2008 ACUB Project Progress Report

1. **Project Title:** Development of captive rearing and translocation methods for Taylor's checkerspot in south Puget Sound

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3. **Project Site (if appropriate):** Captive rearing - Oregon Zoo; Release and monitoring sites – Pacemaker Airstrip and TA 7S on Fort Lewis (2006 releases); Scatter Creek South Unit – 2007 release.

4. **Executive Summary**
Rapid extinction of several Taylor's checkerspot populations in south Puget Sound in 1998 to 2000 illustrated an acute need for a proactive approach to recovery. The species appears to persist at only one site in south Puget Sound at this time. Habitat restoration is proceeding on several sites but natural recolonization is prohibited by the 1) small number of source populations, 2) the small size of source populations, and 3) the large distance between source populations and reintroduction sites. New populations of the species need to be established to stem decline and move toward recovery. A captive rearing program for Taylor's checkerspot was established at the Oregon Zoo in 2004 and has been ongoing since that time, with large annual advances in overall health and survival during all life stages from egg to postdiapause. Cumulative survival from egg to postdiapause was 97.0 percent in 2007-2008, when 598 of 617 hatched larvae emerged from diapause. Current efforts in captivity are primarily aimed at developing and refining methods for rearing Taylor's checkerspot through postdiapause, pupal and adult stages and initiating development of captive mating techniques, which resulted in 25 copulations in 2008 and 10 females producing eggs. Release experiments have made use of captive stock from the Oregon Zoo to test efficacy of release at various life stages. Release during the postdiapause phase has been the most promising so far. We achieved the objectives of the 2008 release, when numerous adult butterflies emerged at the release site during the 2008 flight season, following release of 340 postdiapause larvae. Initial results suggest that releasing larvae in groups rather than as singles may improve survival to adulthood. Adult behaviors observed at the release site include nectaring, territorial displays by males, mating chases, and "routine movement patterns". In contrast to adults that emerged in spring 2007, only one butterfly was observed flying from the site in a direction from which no other butterfly was ever observed. Collectively, these behaviors are suggestive of recognition and acceptance of the release site and confer some measure of site fidelity, and indicate that we our making good progress in our bid to develop methods for re-establish populations of Taylor's checkerspot in South Puget Sound.

5. **Project Goals and Objectives**
The goal of this project is to reduce the probability of extinction of *Euphydryas editha taylori* in south Puget Sound. Funding for this project was awarded for implementation of the Taylor's checkerspot captive rearing program in 2008-2009 and release and monitoring of Taylor's checkerspot at field sites in 2008. The specific objectives of each project are listed below.

Captive rearing

1) Develop and refine methods for rearing Taylor's checkerspot through postdiapause, pupal and adult stages.
2) Increase the scale of Taylor's checkerspot production at the Oregon Zoo.
3) Evaluate the efficacy of adding a second captive rearing facility at a different location (e.g., Woodland Park Zoo).
4) Test hand-mating techniques.
5) Evaluate the capacity for rearing Taylor's checkerspot at the Oregon Zoo and housing needs related to a captive breeding program.

Monitoring the release of prediapause Taylor's checkerspot larvae from 2006

The objective of this component of the project is to conduct flight season monitoring at sites where prediapause Taylor's checkerspot larvae were released in 2006 to document any evidence of emerging adults.

Release and monitoring of postdiapause Taylor's checkerspot larvae in 2008

The objectives of this trial are:

1) Validate the efficacy of releasing Taylor's checkerspot during the postdiapause larval phase for the purpose of maximizing the number of emerging adults.
2) Determine whether releasing postdiapause larvae as groups results in a greater number of adults than larvae released singly.

A controlled test release of postdiapause larvae using enclosures was also to be conducted if time and resources allowed. The purpose was to determine detectability of larvae following release. Unfortunately we were unable to complete this objective.

6. Methods

Captive rearing

As in previous years, eggs were collected in the field in 2007 and brought to the Oregon Zoo for rearing. The rearing protocol followed that described in (Linders 2007) from the egg stage through removal from diapause. All larvae were reared on freshly cut *Plantago* leaves, which were provided twice daily until the larvae stopped eating and entered diapause (like hibernation). Larvae were removed from diapause on 18 February 2008 following increased activity levels. In any given year some proportion of postdiapause larvae opt to return to diapause rather than continue their development to adulthood.

<u>Postdiapause larvae:</u> Postdiapause larvae were ultimately split into three groups according to their destination. The largest group (446 larvae) was intended for release into the wild by the WDFW (Table 1). The second group (147 larvae) was retained for a combination of captive rearing and captive mating trials at the Oregon Zoo (Table 1). A third group of 20 larvae were provided to The Nature Conservancy for work with scent-locating dogs. The rearing methods used for each of these three groups are described below.

Larvae destined for release by the WDFW were divided between 9 glass tanks (10"w x 20"l x 12"h). Each tank housed about 50 individuals (range 46-54). Several potted plants of a variety of host species (*Plantago lanceolata*, *Plectritis congesta*, and *Collinsia parviflora*) were placed in each tank and the remaining spaces filled with loosely crumpled brown paper toweling. Potted plants were replaced every three days or as supplies permitted. The tanks were covered by fabric mesh secured with an elastic band. On 6 March 2008, the WDFW took 340 larvae for release; the balance (99 larvae) remained at the Oregon Zoo until pupation.

The Oregon Zoo used 147 postdiapause larvae to evaluate the effect of host plant quality (cut vs. live) on pupal and adult size (Fig.1). Surviving adults would be used for captive mating trials, so larvae were divided into three breeding "lineages" by combining larvae that originated from different egg clusters; lineages were divided equally between rearing treatments. A control group was reared as in 2006-2007 (Anderson et al. 2007), except cuttings from four host plants (*Plantago lanceolata*, *Plectritis congesta*, *Collinsia parviflora*, and *Castilleja hispida*) were provided twice each day, rather than *Plantago* alone. A maximum of 13 larvae were housed in plastic bins (7.5"w x 12"l x 4.5"h) with paper towels on the bottom, wetted at one end to provide a moisture gradient (Fig. 1). A group of second diapause larvae were also raised using this technique. The second group was reared by placing up to 25 larvae in a glass tank (8"w x 16"l x 10"h) with the same variety of live potted (4" x 4") host plants. Larvae were allowed to feed freely (Fig. 1). Potted plants were replaced every three days as needed, or as supplies permitted. Tanks and bins were covered mesh fabric and secured with an elastic band. Bins and tanks were placed on wire racks with full spectrum fluorescent bulbs on a 12-hour light/12-hour dark cycle maintained by timers. Overhead room lights were turned on during the day to increase light intensity. The lab also has a large (72" x 34") screened southeast-facing window that provides natural sunlight; it was left open in the evening and fitted with a box fan to pull in cool air. Average minimum temperature was 57° F and relative humidity was 52%, the average maximum temperature was 64° F and relative humidity was 61%.

Twenty postdiapause larvae were provided to the Nature Conservancy for training scent-locating dogs in the field. Larvae were housed in a plastic bin and fed cut *Plantago* leaves twice each day, similar to treatment in captivity at the Zoo. The bin was kept outdoors on a covered south-facing porch when not in use.

Fig. 1. Bin (cut leaves) and tank (live plants) methods of captive rearing postdiapause Taylor's checkerspot larvae at the Oregon Zoo, Portland, Oregon, in 2008.

<u>Pupae:</u> Bins were checked daily for pupae. Tanks were checked for pupae every three days when live plants were exchanged. Pupae were weighed using an Acculab Vicon electronic gram scale accurate to 3 significant figures. Up to 10 pupae found on the same day, were placed in a paper towel-lined yogurt cup and dated. Cups were housed in the tank or bin from which they originated. After all larvae had pupated or returned to diapause, tanks were turned to rest on a long side so that the mesh lid was on the side; this prevented adults from escaping when accessing the tanks. Lab temperature was maintained at or below 65° F (ave. min. 56 °F, ave. max. 69 °F; range 52-73 °F) and a moisture gradient was maintained by misting the tanks through the mesh twice daily.

<u>Adults:</u> Pupae were checked several times each day for signs of eclosion. Adults were sexed and those strong enough to eat and move around were assigned a number, weighed and photographed. Pictures of the hind wing and abdomen will be used to provide baseline data. Males were placed into a "bug dorm" tent (23.6"w x 23.6"l x 23.6"h) with other males from the same breeding group and allowed to "age", as they are thought to learn from one another (G. Pratt, pers. comm.).

Too few males eclosed properly to keep the postdiapause rearing treatments separate, however breeding lines were retained. The Oregon Zoo also received 14 wild males collected from Fort Lewis on 8 May 2008. All males were housed in the same manner. Male butterflies were given a variety of fresh-cut flowers in water, placed in both vertical and horizontal orientations. Non-aromatic flowers and species with small inflorescences seemed to be preferred. Males were also offered a 3:1 water/buckwheat honey solution on a cotton-tipped swab regularly. Following eclosion, female butterflies were also fed the honey solution then placed in a labeled yogurt cup in the refrigerator until mating. Butterflies were transferred to and from the honey solution with a cotton-tipped swab then to a wetted sponge to rinse off the honey. The honey solution was left in the enclosure for a maximum of four hours. The lab was maintained so as to exceed 85˚ F in the early afternoon using light from mercury vapor lamps (150 Watt) to simulate sunshine and stimulate activity.

Captive mating

Several techniques were used on a trial and error basis to induce the butterflies to mate. Initially, the following "hand mating" technique described by Gordon Pratt (pers. comm.) was utilized.

"Remove female from fridge, she will be slowed for ~ 15 minutes before she needs to go back. Pick a male that is aggressive to other males or is trying to breed them. He should have an "upright" stance with perfect antennae. 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. When copulating, they will be facing in opposite directions, at this point the female can be grabbed to move the pair into a screened yogurt container and the container placed in partial shade until they finish coupling. Matings shorter than half an hour or longer than 6 hours have generally not been successful."

A second technique involved transferring a male breeding group to a mini bug tent, which was placed in the window in direct sunlight, and then repeating the hand-mating technique. Males displayed breeding behavior when exposed to the sunlight and when forced into close proximity to each other.

In the interest of time and ease of handling, a third technique, referred to as "hands-off mating" was tried simultaneously. This method involved placing the mini bug tent in the window with a group of males and a chilled female and watching for breeding behavior.

All mating attempts were documented. Data recorded included female identification number, male breeding group, weather conditions, technique used, and timing of copulation. When copulation occurred the pair was transferred to a yogurt cup labeled with individual and breeding lineage identification and the time copulation began. Cups were placed near the window and checked frequently to note the time of separation, at which time the male was returned to his breeding group and the female was fed and placed in an oviposition chamber. If no copulation occurred the female was fed and returned to the refrigerator for future breeding attempts.

Monitoring the release of prediapause Taylor's checkerspot larvae from 2006

A total of 608 prediapause larvae were released on 27 June 2006 (Linders 2007). Eighty-one were collected that morning from the Fort Lewis source site; 527 came from the captive-reared Taylor's checkerspot population at the Oregon Zoo. We veered from our original study design, which was to include equal numbers of wild and captive-reared larvae for two reasons. First, survival of prediapause larvae was higher than expected in the lab. Secondly, the mortality rate of eggs and prediapause larvae at a previous release site, seemingly related to inclement weather, underscored the need to minimize impacts to the source populations by erring on the side of caution when removing animals from the source. Larvae were split between four 50-m^2 plots on each of two release sites on Ft. Lewis [Site FL 1 (301 larvae) and Site FL 2 (307 larvae)] with similar habitat characteristics. Release plots were separated by at least 50 m to maintain independence. About 75 larvae were released per plot; they were placed individually in roughly equal numbers on one of two host plants, *Castilleja hispida* or *Plantago lanceolata*. Both host species were available on all plots.

Prediapause larvae were monitored in the days following release and again the following spring (2007) during the postdiapause phase as described in Linders (2007). Release plots were monitored during the 2007 flight season for signs of adult emergence; no adults were observed. Release plots were monitored again during the 2008 flight season because postdiapause larvae have the potential to reenter diapause and postpone adult development. Each plot was monitored for a total of 30 minutes in 10-minute intervals several times during the 2008 flight season (25 April – 25 May) when suitable weather conditions (e.g., >10°C, wind <10 mph, bright sunlight to soft shadows) permitted.

Release and monitoring of postdiapause Taylor's checkerspot larvae in 2008
Three hundred and forty captive-reared postdiapause Taylor's checkerspot larvae were obtained from the Oregon Zoo in Portland on 6 March 2008 and released at Scatter Creek Wildlife Area in South Puget Sound on 7 March 2008. They were maintained at ambient temperature (unheated vehicle or room) until released. The majority (about 95%) of larvae were already in the sixth instar, at which point they are incapable of returning to diapause. A total of 170 postdiapause larvae were released into each of two 15 x 15-m plots. In one plot larvae were placed singly on a host plant, while in the second they were deposited in groups of five. A 10-m buffer was flagged around each plot using 2-m PVC posts set onto rebar stakes, which was tied with three 2-m pieces of colored flagging. Poles and flagging served two purposes: to provide protection from habitat enhancement and recreation activities, and to act as a potential deterrent to butterflies leaving the release site. Postdiapause larvae also go "walkabout" prior to pupation and can move up to 10 meters per day; it was hoped that most of the wandering larvae would be contained within the buffer. The weather at the time of release was warm and seasonal (9.6 °C; 0.0 mph average wind speed; 100% cloud cover); the sun was visible as a bright spot in the clouds.

Time and resources did not permit a controlled test release of postdiapause larvae inside enclosures, however on 8 March 2008 we surveyed the release plots using belt transects spaced 3.0 meters apart to include the plot plus 1 meter on either side. The purpose was to provide some indication of expected detectability rates for postdiapause larvae. Weather during this survey was 12.0-13.8 °C, average wind speed was 2.3 mph, cloud cover was 80% and conditions were partly sunny. Observers surveyed the opposite plot from the one in which they conducted the release to eliminate this source of bias. Observers tried to walk at a pace of about 1 meter per minute, for about a 60-minute search in each plot.

Flight season surveys were conducted as frequently as possible during the 2008 flight season (25 April – 25 May), whenever desired weather conditions (e.g., >10°C, wind <10 mph, bright sunlight to soft shadows) were anticipated. Two observers were employed in monitoring, simultaneously walking the north and south edges of each plot, starting and ending at the outer buffer boundaries and counting all butterflies encountered. A centerline was flagged to reduce confusion and minimize the likelihood of double counting. Observers then switch transects and recounted the same plot again. Both plots were surveyed at least once and sometimes twice a day, weather permitting, in the morning before 1100 and again after 1400 hours. Data recorded included date, weather, observer, plot #, transect #, start/stop time, and number in each group.

To address the question of site fidelity, adult behavior was monitored in the time between transect surveys. Observers scan counted the plot and vicinity prior to following an individual, and then used a random number table (1-4) to scan back across the area counting adults until reaching the appointed number; the origin of the random count was alternated (inside plot vs. in buffer). When few butterflies were present we followed the first one observed, changing the start position to encompass any variation in position origin. For each adult we record scan count, start time, behavior, initial position (inside plot, in buffer, or outside), and gender, if possible. Each change in behavior triggered a new start time, automatically generating the stop time. We watched for responses to encounters with other butterflies, the poles/flagging, and a "nectar bar" consisting of six 1-gallon potted nectar plants placed along one edge of each release plot. We tried to record directional changes relative to the original flight path and position (e.g., inside plot, in buffer, or outside; toward plot vs. outside), and the angle (obtuse, acute). Individual butterflies were tracked as long as possible or until several minutes worth of observations had been collected. Data was collected using digital voice recorders and subsequent transcription. A Lincoln-Peterson Index will be used to analyze the results of the plot surveys.

7. Results and Discussion

Captive rearing

Survival rates from egg through diapause are exceedingly high under captive conditions at the Oregon Zoo (Table 1). Captive rearing methods for these stages are well developed and results have been confirmed across years. In spring 2008, 20 of 31 (64.5 percent) larvae from the 2006-2007 cohort survived a second diapause compared to 99.6 percent (598 of 600) of the 2007-2008 cohort that survived a first diapause.

	2006			2007		
Survival to next life stage	**#**	**% stage survival**	**Cumulative % of total**	**#**	**% stage survival**	**cumulative % of total**
# eggs	895	100.0	0.0			
Egg to hatching	930	na	0.0	617	NA	0.0
Hatching to warm diapause	870	93.5	6.5	602	97.6	2.4
Prediapause release	*527*	*na*	*43.3*	*na*	*na*	*na*
Warm diapause to cold	300	87.5	67.7	600	99.7	2.8
Cold diapause to postdiapause	288	96.0	69.0	598	99.7	3.1
Postdiapause release	*184*	*na*	*80.2*	*340*	*na*	44.9
# that re-entered diapause	31	34.8	96.7	109	42.2	82.3
Postdiapause to pupation	21	23.6	97.7	85	57.0	86.2
Pupal release	*Na*	*na*	*Na*	*40*	*Na*	93.5
Pupae eclosed as adults	17	81.0	98.2	27	60.0	95.6
Percent eclosed Y1 @ Zoo			1.8			4.4

Table 1. Number, stage-specific survival and cumulative percent of total annual cohort for Taylor's checkerspots reared at the Oregon Zoo in Portland in 2006 and 2007. Released animals are removed from successive calculations of stage-specific survival.

Pupae: The first pupa was seen on 18 March 2008, 30 days after breaking diapause. The first pupa from the 2006-2007 cohort (i.e., those coming through a 2nd diapause) was seen on 26 March 2008, 38 days after breaking diapause.

Pupal weights were compared for 3 postdiapause rearing treatments: bins, tanks and dog trials (Table 2). Pupal weights were significantly different between groups (ANOVA, $F = 6.918$, $df = 2$, $p = 0.002$). There was no significant difference between the pupal weights for larvae raised in bins versus tanks (Bonferroni post hoc test, $p = 1.000$) at the Oregon Zoo. However the dog trial group was significantly heavier than pupae from both bins and tanks (Bonferroni post hoc test, $p = 0.011$ and $p = 0.001$, respectively). Pupal weights for 2nd diapause and WDFW groups were not included in this analysis, but there are included in Table 2.

Treatment	**N**	**Mean wt (g)**	**SD**
Bin	29	0.1699	0.0239
Tank	57	0.1653	0.0289
Dog trial	12	0.1992	0.0466
2nd diapause	4	0.1673	0.0081
WDFW	31	0.1431	0.0282

Table 2. Number, mean and standard deviation of pupal weights from five postdiapause rearing groups at the Oregon Zoo in Portland, Oregon, 2008.

Larvae fed cut plants (bins) and those fed live plants (tanks) differed in their development pathways (Table 3). Larvae raised in bins had a significantly higher likelihood of going into 2nd diapause than those raised in tanks ($Chi^2 = 31.04$, $df = 1$, $p < 0.01$; Table 3). Conversely, larvae raised in tanks had a significantly higher likelihood of pupating than those raised in bins ($Chi^2 = 8.57$, $df = 1$, $p < 0.01$; Table 3). Mortality was low for larvae in both rearing treatments (Table 3), but larvae were more difficult to locate in tanks than in bins. Larvae in tanks also had more difficulty pupating than those in bins (IP; Table 3).

	# larvae	**# dead larvae**	**#/% 2nd diapause**	**#/% pupated**	**IP**	**WDFW release**	**# dead pupae**	**#/% eclosed**	**IE**
Bin 1	27	0	16 (59.3)	11 (40.7)	0	0	1	10 (90.9)	4
Tank 1	26	2	0 (0)	22 (84.6)	3	5	5	12 (70.6)	6
Bin 2	25	1	12 (48.0)	12 (48.0)	0	0	1	11 (91.7)	6
Tank 2	25	0	1 (4.0)	23 (92.0)	1	10	1	12 (92.3)	7
Bin 3	22	0	16 (72.7)	6 (27.3)	0	0	1	5 (83.3)	1
Tank 3	22	3	4 (18.2)	13 (59.1)	4	0	5	6 (46.2)	5
All bins	74	1	44 (59.2)	29 (39.2)	0	0	3	26 (89.7)	11
All tanks	73	5	5 (6.9)	58 (79.5)	8	15	11	30 (69.8)	18
Total all	147	6	49 (33.3)	87 (57.8)	8	15	14	56 (77.8)	29

Table 3. Results of bin vs. tank rearing treatments for Taylor's checkerspot from postdiapause larval through adult (eclosion) stages at the Oregon Zoo in Portland, Oregon, 2008. Percent in 2nd diapause and percent pupated are relative to number of larvae; percent eclosed (emerged as adults) is relative to number that pupated; improper pupation (IP) and improper eclosion (IE) are subsets of these and indicate the number of malformations that occurred at these stages of metamorphosis.

Of 99 postdiapause larvae from the WDFW group, 60 (60.6 percent) returned to 2nd diapause and 34 (34.3 percent) pupated. Twenty-five of these pupae were released and four of the remaining nine (44.4 percent) eclosed, although only one (11.1 percent) did so properly. Ten of 20 postdiapause larvae in the dog trial group pupated (50.0 percent) and 4 (20.0 percent) returned to 2nd diapause; nine of 10 pupae eclosed properly.

A total of 74 adult butterflies eclosed; 33 of these were improper eclosions (i.e., some malformation occurred during this stage of metamorphosis). Thirty-three of 74 (44.6 percent) adults were males, 38 (51.4 percent) were females and 3 (4.1 percent) could not be determined with certainty based on visual characteristics. More female butterflies (26 or 68.4%) eclosed properly than male butterflies (14 or 42.4%). Wing and abdominal measurements are still being compiled and analyzed; these will be summarized and included with other outstanding data in a 2008 annual report.

Captive mating

Two of the three mating techniques, notably those that involved hand pairing, were found to be difficult to implement and had lower rates of return relative to effort. The first mating technique involving handholding females was tried using all captive-reared males and 10 of the captive-reared females on the first day of the mating trials. Researchers found it was difficult to see through the clear plastic panels of the bug dorms, which distorted the view inside. No breeding behavior was observed and males became exhausted when harassed frequently with the hand-held females. The second mating technique, involving the mini bug tents, was also difficult to administer because the tents lacked an access sleeve, making them hard to handle and resulting in a lot of escaped butterflies. Hand mating under these conditions was awkward, difficult, and time-consuming for technicians, although a few successful copulations were achieved via this method. The third ("hands off") mating technique proved to be the easiest to implement as well as the most successful in terms of number of copulations.

Of 74 captive-reared butterflies that eclosed as adults, 46 were fit to use for mating trials, including some with wing deformities. Wing formation is highly sensitive to temperature, humidity and wind condition at the time of eclosion and is not necessarily a reflection of genetic fitness. Thirty-two of 46 were (69.6 percent) females; one wild-caught female from Fort Lewis was also used. The 14 captive-reared males were somewhat lacking in vigor, so 14 additional wild-caught males from Fort Lewis were collected and included in the mating trials; two from the Fort Lewis group had wing deformities.

A total of 75 mating trials were conducted, 25 of which resulted in copulation. Ten of the 25 females that copulated laid eggs; we were unable to mate 8 of the females. Of the 10 females that oviposited, one was from the second diapause group, one was reared in a tank, and two were reared in a bin. All six females in the dog trial group, which were reared outdoors, copulated successfully. Only wild caught males mated with captive-reared females. Some males mated more than once, including one male with deformed wings; both females from these latter pairings laid eggs. A successful pairing also occurred with one female that had eclosed improperly.

Sunlight proved to be crucial to success in both mating and oviposition. No mating attempts were successful unless there was enough sun to create shadows. Similarly, females did not begin to oviposit until the oviposition chambers were placed outside in direct sun; days of overcast skies resulted in little oviposition behavior. Additional data on the results of the captive mating trials are still being compiled, including oviposition methods, data and larval survival and will be summarized in a 2008 annual report.

Monitoring the release of prediapause Taylor's checkerspot larvae from 2006
Dates and weather conditions from the 2008 flight season monitoring are still being compiled. Poor weather conditions and higher priority areas for monitoring during the flight season precluded more intensive flight season surveys at these sites. However no adult Taylor's checkerspots were observed at either release site in 2007 or 2008, nor were any larvae observed in 2008. Only 6 postdiapause larvae were observed of the 608 prediapause larvae released in spring 2007, suggesting mortality during and/or prior to diapause in 2006 was likely very high.

The apparently abysmal results of this release are believed to be primarily due to extremely hot dry conditions at the time of release (> 32 °C; RH), coupled with the strategy of releasing larvae individually. While this design attempts to optimize food availability, predator avoidance as well as access to food and other resources may be depend more on group membership. Checkerspots are highly 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 appear to be far more gregarious than previously thought, with some larvae acting as "scouts" in search of food and/or basking sites; other larvae often follow these leaders. 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 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.

Release and monitoring of postdiapause Taylor's checkerspot larvae in 2008
One postdiapause larval survey was conducted on the day following release. Nineteen larvae were observed in West Plot (survey time 75 minutes) and 18 larvae were observed in East Plot (survey time 101 minutes). We had difficulty matching survey rates, in part due to individual search and recording styles, but also likely due to differences in habitat between the two plots. The amount of thatch was considerably higher in West Plot than in East Plot.

Data are still being compiled and analyzed, however anecdotal results suggest that at least 10 or more adults were observed in and around the release plots on any day containing even a moderate amount of sun. This pattern continued throughout the 11 or so days of the flight season at that site. More adults were generally observed in the vicinity of East Plot, where larvae were released in groups, however the greater amount of thatch in West Plot might have contributed to the difference. Adult behaviors observed at the release site include nectaring, territorial displays by males, mating chases, and "routine movement patterns", whereby butterflies could be observed at predictable locations flying to and from other predictable locations. In contrast to adults emerging in the spring of 2007, only one butterfly was seen flying away from the site in a direction from which no other butterfly was ever observed. Collectively these behaviors are suggestive of recognition and acceptance of the release site, and confer some measure of site fidelity. An initial search of host plants at the site during the prediapause larval phase did not reveal any offspring from this effort. However the relatively small population size and very poor weather conditions in spring 2008 would have severely reduced the likelihood of detecting rare, sparsely distributed larval clusters.

The release of late postdiapause larvae has been the most encouraging approach to date and is the only release method we've tested that has produced adult butterflies, which it has done in two consecutive years (2007 and 2008). Our results appear to be better than those achieved by Harrison (1989), who released 100 postdiapause larvae per site at numerous sites and observed 0-3 adults per site. Even without the hard data assessment, we have reaffirmed last year's conclusion that we can successfully rear Taylor's checkerspot from egg to adult, and did so in both lab and field with reasonable certainty.

8. Future Plans

This is the third year of a multi-year project. Additional work remains in compiling and analyzing the data from 2007-2008, for both the captive and field components of this project. A 2008 annual report will be compiled and distributed. It will include the final details and analysis of the 2007-2008 captive rearing effort as well as results-to-date from the 2008-2009 captive rearing and captive mating efforts. Funding appears to be sufficient to complete the existing reporting, however funding for the 2008-2009 captive rearing effort may fall short of expectations due to an unprecedented number of eggs obtained in spring 2008. Funding for the 2008-2009 field season and the 2009-2010 rearing year are also likely to be insufficient due to changes in the scope and detail of the proposed release. Additional funds are being sought from an outside source for the 2008-2009 field release.

9. Further Information Available

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.

Linders, M. J. 2007. Development of captive rearing and translocation methods for Taylor's checkerspot (*Euphydryas editha taylori*) in south Puget Sound, Washington: 2006-2007 Annual Report. Washington Department of Fish and Wildlife, Wildlife Program, Olympia. 29 pp.

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

10. Questions for Further Research

Does releasing larvae in groups increase the likelihood of survival? If so, what are the mechanisms that lead to this result?

What role does the timing and severity of summer drought play in prediapause larval survival of Taylor's checkerspot and how would we expect global warming to affect this relationship?

Would releasing Taylor's checkerspot larvae earlier in the prediapause phase increase their likelihood of survival?

What are the most important factors affecting prediapause larval survival for Taylor's checkerspot in South Puget Sound?

What is the average and range of survival rates for prediapause Taylor's checkerspot larvae in an established population in South Puget Sound? What are the primary sources of mortality?

What are the mechanisms that lead to site fidelity? How do sites with only a few adults in any given year persist over time?