times during the 2007 flight season (20 April-18 May), when suitable weather conditions (e.g., $\geq$10°C, wind <10 mph, bright sunlight) permitted. Flight season monitoring will be repeated in 2008.

Figure 3. Postdiapause larvae foraging on *Plantago lanceolata* immediately after release on 16 March 2007 at the State release site. Larvae were captive-reared at the Oregon Zoo, Portland Oregon from Source site stock.

RESULTS AND DISCUSSION

Captive rearing

The 2006-2007 rearing season was remarkable with regard to the health and survival of Taylor's checkerspots at the Oregon Zoo (Table 2). This was especially impressive because the number of progeny being reared was nearly double that of any previous year. Of 930 hatched larvae, 527 were released into the wild as prediapause larvae and 199 as postdiapause larvae for a total release of 726 or 78.1 percent of all hatched larvae. In addition, 22 larvae pupated in the lab, with 17 eclosing as adults, and up to 11 or more adults eclosed at the State release site.

One objective of egg and prediapause monitoring at the Zoo was to compare survival to that of wild cohorts. However, locating eggs and tracking the fate of wandering larvae was challenging in the environment of the Captive pens (see Experiment #1). Consequently, initial estimates of the number of eggs in the Captive pens were low, and the number of hatched larvae exceeded the number of eggs, confounding comparisons with field trials. To insure sufficient livestock for translocation experiments, an additional 451 eggs were collected from the Source site. Hatch rate for these eggs was 98.0 percent (Table 2), similar to previous years (Appendix C). Based on this hatch rate and the number of larvae that emerged, a post hoc estimate of the number of eggs in the Captive pens is about 498. Survival to diapause was similarly high between groups (Table 2). On 26 June 2006, 527 larvae were returned to WDFW for release (see Experiment #2) and on 5 March and 14 March 2007, 99 and 100 larvae were returned to WDFW for release as part of Management Action #2 (below).

Overall survival during the prediapause phase increased in 2006 (Table 2) compared to 2004 and 2005 (67.9 percent weighted mean, Appendix C) and is believed to result from good moisture balance in the yogurt containers and the benefits of the paper towel liner. In the past, many larvae developed schleritized bristles, a condition resulting from incomplete molt, which compounds with each successive molt and eventually results in death. This condition was not observed in 2006. Larvae left their molts on the paper towels; it is likely that the texture of the towels provided the traction needed for proper molting.

Table 2. Stage-specific survival and cumulative (total) mortality of captive-reared Taylor's checkerspots from egg to adult reared at the Oregon Zoo in Portland in 2006-2007 grouped by egg source. The 726 larvae removed for release (indicated by flags at cell corners), were not included in calculations of percent total mortality. Total number of eggs in 2006 was higher than reported due to uncertainty about numbers in the captive pens; percent hatch rate is based on the portion of the sample for which the number of eggs was known.

	2006 Scatter Creek			2006 Ft. Lewis			2006 Total		
Survival to next life stage	#	% stage survival	% total mortality[1]	#	% stage survival	% total mortality[1]	#	% stage survival	% total mortality[1]
# eggs	444	100.0	0.0	451	100.0	0.0	895	100.0	0.0
Egg to hatching	488	109.9	0.0	442	98.0	2.0	930	NA	0.0
Hatching to warm diapause	458	93.9	6.1	412	93.2	8.6	870	93.5	6.5
Warm diapause to cold	125	78.1	74.4	175	95.6	61.2	300	87.5	67.7
Cold diapause to postdiapause	120	96.0	75.4	168	96.0	62.7	288	96.0	69.0
# that re-entered diapause	21	45.7	95.7	10	17.2	97.8	31	10.8	96.7
Postdiapause to pupation	6	13.0	98.8	16	27.6	96.5	22	21.2	97.6
Pupae eclosed as adults	5	83.3	99.0	12	75.0	97.3	17	77.3	98.2
Percent of cohort eclosed Y1									1.8

[a] more larvae than eggs were located in the captive pens due to difficulty in searching plants.

Two other objectives of the 2006-2007 rearing year were to measure the relative growth and survival of larvae on two different host plants, *Castilleja* and *Plantago*, and to test the benefits of two different container types during diapause. Those results, as reported in Andersen et al. (2007) are summarized here.

Host plants: To control for potentially confounding factors such as source population, cohort and different weigh dates we compared prediapause larval weight from the subset of cohorts originating in Captive pen # 1 (Table 3). Larvae raised on *Castilleja* weighed significantly less than those raised on *Plantago* (Student's t-test, $t = 6.656$, $df = 92$, $P < 0.000$) (Andersen et al. 2007) at the time they were preparing to enter diapause.

Table 3. Average cohort weight (grams) of prediapause Taylor's checkerspot larvae originating in Captive pen #1 by host plant; reared at the Oregon Zoo in Portland 2006-2007 (Andersen et al. 2007).

Host plant	# of larvae	Mean cohort weight (g)	Standard deviation
Plantago	25	.0408	0.01
Castilleja	69	.0250	0.01

Diapause containers: To assess the effect of container (yogurt vs. deli), we measured weight loss over the diapause period using a subset of Source site cohorts fed only *Plantago* to control for observed differences in prediapause larval weights between host plants (Andersen et al. 2007). *Plantago*-feeding larvae were abundant and were therefore chosen for this test. Larvae housed in yogurt containers lost slightly more weight than those in deli containers (Table 4) but the difference was not significant (Students t-test, $t = -1.336$, $df = 11.25$, $P = 0.208$). Even though we could not test it, the same result could be assumed for *Castilleja*-feeders. The lower weight of *Castilleja*-feeders upon entering diapause however could result in container-type having a significant affect their survival.

Differences in larval behavior were observed between container types, but were difficult to quantify. For example, larvae in deli containers changed their position more often during diapause than those in yogurt cups, and were less likely to form webbing. Once in diapause most larvae in yogurt cups formed webbing in the paper towel folds and appeared to make even more webbing once they were outside and cooler weather set in. When active and eating, prediapause larvae were often grouped in the open and moved to more protected areas, such as the "overhang" of the paper towel or between the folds, at other times.

Table 4. Changes in mean larval weight during diapause by container type based on Source site cohorts fed only *Plantago*; larvae were reared at the Oregon Zoo in Portland in 2006-2007 (Andersen et al. 2007).

Container	# cohorts	# larvae	Mean weight (g) prediapause	Mean weight (g) postdiapause	Mean cohort % weight loss	Standard deviation
deli	9	87	0.033	0.018	0.406	0.22
yogurt	8	90	0.035	0.017	0.516	0.10

Diapause survival rates were calculated based on the number of larvae in all cohorts alive from 10 August 2006 (prediapause) to 21 February 2007 (*Plantago*) or 12 March 2007 (*Castilleja*) (Andersen et al. 2007). Survival rates were high for both host plant groups (Table 5) and both container types (Table 6). There was no statistical difference in survival between host plants

(analysis of variance, $F = 0.230$, $df = 1$, $P = 0.635$), in spite of the slightly longer diapause for *Castilleja*-feeding individuals. With tighter controls on the length of diapause, it is possible that *Castilleja*-feeders may have survived better than *Plantago*-feeders during diapause, but we cannot conclude that from this test. There was also no statistical difference in survival between container types ($F = 2.273$, $df = 1$, $P = 0.141$). Larval source ($F = 0.427$, $df = 1$, $P = 0.518$) and the interaction of container and host ($F = 0.043$, $df = 1$, $P = 0.837$) were also not significant.

Table 5. Diapause survival rates by host plant for all Taylor's checkerspot larvae reared in captivity at the Oregon Zoo in Portland in 2006-2007 (Andersen et al. 2007).

Host plant	# cohorts	# larvae prediapause	# larvae postdiapause	Mean cohort % survival	Standard deviation
Castilleja	7	69	66	95.76	6.36
Plantago	32	234	222	96.94	7.73

These results are our best test to date of the effects of host plant choice on larval survival and potential fitness because larvae fed *Castilleja* came from eggs laid on *Castilleja*. Other researchers (Singer et al. 1988, Singer 2004) have found that larvae generally perform better in terms of growth and survival on their local host plant (i.e., that found at the source site) compared to a novel host. The implications are that *Plantago,* the only host plant available in abundance at the Source site, will likely be an important habitat component at potential translocation sites in order to maximize the chance of population establishment, but other traditional hosts, such as *Castilleja*, may also be suitable.

Table 6. Diapause survival rates by container type for all Taylor's checkerspot larvae reared in captivity at the Oregon Zoo in Portland, Oregon in 2006-2007 (Andersen et al. 2007).

Container	# cohorts	# larvae prediapause	# larvae postdiapause	Mean cohort % survival	Standard deviation
deli	20	145	133	94.75	9.62
yogurt	19	158	155	98.82	3.23

While survival through diapause did not appear to be affected, increased activity levels during diapause could have important biological affects in the wild such as exposing the larvae to predation, or reducing overall fitness. Webbing may also reduce predation risk and provide added protection from severe weather during diapause. Female *Euphydryas* grow to twice the size of males. They rely heavily on larval-derived nutrients and emerge with mature eggs that can be laid without additional feeding (Boggs and Nieminen 2004). Weight at emergence is correlated with egg production in female *Euphydryas editha* (Murphy et al. 1983) and a constant fraction of stored nutrients is allocated to reproduction and other uses as adults age (Boggs 1997). Nectar is required to maintain activity and is critical for maximizing egg production in females, but is ultimately a supplement to larval-derived nutrients.

About 62 Taylor's checkerspot larvae that remained in the lab after 16 March 2007, of those 21 pupated and 17 successfully eclosed as adult butterflies (Table 2, Fig. 4, Appendix D). Mean number of days from diapause to pupation was 38.8 $\pm$ 7.8 (SD) (range 32 – 64), with the first pupae forming on 25 March and the last on 15 May. Pupae eclosed after an average of 20.1 $\pm$ 3.7 (SD) days (range 11 – 27); eclosion dates ranged from 15 April to 26 May. Adult survival ranged from 7 to 36 days, averaging 24.6 $\pm$ 9.3 (SD) days (Fig. 4 and Appendix D). The first

adult to die did so on the 28 April; the last butterfly died 2 June 2007 (Andersen et al. 2007). A subset of mounted adults from those that eclosed at the Zoo were recently observed and were notably smaller than those typically seen at the Source site or elsewhere. However there was no notable difference in the size of adults eclosing at the release site (see report cover) relative to those seen elsewhere. Small adults are a concern in captive-reared/captive-bred populations and it is especially important not to select for lab-adapted individuals. Post-diapause larvae released in Management action #2, particularly those in the later release, appeared equally as big as larvae seen elsewhere, so it appears that this discrepancy may have developed in the phase just prior to pupation. The factor most likely responsible for producing small adults in captivity is poor quality food during the postdiapause phase (G. Pratt, pers. comm.).

Results of the 2006 captive rearing trials are promising and suggest that methods development may be nearing completion. Developments for the 2007-2008 rearing season will focus on care in the post-diapause stages, particularly improvements in food quality and added light. Food quality can be improved by providing live plants rather than cut leaves, as well as diversifying the species offered so that larvae are free to choose their own food. Hand mating of adult butterflies will proceed on a trial basis in 2008. The high survival demonstrated by the current rearing methods (98.2 percent survival of 611 hatched larvae went into diapause in 2007) helps to maximize the number of checkerspots available for release trials.

More work is needed to move from captive rearing to captive breeding. While this is a sensitive topic among some researchers, there are few populations of Taylor's checkerspot (or other rare species) large enough to provide the number of livestock needed for population establishment through direct translocation on more than a few sites without causing unacceptable risk to source populations. Furthermore, incorporating captive rearing and breeding techniques provides an opportunity to learn about other key factors, such as parasites, that may be associated with these populations. Knowledge of these factors can be critical to the establishment and long-term persistence of checkerspot populations. Captive breeding can be conducted with sufficient caution and consideration to make a significant contribution to recovery.

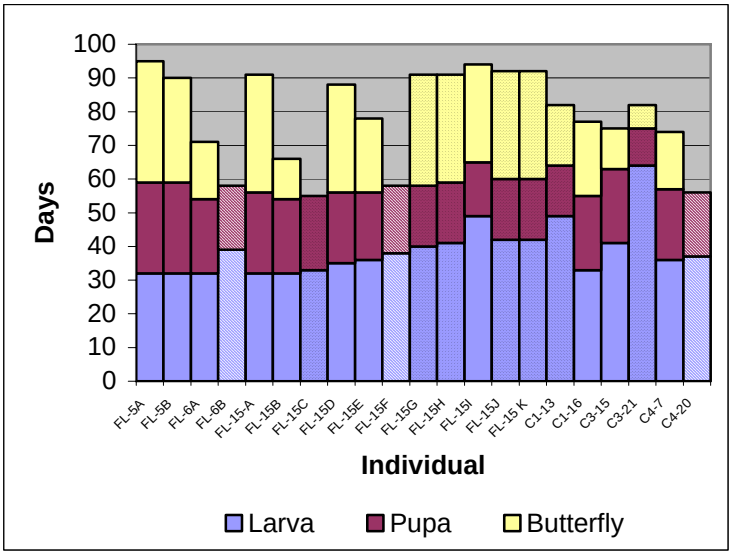

Figure 4. Postdiapause life stage durations for captive-reared Taylor's checkerspots at the Oregon Zoo in Portland in 2006-2007; solid bars denote males, dots females, stripes unknown (Andersen et al. 2007).

Experiment #1: Translocation of eggs via wild females
A total of 8 wild adult female Taylor's checkerspots were captured from the source site in early May and placed in net pens on the release site where they laid eggs on both potted and wild host plants (Fig. 5). Potted plants with eggs were moved to the Oregon Zoo on 16 May 2006. This approach established the following:

1) females recognize both *Castilleja* and *Plantago* as host species, although only the latter is available in any quantity at the source site;
2) females will lay eggs in a controlled setting;
3) females can live several to many days after they stop producing eggs;
4) average female life span was 12.9 $\pm$ 4.9 (SD) days (range = 5-19 days; n = 8);
5) minimum egg production averaged 142 eggs per female (projected range was 120-300 eggs per female based on large size of *taylori* eggs and data for other subspecies); and
6) survival from egg to diapause in the wild can be very low (0.8 percent; 5 larvae from 639 eggs), while in the lab it can be very high (91.4 percent; 412 larvae from 451 eggs).

The initial methodology specified a detailed comparison of survival from egg to 3rd instar for both captive-reared and wild cohorts. However, staff at the Oregon Zoo had some difficulty locating eggs in large, dense potted host plants, and larvae were observed crawling out of the plants even after all egg clusters were thought to have been located (Andersen et al. 2007). As a result, there was a net gain in numbers from egg to hatching (Table 2). Given the marked difference in final outcome between wild and captive reared larvae, however, there was little to be gained by a more detailed comparison in this particular trial.

Many of the results of Experiment #1 are consistent with those reported by other researchers (Moore 1989, Cushman et al. 1994). Recognition of host plant species by females for the purpose of oviposition is a genetically heritable trait in checkerspots. In this case, the recognition of the native host may indicate that important genetic diversity is still present in the source population even though they have been maintained on non-native *Plantago* for many generations. Life span and egg production are important variables in calculating impacts to source populations when removing individuals for captive rearing and translocation. These data will be used to refine our existing model, but more detailed work needs to be done on this issue because most females deposit a large percentage of their eggs in the first few days after eclosing and most captured females showed at least some wing wear. Prolonged hard rains struck ripe eggs and newly hatched larvae in late May and appeared to be the greatest source of mortality at the release site; rain was also listed as a factor in egg mortality by Moore (1989). Inclement weather is one of the primary factors affecting the success of invertebrate reintroductions (Oates and

Figure 5. Eggs of Taylor's checkerspot laid by females in enclosures on *Plantago lanceolata* at the State release site in 2006. Eggs turn red within about 48 hours of hatching.

Warren 1990). It is not known whether the effects of rain on egg and early instar mortality were greater at the release site than at the source site or an alternate site with more habitat structure (e.g., larger host plants and more tall forbs). Moore (1989) reported cumulative egg to diapause survival rates of 5 to 31 percent. In the future, photographing egg clusters would provide a more detailed assessment of egg development, condition, and mortality, while reducing damage to host plants from handling. The primary sources of mortality in south Puget Sound may also differ from those in other *E. editha* populations, but likely also vary between years on any given site. Moore (1989) found mortality rates to be relatively consistent between the 3 years of her study. My results indicate that rearing Taylor's checkerspot from egg to warm diapause in captivity can greatly increase the number of individuals available for translocation, while minimizing impacts to source populations.

This site was also monitored for four 30-minute periods during the 2007 flight season. No adult checkerspots were observed. No flight season monitoring will occur in 2008 due to survival of only a few prediapause larvae in 2006.

Experiment #2: Release of wild and captive late prediapause larvae
Both overwinter survival, and survival to adulthood appeared to be extremely low regardless of whether larvae were reared in the wild or in captivity. Five intensive larval searches were done to monitor for postdiapause larvae. A total of 6 larvae were observed among the 8 release plots. Three larvae were observed in two visits to the plot that received the wild larvae on Site FL1; two larvae were observed on a second plot at Site FL1. One larva was observed in a plot on Site FL2. Sample sizes are too low to warrant a meaningful comparison between groups. No adults were observed during flight season monitoring (Table 7).

The weather on the day of release was >32 °C and clear; these hot dry conditions set in 1-2 days prior. Weather during and immediately following a release is the most important factor influencing the success of a butterfly reintroduction (Oates and Warren 1990). Site FL1 felt noticeably warmer and dryer than Site FL2 on the release day, and monitoring 3 and 2 days post-release revealed 5 and 34 larvae visible on Site FL1 and Site FL2. Plants on Site FL1 also were notably more desiccated than on Site FL2. Larvae were easier to observe on *Castilleja* than on *Plantago*, as they were often near the tips of the plant. No larvae were visible on Site FL2 6 days post-release (access to Site FL1 was prohibited that day).

The apparently abysmal results of this release are believed to be primarily due to extremely dry conditions at the time of release, coupled with the strategy of releasing larvae individually. Other researchers have released larvae individually (Harrison 1989). While this design attempts to equalize opportunities for survival relative to food availability, access to food and other important resources may be more critical and more closely linked to group membership. Checkerspots are very gregarious, particularly during the larval stages. While it is generally believed that larvae disperse prior to diapause observations in captivity at the Oregon Zoo indicate that larvae diapause in groups of one to many. Furthermore, even postdiapause larvae have been observed to be far more gregarious than previously thought with some larvae acting as "scouts" in search of food and/or basking sites. Other larvae follow this leader. Observations of prediapause larval groups also indicate that some larvae spin more webbing than others. Webbing is a protective covering that is likely to reduce risk of predation, and may also increase

Table 7. Summary of 2007 flight season surveys conducted for Taylor's checkerspot at translocation sites in south Puget Sound, Washington. Larvae were released either pre- or postdiapause.

Site name	20-Apr	23-Apr	25-Apr	30-Apr	01-May	03-May	04-May	07-May	08-May	09-May	10-May	11-May	13-May	15-May	17-May	18-May	# surveys	# w/ good weather	# larvae released	larval stage	# adults obs	flight year
County site		0			0			0		0							4	4	30	post	0	2
FL1	0			0			0		0		0					0	6	5	301	pre	0	1
FL2	0			0		0			0		0					0	6	6	307	pre	0	1
State site		0	0		0		0	7		3	1	0	0	0	0		10	7	199	post	11	1

[a] Not part of an official survey.

survival during diapause by moderating the effects of weather. Earlier release dates may improve survival to and during diapause and could be explored relative to the onset and severity of summer drought. Maintaining maternal cohorts and releasing larval groups wherever possible to maximize likelihood of survival, should be the strategy used in future release testing. This research is considered a low priority at this time relative to other aspects of release methodology, but may be of critical importance to the long-term persistence of Taylor's checkerspot populations and the ranking of potential release sites.

Management Action #2: Release captive-reared post-diapause larvae

The release of late postdiapause larvae has been the most encouraging approach to date. Post-release surveys on 18 March revealed 15 and 22 larvae on Plots A (release 6 Mar 2006) and B (release 16 Mar 2006). Surveys on 27 March 2007 revealed 5 and 4 larvae on Plots A and B. Expected survival rates, with and without parasitism, for postdiapause and pupal stages are presented in Table 8. Due to the discovery of larval parasites between release dates, survival rates in Plot A may resemble those with parasitism whereas those in Plot B may resemble those without parasitism, but there is no way to know for sure.

The most significant result of this release is the observation of 11 adult Taylor's checkerspots at the State release site (Table 7). Two observations of adults were made at Plot A during surveys on 7 May, and 5 adults were observed at Plot B, although it is not known exactly how many individuals this represents. On 9 May, 3 adults were observed at Plot A, but none at Plot B. A single adult was observed near Plot A on a cursory visit to the site on 10 May. No adults were confirmed on any part of the site after that date. While adults appeared outside of the plots, they were always in relatively close proximity when sighted, suggesting that the 50-m distance separating plots is adequate for quantifying results from different treatments. Surveys were conducted on nearly all sunny days available, but the spring was cool and cloudy in general, which delayed the flight season slightly. Adults were observed between 1039 and 1340 hours, and seemed to move away from the release site fairly soon after initial observations. All of the checkerspots we observed appeared fresh and one was still wet from eclosing. Several female checkerspots were also confirmed, with the balance being of unknown gender. Behaviors included flying, perching, basking and nectaring. Nectaring was observed one time each on *Balsamorhiza deltoidea* and *Lomatium triternatum* and possibly on *Ranunculus occidentalus*.

The limited number of observed adults precludes any conclusions about release dates or parasites except that if captive larvae are likely to be hosting parasites a later release date will control for

this factor. On the one hand fewer adults emerged than might be expected based on Moore's (1989) observations of an established population, especially given the robust appearance of the larvae, the late dates they were released relative to pupation, and the large number of larvae released. On the other hand, with nearly half of larvae released in Plot B appearing ready to re-enter diapause, the results may be better than expected, especially considering that Harrison (1989) released 100 postdiapause larvae per site at numerous sites, which resulted in 0-3 adults per site. We answered the most basic question about whether we can foster Taylor's checkerspot from egg to adult, and did so in both lab and field with reasonable certainty. However, we have generated many new questions in the process. For example, did the emerging adult checkerspots disperse in response to low population density, as they are prone to do? If so how do sites with only a few adults in any given year persist over time?

In considering alternative approaches to release, the high rate of pupation and eclosion in captivity is notable. When the sample is restricted to the balance of the 3 cohorts retained because of potential for hosting parasites (other larvae were retained because they were small and appeared to be re-entering diapause). Of 60 postdiapause larvae in these 3 cohorts 24 (40.0 percent) died, 10 (16.6 percent) were released, 16 (26.6 percent) pupated, and 10 (16.6 percent) re-entered diapause; 13 of 16 pupae (81.3 percent) properly eclosed. This suggests that releasing pupae in the manner Pickering (2001) has used with Oregon silverspot (*Speyeria zerene hippolyta*) may be worth testing.

Table 8. Potential survival rates and numbers for postdiapause larvae released into the wild and followed through eclosion. Range of survival rates are presented with and without parasitism based on Moore's (1989) observation of *E. editha* in California. We assume captive-reared larvae are protected from parasites in captivity. Numbers of progeny were rounded down to the nearest whole number.

with parasitism	**% larval survival**	**# larvae**	**58 % pupal survival**	**93 % pupal survival**
larval survival – lo	3	3	1	2
larval survival – hi	88	88	51	81
without parasitism				
larval survival – lo	36	36	20	33
larval survival – hi	93	93	53	86

CONCLUSIONS

Captive rearing

- Captive rearing appears to be a beneficial strategy for increasing the number of individuals available for translocation by increasing stage-specific survival throughout the life cycle. Successful development of rearing methods for postdiapause life stages will determine the potential of captive rearing to contribute to population re-establishment.
- At the start of diapause larvae raised on *Castilleja* weighed significantly less than those reared on *Plantago*, but there was no difference in larval survival during diapause regardless of host plant.
- Attempts to compare relative survival of postdiapause larvae by host plant were impaired by an inability to produce significant quantities of *Castilleja* by the time larvae emerge from diapause in February. Field observations of *Castilleja* corroborate these results.

- Larvae housed in yogurt containers lost slightly more weight than those in deli containers but the difference was not significant; survival was high for both groups and did not differ between container types.
- Larvae housed in deli containers during diapause made more webbing but no differences in survival were observed at this stage. However better protection from inclement weather and reduced weight loss may confer increased survival in later stages and/or increased reproductive potential, particularly for females.
- Group behavior may be critical to larval survival in both pre- and postdiapause phases.
- Effective captive rearing methods for Taylor's checkerspot have been established for all stages from egg to postdiapause; development of methods for rearing from the postdiapause to adult stages are nearly complete with only fine-tuning required.
- We demonstrated that Taylor's checkerspot can be fostered from egg to adult and did so in both lab and field with reasonable certainty.
- The program at the Oregon Zoo will need to move from captive rearing to captive breeding to accommodate recovery of Taylor's checkerspot throughout the state. Captive rearing can only supply enough livestock for population re-establishment if a large (>300 adults) and stable source population of the correct genetic origin is available.

Translocations

- Female Taylor's checkerspots will readily lay eggs in a controlled setting.
- Females recognized both *Castilleja* and *Plantago* as host species, although only the latter is available in any quantity at the Source site.
- Minimum egg production averaged 142 eggs per female and is expected to be somewhat higher because most females had likely eclosed more than 1 day prior to capture. This is consistent with the expectation that *taylori* would lay fewer eggs than other subspecies because the eggs are larger.
- Survival from egg to diapause in the wild is very low; in the lab it has been very high.
- Both overwinter survival, and survival to adulthood appeared to be very low for translocated prediapause larvae regardless of source (wild vs. captive).
- Extremely dry conditions at the time of release coupled with the strategy of placing larvae individually rather than in groups on the host plant is believed to account for the seemingly poor survival observed in the prediapause larval release.
- Postdiapause larvae were easier to observe on *Castilleja* than on *Plantago*, as they were often near the tips of the plant.
- Release of postdiapause larvae appears to be the most promising approach so far but the limited number of observed adults precludes any conclusions about release dates or parasites. Assuming that all adults observed were unique individuals, our results exceeded those expected based on Harrison's (1989) translocations but fell far short of expected results based on Moore's (1989) observations of an established population.
- Methods to increase the number of adults eclosing will require further exploration.
- A seemingly high rate of pupation and eclosion in the lab suggests that releasing pupae in the manner Pickering (2001) has used with Oregon silverspot (*Speyeria zerene hippolyta*) may be worth testing.
- Rapid dispersal of adults at the release site raises additional questions that will need to be addressed in future release trials.

QUESTIONS GENERATED BY THIS RESEARCH

- Parasites aside, is prediapause survival a function of group size and food availability?
- Does *Castilleja* ever occur in sufficient amounts in February to be a sole food source for postdiapause larvae?
- What is the egg-laying potential of an adult female Taylor's checkerspot?
- Do female *taylori* from the Source site recognize other hosts such as *Collinsia grandiflora*, which is also found at the Source site?
- Given the robust appearance, number of larvae and late release date relative to pupation, why did seemingly few adults eclose on the State site?
- What percent of released postdiapause larvae are returning to diapause as opposed to pupating and/or eclosing? How does this compare to results in the lab?
- What rate of eclosion is reasonable to expect?
- How many postdiapause larvae or pupae will be required for population establishment?
- Did the adult checkerspots disperse in response to low population density, as they are prone to do? If so, how do sites with only a few adults in a given year persist over time?
- Was dispersal the result of a lack of physical barriers OR are there other chemical factors that may signal to emerging adults that a site is suitable for occupation?
- What is the definition of an established population?

NEXT STEPS

- Determine the eclosion rate of released of postdiapause larvae compared to released pupae. If possible, then compare these to stage-specific survival rates in the lab.
- Refine postdiapause larval care.
- Extend development of captive rearing methods to pupal and adult stages.
- Test hand-mating techniques in captivity.
- Determine the egg-laying potential of female *taylori* in captivity; weigh pupae or newly emerged females to confirm correlation reported in the literature and as an indication of health.
- Measure wing length of adults eclosing in the lab and if possible in the field to insure healthy animals are being released.
- Determine whether adults are successfully reproducing at the release site by monitoring adult behavior and searching for egg masses and larvae.
- Determine whether female *taylori* at Source site will oviposit on *Collinsia grandiflora* (Fig. 6).
- Determine the minimum amount of time adult checkerspots remain in the vicinity of the release site. Does this increase with the number of adults present?
- Determine the number of adults that need to eclose to result in a high likelihood of population establishment.

Figure 6. *Collinsia grandiflora*, a potential Taylor's checkerspot oviposition plant found at the Source site in 2006.

LITERATURE CITED

Andersen, M., D. Shepherdson, S. Rosen, E. Barclay. 2007. Oregon Zoo Taylor's checkerspot (*Euphydryas editha taylori*) rearing 2006-2007. Final report. Oregon Zoo, Portland, Oregon. 11 pp. + appendices.

Boggs, C. L. 1997. Reproductive allocation from reserves and income in butterfly species with differing adult diets. Ecology 78: 181-191.

Boggs, C. L., and M. Neiminen. 2004. Checkerspot reproductive biology. Pp. 92-111 *in* Ehrlich, P. R., and I. Hanski, eds. On the wings of checkerspots: a model system for population biology. Oxford University Press New York City, New York.

Cushman, J. H., C. L. Boggs, S. B. Weiss, D. D. Murphy, A. W. Haney, and P. R. Ehrlich. 1994. Estimating female reproductive success of a threatened butterfly: Influence of emergence time and host plant phenology. Oecologia 99:194:200.

Grosboll, D. 2004. Captive rearing the Endangered Mardon skipper (Polites mardon) and Taylor's checkerspot (Euphydryas editha taylori) butterflies: initial results. Hooper, T. D., ed. Conference proceedings for Species at Risk 2004: Pathways to Recovery, held Victoria, BC. Available online at: http://www.speciesatrisk2004.ca/pdf/grosboll_edited_final_march_11.pdf

Harrison, S. 1989. Long-distance dispersal and colonization in the bay checkerspot butterfly, Euphydryas editha bayensis. Ecology 70: 1236-1243.

Linders, M. J. 2006. Captive rearing and translocation methods development for Taylor's checkerspot (*Euphydryas editha taylori*), South Puget Sound, Washington. Washington Department of Fish and Wildlife report, Olympia, Washington. 26 pp. + appendices.

Murphy, D. D., A. E. Launer, and P. R. Ehrlich. 1983. The role of adult feeding in egg production and population dynamics of the checkerspot butterfly Euphydryas editha. Oecologia 56:257-263.

Moore, S. D. 1989. Patterns of juvenile mortality within an oligophagous insect population. Ecology 70: 1726-1737.

Oates, M. R., and M. S. Warren. 1990. A review of butterfly introductions in Britain and Ireland. JCCBI/WWF, Godalming. 95 pp.

Pickering, D.L. 2001. Fifth Annual Report on Permit #TE804885-5, Enhancement of survival permit for threatened wildlife, Oregon silverspot butterfly (*Speyeria zerene hippolyta*). Report to US Fish and Wildlife Service, Portland Field Office, Portland, Oregon.

Pratt, G. 2005. 2005-2006 synopsis of Quino Checkerspot rearing methods. Unpublished report. University of California- Riverside.

Singer, M. C. 2004. Measurement, correlates and importance of oviposition preference in the life of checkerspots. Pages 112-137 *in* Ehrlich, P. R., and I. Hanski, eds. On the wings of checkerspots: a model system for population biology. Oxford University Press New York City, New York.

Singer, M. C., D. Ng, and C. D. Thomas. 1988. Heritability of oviposition preference and its relationship to offspring performance within a single insect population. Evolution 42:977-985.

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APPENDIX A. TAYLOR'S CHECKERSPOT CAPTIVE REARING PROTOCOL FOR 2007-2008 AT THE OREGON ZOO, PORTLAND, OREGON.

Eggs: *Plantago* leaves containing egg clusters will be brought to the Oregon Zoo from the field site in Washington. At the Zoo, egg clusters and larvae will be moved into 8 oz. Dannon yogurt containers lined with paper toweling. Regular white kitchen paper towels will be cut into quarters, then folded into quarters and placed in the cup with the folded edges pointed downward to form an upside-down cone with one of the top edges folded over to form an overhang for the larvae to use as a shelter. The lids of these specific yogurt containers allow air movement, which reduces mold. These containers will not require misting, as plant parts provide the necessary moisture. Egg clusters will be left on the leaves until hatching, at which time fresh *Plantago* leaves will be provided for food (see prediapause larvae for details). Eggs and larvae will be checked daily and the following data collected regularly: total number of eggs, date of hatching, total # of larvae, and instar of larvae.

Prediapause larvae: Larvae will be checked and mortality assessed daily as indicated above (see Eggs). Containers will be cleaned of frass every 3 to 4 days, or as appropriate. All larvae will be allowed to molt to the 5^{th} instar (just prior to diapause), then all larvae in a random subset of the larval groups will be weighed individually.

Diapause: Larval groups will be housed in clean yogurt containers lined with paper toweling as described above (see Eggs); it is important not to disturb larval diapause shelters during this process. Once all larvae have entered diapause, yogurt containers will be placed inside inverted clay pots located outside under the Animal Management building overhang, as in 2005 and 2006. Larvae will be checked weekly for condition, mortality and indications of movement. No moisture will be added to the yogurt containers; however clay saucers will be wetted during weekly checks.

Postdiapause: Larvae will be removed from diapause in February following increased activity levels and corresponding emergence of wild progeny. The same cohorts that were individually weighed prediapause will be weighed postdiapause. From diapause, larvae will be placed in clear sandwich-sized (4"x4") plastic containers with lids and a moist blue paper shop towel in the bottom. This procedure puts the larvae in an environment of increased humidity, allows them to drink, and aids with the logistics of setting up and moving a large number of larvae from the diapause site to the postdiapause site (mezzanine of the butterfly lab). Larvae will be provided with fresh *Plantago* leaves on a daily basis. The containers need to have the lids removed daily for a few minutes to allow for exchange of fresh air. Within the next few days, larvae will be moved into clear plastic Sterilite shoebox bins (12" long x 7.5" wide x 4.5" deep) lined with shop towels. The bins will be covered with netting (tulle) and secured with a rubber band to maximize direct light and airflow. Overhead lights will be turned on during the day to increase light intensity. The temperature of the lab will be maintained at 65 ºF or lower, with a relative humidity of about 55%. Lids will be placed askew over the bins (90% of bin covered) overnight and removed the following morning to prevent dehydration. A maximum of 10 individuals will be housed in a bin. Bins will be cleaned every other day or as needed. Fresh food will be placed on one end; a mounded paper towel ("mima mound") on the other end will be provided as a basking structure. A moisture gradient will be maintained across the paper towel. Bins will be

placed on wire racks with full spectrum fluorescent bulbs on a 10-hour light/14-hour dark cycle maintained by timers. Larvae will also be provided with one or two 75-watt tungsten lamps for 4 hours/day to provide extra light and heat. Larvae will be released into the field about a week prior to pupation (about mid-March) to allow time to acclimate before pupating.

Larvae showing signs of returning to diapause will be fed as needed and allowed to diapause in the lab. They will be kept at the Oregon Zoo in hopes of releasing them the following spring.

About 50 larvae, depending on availability, will be allowed to pupate in the lab and eclose as adults for the purpose of testing hand-mating techniques. It is not uncommon to have biased sex ratios and many males will simply not mate in captivity. Sometimes males hatch too early, females hatch too early, etc., with small numbers. There is also a process by which males learn from each other. Males housed separately seem to be ignorant when it comes to mating compared to those that are housed in groups.

Pupation:
Bins will be checked daily for pupae. When found, pupae will be removed, labeled, and placed in a new bin. If larvae pupate on a "mima mound", a piece of the paper towel will be cut and taped to the side of a bin. Alternatively, pupae will be hung with gauze or spider webbing and attached to the side of a bin. Pupae will be housed only with members of their original cohort. Pupae will be kept in a location where the temperature and humidity can be controlled. Bins will be placed on a rack and allowed to receive lots of diffuse sunlight. Temperatures will be maintained between 75-80 ºF. A moisture gradient will be maintained in the bins where the pupae are housed by wetting a blue shop towel in the bottom of the bin.

Adults and mating:
Butterflies will be housed in the same shoebox bins and given a variety of flowers especially those that bees have been observed using. Flowers will be placed in vials of water secured with florists foam and at least some fresh flowers will be added daily. Non-aromatic flowers and species with small inflorescences seem to be preferred. Flowers will be placed such that some are in a vertical orientation and others in a horizontal orientation. Gatorade (melon flavored) will be offered daily on a sponge. The sponge should be tall enough to reach above the edge of the lid of a small Petri dish. The sponge should be saturated with Gatorade so that butterflies can feed off the sides. Butterflies will be placed on the Gatorade sponges once each day as early in the day as possible. Butterflies will be transferred to and from the sponges by gentling lifting them with a cotton-tipped swab. When placing a butterfly on the Gatorade sponge, the cotton-tipped swab should be saturated with Gatorade to stimulate nectaring. Gatorade-soaked sponges will be left in the boxes for a maximum of four hours. After feeding, the butterfly will be transferred with a cotton-tipped swab saturated with water, to a water- saturated sponge to rinse the Gatorade off. Gatorade sponges will be soaked overnight in water after each use to cleanse them thoroughly. The lab will be maintained so the temperature is at least 85 ºF in the early afternoon to stimulate activity. Adults will be sexed after they eclose and males will be housed together and observed for chasing behavior. Males exhibiting chasing behavior will be housed with a female and observed for breeding behavior. Records will be kept to document which individuals have mated and the results of the mating. If no mating behavior is observed, an exploration of other breeding

techniques that have been successfully used in a captive environment may be attempted, such as the “hand mating” technique used by Pratt (2005; see below).

“Prior to mating her put the female in the fridge (or a cooler) for at least 5 minutes to reduce her ability to reject a male. Immediately after removing her from the fridge or cooler, bring the males into the warm sunlight and rub the female's posterior end against the male's antennae until the male responds by fanning his wings and twisting his abdomen. It may be necessary to try several males to get a response. At this point aim the female's posterior end into the male's posterior end until he clasps her genitalia. Once done, set them aside in a screened yogurt containers in partial shade until they finish coupling. Matings shorter than a half an hour or longer than 6 hours have generally not been successful.” (Pratt 2005)

If mating occurs, some females will be housed in the same shoebox bins as the post diapause larvae, but provided with cut Plantago leaves in a vial for the female to use for egg laying. Other females will be housed in terrariums screened on top with one potted host plant (Plantago and/or Castilleja) for egg laying. The cage containing the female and plants will be kept in a bright location with diffuse sunlight. Enclosures will be checked daily for any signs of egg laying. Once eggs darken they are within 24-48 hours of hatching. Plant parts containing egg clusters can be harvested and the eggs cared for as described above. The lab-reared male will either be frozen or returned to his cage for future matings.

NOTE: Also pay attention to host plant condition and nutrition, as these are crucial factors in triggering and maintaining healthy diapause.

APPENDIX B. AVERAGE WEIGHT OF CAPTIVE REARED TAYLOR'S CHECKERSPOT LARVA BY COHORT IN CAPTIVITY AT THE OREGON ZOO, PORTLAND, OREGON. PREDIAPAUSE LARVAE WERE WEIGHED BETWEEN 29 JUNE AND 5 JULY 2006. POSTDIAPAUSE LARVAE WERE WEIGHED 21 FEBRUARY 2007 (HOST PLANT PLLA) AND 12 MARCH 2007 (CAHI). FROM ANDERSEN ET AL. (2007).

Cohort	Container	Host Plant	# in cohort prediapause 26 July 2006	Average prediapause weight	# in cohort postdiapause Feb-Mar 2007	Average postdiapause weight
C1-13B	deli	CAHI	1	0.0264	1	0.0170
C1-2B	deli	PLLA	6	0.0425	6	0.0248
C1-16B	deli	PLLA	5	0.0459	5	0.0199
C3-5C	deli	PLLA	3	0.0440	3	0.0247
C3-15AB	deli	PLLA	10	0.0342	7	0.0155
C3-20B	deli	PLLA	5	0.0404	5	0.0211
C4-17A	deli	CAHI	8	0.0297	7	0.0146
C4-18	deli	PLLA	1	0.0358	1	0.0198
Fl-3B	deli	PLLA	1	0.0153	1	0.0090
Fl-5	deli	PLLA	15	0.0337	15	0.0193
Fl-6BA	deli	PLLA	9	0.0429	8	0.0211
Fl-8	deli	PLLA	17	0.0378	12	0.0193
Fl-9	deli	PLLA	3	0.0166	3	0.0196
Fl-14A	deli	PLLA	8	0.0340	8	0.0169
Fl-15BAB	deli	PLLA	13	0.0393	13	0.0203
Fl-16BA	deli	PLLA	8	0.0384	8	0.0200
Fl-17AA	deli	PLLA	13	0.0388	12	0.0180
C4-20A	deli	PLLA	1	0.0523	1	0.0177
C1-14A	deli	CAHI	7	0.0249	6	0.0174
C1-22	deli	CAHI	11	0.0241	11	0.0243
C1-13A	yogurt	CAHI	35	0.0253	34	0.0169
C1-1	yogurt	PLLA	11	0.0362	11	0.0165
C1-16C	yogurt	PLLA	1	0.0449	1	0.0217
C2-23	yogurt	PLLA	2	0.0363	2	0.0194
C3-5D	yogurt	PLLA	5	0.0444	5	0.0227
C3-15B	yogurt	PLLA	1	0.0425	1	0.0219
C4-20B	yogurt	PLLA	1	0.0523	1	0.0224
Fl-4	yogurt	PLLA	6	0.0320	6	0.0186
Fl-6BB	yogurt	PLLA	10	0.0385	10	0.0176
Fl-10	yogurt	PLLA	15	0.0438	15	0.0180
Fl-12	yogurt	PLLA	14	0.0373	14	0.0145
Fl-14B	yogurt	PLLA	9	0.0301	9	0.0131
Fl-15	yogurt	PLLA	14	0.0388	14	0.0182
Fl-16BB	yogurt	PLLA	8	0.0267	7	0.0182
Fl-17BA	yogurt	PLLA	14	0.0386	13	0.0172
C3-21	yogurt	CAHI	1		1	0.0254
C2-12B	yogurt	CAHI	6		6	0.0197
C4-6A	yogurt	PLLA	4		4	0.0184
C4-7C	yogurt	PLLA	1		1	0.0150
Total			**303**		**288**	

APPENDIX C. STAGE-SPECIFIC SURVIVAL AND CUMULATIVE (TOTAL) MORTALITY OF CAPTIVE-REARED TAYLOR'S CHECKERSPOTS FROM EGG TO ADULT AT THE OREGON ZOO, PORTLAND, OREGON ORGANIZED BY EGG COLLECTION YEAR AND SOURCE LOCATION. ANIMALS REMOVED FROM THE SAMPLE FOR RELEASE OR ALTERNATE TREATMENT (INDICATED BY FLAGS AT CELL CORNERS), WERE NOT INCLUDED IN CALCULATIONS OF PERCENT TOTAL MORTALITY. ACTUAL NUMBER OF EGGS IN 2006 WAS HIGHER THAN REPORTED; PERCENT HATCH RATE IS BASED ON THE PORTION OF THE SAMPLE FOR WHICH THE NUMBER OF EGGS WAS KNOWN.

	2003 Clallam County			2004 Clallam/Thurston			2005 Thurston/Pierce			2006 Pierce County		
Survival to next life stage	#	% stage survival	% total mort.	#	% stage survival	% total mort.	#	% stage survival	% total mort.	#	% stage survival	% total mort.
# eggs	94	100.0	0.0	na	na	na	490	100.0	0.0	895	100.0	0.0
Egg to hatching	92	97.9	2.1	386	100.0	0.0	467	95.3	4.7	930	98.0	0.0
Hatch to warm diapause	75	91.5	10.6	253	65.5	34.5	326	69.8	33.5	870	93.5	6.5
Warm diapause to cold	72	96.0	14.3	152	60.1	60.6	106	32.5	78.4	300	77.1	67.7
Cold to post-diapause	50	69.4	41.1	112	73.7	71.0	46	43.4	90.1	288	96.0	69.0
# to 2nd diapause	20	27.8	na	31	20.4	92.0	2	4.3	99.4	31	10.8	96.7
Postdiapause to pupa	12	24.0	55.7	4	4.9	99.0	na			22	21.2	97.6
Pupa to adult	10	83.3	67.9	0	0.0	100.0				17	77.3	98.2
% of cohort eclosed		**10.6**									**1.8**	

APPENDIX D. NUMBER OF DAYS AND DATES SPENT IN POSTDIAPAUSE LIFE STAGES FOR 21 TAYLOR'S CHECKERSPOTS REARED IN CAPTIVITY AT THE OREGON ZOO, PORTLAND, OREGON IN 2006-2007 (ANDERSON ET AL. 2007). IE INDICATES IMPROPER ECLOSION; * INDICATES ACCIDENT DURING ECLOSION.

ID	Sex	Host plant	Date out of diapause	Pupation date	# days to pupation	Eclosion date	# days to eclosion	Date of death	Adult lifespan # days
C1-13	F	CAHI	12-Mar	11-Apr	49	26-Apr	15	14-May	18
C3-21	F	CAHI	12-Mar	15-May	64	26-May	11	02-Jun	7
FL-15C	F	PLLA	21-Feb	26-Mar	33	17-Apr	22	IE	na
FL-15G	F	PLLA	21-Feb	02-Apr	40	20-Apr	18	23-May	33
FL-15H	F	PLLA	21-Feb	03-Apr	41	21-Apr	18	23-May	32
FL-15J	F	PLLA	21-Feb	04-Apr	42	22-Apr	18	24-May	32
FL-15K	F	PLLA	21-Feb	04-Apr	42	22-Apr	18	24-May	32
FL-5A	M	PLLA	21-Feb	25-Mar	32	15-Apr	27	21-May	36
FL-5B	M	PLLA	21-Feb	25-Mar	32	15-Apr	27	16-May	31
FL-6A	M	PLLA	21-Feb	25-Mar	32	16-Apr	22	03-May	17
FL-15A	M	PLLA	21-Feb	25-Mar	32	18-Apr	24	23-May	35
FL-15B	M	PLLA	21-Feb	25-Mar	32	16-Apr	22	28-Apr	12
FL-15D	M	PLLA	21-Feb	28-Mar	35	18-Apr	21	20-May	32
FL-15E	M	PLLA	21-Feb	29-Mar	36	18-Apr	20	10-May	22
FL-15I	M	PLLA	21-Feb	11-Apr	49	27-Apr	16	26-May	29
C1-16	M	PLLA	21-Feb	26-Mar	33	17-Apr	22	09-May	22
C3-15	M	PLLA	21-Feb	27-Mar	41	18-Apr	22	30-Apr	12
C4-7	M	PLLA	21-Feb	29-Mar	36	19-Apr	21	06-May	17
C4-20	U	PLLA	21-Feb	30-Mar	37	18-Apr	19	IE*	na
FL-6B	U	PLLA	21-Feb	01-Apr	39	20-Apr	19	IE	na
FL-15F	U	PLLA	21-Feb	31-Mar	38	20-Apr	20	IE	na