IMPORTANCE OF ACCURATE ENVIRONMENTAL CONDITIONS DURING CAPTIVE REARING OF AN ENDANGERED BUTTERFLY (*EUPHYDRYAS EDITHA TAYLORI*): COLLABORATIVE RESEARCH WITH SUSTAINABILITY IN PRISON PROJECT AND INCARCERATED TECHNICIANS

by
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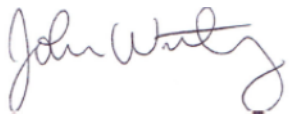

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ABSTRACT

Importance of Accurate Environmental Conditions During Captive Rearing of an Endangered Butterfly (*Euphydryas editha taylori*):
Collaborative Research with Sustainability in Prisons Project & Incarcerated Technicians

Carly M Boyd

Captive rearing is increasingly used as a method to prevent the demise of critically endangered species. If the conditions under which the captive rearing takes place do not mimic conditions in the wild, one result may be low productivity and survival of the species in question. Taylor's checkerspot butterfly (*Euphydryas editha taylori)* is an endangered species endemic to the Willamette Valley-Puget Trough-Georgia Basin ecoregion of the Pacific Northwest. In 2003, *ex-situ* conservation programs for *E. e. taylori* started at the Oregon Zoo, and expanded to Mission Creek Corrections Center for Women (Washington) with the Evergreen State College's Sustainability in Prisons Project in 2011. The environmental targets for *E. e. taylori* in captivity were established based on what is understood to be optimal wild conditions without the extreme that can occur in the field. To determine the frequency in which the environmental targets were met, a thorough examination of measured temperature and relative humidity in captivity was performed. The actual environmental conditions at MCCCW during seven rearing seasons (2013-2014 to 2018-2019, and 2020-2021) were compared to the environmental rearing targets to find the percent of time the environmental targets were met and the percent of days outside of environmental targets for each life stage. Data collected on productivity and survival—including copulations, oviposition success, egg estimates, and larval counts at different life stages—were converted to rates and correlated with how often environmental targets were met in captivity. In addition, since temperature impacts morphological traits, seasonal morphometric data (adult females' weight and wing area of captive and wild butterflies were compared. Environmental targets were infrequently met, depending on life stages. However, the percentage of time targets were met typically did not correlate with butterfly productivity, i.e., survival from one life stage to the next. In addition, captive females weighed more than wild females on average, and had slightly less wing area. There was also evidence for a slight decreasing trend in wing area, but not mass, of females over time.

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ACRONYMS

JBLM – Joint Base Lewis-McChord

MCCCW – Mission Creek Corrections Center for Women

PNW – Pacific Northwest

SPP – Sustainability in Prisons Project

USFWS – U.S. Fish and Wildlife Service

WPG – Willamette Valley-Puget Trough-Georgia Basin

WADOC – Washington Department of Corrections

WDFW – Washington Department of Fish and Wildlife

INTRODUCTION

Prairies of the Pacific Northwest (PNW) are home to one of the scarcest endemic butterflies in one of the rarest ecosystems in North America. Not expected to recover without human intervention, the endangered Taylor's checkerspot butterfly (*Euphydryas editha taylori)* provides an example of a specialist species diminished beyond the point of natural reestablishment due to climate change and habitat loss (Stinson, 2005; New, 2014b). Pacific Northwest prairies previously teemed with butterflies in flight, including *E. e. taylori*, indicating an abrupt transformation to PNW prairies of the modern-day (Stinson, 2005; Balke and Fyson, 2014). A prime candidate for captive rearing and habitat restoration, a single extant South Puget Sound prairie population of *E. e. taylori* remains on the Joint Base Lewis McChord artillery range 76 in western Washington. Due to their diminished status, *E. e. taylori* receiving listing as endangered in Washington State in 2006 and under the U.S. Endangered Species Act in 2013 (USFWS, 2013).

Captive rearing for this species was initiated at the Oregon Zoo in 2003, with the first release occurring on Joint Base Lewis McChord (JBLM) in 2006, continuing annually thereafter (Grosboll, 2004; Linders, 2007; Linders *et al.*, 2019). Official husbandry protocols were developed and implemented in 2009, and in 2011 the program expanded to Mission Creek Correctional Center for Women (MCCCW) through a partnership with the Sustainability in Prisons Project (Barclay *et al.*, 2009). With much to uncover about the specific life-history traits and habitat needs of *E. e. taylori* when conservation initiated, success prevailed thanks to the vast knowledge established from studies that focused on other *Euphydryas editha* subspecies—namely *E. e. bayensis* in

California—and dedicated researchers (Ehrlich and Hanski, 2004b; Grosboll, 2004; Linders, 2007). While the Oregon Zoo experienced challenging seasons with high levels of larval mortality during and after the dormant life stage in more recent years, MCCCW continually sees success which has been partially attributed to captive rearing being carried out predominantly in greenhouses, providing near-ambient conditions during all life stages (Lewis *et al.*, 2018). Even with many successful releases, this species still requires captive rearing to prevent extinction.

Employed to prevent the total loss of critically imperiled species, captive rearing—when aptly coupled with habitat restoration—provides a recovery opportunity for at-risk species which may not naturally reestablish in the wild. Invertebrates make excellent candidates for captive rearing programs, frequently with short life spans, quick reproductive cycles, and small physical sizes, allowing for smaller captive rearing facilities and shorter rearing seasons compared to most vertebrates (Hughes and Bennett, 1991; Pearce-Kelly *et al.*, 2007). Concerns around the overall effectiveness of captive rearing programs arise regarding risks associated with small populations sizes, rearing conditions diverging from wild conditions, and the potential diseases and illnesses to spread to the wild population (Snyder *et al.*, 1996; Norberg and Leimar, 2002; Adamski and Witkowski, 2007). If captive rearing proves successful enough to cease, programs frequently require continued *in-situ* conservation intervention to maintain the population (Pau and Holman, 2019).

Consequences of ineffective captive rearing can include loss of genetic material, adaptation to the captive environment, inbreeding, decrease in overall survivorship, and mortality (Snyder *et al.*, 1996; Ballou *et al.*, 2010; Miller *et al.*, 2014). The advantages

allotted to invertebrates for successful captive rearing also can allow for rapid consequences if captive conditions do not adequately mimic wild conditions necessary for that species to survive and reproduce once released back into the wild (Lewis and Thomas, 2001; Schultz, Dzurisin and Russell, 2008; Christie *et al.*, 2012). While short lifespans can be beneficial in producing many individuals for reintroduction from an endangered invertebrate species, procedures must provide accurate life-history information and be followed precisely to ensure captive conditions mimic wild conditions.

Temperature can critically impact the morphological and life-history traits of butterfly species (Nicholls and Pullin, 2000; Berwaerts, Van Dyck and Aerts, 2002; Norberg and Leimar, 2002). Maintaining environmental conditions that mimic the natural conditions a species would experience in the wild is exceedingly crucial for captive wildlife breeding. For *E. e. taylori*, captive conditions are maintained based on environmental targets developed to mimic wild conditions in the prairies while eliminating extreme conditions that may occur in the wild. Captive rearing conditions get reported based on environmental captive rearing targets that were officially established during the 2014 Captive Rearing Meeting—held between Washington Department Fish and Wildlife, Oregon Zoo, and Sustainability in Prisons Project program partners—and have been adjusted over the years with current targets found in MCCCW captive rearing procedures (Lewis *et al.*, 2018; Curry *et al.*, 2020).

No study has yet determined if captive rearing conditions at MCCCW meet these targets, nor the impacts of the actual environmental conditions on captive rearing outcomes such as *E. e. taylori* productivity and survival through all life stages and

morphological measurements for adult females. This study will determine how often these current environmental targets are realized by analyzing the actual conditions during captive rearing. This information will be used to indicate if correlations exist between *E. e. taylori* survival—from copulation and egg-laying through to post-diapause, pupation, and eclosion—and environmental conditions experienced in captivity. Additionally, the morphological measurements of the wild adult females—brought to the facility to produce larvae for captive rearing—and captive-bred adult females will be compared to support or oppose if selective pressure is acting upon the captive population.

LITERATURE REVIEW

LEPIDOPTERA PRESERVATION

Biodiversity Loss and Conservation

Biodiversity loss disrupts internal structures that uphold diverse ecosystems and contemporary societies, constituting biodiversity preservation an integral and obligatory part of conservation management plans. Unprecedented rates of biodiversity loss hasten extinction rates and drove 200 invertebrate species to extinction in one century—conversely, background extinction rates indicate average loss incurring with one species lost every 50 years (Ceballos, Ehrlich and Dirzo, 2017). This sixth mass extinction event—named the Anthropocene extinction due to the overwhelming evidence designating humanity's pursuit of growth and development primary contributors—necessitates the commitment to preserve the biodiversity that remains (Agrawal and Redford, 2009). Worldwide exponential population growth and increased urbanization coupled with the expanding access to technology and divergence from historically less invasive resource management tactics in the global north fuels this excessive consumption and degradation of biodiversity (Wood *et al.*, 2000).

Primary outcomes of this ramped development include land use and transformation, overexploitation of resources, introducing invasive species, and climate change, concurrently deteriorating habitat and accelerating loss (Ehrlich and Wilison, 1988; Vijeta, Shikha and Anamika, 2021). Habitat degradation principally drives biodiversity vulnerability and narrows the prospects for species recovery since underlying impacts may not immediately present themselves (Tilman *et al.*, 1994; Guardiola *et al.*,

2018). In addition, small or fractured populations have a higher chance of having lower genetic diversity with no connectivity to other populations' genetic material: a small population's gene pool, when cut off from any other source of genetic material, increases the risks of inbreeding depression and genetic drift which can result in lower fitness for the populations (Gilbert and Singer, 1973; Frankham, Briscoe and Ballou, 2002; Rochat *et al.*, 2017).

Modern biodiversity loss prevention methods tend to function retroactively and the need for intervention generally presents itself once a species reaches a point of critical concern. The preeminent method for combating extinction is to prevent endangerment and habitat loss, though, for at-risk species in the present, this alone likely will not lead to recovery. Habitat degradation often impacts the ecosystem's composition indeterminably at first, and once species loss accelerates, the impacts of habitat deterioration become apparent (Kuussaari *et al.*, 2009). This phenomenon, termed an extinction debt, insidiously delays recovery response time and action, as the impact of the environmental changes on the residing population do not immediately present themselves yet will rapidly become apparent as the habitat continues to deteriorate (Tilman *et al.*, 1994; Kuussaari *et al.*, 2009; Guardiola *et al.*, 2018). Although extinction debts often occur, New (2014b) discusses how recovery prospects through conservation and habitat management plans remain possible if the species still endues.

Lepidoptera Loss and Conservation

Lepidoptera—the order of butterflies and moths—hold no immunity to the unprecedented extinction rates plaquing global biodiversity. In one study of 435 butterfly species native to Europe, about 83 butterflies (19%) were considered threatened or near-

threatened, 34 species (8%) vulnerable or endangered, and four species (1%) critically imperiled or extinct (van Swaay *et al.*, 2010). Research conducted at a nature reserve in Sweden over 50 years revealed that 159 of 597 species (27%) could no longer be found by 2004, declaring these species extinct (Franzén and Johannesson, 2007). These examples represent a small proportion of studies that collectively reveal an alarming downward trend in butterfly diversity worldwide (Sánchez-Bayo and Wyckhuys, 2019). Efforts to preserve insect biodiversity increased over time, corresponding with an increase in understanding of the pivotal role many insects play in their ecosystems. The sheer quantity of insects indicates their importance, suggesting that if insect species started vanishing at accelerating rates, the impacts would ripple throughout entire ecosystems (Black, Shepard and Allen, 2001). Ecologists and restorationists recognize the importance of insects in their ecosystem, however, the average person may not validate this reality, and minimal public advocacy for insect preservation can lead to diminutive political and financial support. For example, out of 720 animal species listed under the U.S. Endangered Species Act, only 13% are insects, even though research indicates insects make up at least 70% of animal species richness (Ehrlich and Hanski, 2004b; USFWS, 2021).

One of the most popular insect groups, butterflies possess an immense aesthetic value that contributes to their status of charismatic ambassadors to their ecosystems, holding the tremendous potential to bring awareness to declining habitats with their absence (Ehrlich and Hanski, 2004b; New, 2014a). Congruent with this level of attention, butterflies make up 33% of insect species and 86% of Lepidopteran species, listed under the Endangered Species Act (T.R. New, 1997; Sharma and Sharma, 2017; USFWS,

2021). Some consider butterfly species indicators of their ecosystems' current health status. Given Lepidoptera species' high vulnerability to deteriorating habitat, research shows a decline or change in ecosystem operations corresponding to loss of Lepidoptera species (Erhardt and Thomas, 1991; Cleary, 2004; Sánchez-Bayo and Wyckhuys, 2019). This preferential treatment allows for faster responses to at-risk butterfly population declines. At the same time, this promotes further development of insect conservation studies and model systems for populations through detailed research that can be applied to other populations outside that species and order (Hanski, Hellman, *et al.*, 2004; New, 2014c).

Industrial agriculture and the resulting pollution from pesticides and herbicides contribute to Lepidoptera risks, in conjunction with pathogens, host-species loss, invasive species, and climate change (Pyle, 1976; McLaughlin *et al.*, 2002; New, 2014b). The combination of Lepidopterans being ectotherms that behaviorally thermoregulate and one of the most well-studied insect taxa has made many Lepidoptera species indicators of the impacts that the current climate crisis is having on their habitat and ecosystem. One of the most prominent phenological responses to climate change involves the asynchrony between animal development times and host plant development and senescence (Hill *et al.*, 2021).

Many generalist species are better able to adapt to climate change through dispersal ability and limited restrictions to specific hosts or nectaring plants. For example, in response to warming winters, sachems (*Atalopedes campetris),* a small skipper butterfly, shift poleward to more suitable habitat amid the climate crisis (Crozier, 2003, 2004). Far from positive, this effect, referred to as ecological drift, leads to generalists

replacing specialists, decreasing the biodiversity within the ecosystem (New, 2014b). For more specialized butterfly species, this level of adaptation to climate change is not ordinary. Intrinsic barriers often restrict specialists' ability to disperse along with physiological restrictions regarding suitable habitat and host plant species (Parmesan *et al.*, 2015; Hill *et al.*, 2021). All-encompassing *in-situ* and *ex-situ* conservation methods used in conjunction make the optimal method for preventing the total demise of these specialist species.

BACKGROUND ON CHECKERSPOTS

Description of Checkerspots

First described by Boisduval in 1852, 26 different *Euphydryas editha* subspecies reside throughout North America (Boisduval, 1852; Pelham, 2012). Named for their appearance, these graceful butterflies brandish a checkered pattern on their wings that demands adoration and captivates the imagination (Murphy et al., 2004, p. 18). Medium in size, checkerspots wingspan average 1.5-3 cm long and primarily have orange, red, black, or brown colored checkered-pattern (Murphy et al., 2004, p. 22; Pyle and LaBar, 2018, p. 6348). Checkerspots (Euphydryas editha) belong to the Nymphalid family, commonly referred to as the "brush-foot" butterflies. In adult females, the two front legs developed over time into sensory organs that assess the acceptability of potential host plant based on their chemical properties and condition for oviposition (Murphy et al., 2004, p. 18; Willmott, 2004). Not unique to checkerspot butterflies, it is common to see checkered patterns in various colors on other species in the *Nymphalid* family, and the checkered pattern seems to make prey tracking an arduous task for predators during flight

(Nijhout, 1991; Pyle and LaBar, 2018). However, the wings of checkerspot butterflies do bear a distinct "*editha* line" that runs between the red or orange bands, distinguishing them from other similar-looking *Nymphalidae* species (Murphy *et al.*, 2004, p. 18).

History of Checkerspot Butterflies

About 5 miles from Stanford University campus, on Stanford's Jasper Biological Preserve, Paul Ehrlich identified a small, checkered butterfly on a hilltop containing a fragmented patch of grassland encompassed by a border of dense chaparral hillside. Later named the bay checkerspot butterfly (*E. e. bayensis*), this subspecies eventually became a model system for population biology after extensive studies that transpired over time (Ehrlich, 1961; Agrawal and Redford, 2009). This research exposed the immense cost resulting from population extinctions; genetic diversity held within distinct populations grant survival advantages when habitat corridors allow for an exchange of genetic material between them (Ehrlich and Hanski, 2004a, p. 7). One instance of utilizing *E. e. bayensis* research to inform relationships in other species details how the microclimate impacts dictate phenological relationships between development times of *E. editha* larvae and their host plant. This association leads to variation in population development and survival under a diversity of microclimates and applies to numerous *E. editha* ecotypes and other butterfly species that have phenological relationships with their host species (Hanski, Hellman, *et al.*, 2004). Not only did this species become the cornerstone for metapopulation research for that time, but the data collected from these studies have also informed initial life-history traits of checkerspot species across North America (Ehrlich and Hanski, 2004a; Warren, 2005).

Emphasizing the significance of population biology, the sedentary nature of these butterflies led to disparities in the populations that reside in separate geographical regions and variation within a single population. Intrinsic barriers to dispersal in checkerspots limit the flow of genetic material between isolated populations in fragmented habitats. Studies revealed initial colonies consisting of three populations showed virtually no migration, and therefore no flow of genetic material, between the populations in question (Ehrlich, 1961; Singer and Hanski, 2004, p. 184). A more recent study clarified these findings, showing female Taylor's checkerspots (*E. e. taylori)* seldom in continuous flight. Though they found males to be much less sedentary and able to move across habitat boundaries, they only do so under favorable circumstances and will not disperse to suitable sites over 100 miles away (Bennett *et al.*, 2013). This inability to maneuver between fragmented habitats isolates populations further by preventing the establishment of metapopulations and inhibiting reinstatement after stochastic events (Brückmann, Krauss and Steffan-Dewenter, 2010; Hanski, 2011).

Selection pressures fluctuate depending on habitat types influencing a population's genetic makeup, and habitat uniformity varies, leading to genetic phenotypic divergencies even within a single population (Ford and Ford, 1930; Ehrlich, 1984; Murphy *et al.*, 2004). At-risk checkerspot ecotypes benefit from their subspecies classifications, allowing them to receive federally listed under the ESA, however, this often occurs after populations become critically at-risk. Currently, the federal listing status for *E. e. bayensis* is threatened, *and E. e. taylori* and *E. e. quino* obtained endangered listing statuses, all of which benefit from protections that prohibit direct harm

and preserve habitats, intending to reestablish metapopulations (Murphy *et al.*, 2004, p. 24; USFWS, 2013, 2021).

Life History Overview of Checkerspots

Recognition and follow-up from the scientific community subsequent to initial research allowed *Euphydryas editha bayensis* to become an extensively studied subspecies; this research constituted most of the early life-history framework for other checkerspot subspecies (Ehrlich, 1992; Ehrlich and Hanski, 2004b; Pyle and LaBar, 2018). The flight season for checkerspots in lower elevations commences around March to May, while flight seasons in higher elevations begin around June to August (Ehrlich and Hanski, 2004b). Mating behaviors vary among subspecies, but males often exhibit patrolling and perching behaviors to secure a mate, while females remain relatively sedentary during their search for suitable host plants for oviposition (Bennett, Smith and Betts, 2012; Bennett *et al.*, 2013). Checkerspots predominantly utilize host plant species from four families within the subclass *Asteridae*: *Acanthaceae, Asteraceae, Scrophulariaceae,* and *Plantaginaceae,* with the latter two families containing iridoid glycosides that, when ingested, will ultimately produce unpalatable individuals as a predator defense (Murphy *et al.*, 2004, p. 22; van Nouhuys and Hanski, 2004). Checkerspots use these preferred host plants for ovipositing and larval development, though larvae host plant preferences tend to be less stringent than adult checkerspots depending on the subspecies and dispersal abilities (Kuussaari *et al.*, 2004, p. 142).

Ovipositing females lay eggs in masses, on or around host plant leaves. Studies report *E. e. bayensis* cluster sizes to vary from 20 to 350 eggs, averaging 40-50 eggs per cluster, with preliminary research showing that a single female *E. e. bayensis* can lay up

to 1,200 eggs in a lifetime (Labine, 1966, 1968; Singer, 1972; Murphy *et al.*, 2004, p. 25) Eggs develop over a 13 to 15 day period before hatching into first instar larvae. Larvae of the same egg clusters create tents of webbing to live collectively and consume host plant leaves. Larvae continue to feed and develop through instars, the quantity of which varies depending on the subspecies, with *E. e. bayensis* larvae known to develop through 3 instars prior to an obligatory diapause. The larvae physically mature and darken in color over this 3-to-5-week prediapause period, demonstrating their micro-climate requirements and need for basking behaviors for thermoregulation during adult and larval life stages (Murphy *et al.*, 2004, p. 22). Larvae also produce setae as they develop and darken, delicate hair structures all over their bodies that mitigate heat loss (Weiss, Murphy and White, 1988; Hellmann *et al.*, 2004, p. 47).

Having developed sufficiently before all available host plants senesce—and maintaining their gregarious lifestyle—checkerspot larvae enter the obligatory dormancy period in mid-summer or early fall to forgo and survive extreme environmental conditions (Kuussaari *et al.*, 2004, p. 139). Larvae break diapause—concluded by late winter rains or melting snow depending on the species and environment—and the surviving postdiapause larvae continue feeding for a few weeks. Postdiapause larvae can migrate 10-20 meters per day to find acceptable host plants and foraging conditions to grow from 3 mg at wake up to the necessary size of 300-500 mg for pupating (Hellmann *et al.*, 2004, pp. 46–47). If adverse weather conditions arise, checkerspots may reenter diapause and extend their lifecycle into the next year instead of pupating in the hope of a higher chance of reproductive success (Kuussaari *et al.*, 2004, p. 139). Postdiapause

larvae eventually pupate, often found on vegetation right above the ground, and after a period eclose as adults to complete their lifecycle (Kuussaari *et al.*, 2004; Potter, 2016).

Environmental Conditions and Checkerspots

Analogous with other insects, checkerspot life-history traits hinge on the phenological circumstances influencing reproductive success (Taylor, 1981; Hellmann *et al.*, 2004). Contingent upon synchrony between resource availability and adult butterfly emergence, the reproductive success of checkerspots depends upon an individual's opportunities to mate, availability and condition of host plants, ability to circumvent extreme weather conditions, and offspring survival (Weiss *et al.*, 1993; Kuussaari *et al.*, 2004). Variation in adult emergence times primarily impacts these phenological factors and therefore reproductive success; larvae produced by adults that eclose early in the flight season experience higher survival in most habitat conditions, whereas females that eclose mid-to-late in the flight season produce larvae with more habitat restrictions and lower prospects for survival, if at all (Weiss, Murphy and White, 1988; Weiss *et al.*, 1993; Hellmann *et al.*, 2004, pp. 51–53). In homogeneous habitat settings, emergence times depend on weather conditions and how rapidly larvae develop, established primarily by larvae's genetic and phenotypic make-up given the minimal variation in host plant species availability or microclimate from one host plant to another. However, in heterogeneous habitat settings, emergence times are more influenced by microclimates that determine development rates and therefore development timing; in cooler temperatures, the likelihood for larvae to develop appropriately and in time for winter diapause decreases, explaining why individuals that enclose later in the season have lower reproductive success (Hellmann *et al.*, 2004).

Emerging early and arduously securing a satisfactory host plant for oviposition alone does not guarantee larval survival. Two primary causes of larval death arise, one affecting the species disproportionally more than the other. Predation by spiders, insects, parasitoids, and vertebrates such as birds are known to feed on checkerspot species, however, there is little evidence that predation or parasitism contributes to significant quantities of larval mortality (Boggs and Nieminen, 2004; Kuussaari *et al.*, 2004). The gregarious lifestyle of checkerspot larvae, the unpalatable nature of larvae if primary host plants contain iridoid glycosides, and the deterrent of external coloration increase this species' likelihood of evading predation (Kuussaari *et al.*, 2004, p. 149). Far more prevalent in *E. editha*, larval mortality by starvation critically influences population dynamics. Preventing starvation in prediapause larvae becomes contingent on meeting microclimate requirements under which host plants are grown, influencing survival and development times (Kuussaari *et al.*, 2004, p. 138). For checkerspots, these optimal microclimate conditions tend to be warm and dry.

The causes and impacts of starvation range depending on the ecosystem and habitat types, varying by subspecies: in areas where summers are dry and warm, early host plant senescence and the subsequent pre-diapause larval mortality show less than 10% of offspring making it to adulthood (Kuussaari *et al.*, 2004, p. 149; Singer and Hanski, 2004). A study conducted by Hellmann (2002) showed high surface temperatures directly influencing the rate of senescence in *Plantago erecta* and *Castilleja*—two host species utilized by *E. editha*—though *Plantago* senesced more rapidly than *Castilleja.* In these climates, a conflict arises between larval and host plant requirements, where sunny, warm weather that accelerates larval development also accelerates host plant senescence.

Alternatively, cool and rainy weather that prolongs host plant senescence slows larval development, delaying diapause and postdiapause. A delicate relationship between adult emergence times, larvae development, host plant senescence, and environmental conditions becomes apparent. This correlation principally influences adult emergence timing for the following season and, therefore, a primary influence on population size fluctuations in checkerspot species (Singer, 1971, 1972; Kuussaari *et al.*, 2004). Alternatively, where early senescence infrequently occurs, restricted host plant availability may lead to larval competition causing overconsumption and eventual pre-diapause mortality. Larval-host plant relationships often phenological strain, depending on subspecies and habitat types, increasing in frequency as climate change pushes them farther out of sync (Singer, 1972).

In addition to temperatures impacting larval and host plant development, other weather patterns notably influence larval survival by expediting or decelerating larval maturation in relation to the host species. Early studies of five specific *Euphydryas editha bayensis* populations observed distinct waning through all populations following a drought in CA that spanned from 1975 to 1977 (Ehrlich and White, 1980). While dry and warm conditions are often optimal for developing many *E. editha* subspecies, similar to high temperatures, extreme arid conditions accelerate host plant senescence, leading to larval starvation (Singer, 1972; Hellmann *et al.*, 2004, pp. 51–53). Desiccation of eggs and larvae may occur in exceedingly dry conditions, though this is less likely than prediapause larval starvation and this threshold for desiccation depends on the subspecies (Kuussaari *et al.*, 2004). In contrast, the research found excessive rainfall spurs population declines due to considerably increasing development times in prediapause

larvae (Dobkin, Olivieri and Ehrlich, 1987). Moisture and precipitation, much like temperature, create a trade-off between the relatively dry requirements needed for larval development and the moist conditions that would slow larval development and prolong host plant senescence.

Habitats with diverse topography result in various microclimates, likewise prompting severance between larval development and host plant senescence, burdening postdiapause larvae and pupation development times (Singer, 1972; Weiss, Murphy and White, 1988; Hellmann *et al.*, 2004). An early study examining the impact of slope direction on larval survival showed significant differences in temperatures on the south and north-facing slopes versus flat ground. While north-facing slopes saw ground conditions analogous to air temperatures, areas of flat ground surpassed air temperature by 41º-54ºF, and south-facing slopes recording temperatures 68º-86ºF higher than air temperatures (Singer, 1972; Weiss, Murphy and White, 1988; Hellmann *et al.*, 2004), showing south-facing slope temperatures transcending flat ground conditions by 27º-32ºF. Contrary to initial thoughts, the study predominantly found surviving postdiapause larvae on cooler north and east-facing slopes. Although the cooler conditions slowed prediapause larval development, it prolonged host plants' senescence and prevented larval starvation, allowing larvae to reach diapause eventually. Postdiapause larvae would emerge on these cooler slopes and exhibit basking behavior, utilizing their black color and setae to increase body temperatures as much as 50-54ºF above-ground air temperatures and prevent heat loss (Weiss, Murphy and White, 1988; Hellmann *et al.*, 2004).

In alignment with other life stages, once postdiapause larvae pupate, pupae development rate corresponds to the microclimate of the slope and the ability efficiently thermoregulate; the success of pupal development is contingent upon prediapause dispersal ability and proximity to warmer microclimates (Weiss *et al.*, 1987, 1993; Weiss, Murphy and White, 1988). Similar to other life stages—specifically more sensitive ones including adults, eggs, and early instar prediapause larvae—extreme heat can lead to pupal mortality, with an unpublished study observing wide-spread mortality in pupae on south-facing slopes during a heatwave where ground temperatures, at times, exceeded 105ºF (Hellmann *et al.*, 2004, p. 47).

All of these environmental factors that influence larval and pupal development times contribute to the timing of adult emergence, which, as previously discussed, impacts reproductive success more than any other factor. The advantages allotted to larvae produced by early-emergence females allows these larvae to circumvent most other elements that influence survival and population fluctuations (Weiss *et al.*, 1993; Cushman *et al.*, 1994). This study went on to show that not only does annual average emergence timing vary by 28 days on the same slope, adult emergence varies nearly 43 days between north and south-facing slopes, with postdiapause larval distribution impacting the average emergence time for adult butterflies by 10 to 12 days (Weiss *et al.*, 1993; Hellmann *et al.*, 2004, p. 48).

The severe impacts of extended adverse environment conditions spanning multiple seasons inevitably lead to severe population declines; prolonged years of extraordinarily cool and moist or warm and dry conditions destabilize the already delicate relationship between larval and host plant development so significantly and can bring

about regional extinctions (Singer, 1972; Hellmann, 2002; Hellmann *et al.*, 2004, p. 51). Any outside variable that could impact this dependency may have a similar outcome. For example, climate change increases variability in weather patterns, and a study looking into the ramifications of increased inconsistent precipitation patterns on *E. e. bayensis* populations indicated a corresponding surge in population size variability that can quickly induce local extinction (McLaughlin *et al.*, 2002). The primary preventative method for regional population extinctions involves ensuring heterogeneous habitats have numerous host species to ensure sufficient host overlap and preventing the degradation of or reestablishing metapopulations (Singer, 1971, 1972; Fleishman *et al.*, 2000; McLaughlin *et al.*, 2002; Hellmann *et al.*, 2004).

EUPHYDRYAS EDITHA TAYLORI BACKGROUND

Euphydryas editha taylori *Description & History*

First described by W. H. Edwards in 1988, *Euphydryas editha taylori* was named after a well-known Lepidopterist, George W. Taylor (Guppy and Shepard, 2001). This subspecies was previously found in prairie habitats from the Willamette Valley in OR through the Salish Lowlands in WA up to the Georgia Basin in BC—the Willamette Valley-Puget Trough-Georgia Basin (WPG)—and is one of 15 at-risk species found throughout this region (Schultz *et al.*, 2011; Pyle and LaBar, 2018). Six of these at-risk species received federal recognition as candidates for listing, or received listing status as threatened or endangered under the ESA (Pyle and LaBar, 2018; USFWS, 2021). Not the only checkerspot species residing in the PNW, the petite *E. e. taylori* is most similar in size to the *E. e. edithana* in comparison to the larger *E. e. beani* and *E. e.*

colonia subspecies, the latter of which being the largest of the four (Guppy and Shepard, 2001; James and Nunnallee, 2011; Pyle and LaBar, 2018). Possessing modest rounded wings spanning 2.6-4.3 cm, *E. e. taylori* also has the darkest coloration of all four PNW subspecies, with alternating predominantly orange, black, and white bands with a distinct, primarily orange editha line running through them (Heron, 2011; Potter, 2016).

The only PNW checkerspot subspecies that do not inhabit the cascades, *E. e. taylori's* historic range once occurred throughout the WPG Today, most of the remaining populations reside in Washington. The population of primary focus in this research occupies Joint Base Lewis McChord artillery range 76 in Pierce County, WA (Stinson, 2005; Potter, 2016). One of only 47 butterfly species previously found throughout this habitat, this *E. e. taylori* population dwells within the cool and wet South Puget South Prairie landscape, an inadequate habitat type for most butterfly species (Dunn and Fleckenstein, 1997; Pyle and LaBar, 2018). *E. e taylori* habitat requirements remain consistent even within a range of habitat types and elevations; suitable habitat requirements include copious host and nectar plants—preferably from various species—native grasses, patches of bare terrain, and open structure forbs (Stinson, 2005; Potter, 2016). Defined as prairie-oak habitats, these prairies predominantly contain grasses and white oak *(Quercus garryana). E. e. taylori* are not restricted to prairie-oak habitats, found in glacial outwash prairies, forest balds, oak woodlands, coastal bluffs, and stabilized dunes (Guppy and Shepard, 2001; Stinson, 2005; Schultz *et al.*, 2011; Potter, 2016; Pyle and LaBar, 2018).

E. e. taylori historically utilized the golden paintbrush (*Castilleja levisecta*) as a host plant. Though today they primarily utilize the introduced English plantain (*Plantago*

lanceolata) in addition to harsh paintbrush (*Castilleja hispida*), slender plantain (*Plantago elongate*), sea blush (*Plectritic congesta*), dwarf owls-clover (*Triphysaria pusilla*), blue eye Mary (*Collinsia spp*.), and owl's clover varieties *(Orthocarpus spp*.) (Guppy and Shepard, 2001; Severns and Warren, 2008; Schultz *et al.*, 2011; Buckingham *et al.*, 2016; Haan, Bowers and Bakker, 2021). An unlikely relationship arose between the non-native *P. lanceolata* and endangered *E. e. taylori,* taking the place of a primary host species. However, *E. e. taylori* will utilize native host plants if available, climate change hastens senescence in many of these native species, causing misalignment in life-history time-frames between former host species and *E. e. taylori* (Buckingham *et al.*, 2016; Haan, Bowers and Bakker, 2021). Primary nectaring species for *E. e. taylori* include common camas (*Camassia quamash*), nineleaf biscuitroot (*Lomatium trieratum*), and Puget balsamroot (*Balsamorhiza deltoidea*) (Stinson, 2005; Potter, 2016).

Euphydryas editha taylori *Life History*

Akin to other checkerspot ecotypes, *Euphydryas editha taylori* has life-history traits comparable to the well-researched *E. e. bayensis*: *E. e. taylori* adults emerge around mid-April to late-May, and fly until about mid-June, depending on weather and resource availability (Stinson, 2005; Potter, 2016). As previous studies indicated for *E. e. bayensis*, *E. e. taylori* emergence times also vary greatly depending on weather and microclimate conditions, host plant availability, geography, and the ever-changing conditions of climate change that influence larval development times. Females search for acceptable host plants for oviposition during this flight period—predominantly utilizing *P. lanceolata*—while males perch and patrol for females to mate with (Murphy

et al., 2004; Bennett, Smith and Betts, 2012). Once females have selected a host plant for oviposition, they will lay eggs in clusters on or around host plant leaves. *E. e. taylori* females usually lay multiple egg clusters over the flight season and conclude their life soon after, completing the species' one-year life cycle, making them univoltine (Potter, 2016). Eggs develop over 8-14 days before hatching around mid-June (Barclay *et al.*, 2009; James and Nunnallee, 2011; Curry *et al.*, 2020).

Gregarious larvae feed on host plants and disperse minimal distances, as needed, in search of more food or optimal conditions for development. They develop from first instar at hatch through to fifth instar, slowing eating, molting, and developing new skins at the onset of each instar (Barclay *et al.*, 2009; Potter, 2016). Once larvae reach fifth instar—about a month after hatching, around July-August—larvae will soon stop eating entirely and enter diapause, a dormant phase that, depending on development times, aligns with host plant senescence to prevent larval starvation and allow them to survive through the winter (Hellmann *et al.*, 2004; Kuussaari *et al.*, 2004). Around late February to early March, environmental conditions trigger larvae to break diapause and resume feeding for development as postdiapause larvae. Postdiapause larvae have a higher capacity to disperse in search of proper host plant and microclimate conditions if needed, and about late-March to early-May, larvae will pupate (Hellmann *et al.*, 2004; Stinson, 2005; Potter, 2016). Pupation development times can vary greatly depending on environmental conditions, and adults will eclose anywhere from two to six weeks after pupation, emerging for the flight season (Barclay *et al.*, 2009; Potter, 2016).

Euphydryas editha taylori *Habitat Loss & Decline*

Conservationists witnessed drastic declines in *Euphydryas editha taylori* populations following the dramatic loss of grasslands state and nationwide due to this species' immense sensitivities to habitat changes and disturbances. In Washington state alone, prairies once covered over 150,000 acres of land and a meager 8% of grasslands remaining today, with most composed of only 25% native grasses (Stinson, 2005; Peter and Harrington, 2014). Nationally, the primary reason for grassland loss occurs through land transformation for other uses, primarily urban spread and conversion to farmland and pastures; known for their productive soils, prairies require little effort to convert for agricultural purposes since they do not require clearcutting, allowing the minimal barrier to entry for development (Dunwiddie and Bakker, 2011). Urban development also contributes to the loss of prairie-oak habitats and grasslands. The dominant habitat type throughout the Willamette Valley-Puget Trough-Georgia Basin consists of prairies and grasslands. Though the WPG accounts for less than 4% of land in the PNW region, 75% of the population in this region occupy counties or districts that entirely or partially overlap with the WPG (Dunwiddie and Bakker, 2011).

Wildfire suppression accounts for much of the remaining prairie deterioration, prior to colonization throughout North America, many First Nations managed grasslands within the Coast Salish region of what is now known as the northern portion of the PNW in the United States and BC in Canada (Wonders, 2008). One of the most notable First Nations management practices included regular controlled burns of grasslands which allowed game management, hunting, traveling, and cultivations of plants for food, medicine, and basketry, contributing significantly to maintaining cultures and economies

(Agee, 1989, 1996; Boyd, 1999; Hamman *et al.*, 2011; Ryan, Knapp and Varner, 2013). Following colonization that brought disease, war, genocide, and the imposition of Euro-American culture on all remaining native peoples, controlled burns ceased in the 1800s, and the succession and eventual occupation by Douglas-firs that followed turned many grasslands into forests (Hamman *et al.*, 2011; Peter and Harrington, 2014).

In recent years, western cultures gained a better understanding of prescribed burns as a restoration tool for managing ecosystems by looking to these historical methods. Western restorationists worked with and learned from First Nations people to reintroduce these management tools under the designation "Traditional Ecological Knowledge" to reverse some prairie succession and degradation (Hamman *et al.*, 2011; Trager and Daniels, 2011). Other causes of prairie loss nationwide include habitat fragmentation, loss of native fauna, the introduction of non-native and invasive fauna, resource extraction, and military training (Stinson, 2005).

Historically, 45 *E. e. taylori* population sites existed in WA, and 25 other population sites existed between OR and BC. In response to habitat loss and fragmentation, *E. e. taylori* populations began to decline dramatically by 1991, while just 11 years prior, reports describe dense clusters of *E. e. taylori* found in OR, providing an example of an extinction debt evoked by habitat degradation beginning to catch up to the subspecies by the early 1990s (Dornfeld, 1980; Stinson, 2005). Populations in WA and BC saw analogous declines in *E. e. taylori* populations, and information available today shows only eight extant populations persist in WA (Holtrop, 2010; Holtrop, Hays and Potter, 2013; Potter, 2016), three populations in OR (Warren, 2005; Page *et al.*, 2008) and one population in the Denman Island of BC (Guppy and Shepard, 2001; Balke and

Fyson, 2014). In WA, most extant *E. e. taylori* populations dwell around the forests of the northeastern Olympic Peninsula, utilizing habitat and host plants that grow in forest clearings and forest balds (Potter, 2016). These forest populations comprise 6 of the eight total WA populations with one additional population off the Sequim coast, north of the forest populations, utilizing dunes for habitat. The remaining singular population inhabits the prairies of JBLM, approximately 110 miles away from the closest extant populations (Holtrop, Hays and Potter, 2013; Potter, 2016; Linders *et al.*, 2019). A stark contrast to the 32 historic populations throughout the South Puget Sound prairies, this isolated population would perish without intensive intervention (Stinson, 2005; Holtrop, 2010). This dire situation culminated in the listing of *E. e taylori*, not only as an endangered species under the Endangered Species Act in 2013 but prior to that, *E. e. taylori* acquired endangered listing status in Canada by 2011 and WA by 2006 (Heron, 2011; USFWS, 2012; Potter, 2016). Considering this species significantly reduced population size and unfeasibility to repopulate naturally, the best option to prevent the total demise of this delicate butterfly is through all-encompassing *in-situ* and *ex-situ* conservation methods.

Management & Restoration of Euphydryas editha taylori *and their Habitat*

Once the threat to *Euphydryas editha taylori's* persistence was deemed severe enough for state and federal agencies to enable listing status, recovery plans for *E. e. taylori* and their habitat began development. A massive undertaking, numerous organizations and agencies collaborate to form partnerships, including the Cascadia Prairie Oak Partnership, which works throughout western WA and OR to restore and preserve WPG prairies (Dunwiddie and Bakker, 2011). This cooperative includes state and federal agencies, universities, municipalities, non-profit organizations, and private

landowners, including First Nations peoples, USFWS, WDFW, Center for Natural Lands Management, Department for Natural Lands Management, The Nature Conservancy, and Sustainability in Prisons Project (Hamman, 2018). Primary tenets of prairie restoration focus on preventing succession and infringement of invasive species through mowing, prescribed burns, and the practice of flagging and removing or spraying invasive and non-native species with herbicides (Hamman *et al.*, 2011; Schultz *et al.*, 2011).

Balancing habitat restoration efforts and endangered species recovery proves arduous, especially in the case of sensitive or specialized native plant and animal species. Considered one of *E. e. taylori's* historic host species, *E. e. taylori* will utilize available *C. levisecta* for oviposition and larval development, however, *E. e. taylori* shows lower survival in early instars when utilizing this species (Buckingham *et al.*, 2016; Haan, 2017). Some speculate whether a loss of adaptation occurred, limiting *E. e. taylori's* ability to utilize *C. levisecta* effectively and restricting habitat management efforts. However, earlier studies show little evidence supporting this other than limited interactions in the wild between the two species, which suggests these two species possibly are not historically linked to the extent previously described (Haan, 2017). A more recent study showed lower oviposition preference for *C. levisecta,* indicating a change from previous studies, with larval growth limited on *Castilleja* when they consumed predominantly leaves instead of bracts (Haan, Bowers and Bakker, 2021). Presently, captive and wild *E. e. taylori* primarily utilize *P. lanceolata* for oviposition and larval development, creating contention between *E. e. taylori* recovery that relies on this non-native species and prairie recovery that revolves around returning South Puget Sound prairies to native plant communities (Severns and Warren, 2008; Dunwiddie and Rogers,

2017). For this reason, efforts to restore prairies are strategic, careful, and never entirely based around the threatened species in question also considering native species in other portions of the ecosystem.

Essential components of prairie habitat requirements exist to ensure *E. e. taylori* recovery, and specific habitat management plans were established and implemented to benefit the endangered butterfly and the prairie ecosystem. These include large, open areas that are well-connected to itself and other suitable adjacent sites, bare ground with surrounding herbaceous vegetation, high-density patches of acceptable host and nectar plant, and some topographic variety to allow for minor microclimate differences (Stinson, 2005; Severns and Grosboll, 2011; Potter, 2016). Other management efforts revolve around propagating and planting native grasses and forbs and non-native English plantain and removing invasive species such as Scotch broom (*Cytisus scoparius*) and Himalayan blackberry (*Rubus armeniacus)* (Potter, 2016). Prairies open-areas make them especially at risk for invasive species to move in, such as false brome (*Brachypodium sylvaticum)* reported on JBLM lands for the first time in 2021 (Hamilton-Wissmer, 2021).

Weather events and climatic change alter the relative timing of *E. e. taylori* and host plant development, causing them to be out of sync, which shows a need to establish multiple host species in South Puget Sound prairies to serve as a buffer for early, mid, and late *E. e. taylori* emerging into flight season (Singer, 1972; Schultz *et al.*, 2011). Previously, little was known about the distribution and abundance of *E. e. taylori* or their habitat requirements, but in-depth surveying and population monitoring began in the late 1990s and continues today (Morgenweck and Dunn, 2003; Stinson, 2005). There is a

better understanding of *E. e. taylori's* current status and habitat needs, which represents the best idea of what restoration to a natural landscape should look like in this ecosystem. However, there is still no definite characterization of what ideal habitat restoration should look like for this species, this habitat, and other species present in it.

Euphydryas editha taylori *Captive Rearing Background*

Not expected to repopulate historical occupation sites naturally due to how fragmented and diminished *Euphydryas editha taylori* populations became, captive breeding and captive rearing began work in conjunction with habitat restoration. *Ex-situ* methods supplement the wild *E. e. taylori* population to prevent complete extinction. In 2004, WDFW and the Oregon Zoo partnered to develop initial captive rearing and translocation methods for *E. e. taylori*, eventually finding that post-diapause larvae are the most viable life stage for translocation and reintroduction (Grosboll, 2004; Linders, 2007; Potter, 2016). Since only a single naturally-occurring *E. e. taylori* population remains in the South Sound prairies, original trials and following efforts rely entirely on founding individuals from the population on JBLM artillery range 76 (Potter, 2016; Linders *et al.*, 2019).

These initial captive rearing trials showed success and informed most of the original captive rearing protocols, demonstrating an increase in survival and production (Grosboll, 2004). The first reintroduction occurred on JBLM in 2006, occurring annually thereafter (Linders *et al.*, 2019). These reintroductions occur on four sites within South Puget Sound prairies—two located on JBLM and two in Thurston County—in the hopes of eventually developing into a metapopulation. Eventually, established methods for breeding and rearing *E. e. taylori* were incorporated by the Oregon Zoo conservation

research team working with WDFW under the approval of USFWS (Barclay *et al.*, 2009). Captive rearing at the Zoo continued from its initial start in 2003 until 2020, when the program ceased due to funding, impacts of COVID-19, and the seeming improvements of wild *E. e. taylori* populations. In 2011, the Sustainability in Prisons Project—a partnership between The Evergreen State College and WA Department of Corrections—collaborated with WDFW, the Zoo, and USFWS to begin a captive rearing program for *E. e. taylori* in Mission Creek Corrections Center for Women.

Bringing incarcerated individuals closer to nature in it of itself is a net positive. SPP provides education opportunities, job training, and networking connections—all in relation to sustainability and the environment—to the carceral system in WA. SPP's contributions carry such influence, other states followed suit throughout the country (Kaye *et al.*, 2015). SPP awards certifications to incarcerated individuals who meet specific education and experience criteria for some programs, which designates them 15 college credits at The Evergreen State College upon enrollment after release from prison. SPP and the co-director, Kelli Bush, strive to expand education opportunities and currently, the SPP team of managers and master's student employees are working to establish a consistent education curriculum that will allow incarcerated program partners to earn college credits from TESC while incarcerated (*pers. communication*, Kelli Bush).

Research carried out by TESC masters students investigating the impacts of SPP programming on current and formerly incarcerated individuals continues to grow, and findings indicate positive participant experiences (Clarke, 2011; Weber, 2012; Gallagher, 2013; Webb, 2016; Gilliom, 2017; Passarelli, 2017). However, Webb (2016) expressed skepticism of the overall influence by discussing the continued existence of the Prison

Industrial Complex: a system described by Alexander (2010) as derived from a history of Jim Crow policy and slavery, and one that disproportionately impacts black people and all people of color and allows various institutions to benefit from mass incarceration (Alexander, 2010; FBP, 2022). This context leads some to believe efforts to achieve sustainability more closely resemble greenwashing, for example Jewkes and Moran (2015) state that reducing prison population sizes would have a more substantial environmental and social impact while green prisons become more productive and competitively efficient. Given the historical and ethical background and the variety of opinions on the carceral system, the impacts programs have on the individual participants cannot go overlooked. In addition to growing reports of positive experiences, the job training, and education opportunities, SPP contributes to post-release support systems, all of which has been found improve employment opportunities upon release and to reduce recidivism (Kaye *et al.*, 2015).

At MCCCW in 2011, the first captive rearing season took place in a 3 x 7.3 m glass greenhouse, and the program saw success comparable to the Zoo (Linders and Lewis, 2013). Incarcerate butterfly technicians—usually trained by Oregon Zoo keepers or graduate students employed as Butterfly Program Coordinator with Sustainability in Prisons Project—carry out daily care of the endangered *E. e. taylori*. The MCCCW captive rearing program has expanded over the years; currently carried out in two large greenhouses with the goal of producing 5,000 postdiapause larvae per greenhouse, though the program has not yet reached this capacity due to the continuing impacts of COVID-19. Population supplementation has prevented the total demise of this species thus far; however, the long-term fate of *E. e. taylori* is not yet secure. In order for

reintroduction sites to be considered reestablished, during the flight season, surveyors must see over 250 adults in one day over 50 acres of space for five consecutive years, happening in conjunction with five years of successful releases, followed by five years of surveilling the prosperous population (Stinson, 2005; Potter, 2016).

CAPTIVE REARING

Captive Rearing & its Risks

Some at-risk populations regress beyond the point of natural recovery, requiring off-site management. Conservationists typically only resort to captive rearing when a species becomes critically at-risk in attempt to prevent unnecessary extinction (Engelmann and Engels, 2002). *Ex-situ* conservation—which involves rearing individuals in captive conditions that mimic wild conditions—became common practice over the years to mitigate the unprecedented biodiversity loss occurring worldwide. In this practice, the captive stock is either reared or bred to produce offspring with the goal of eventual release during the life stage with the highest chance to survive such reintroduction back into that species' wild habitat (Engelmann and Engels, 2002). However, not a catch-all solution for wide-spread species endangerment, the implementation of captive rearing remains reserved for dire circumstances of critical endangerment. It provides a short-term solution to prevent the total eradication of at-risk species due to dramatic declines caused by habitat fragmentation and degradation (Hughes and Bennett, 1991; Caughley and Gunn, 1996; Crone, Pickering and Schultz, 2007).

Reintroductions fail to find success when restorationists fall short when attempting to address habitat degradation concerns alongside implementing *ex-situ* conservation methods; captive rearing efforts have the highest chance for success when coupled with habitat restoration and management plans (Morton, 1983; Adamski and Witkowski, 2007). *In-situ* and *ex-situ* conservation methods used congruently allow restorationists to invest in and ascertain valuable life history information of other species throughout the habitat, leading to more inclusive management plans (Ehrlich and Hanski, 2004b; Grosboll, 2004; Daniels *et al.*, 2020). Since primary resources initially tend to go towards charismatic megafauna, this allows for the chance to increase education and outreach into the surrounding community that helps support public engagement in conservation efforts to benefit more than the species in question.

Risks heighten when implementing *ex-situ* conservation methods with significantly diminished endangered species populations, and these methods alone cannot assume to completely fix these dire situations once a population's genetics become severely constrained (Rahbek, 1993; Sigaard *et al.*, 2008). Primary constraints of this practice that could lead to increased risk include financial and physical resources restrictions, the possibility of spreading diseases and parasites from captive to wild populations, and genetic hazards derived from small population sizes. These practices frequently initiate with a diminished founder population and, therefore, a small gene pool given the nature of critically endangered species (Ballou *et al.*, 2010). Usually, constraints limit the understanding of the diminished founder population's genetic makeup, impeding the ability to track lineages and relatedness to prevent inbreeding (Hedrick and Hurt, 2012; Miller *et al.*, 2014). In order to feasibly circumvent inbreeding

with minimal genetic information, best practices comprise translocation from larger, more established populations to be reintroduced into smaller, less stable populations (Crone, Pickering and Schultz, 2007). However, minor or significant genetic differences often arise between segregated populations depending on habitat types that could affect fitness if translocated to other habitat types (Frankham, Briscoe and Ballou, 2002; Crone, Pickering and Schultz, 2007).

Traits that allow successful survival and reproduction in the wild can be unintentionally subject to selection during captivity (Nylin and Gotthard, 1998; Norberg and Leimar, 2002; Frankham, 2008). Once a critically endangered population is deemed suitable for captive rearing and eventual reintroduction, captive conditions must mimic wild conditions sufficiently to prevent selective pressure from causing adaptations to captive conditions (Frankham, 2008). Selection pressure can lead to naïve individuals, less adept at surviving upon release (Sutherland, 1998; Bryant and Reed, 1999; Stockwell and Weeks, 1999; Frankham, 2008). These genetic adaptations can occur exceedingly rapidly, observed to occur in as little as one generation (Dzurisin, 2005; Crone, Pickering and Schultz, 2007; Christie *et al.*, 2012). Due to financial and resource constraints, it is rare to assess and compare captive and wild individuals of a species to understand if adaptations to captivity are occurring and how that impacts survival (Schultz, Dzurisin and Russell, 2008). The risks of working with a small gene pool are compounded and can lead to further loss of genetic materials in the captively reared population, including an increase in genetic homogeneity and loss of rare alleles (Snyder *et al.*, 1996; Crone, Pickering and Schultz, 2007; Schultz, Dzurisin and Russell, 2008).

Unfortunately, multiple captive rearing and captive breeding programs show decreased fitness within the reintroduced individuals when post-release monitoring occurs (Snyder *et al.*, 1996; Christie *et al.*, 2012). In order to maintain the genetic integrity of the captively reared population and prevent the production of less-fit individuals, captive conditions must mimic wild conditions as closely as possible, including seasonal habitat environmental conditions (Frankham, 2008). Challenges arise when working to understand what exact environmental requirements entail, in addition to other factors—habitat size, conditions, connectivity, and weather conditions that determine the success of that captive rearing reintroduction—and it becomes difficult to understand how minor misalignments could impact the likelihood of reintroduction success after captive rearing (Oates and Warren, 1990; Hanski, Ehrlich, *et al.*, 2004). The extent of this trial-and-error period prolongs the length of the captive rearing program, which can lead to long-term supplementation of captive individuals into wild populations as factors of captive rearing and habitat management get refined. These long-term programs go against long-standing recommendations that these practices are interim programs to prevent the total demise of the population and only be used as a last resort (Pyle, 1976; Hughes and Bennett, 1991).

Examples of Captive Rearing Programs

Even with the inherent risks, captive rearing holds considerable potential to save invertebrates specifically from extinction for a plethora of reasons. Invertebrates tend to have diminutive sizes, brief life spans, and rapid reproduction times that allow captive rearing programs to be more financially attainable and swiftly produce wildlife for release (Hughes and Bennett, 1991; Pearce-Kelly *et al.*, 2007; Linders and Lewis, 2013)

However, genetic risks quickly compound in invertebrates due to these life history traits, allowing for rapid onset of genetic divergences that could affect the captive population. Even with the risks, the benefits of preventing the total demise of endangered species outweigh potential hazards; butterfly captive rearing and breeding programs have increased exponentially since the early 2000s, with the expansion occurring at a faster rate in the U.K. compared to the U.S. (Schultz, Russell and Wynn, 2008). *Ex-situ* conservation is recommended for about 50% of threatened or endangered butterfly species under the ESA, with half focusing on captive rearing and the other half on captive breeding (Schultz, Russell and Wynn, 2008).

A remarkably high-profile species, monarch butterflies, *Danaus plexippus*, inspire and enlighten the public given their beauty and fascinating life history. Though infrequent, a charismatic species occasionally benefits from the public widely supporting its conservation. Often considered flagship species, these designations and public awareness help restoration progress and research by accumulating financial aid and resource support towards the species recovery (Tim R. New, 1997; Barua *et al.*, 2012; New, 2014c). Public concern also leads to outreach, including volunteers participating in the species recovery process. Many citizens began rearing or purchasing monarch butterflies from commercial breeders to eventually release them in the wild when this species became unstable and at-risk in some areas (Davis, Smith and Ballew, 2020). Studies show that monarch butterflies captively reared under insufficient conditions led to weaker, smaller—which tend to impact flight ability—and more physically-pale individuals, indicating inadequate captive rearing conditions (Tenger-Trolander *et al.*, 2019; Davis, Smith and Ballew, 2020). However, a study from 2021 shows that, when

using more accurate environmental targets and adding clarity to the captive rearing procedures, migratory orientation was relatively unimpacted upon release of monarch butterflies compared to other studies (Wilcox *et al.*, 2021). This success shows promise for captive rearing practices, though standardizing these practices becomes ambitious in the case of *Danaus plexippus*, given the public participation in captive rearing.

Often long-standing captive rearing projects are considered successful in that they prevent the demise of an at-risk species and, in some cases, re-establish populations, however, species infrequently return from a critical point due to captive rearing that no longer requires any intervention and supplementation. An *ex-situ* conservation effort initiated in 1994 with the Karner blue butterfly (*Lycaeides melissa samuelis*) found exceptional success in producing fit individuals compared to their wild counterparts (Herms et al., 1996). Rearing of these butterflies occurred within environmental chambers in a lab setting that achieved optimal environmental conditions by imitating the species' wild conditions; chambers maintained temperatures from 24º - 26ºC with an 18:6 hour light-dark period and humidity at 57-68% (Herms et al., 1996; Webb, 2010). After initial success, established propagation procedures include these valuable environmental guidelines.

On a wildlife refuge in New Hampshire, reports showed less than 50 Karner blue butterflies in the early 2000s, with one location becoming completely extirpated (Pau and Holman, 2019). Through habitat restoration and population augmentation using proven captive rearing methods, this site was reestablished in addition to other sites becoming fortified, meeting the population goal of over 3000 individuals in a 2018 survey and qualifying the species for delisting (Pau and Holman, 2019). While this species does still

require maintenance in the form of habitat restoration and monitoring and has not yet been removed from the state or federal endangered species list, captive rearing did eliminate the immediate risk of extinction and allowed for more time to research ecosystem and life history requirements, likely contributing to the successful outcome of this program and others for this species.

The listing status varied throughout the range of another important flagship and indicator species, the Apollo butterfly (*Parnassius apollo)*, with different European countries experiencing different rates of loss initiating in the early 20th century (Pierzynowska, Skowron Volponi and Węgrzyn, 2019). Regional extirpation of this species resulted in total extinction in a number of countries, and the species, and the Convention on International Trade in Endangered Species considers Apollo butterflies near-threatened throughout their range in Europe (CITES, 2021). However, the IUCN Red List previously listed the Apollo butterfly as near threatened throughout its entire range, and as of 2021, the IUCN downgraded this status to least concern, though still considered declining (Nadler *et al.*, 2021). Delisting likely occurred due to the various recovery actions taken throughout Europe, including *ex-situ* conservation methods.

One example of implementing these practices includes an exceedingly diminished population in the Pieniny National Park in Poland; captive rearing significantly assisted in increasing a critically at-risk population of 30-50 Apollo butterflies into a metapopulation of 100-1200 individuals over 12 years (Adamski and Witkowski, 2007; Pierzynowska, Skowron Volponi and Węgrzyn, 2019). However, initial trials of captive rearing saw high mortality, and later witnessed adults eclosing with malformed or sometimes missing wings (Witkowski and Adamski, 1996; Adamski and Witkowski,

1999; Pierzynowska, Skowron Volponi and Węgrzyn, 2019). Though this phenomenon occurred in the wild, Witkowski and Adamski (1999) observed this at a higher rate in female Apollo butterflies in captivity, instead of almost exclusively observed in males in the wild.

Recent research seems to indicate that these malformations correlate with several factors: genetic bottleneck effect leading to mutations, the insufficient chemical make-up of the host plant, lack of exposure to an intracellular symbiont, or bacterial infection (Łukasiewicz and Węgrzyn, 2015; Łukasiewicz, Sanak and Węgrzyn, 2016a, 2016b; Pierzynowska, Skowron Volponi and Węgrzyn, 2019). Some describe that this information could better inform captive rearing procedures to increase *ex-situ* success, while others attempt to shift focus towards habitat corridors and wild translocation to bolster the populations genetic integrity (Adamski and Witkowski, 2007; Dabrowski, 2008; Fred and Brommer, 2015). Though there appears to have been a trial-and-error period to ascertain best practices for captive rearing of the Apollo butterfly, the *ex-situ* program seemingly achieved great success in increasing population numbers even with these trying aspects. This metapopulation allows for the opportunity to shift towards *in-situ* conservation methods without immediate risk of extirpation.

Environmental Conditions & Captive Rearing

Staunchly contingent on temperature, development times and body size tend to decrease in ectotherms as temperature increases until a certain point at which mortality will likely occur if temperatures become extreme (Atkinson, 1994; van der Have and de Jong, 1996; Angilletta, Steury and Sears, 2004; Fischer and Karl, 2010). Studies indicate environmental conditions experienced in captivity contribute to morphological

discrepancies within captively reared butterflies, and as a notably influential factor of development times, it could lead to severe survival repercussions (Nicholls and Pullin, 2000; Woodworth *et al.,* 2002). Changes in environmental conditions encountered during developmental stages may trigger phenotypic responses, expressed as variation in proportions, especially if conditions continue to diverge through other life stages and generations with the potential to act as a prominent selective pressure (Atkinson, 1994; Loeschcke, Bundgaard and Barker, 2000; Steigenga and Fischer, 2009).

Contingent on the relationship between environmental conditions, development times, productivity, and survival, the temperature-size rule supports findings of larger body sizes at lower temperatures (Atkinson, 1994; Angilletta, Steury and Sears, 2004). Morphological changes in butterflies explicitly impact an individual's ability to fly, consequently affecting the ability to properly disperse adequately and successfully reproduce, in addition to diminishing competitive capability and fecundity (Berwaerts, Van Dyck and Aerts, 2002; Norberg and Leimar, 2002). However, this rule cannot be generally applied to ectotherms, and discerning whether this is a genetic or phenotypic response to temperature depends on the species and requires research to determine (Loeschcke, Bundgaard and Barker, 2000; Angilletta, Jr., and Dunham, 2003). Variation in environmental conditions during captive rearing tend to have a reduced impact on species living with greater environmental stochasticity; this can pose a challenge for more specialized species, though insects often possess a certain margin of safety against stochastic events (Inchausti and Halley, 2003; Crone, Pickering and Schultz, 2007).

A number of investigations into acclimation capacity uphold an optimal temperature development hypothesis that indicates a threshold of moderate temperatures

produce more successful individuals, while success likely decreases outside of this moderate temperature threshold (Huey and Berrigan, 1996; Huey *et al.*, 1999; Woods and Harrison, 2002). Moderate environmental conditions that mimic wild conditions without extreme weather events that can occur out in the wild often become the goal when captively rearing wildlife. Challenges can arise when these optimal environmental conditions are not well understood for a species.

The endangered Puget blue butterfly, *Icarcia icarioides blacmorei*, also found in WA South Puget Sound prairies, requires captive rearing to recover to prevent extinction. Wild individuals were collected over two generations for rearing in captivity (Schultz, Dzurisin and Russell, 2008). Studies compared survivorship, development rates, sex ratio, biomass, size, and adult morphology between individuals reared in differing captive circumstances to identify which conditions appropriately mimic wild conditions. To determine this, the study then compared the morphology of captively reared adults to wild adults to understand if divergencies occurred under any of the three captive rearing conditions (Schultz, Dzurisin and Russell, 2008). One experimental group of Puget blue butterflies was kept in refrigerators at the Oregon Zoo, a standard practice for other butterfly rearing programs. The second group and third group, held at Washington State University-Vancouver, were either reared in outdoor enclosures experiencing relatively ambient conditions reared indoors in diapause chambers and experiencing temperature, humidity, and light structures in a way to mimic best judgments of optimal conditions (Dzurisin, 2005; Schultz, Dzurisin and Russell, 2008).

Results show survival and sex ratios were similar across all captive sites, and other findings were consistent with the results of a long-term study that shows captive-

bred cabbage butterflies (*Pieris brasicae)* developed into smaller individuals than their wild counterparts (Lewis and Thomas, 2001; Schultz, Dzurisin and Russell, 2008). These findings are comparable to initial conversations with Mary Linders regarding the start of the *E. e. taylori* captive rearing program where captively reared individuals appeared smaller than wild individuals (Schultz, Dzurisin and Russell, 2008). However, reports indicate initial success with the *E. e. taylori* captive rearing program at the Oregon Zoo and continued success since the program expanded to Mission Creek Corrections Center for Women, and these trials helped to determine life-history traits and develop appropriate captive rearing methods and conditions utilized today (Grosboll, 2004; Linders, 2007, 2011).

Depending on how long this trial-and-error period takes to determine appropriate captive rearing conditions, risks imply that captive rearing as a primary method for increasing population abundance may not always lead to enriching populations with fit individuals (Lewis and Thomas, 2001; Norberg and Leimar, 2002; Schultz, Dzurisin and Russell, 2008). If captive rearing must take place, one of the most critical factors to ensure success is to mimic wild conditions in whatever way possible. Preferred practices often include the use of controlled environmental chambers, which have seen continued success at producing butterflies that are fit compared to their wild counterparts (Herms *et al.*, 1996). Studies found that outdoor enclosures during diapause allow environmental conditions to mimic wild conditions and minimize the risks of selection pressures changing because of divergent captive conditions (Nicholls and Pullin, 2000; Lewis and Thomas, 2001).

To ensure selection is not occurring and promote the longevity of these successful programs, comparisons and assessments between captive and wild individuals and their environmental conditions must be carried out regularly to ensure they develop similarly. Recommended methods of assessment include tacking morphological data which could help reveal population disparities (Crone, Pickering and Schultz, 2007; Schultz, Dzurisin and Russell, 2008). Assessments become especially important as the climate changes at an increasing pace. However, research indicates high survival and productivity in captivity leading to large-scale reintroductions may not augment populations until any potential genetic and habitat concerns—including restoration and improving connectivity to other populations— are addressed, and reintroductions occur during optimal weather conditions (Oates and Warren, 1990; Lewis and Thomas, 2001; Nieminen *et al.*, 2001; Hanski, Ehrlich, *et al.*, 2004).

METHODS

CAPTIVE REARING PROCESS

Butterfly Technician Environmental Management

Captive rearing at MCCCW is carried out in a 7.3 x 3.1 m glass greenhouse, called greenhouse Raven (Figure 1). The design of this captive rearing greenhouse is expected to provide relatively ambient environmental conditions while minimizing extremes. There are two rooms in Raven—one smaller room (3 x 2.4 m) and one larger room (3 x 4.9 m)—that can be partitioned by a glass door, the windows of which can open and allow airflow between the two rooms. Raven has UV-transmitting glass panels to allow for maximum sunlight and heat in addition to heaters attached overhead either entrance to prevent freezing in the winter or help maintain environmental targets for larvae in the spring. To avoid overheating in the summer, cooling is carried out by ceiling exhaust fans, motorized dampers, and automated roof windows scheduled to open and close, allowing passive ventilation when temperatures go above 86°F.

Two knit aluminum 50% reflective shade cloths cover the top of the greenhouse during the summer months to prevent extreme heat caused by excessive sunlight exposure. Butterfly Technicians Rearing Specialists monitor and maintain environmental parameters within the greenhouse to reach environmental targets during each life stage by increasing or decreasing the heat or fans, hanging sheets to block intense sunlight, using humidifiers, and using icepacks, among other tactics. Technicians monitor environmental conditions based on environmental targets (Table 1) using classic and digital thermometers and relative humidity gauges showing minimum, maximum, and current

environmental conditions. Technicians record minimum and maximum temperature and relative humidity daily onto a data form and graph this data compared to the targets.

A majority of the diapause stage occurs outside the greenhouse, in an 8’ x 10’ x 8’ shed with three 2’ x 2’ windows (Figure 1) and plenty of ventilation that allows for ambient but not extreme environmental conditions. The shed has eight 4” x 10” grille vents—six along the bottoms of three walls and two on opposing walls along the edge of the ceiling—four 4” circular screen vents along the peak of the ceiling, and six panels along the midpoint of the 12 m walls, with three 1” circular screen vents per panel. Minimal maintenance is required of the Technicians to manage environmental conditions during cold diapause.

Figure 1. MCCCW captive rearing greenhouse (left, photo credit: former Butterfly Program Coordinator) and cold diapause shed (right).

Collecting Wild Adults

Collection of wild *Euphydryas editha taylori* adults occur from mid-April to early May, although this period can vary by 2-4 weeks based on rain and other weather patterns of that year (Linders *et al.*, 2019). Usually occurs on Range 76 located at JBLM,

biologists search for and collect adults in the morning hours during the peak of *E. e. taylori* flight season. Once captured, adults are placed in insect jars with proper ventilation. The insect jars are then stored in small coolers (Figure 2) until they can be transported to the captive rearing facility at MCCCW and processed by the Butterfly Technicians.

Figure 2. Pictured are the containers in which wild females are transported to MCCCW, inside a cooler for transport (left) and a wild female feeding (right).

Butterfly Technician Daily Care Procedures

All *E. e. taylori* daily care practices follow established husbandry manual protocols developed by Oregon Zoo in 2009 for captive rearing and propagation (Barclay *et al.*, 2009). Specific captive rearing procedures for MCCCW have been created based on approved protocols from the husbandry manual and refined over the years to accommodate the differences in captive rearing facilities between the Oregon Zoo's lab and the MCCCW's greenhouses (Curry *et al.*, 2020). Procedures are currently in place for *E. e. taylori's* life cycle in captivity: oviposition, eggs to third instar, third instar to fifth instar, warm diapause, cold diapause, wake up, post diapause, and release (Curry *et*

al., 2020) while older renditions of the procedures include pupation, adult eclosion, captive females and males, and breeding. Note that captive breeding ceases in 2020 and no longer take place at MCCCW. The goal the program is to produce at least 5,000 eggs total that will result in 2,500 postdiapause larvae (Appendix A. Table 15).

Incarcerated Butterfly Technicians receive training from program partners prior to the active rearing season to follow these protocols and procedures with minimal supervision. Butterfly Program Coordinator visits the program a minimum of once a week during the active rearing season to gauge program progress and facilitate communication between partners. Technicians must be escorted out of the prison fence around 0900 to carry out daily care tasks and remain there until they are brought back inside around 1300, depending on the time of year.

Captive males were kept in large, mesh tents with several "brothers" and had access to nectar plants, while captive females were stored in 16 oz deli cups inside a refrigerator until breeding introductions occurred. Introductions were carried out in portable popup tents, and once copulation occurred, Technicians moved females to an oviposition chamber (Figure 3), and males retired to their large mesh tents. Lineages were recorded using matrilines and patrilines to keep track of introductions and copulations.

Technicians process the wild females by creating a matriline ID and placing them into an oviposition chamber. Chambers are designed around a one-gallon potted *Plantago lanceolata,* where they have access to a cotton ball soaked in a honey-water solution and a water-soaked sponge. Technicians search oviposition chambers for eggs daily while females have the opportunity to feed under a 16oz deli cup.

Figure 3. Females inside oviposition chamber, on the mesh closure (left), laying eggs (middle), and feeding on honey-water solution (right).

If eggs are found, Technicians cut the leaf from the plant, remove as much of the excess leaf as possible, perform and record egg estimates, and place eggs in a prepared five oz deli cup lined with a folded paper towel. Females have the opportunity to oviposit until they expire. Eggs and larvae are tracked and stored based on the matriline and cup IDs. Eggs develop over 10-14 days, darkening from yellow to brown to purple, at which point Technicians begin placing small leaves inside the cups preparing for eggs to hatch. Eggs will hatch into first instar, and Technicians give larvae an increasing number of leaves and replace paper towel liners as needed as larvae develop through first and second instar (Figure 4).

Figure 4. First instar larvae at hatch (left), third instar larvae (middle), and egg and larval cup set-up (right).

Once larvae reach third instar, Technicians officially count and record the first hard larval count of the season; Technicians count about 15 larvae per cup into 16 oz deli cups lined with paper towels (Figure 4). Technicians continue prediapause larval care procedures as larvae develop through third and fourth instar. Once larvae reach fifth instar, warm diapause has officially started. About two weeks after allocation, Technicians count and allocate larvae into 16 oz cups of 50 larvae per cup in-between folded paper towels (Figure 5). Around mid-September, Technicians move larval cups from the greenhouse outside to the diapause shed, and larval cups are stored on 12” terra cotta dishes under 10” terra cotta pots with the drainage hole plugged with a cloth. Larvae remain in cold diapause requiring minimal maintenance until mid-February.

Figure 5. Diapausing larvae (left) and cold diapause shed set up (right).

Wake-up occurs when the Technicians report high percentages of larval movement following prolonged days of warmth. Larvae are brought into the greenhouses and, Technicians allocate them into 16 oz release cups with about 15 larvae each. Most

postdiapause larvae will be released, which occurs two-three weeks after wake up, depending on what the partner Biologist observes in the field. Retained larvae have the opportunity to develop into sixth instar, where most pupate (Figure 6), but some will reenter diapause, depending on environmental conditions. Pupae are stored in five oz deli cups with a mesh covering until adults eclose about two-six weeks after pupation, at which point Technicians record morphometric data and employ captive adult care procedures.

Figure 6. 6th instar larvae (left) and pupae (right). Photo credit: Keegan Curry.

CAPTIVE REARING DATA COLLECTION

Productivity Outcomes Data Collection & Reporting

Captive rearing data—including breeding introduction data, oviposition data, egg estimates, hatch rates, development rates and dates, number of larvae into and out of diapause, and number of larvae to release—are recorded on a variety of data forms by Butterfly Technicians (Appendix B, Figure 10). While continuing to capture the same necessary information for reporting, these data collection forms were simplified by

Keegan Curry in 2019. This thesis used data from seven seasons, 2013-2014 through 2020-21. Note that the postdiapause larvae life stage was broken down into three different groups for this study:

1. Postdiapause to Release
2. Postdiapause to Second Diapause
3. Postdiapause to Pupation

Environmental Data Collection

Honest Observer by Onset (HOBO) loggers are external data loggers that record temperature, relative humidity, and light intensity at various intervals. These HOBO data loggers are used to record relative humidity and temperature within the MCCCW *E. e. taylori* captive propagation facility. These are placed in the same or similar settings as *E. e. taylori* at various life stages to monitor environmental conditions during captive rearing, see photos (Barclay *et al.*, 2009). The life cycle for environmental conditions is broken into six stages (Table 1).

HOBO loggers were programmed by connecting them to the Onset HOBOware software using a USB cable and selecting launch logger. They are then programmed to collect temperature and percent humidity every 1-3 hours, in addition to programing them to collect minimum, maximum, and average temperature and humidity once daily. Hourly data is used to calculate daily min/max/avg daily environmental data for seasons where loggers were not programmed to collect daily data, or we are missing some of this daily data. Historically, these HOBO loggers were deployed at the beginning of the season or a new life stage and remained deployed until the start of another life stage or longer.

To keep environmental logging continuous, new loggers are brought in to MCCCW to replace existing loggers, and previously deployed loggers are brought back to The Evergreen State College and read using HOBOware software. The data is exported to a CSV file then saved as an Excel workbook. Workbooks from the individual launches are combined and organized into life stages by date for that season. The Butterfly Program Coordinator summarizes each life stage's minimum, maximum, and average environmental data in Table 2. These summaries are compared to captive rearing targets in Table 1 and reported on annually by SPP and WDFW to understand program success.

Table 1. Euphydryas editha taylori *captive propagation environmental targets for all life stages.*

Life stage	Target	
	Temp (°F)	*%RH*
Males	50°-85° 78°-85° for 2-6 h/day	≥50%
Females & Oviposition	50°-90° 78°-90° for 4-8 h/day	≥50%
Eggs & Prediapause	50°-90° avg min ≤65°	≤65%
Warm Diapause	50°-90°	≥45%
Cold Diapause	≤35° for ≥60 days	≥50% avg max ≤90%
Post- Diapause	≤45° night ≥65° daytime	≥50%
Pupation	>50° night ≥65° daytime	≥65%

Morphometric Data Collection

Butterfly Technicians measured adult butterflies' weight (g) after captive eclosion occurred or after wild females were delivered. Technicians placed butterflies inside an enclosed scale and measured to the nearest 0.0001 gram (Figure 7). Weights were recorded by Butterfly Technicians, then transcribed and summarized in Excel by the Butterfly Program Coordinator for the annual report. Technicians measured butterfly wing area (cm2) by delicately placing butterflies with their left side up inside a petri dish and setting the dish on a 4 x 4 graphing paper. The butterflies ID tag is placed near it and was photographed and used later to identify and double-check measurements. Butterfly technicians recorded measurements, then the Program Coordinator transcribed and summarized this data for annual reporting (Appendix A. Table 24). Wild populations had smaller sample sizes since fewer wild females were available for measurements. Note that morphometric data collection ceased in 2020.

Figure 7. Wing area measurements (left) and weight measurements (right) for adult females. Photo credit: former Butterfly Program Coordinator.

DATA ANALYSIS

Productivity Data

Data is collected per matriline or larval ID, with a form created for every matriline produced and brought into the greenhouse. Data forms inform each season's annual report to understand program success based on productivity targets; historically, captive rearing targets at MCCCW have been for captive and wild females to jointly produce a total of 2,500 postdiapause larvae from up to 5,000 eggs (Hamilton *et al.*, 2013; Curry *et al.*, 2018). In the 2018-2019 season, this target changed to producing 1,800 postdiapause larvae from up to 5,000 eggs (Curry *et al.*, 2019). Reporting data was organized by life stage to find where I used this data on *E. e. taylori* in captivity to find percent productivity and survival from one life stage to the next, as follows:

1. Percentage of productive males were found by dividing successful copulations by the total number of introductions that occurred that season.
2. Percentage of productive females were found by dividing the number of females that have successfully oviposited by the total number of females brought to the rearing facility that season.
3. Hatch rate was found by dividing the first hard larval count, executed once all larvae have reached 3rd instar (prediapause count), divided by egg estimates at oviposition.
4. Percent into diapause was found by dividing the number of larvae counted into cold diapause cups by the first hard count of larvae at 3rd instar.
5. Percent out of diapause was found by dividing the larvae count out of diapause at wake up by the number of larvae allocated to cold diapause.

6. Percent to release or retainment was found by dividing the total number of surviving larvae at the end of the season by the number of larvae at wake up from cold diapause.
7. Percent return to diapause was found by dividing the number of larvae that reentered diapause by the number of retained larvae.
8. Percent to pupation was found by dividing the number of larvae that pupated by the number of retained larvae.
9. Percentage of successful pupations and eclosions was found by subtracting the number of unsuccessful eclosions from the number of pupations, then dividing that number by the number of pupations.

Environmental Data

Hourly environmental data—organized by season and life stage—was accessed from the SPP server, with 2013-2014 being the first season HOBO loggers were consistently used to collect hourly temperature and humidity data. The 2019-20 season was excluded from this study due to missing environmental data. Hourly data was rounded to the nearest whole number and was then used to calculate daily minimum, maximum, and average temperature and humidity for seasons or life stages where loggers weren't programmed to collect or were missing some of this daily data. Minimum, maximum, and average environmental data was then summarized for reporting (Table 2). These summaries for each season were averaged by life stage to produce an overall average and overall range that was compared to the environmental targets, providing an idea of environmental conditions experienced for all the seasons in this study (Appendix A. Tables 16-22),

Rounded hourly data was compared to the targets using IF statements in Excel to determine if they were within the target range or above/below the absolute target. When looking to see if hourly data ever met the temperature targets during the eggs & pre-diapause life stage, targets for this life stage are temperatures between 50° to 90° for 90% of the day. After determining whether a day was within the life stages environmental parameters, a one or zero was used to indicate whether that day met the target (1) or not (0). Ones were counted and divided by the number of days in that life stage for that season, then converted to a percent. This was used as the percent of time targets were met, with high percentages meaning targets were more frequently met.

Minimum and maximum daily temperatures were used to determine the percent of days outside of temperature targets. IF statements were used to detect if temperatures in a day went below or above absolute temperature targets by comparing the absolute minimum temperature target to the minimum daily rearing temperatures and the absolute maximum temperature target to the daily maximum rearing temperatures (Appendix B Figure 11). This was done for all life stages except cold diapause, which is the only stage without a temperature target range. The number of days outside the temperature targets was summed and divided by the total number of days in the season to find the percent outside the temperature target. For this methodology, high percentages mean targets were less frequently met.

Table 2. Example summary of overall minimum, maximum, and average temperature (ºF) and relative humidity (%) during captive rearing. This summary is produced for every life stage in each season.

	Min	Max	Avg
Min Temp	49º	59º	53º
Max Temp	69º	89º	81º
Avg Temp	57º	69º	64º
Min RH	26%	57%	38%
Max RH	66%	98%	82%
Avg RH	48%	81%	63%

Spearman's Rho

Spearman's rho (a non-parametric correlation coefficient) was calculated in JMP Pro (version 16.1.0). Spearman's rho was calculated between percent productivity and survival rates and the percentage of time the relative humidity target was met, and between percent productivity and survival and the percentage of time the temperature target was met. This was done for all life stages in both wild and captive populations for all seasons in this study. An alpha of 0.10 was used for statistical significance, to add to the power of statistical tests, and p-values between 0.05 and 0.10 are pointed out distinctly for the reader.

Morphological Data Analysis

Morphological data from the SPP server included 2013-14 to 2018-19 for weight, and 2014-15 to 2018-19 for wing area. Only data on wild and captive female adults were used. Raw data from area season was organized categorically depending on if the female was wild or captive bred. This data was input in JMP and the Wilcoxon rank-sum test

was used to determine if the captive and wild morphometric data differed in each season. Wilcoxon rank-sum was used to keep the analysis uniform because some seasons' distributions were not normally distributed. Each season, wing area has smaller sample sizes because individuals with damaged wings are excluded from the wing area measurement.

In addition, in JMP a multiple linear regression was run using weight as a response variable, and separately using wing area as a response variable, with both captivity status (captive v. wild) and season (year) as predictor variables. In both cases the overall model was statistically significant (for weight, $F_{2,730} = 105.9$, $p < 0.001$, for wing area, $F_{2,342} = 20.7$, $p < 0.001$) albeit with relatively low adjusted R^2 values (0.22 for weight and 0.10 for wing area).

RESULTS

ENVIRONMENTAL DATA

Overview

Overall averages and average ranges summarize actual captive rearing conditions at MCCCW for 2013-2020 (Table 3). Overall average temperature meets the respective targets for males, females, prediapause, warm diapause, and pupation for nighttime. Overall average relative humidity meets the targets for every life stage. Overall average ranges are often outside of temperature target ranges and often meet relative humidity targets.

Temperature

No life stages consistently met the targets 100% of the time (Table 4); pupation met nighttime temperature targets 100% for 2/6 seasons and prediapause and warm diapause for 1/7 seasons. Eggs and prediapause (4/7) and warm diapause (3/7) often met the target 81-99% of the time. A majority of the targets are completed between 1-20% of the time; males (2/6), females & oviposition (4/7), cold diapause (7/7), postdiapause (3/7 for nighttime, 4/7 for daytime), and pupation (2/6 daytime) having multiple seasons in this range. Postdiapause (4/7 nighttime, 1/7 daytime) and pupation (2/6 daytime) both have seasons where the target is never met (0%) (Appendix B. Figures 12-19).

Table 3. Overall averages and average ranges of environmental conditions compared to life stage targets for all life stages in seasons 2013-2014 to 2018-2019 and 2020-2021.

Life Stage	Temp(°F) Targets (abs)	Overall Average (Overall Avg Range)	%RH Targets	Overall Average (Overall Avg Range)
Males	(50°-85°) 78°-85° for 2-6 h/day	64 (47-92)	≥50%	60 (27-89)
Females & Oviposition	(50°-90°) 78°-90° for 4-8 h/day	65 (46-94)	≥50%	62 (27-88)
Eggs & Prediapause	(50°-90°) avg min ≤65°	67 (48-97)	≤65%	64 (29-91)
Warm Diapause	(50°-90°)	70 (52-97)	≥45%	65 (28-91)
Cold Diapause	≤35° for ≥60 days	44 (19-84)	≥50%	86 (40-94)
Postdiapause	≤45° night ≥65° daytime	58 (40-85)	≥65%	68 (29-94)
Pupation	>50° night ≥65° daytime	62 (47-90)	≥50%	69 (31-95)

Relative Humidity

No life stages consistently met the targets 100% of the time (Table 4); warm diapause and cold diapause met the relative humidity target 100% for 1/7 seasons each. A majority of the targets are either met 21-40% of the time—males and postdiapause (2/7)4/6), females & oviposition (3/7), eggs & prediapause (2/7), and pupation (2/6

daytime)—or met between 81-99% of the time; females (2/7), cold diapause (6/7), and postdiapause (2/7). Eggs & prediapause (4/7) typically met the target 1-20% of the time. No life stages never met the relative humidity target (0%) (Appendix B. Figures 12-19).

Table 4. Average and range of the percent of time the environmental targets are met for all life stages for seasons 2013-2014 to 2018-2019 and 2020-2021.

Life Stage	Target Temp (°F)	%RH	%Time Targets Met Temp (°F) Average (Range)	%RH Average (Range)
Males	(50°-85°) 78°-85° for 2-6 h/day	≥50%	34% (2%-67%)	44% (21%-93%)
Females & Oviposition	50-90 (50°-90°)	≥50%	18% (0%-48%)	51% (14%-95%)
Eggs & Prediapause	78°-90° for 4-8 h/day avg min ≤65	≤65	85% (47%-100%)	26% (1%-96%)
Warm Diapause	(50°-90°) avg min ≤65°	≤65%	85% (59%-100%)	74% (57%-100%)
Cold Diapause	≤35 for ≥60 days	≥45%	7% (2%-16%)	97% (94%-100%)
Post-Diapause	≤45° night >50 night	≥50%	4% (0%-15%) 21% (0%-72%)	69% (47%-88%)
Pupation	≤45° night ≥65° daytime	≥50%	86% (41%-100%) 16% (0%-36%)	36% (8%-71%)

Outside Temperature Targets

Overall, the percent of time the minimum daily temperature is below the absolute minimum temperature target for each life stage is relatively consistent from season to season except for 2013-2014 (Table 5). Males (2/6), females (3/7), prediapause (3/7), warm diapause (6/7), and pupation (2/6) were often never outside of the target. Males (3/6), females (2/7), warm diapause (3/7), and pupation (2/6) were often only outside of the target 1-21% of the time. Postdiapause (3/7) was often outside the target 81-99% of the time. During the 2018-2019 season, postdiapause was always outside the nighttime target (100%) (Appendix B. Figures 20-27) The minimum target was frequently greater than the absolute minimum target, either 61-80% (3/7) or 81-99% (4/7) of the time.

Overall, the percent of time the maximum daily temperature is above the absolute maximum temperature target for each life stage is relatively consistent from season to season (Table 5). Males (1/6), females (2/7), warm diapause, postdiapause, and pupation (1/7) were occasionally never outside of the target. However, males (2/6), females (4/6), prediapause (6/7), warm diapause (3/7), postdiapause (4/7), and pupation (4/7) were often only outside of the target 1-20% of the time. During the 2020-2021 season, pupation was the only life stage to be between 81-99%. (Appendix B. Figures 19-26)

Table 5. Average and range of the percent of days outside of the environmental targets for all life stages for seasons 2013-2014 to 2018-2019 and 2020-2021.

Life stage	Target	% Outside Target	
	Temp (°F)	Below Target Average (Range)	Above Target Average (Range)
Males	(50°-85°) 78°-85° for 2-6 h/day	19% (0%-84%)	22% (0%-38%)
Females & Oviposition	50-90 (50°-90°)	16% (0%-58%)	12% (0%-26%)
Eggs & Prediapause	78°-90° for 4-8 h/day avg min ≤65	7% (0%-40%)	10% (2%-26%)
Warm Diapause	(50°-90°) avg min ≤65°	1% (0%-8%)	21% (0%-53%)
Cold Diapause	≤35 for ≥60 days (50°-90°)	--	88% (75%-97%)
Post- Diapause	≤45° night ≥65 daytime	53% (0%-100%)	21% (0%-69%)
Pupation	≤35° for ≥60 days ≥65 daytime	18% (0%-59%)	14% (0%-50%)

PRODUCTIVITY & SURVIVAL DATA

Captive Population

Male productivity was consistently low until the 2018-19 season, when it peaked at 62.7% (Table 6). Female productivity was at or above 90% for the first three seasons, then dropped significantly for 25% in the 4th season, and remained below 75% productivity for the last two seasons. Hatch rates vary greatly by season. Percent into diapause remained above 80% for all six seasons, and percent out of diapause and percent to release/retainment never went below 96%.

Table 6. Percentage of productivity & survival of captive E. e. taylori *populations at MCCCW for seasons 2013-2014 to 2018-2019.*

Season	% Males Productive	% Females Productive	Hatch Rate	% Into Diapause	% Out of Diapause	% To Release
2013-14	10.6%	93.8%	62.0%	99.0%	99.9%	99.8%
2014-15	27.8%	95.2%	29.8%	94.6%	99.6%	100.0%
2015-16	16.0%	90.0%	33.3%	86.7%	98.0%	99.8%
2016-17	10.7%	25.0%	49.2%	98.2%	100.0%	99.4%
2017-18	7.7%	71.4%	24.8%	96.7%	100.0%	100.0%
2018-19	62.7%	66.7%	96.1%	97.1%	96.7%	96.2%
Average	23%	73.7%	49.2%	95.4%	99.0%	99.2%
Range	8%-63%	25%-95%	25%-96%	87%-99%	97%-100%	96%-100%

Wild Population

Females' productivity varied between 70-100% for all 7 seasons, with an average of 85.5% (Tables 7 & 8). The hatch rate remained above 80%, going above 100% for 3 seasons since egg counts are estimates. Percent into diapause is its lowest in the 2014-15 seasons but stays above 97% for every other season. Percent out of diapause remained above 96% for all seasons and percent to releases/retained remained above 98% for all seasons. Return to diapause is highest from in the last three seasons (2013-2018), and pupation is lowest in 2013-14, 2016-17, and 2017-18. Eclosion is consistently high, with 2015-2016 having the lowest percent success (79%).

Table 7. Productivity and survival of the wild E. e. taylori *populations at MCCCW for adults to postdiapause stages during seasons 2013-2014 to 2018-2019 and 2020-2021.*

Season	% Females Productive	Hatch Rate	% Into Diapause	% Out of Diapause
2013-14	91.7%	97.1%	99.5%	99.8%
2014-15	75.0%	92.5%	90.4%	98.1%
2015-16	85.0%	80.3%	98.2%	96.6%
2016-17	70.0%	114.4%	99.2%	99.7%
2017-18	91.3%	104.9%	98.1%	99.8%
2018-19	100.0%	101.2%	97.1%	99.8%
2020-2021	85.7%	95.2%	99.0%	99.9%
Average	86%	98%	97%	99%
Range	(70%-100%)	(80%-114%)	(90%-100%)	(97%-100%)

Table 8. Productivity and survival of the wild E. e taylori populations at MCCCW for release/retainment to eclosion stages during seasons 2013-2014 to 2018-2019 and 2020-2021.

Season	% Released/ Retained	% Return to Diapause	% Wild to Pupation	% Successful Pupation & Eclosion
2013-14	100.0%	12.0%	58.0%	92.0%
2014-15	100.0%	8.3%	76.3%	88.5%
2015-16	99.9%	13.2%	85.4%	78.5%
2016-17	98.9%	37.4%	52.9%	86.8%
2017-18	99.9%	27.0%	57.6%	93.6%
2018-19	99.1%	25.1%	60.6%	87.2%
2020-2021	100.0%	--	--	--
Average	100%	20%	65%	88%
Range	(99%-100%)	(8%-37%)	(53%-85%)	(79%-94%)

SPEARMAN'S RHO CORRELATION

Captive Population – Productivity/Survival vs. Environmental Targets

The percent success of different life stages for the captive population was not typically correlated with the percentage of time the environmental targets were met. Using Spearman's rho, the percent of ovipositing females was positively correlated with percentage of time %RH is meeting the target (rho = 0.77, p = 0.07, Table 9). However, the percentage of successful ovipositing females and % of time temperature met the target was negatively correlated (rho = -0.75, p = 0.08, Table 9). Also note that these p-values were between 0.05 and 0.10 (Appendix B. Figures 28-33).

Table 9. Spearman's rho coefficient for the percent productivity/survival of the captive population versus percent of time the environmental targets were met for seasons 2013-2014 to 2018-2019 and 2020-2021.

Captive Population		Spearman's Rho Coefficient		
Life Stage	% Productivity /Survival Rates	% Time RH Target is Met	% Time Temp Target is Met	
Males & Breeding	% Successful Copulations	0.17	-0.08	
Females & Oviposition	% Females Productive	0.77*	-0.75#	
Eggs & Prediapause	Hatch Rate	-0.26	-0.26	
Warm Diapause	% Into Diapause	0.31	-0.20	
Cold Diapause	% Out of Diapause	-0.25	0.07	
Postdiapause	% From Wake Up to Release	0.00	Daytime -0.24	Nighttime 0.31

* p = 0.07
# p = 0.08

The percent success of different life stages for the captive population was not typically correlated with the percentage of days outside the temperature targets. Using Spearman's rho, the percent of prediapause larvae was positively correlated with the precent of days with temperatures below the minimum target (rho = -0.88, p = 0.02, Table 10). However, the percent of successful ovipositing females was negatively correlated to the percent of days with temperatures above the maximum target (rho = -0.78, p = 0.07, Table 10) in addition to the percent of larvae out of diapause being negatively correlated to the percent of days with temperatures above the maximum target (rho = -0.75, p = 0.08, Table 10). Also note that these p=values were between 0.05 and 0.10 (Appendix B. Figures 42-47).

Table 10. Spearman's rho coefficient for the percent productivity/survival of the captive population versus the percent of time outside the temperature targets for seasons 2013-2014 to 2018-2019 and 2020-2021.

Captive Population		Spearman's Rho Coefficient	
Life Stage	% Productivity /Survival Rates	% Below Min Temp Target	% Above Max Temp Target
Males	% Males Productive	-0.66	-0.06
Females & Oviposition	% Females Productive	-0.17	-0.78*
Eggs & Prediapause	Hatch Rate	0.88^	0.58
Warm Diapause	% Into Diapause	0.65	0.20
Cold Diapause	% Out of Diapause	--	-0.46
Postdiapause	% To Release	-0.29	-0.09

^ p = 0.07
*p = 0.02

Wild Population – Productivity/Survival vs. Environmental Targets

The percent success of different life stages for the wild population was not typically correlated with the percentage of time the environmental targets were met. Using Spearman's rho, the percent of larvae that pupated was negatively correlated to the percentage of time night temperature targets are met (rho = -0.76, p = 0.08, Table 11). However, the percent of successful ovipositing females was positively correlated to the percent of time outside the percent females productive (rho = 0.88, p = 0.02, Table 11) Also note that these p=values were between 0.05 and 0.10. (Appendix B. Figures 34-41)

Table 11. Spearman's rho correlation for the percent productivity/survival of the wild population versus the percent of time the environmental targets were met for seasons 2013-2014 to 2018-2019 and 2020-2021.

Wild Population		Spearman's Rho Coefficient		
Life Stage	% Productivity /Survival Rates	% Time RH Target is Met	% Time Temp Target is Met	
Females & Oviposition	% Females Productive	-0.25	0.23	
Eggs & Prediapause	Hatch Rate	-0.11	-0.44	
Warm Diapause	% Into Diapause	0.39	0.07	
Cold Diapause	% Out of Diapause	-0.68^	-0.28	
Postdiapause to Survival	% To Release or Retained	0.22	Daytime -0.12	Nighttime -0.11
Postdiapause to 2nd Diapause	% Wilds Return to 2nd Diapause	-0.03	Daytime -0.20	Nighttime 0.39
Postdiapause to Pupation	% Wilds to Pupation	-0.29	Daytime -0.12	Nighttime -0.76*
Pupation	% Wilds Enclosed	0.31	Daytime -0.12	Nighttime -0.09

^ p=0.09

* p=0.08

No correlations occurred for any life stages in the wild population compared to the percent of days outside the temperature targets (Table 12; Appendix B. Figures 48-55).

Table 12. Spearman's rho coefficient between the productivity/survival of the wild population versus the percent of time outside the temperature target for seasons 2013-2014 to 2018-2019 and 2020-2021.

Wild Population		Spearman's Rho Coefficient	
Life Stage	% Productivity /Survival Rates	% Below Min Temp Target	% Above Max Temp Target
Females & Oviposition	% Females Productive	0.04	0.13
Eggs & Prediapause	Hatch Rate	0.19	-0.19
Warm Diapause	% Into Diapause	0.61	-0.04
Cold Diapause	% Out of Diapause	--	-0.44
Postdiapause to Survival	% To Release or Retained	-0.26	0.26
Postdiapause to 2nd Diapause	% Wilds Return to 2nd Diapause	0.03	0.09
Postdiapause to Pupation	% Wilds to Pupation	0.26	-0.31
Pupation	% Wilds Successfully Enclosed	-0.06	-0.26

MOPHOLOGICAL RESULTS

Weight & Wing Area

Captively bred adult females weighed significantly more than wild females in all six seasons (Table 13, Figure 8, Appendix B Figures 56-61). There was not an effect of season on butterfly weight (over time, Table 13). Differences in wing area between the captive and wild females were more variable, as wild adults had significantly larger wings during the 2014-2015 and 2017-2018 seasons but not others (Table 14, Figure 9, Appendix B. Figures 62-66). Both captivity status (wild vs. captive) and season had significant effects on wing area (Table 14), with a slight decrease in wing area across the seasons (visible in Figure 9).

Table 13. Median adult female E. e. taylori *weights by season at MCCCW, with p-values from a Wilcoxon rank sum test (see also Appendix B Figures 36-61).*

Median Weight (g)				Multiple Regression on Weight		
Season	Captive	Wild	p-value	Parameter	Estimate	p-value
2013-14	0.18	0.14	0.03			
2014-15	0.15	0.09	<0.0001	Status[Captive]	0.022	<0.001
2015-16	0.17	0.11	<0.0001	Season	-0.001	0.114
2016-17	0.17	0.12	<0.0001			
2017-18	0.15	0.12	<0.0001	$R^2_{adj} = 0.22$		
2018-19	0.17	0.12	<0.0001			

Table 14. Median adult female E. e. taylori *wing area by season at MCCCW, with p-values from a Wilcoxon rank sum test (see also Appendix B Figures 62-66).*

Median Wing Area (cm^2)				Multiple Regression on Area		
Season	Captive	Wild	p-value	Parameter	Estimate	p-value
2014-15	1.43	1.68	0.0118			
2015-16	1.62	1.61	0.5535	Status[Captive]	-0.037	0.011
2016-17	1.51	1.63	0.4588	Season	-0.065	<0.001
2017-18	1.34	1.51	0.0035			
2018-19	1.24	1.28	0.273	$R^2_{adj} = 0.10$		

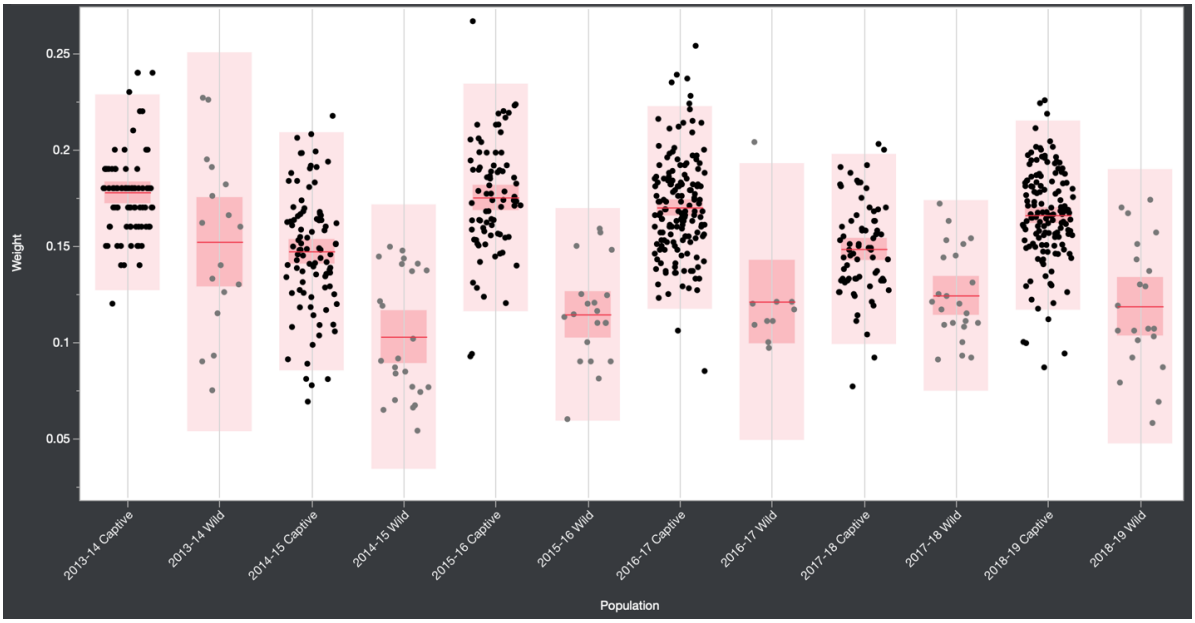


Figure 8. Data points and boxplots of adult female weight (g), by captive (black points) or wild (grey points) status and across the 2013-14 to 2018-19 seasons (x-axis labels). The light pink boxes represent 10th and 90th percentiles, the red line the mean, and the reddish boxes the 95% CI around the mean.

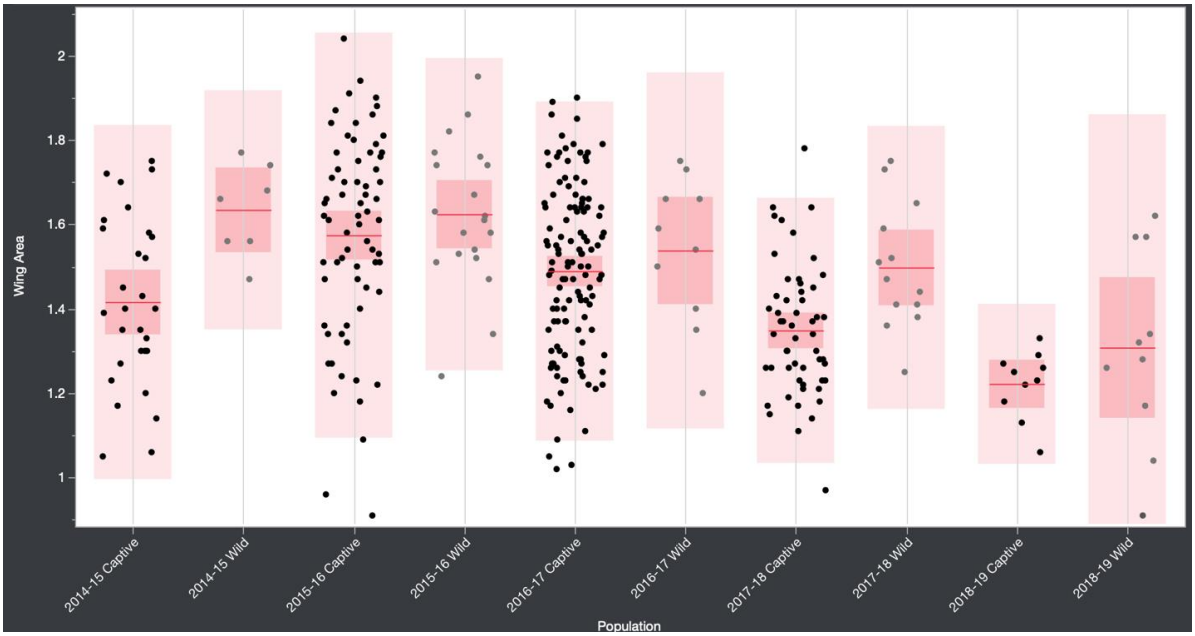


Figure 9. Data points and boxplots of adult female wing area (cm^2), by captive (black points) or wild (grey points) status and across the 2013-14 to 2018-19 seasons (x-axis labels). The light pink boxes represent 10th and 90th percentiles, the red line the mean, and the reddish boxes the 95% CI around the mean.

DISCUSSION

Adult Butterflies

Both the percentage of time the environmental targets were met during different stages of captive reading, and butterfly productivity and survival rates, were variable. For different life stages, there was a lack of correlation between the percent productivity and survival and the percent of time environmental targets were met (Table 9) in addition to a lack of correlation to the percent of days outside the temperature targets (Table 10) throughout the seasons in this study. Whether or not males successfully copulate (% males productive) experienced variation throughout the seasons but was relatively low, with average productivity of 23% (Table 6). Environmental conditions could influence these outcomes, given the need for adult butterflies to bask for thermoregulation and capacity to become exhausted by high heat (Porter, 1982; Weiss, Murphy and White, 1988; Hellmann *et al.*, 2004). However, these factors lack any correlations in this study.

Females and oviposition success (% females productive) also experiences variation, particularly in captive females yielding a range of 25%-95% productivity (Table 6). The percent of captive females productive and the percent of time the temperature target is between 78º-90º for two to six hours per day (Table 9) was negatively correlated, in addition to a negative correlation to the percent of days the maximum daily temperature was above the maximum target of 90º (Table 10). However, no such correlations exist for wild females. Captive females could be experiencing diminished environmental plasticity compared to their wild counterparts. However, parameters often used to compare phenotypic plasticity between the wild and captive

populations would be challenging to collect for this program; ages and first-reproductions of wild individuals are unknown, limiting insight into fecundity (Stillwell and Fox, 2005; Steigenga and Fischer, 2009). Current research on fitness of captively reared and captively bred *E. e taylori* has focused on high larval mortality experienced during diapause and postdiapause at Oregon Zoo and investigating oviposition outcomes and larval host plant preferences (Lewis *et al.*, 2018; Haan, Bowers and Bakker, 2021). Parameters available to investigate captive fitness within this program include reintroducing morphometric data collection, adding egg measurements via microscopes, recording and quantifying development times seasonally (Fischer, Brakefield and Zwaan, 2003; Stillwell and Fox, 2005; Crone, Pickering and Schultz, 2007).

Captive female wing areas were similar to or smaller than wild female wing area (depending on the season), with wild wing area on a downward trend overall (Table 14, Figure 9). For one of these seasons (2014-2015), the wild sample size was only seven compared to the captive sample size of 29. Similarly, the 2016-2017 season, which did not show a significant difference (Table 14), also had sample size disparities with the wild sample size at ten versus the captive sample size of 124. Differences in sample sizes between captive and wild populations always exist given the quantity of postdiapause larvae retained and because morphometric data is recorded for all captive adults that eclose even if they get released. This disparity could skew results, so a larger data pool, including Oregon Zoo morphometric results, could help inform this research. These results are consistent with research trialing optimal rearing conditions for the Puget blue butterfly, which resulted in smaller wings and body lengths, and other earlier findings

showing similar outcomes (Lewis and Thomas, 2001; Schultz, Dzurisin and Russell, 2008; Altizer and Davis, 2009).

Captive and wild females' weights were significantly different for all seasons (Table 13), with captive females consistently being heavier than wild females (Figure 8). This appears to be consistent with research that found larger or heavier body sizes in captive butterflies (Lewis and Thomas, 2001; Schultz, Russell and Wynn, 2008) but inconsistent with a study showing smaller body sizes in captive individuals (Schultz, Dzurisin and Russell, 2008). Larger sizes do tend to be linked to lower temperatures (Atkinson, 1994; Angilletta, Steury and Sears, 2004), and some studies indicate selective pressure towards lower temperatures could decrease productivity during warmer conditions (Berger, Walters and Gotthard, 2008; Fischer and Karl, 2010) however this is not a general rule for all ectotherms (Loeschcke, Bundgaard and Barker, 2000; Angilletta, Steury and Sears, 2004; Stillwell and Fox, 2005). Research also suggests a positive relationship between temperature, body size, and fecundity (Blanckenhorn, 2000; Gotthard, Berger and Walters, 2007; Berger, Walters and Gotthard, 2008) however the outcomes of this limited study are not consistent with these findings. Studies of temperature variation impacts on checkerspots and *E. e taylori* focus predominantly on the relationship between larval development times and how that relates to host plant availability and adult emergence timing (Weiss, Murphy and White, 1988; Weiss *et al.*, 1993; Hellmann *et al.*, 2004; Bennett, Betts and Smith, 2014). In addition, some studies comparing body size look at body area or length rather than body mass, making it unclear if these are comparable.

Egg & Larval Stages

Captive hatch rates had greater variation than wild hatch rates (Tables 6 & 7). However, there was a lack of correlation between prediapause survival and whether or not the environmental targets were met (Tables 10 & 12). It's unclear what caused the captive population to have more variation in hatch rates than the wild population, given that primary causes of egg loss in the wild are predation—which was not a factor in captive rearing—or desiccation by extreme temperatures (Kuussaari *et al.*, 2004). However, egg clusters can be exceedingly challenging to count depending on how many eggs were laid in a single cluster, so egg counts are considered estimates. Wild and captive hatch rates would still see similar variation if this were the sole factor.

Prediapause (% into diapause), warm and cold diapause (% out of diapause), and postdiapause (% to release/retained) life stages do not have a lot of variation between seasons for either captive or wild populations (Tables 6-8). The success of these life stages and the percentage of time environmental targets were met lack any correlation for the captive population (Tables 10 & 12). However, a positive correlation was indicated for the captive-bred population between eggs and prediapause larvae (hatch rate) and the percent of days below the minimum temperature target of met (Table 11). Exceedingly high temperatures can lead to egg desiccation and larval death, while low temperatures slow development (Hellmann *et al.*, 2004; Kuussaari *et al.*, 2004). There has a positive correlation between temperatures below 50° and prediapause larval survival for the captive population (Table 11), which could support acclimatization to cool conditions (Blanckenhorn, 2000). Seasons with the highest captive egg production, 2013-2014, 2016-2016, and 2018-2019, interestingly have the lowest minimum temperature,

with two of those seasons also having the highest maximum temperatures for all of the seasons in this study (Appendix A. Tables 17-22), making these findings vague.

It's unclear how the percentage of time environmental targets were or were not met during prediapause larval stages impacts the timing of other life stages and how that impacts success once released. Prediapause, postdiapause, and pupation development times strongly rely on microclimate conditions. During any of these stages, development times influence the timing of other life stages, which can impact adult emergence time, a primary determinant of reproductive success (Weiss *et al.*, 1987, 1993; Hellmann *et al.*, 2004; Kuussaari *et al.*, 2004). Tests indicated a negative correlation exists for postdiapause larvae to second diapause (% Return to diapause) and the percent of time nighttime temperature target is met (Table 11). Postdiapause development is more strongly reliant on the ability to thermoregulate within the microclimate the larvae are in (Weiss, Murphy and White, 1988; Hellmann *et al.*, 2004).Wild larvae also have a negative correlation to the percent of time the humidity target being met during diapause; however, the direct impacts of humidity on captive rearing are less understood given humidity's usual relation to host plant senescence in conjunction with temperature (Hellmann *et al.*, 2004; Klockmann and Fischer, 2019; Reed *et al.*, 2019).

Program Success

Euphydryas editha taylori captive rearing seasons at MCCCW tend to be successful in producing enough eggs that hatch larvae that survive through all life stages in captivity and make it to be released in the wild (Tables 6-8). A 2016 periodic update of *E. e. taylori* and their habitat showed a prosperous population on JBLM range 76, near where MCCCW captively reared larvae are released (Potter, 2016). However, this

population was not observed during brief inspections in the 2021 *E. e. taylori* flight season. Access to this site was restricted following the impacts of COVID-19 and official surveys and reports have yet to officially confirm this population's continued presence in the site (*pers. communication*, Mary Linders). This development isn't necessarily an indication that captive rearing is producing unsuccessful individuals since bounteous flight seasons have been observed on range 76 weeks after seemingly successful translocations of captive populations from MCCCW to release sites have taken place. Poor host plant availability and unprecedented weather events in the field are thought to play a role in why butterflies were not observed this past year. *Euphydryas* editha subspecies are also notorious for disappearing and reappearing depending on seasonal success (Hanski, Ehrlich, *et al.*, 2004; Hellmann *et al.*, 2004). When compared to the captive rearing program for the critically endangered *Euphydryas editha quino*—which was able to release a total of 1,513 larvae over two captive rearing seasons (CBI Blog, 2020)—*E. e. taylori* program goals of harvesting 2,500 postdiapause larvae from 5000 eggs seem substantial (Appendix A. Table 16). MCCCW, though it has only passed the threshold of 5000 eggs harvested once in the 2018-2019 season, exceeds the postdiapause larvae target every season, excluding 2017-2018 and 2020-2021 (Appendix A Table 23). When compared to the trials of inexplicable larval mortality experienced during diapause and postdiapause at the Oregon Zoo, MCCCW captive rearing program was highly prosperous; per the Zoos 2017-2018 report, 56% of the near 1500 larvae into diapause survived to postdiapause, and 28% of the larvae that entered diapause survived to release (Lewis *et al.*, 2018).

The other remaining *E. e. taylori* captive rearing program in OR—separate from this program and Oregon Zoo, and located in Coffee Creek Corrections Facility (CCCF)—set a goal for 2020-2021 to exceed the previous season postdiapause release of 1100, exceeding that goal by 136 (Naseth, 2021). However, mortality experienced after postdiapause was relatively high compared to MCCCW, with 66% survival from diapause to release. For comparison, MCCCW experienced 90% survival from the start of diapause to release for 2017-2018 and 99% survival for 2020-2021. The Zoo and CCCF rear in a lab setting, excluding cold diapause where larvae are moved outside to an overwintering area per research and protocol (Schultz, Dzurisin and Russell, 2008; Barclay *et al.*, 2009) However, providing a significant number of larvae for release alone is not related to reintroduction success (Oates and Warren, 1990; Hanski, Ehrlich, *et al.*, 2004, p. 281). In the labs, wild conditions need to be replicated, as opposed to the greenhouses at MCCCW, where near-ambient conditions need to be manipulated. Comparisons could be carried out between these three facilities to fully understand survival differences and the impacts of environmental conditions, respectively. Ongoing efforts to improve habitat connectivity, remove invasive species, and restore prairies strengthen chances of captive rearing success (Potter, 2016). Coupling habitat progress and conditions with program outcomes and conditions is likely the next step in future research that would provide the greatest benefit to future program, and species recovery, success.

Recommendations to Environmental Targets & Procedures

It is imperative for those working with the Butterflies Technicians to focus on instilling the importance of managing and recording the environmental conditions. Tying

the control of the environmental conditions firmly to the productivity and survival of the butterfly brings this into the purview of *E. e. taylori* daily care needs, rather than an isolated task that may appear nonessential or may become easily forgotten. Once the Technicians receive the necessary training and educational background, they should receive clear procedures dedicated to detailing the environmental targets for each life stage and what the Technicians can do to adjust conditions and bring them closer to the objective conditions. Studies show that when captive rearing procedures—including environmental parameters—are detailed and comprehensive, there is a higher chance for success (Adamski and Witkowski, 2007; Crone, Pickering and Schultz, 2007; Wilcox *et al.*, 2021). This empowerment through education allows Butterfly Technicians to work as a team to command the decision-making for the greenhouse based on the tools available to them. Rapid response by technicians could minimize loss of butterfly production or larval mortality that may otherwise occur while waiting for communication between Technicians and the Butterfly Program Coordinator.

Established parameters for maintaining environmental conditions were initiated but could be expanded upon and developed into Captive Rearing Environmental Target Procedures. An example could be females and oviposition, where the temperature should be between 78º-90º for 4-8 hours of the day. This translates to moving the ovipositing chambers into sunlight within the greenhouse but still providing the option of shade—either through the plantain leaves or adding a sticky note to the top of the chamber—to promote egg-laying but prevent overheating (*pers. communication,* Mary Linders). This allows thermoregulation but prevents exhaustion and early mortality. In addition, there is a very brief description in the husbandry manual explaining basking and the need to

provide a variety of light, temperature, and humidity microclimates for basking adults and postdiapause larvae. Basking information has not been elaborated on in the MCCCW procedures, making it unclear what basking behaviors to expect.

Photos throughout the procedures also show ceilings or shelves draped in sheets during oviposition, eggs and early instar larvae, and postdiapause larvae life stages (Barclay *et al.*, 2009; Curry *et al.*, 2020). This could be a tactic to prevent extreme heat, or the set-up technicians use to block the intense setting sun before leaving for the day. However, this is unclear and conflicts with the directive in the husbandry manual and the basking behavior that this species is known to carry out during multiple life stages (Weiss, Murphy and White, 1988). This information has been briefly described in captive rearing procedures, but incorporating and situating this kind of information into environmental procedures emphasizes the importance of environmental conditions during captive rearing. Covering portions of the greenhouse with shade cloths allows for more control and providing temperature thresholds in which these should be erected could be imperative to productivity and development. Providing explicit instruction on how to achieve—or get close to achieving—environmental targets in relation to the outcomes—or consequences—of carrying out these tasks can prevent conditions from swinging from one extreme to another.

Environmental targets for reporting and rearing could be slightly different to prevent overreporting but still provide the Butterfly Technicians with explicit instructions for managing environmental conditions. Life stages where targets are rarely met could cause misalignment in the Butterfly Technicians' understanding of why this is important and lead to despondency at their work. Cold Diapause, as an example, has an average

mean temperature of 44 and an average minimum temperature of 19 (Table 3), with the 2016-2017 season having the highest percentage of time meeting the target at 16% (Table 4). This temperature target could be slightly increased since the lack of meeting the target doesn't appear to impact larval survival due to the lack of mortality out of diapause. This is especially true since average temperatures in Belfair remain above 35 degrees for 11 months of a year (Appendix B. Figure 67) based on records available online (Weather Spark, no date). An absolute minimum temperature could be applied to females and oviposition—and males if breeding returned—so Technicians know to readily implement adjustments to conditions if the overnight temperature dipped below the specified absolute minimum. Recording and properly managing ambient conditions using HOBO loggers at MCCCW could better inform this process.

Providing an absolute range for humidity during all life stages may allow Technicians to better understand what steps they should take to adjust conditions and prevent loss of production or larval mortality that may occur from females becoming waterlogged or egg and larval cups becoming moldy due to high humidity. Currently, there's no indication that high humidity could be unfavorable with these targets. This lack of clarity could prolong daily care when even clean larval cups need fresh liners due to saturation by condensation. Providing an absolute range for humidity—such as removing shoe bin lids, moving shelves near open windows and vents during the day, and ensuring plantain leaves are thoroughly dry for all larval stages—could keep humidity closer to the targets and reduce the amount of time Technicians spend on *E. e. taylori* daily care during transition periods. Providing an absolute range for temperature during all life

stages allows for a similar response by technicians; the range indicates when action should be taken to prevent temperatures from going to an extreme.

Once there is a better capacity for maintaining these targets, a qualitative aspect could be added to the adult life stages in addition to the numeric temperature targets. The Technicians are required to put adults in light for thermoregulation for a portion of the day per protocols and procedures, seemingly indicated by the specificity of the targets during those life stages in comparison to other targets (Boggs and Nieminen, 2004; Barclay *et al.*, 2009; Curry *et al.*, 2020). Since breeding no longer occurs—when carrying out and checking ups on copulation tents took up a significant amount of the Technicians' time—maintaining these targets for ovipositing females could involve Technicians regularly checking on activity in-between searching chambers for eggs. Technicians could record behavioral observations, and productivity and environmental conditions will be documented. Activity for the day could be summarized by the team and discussed with the Butterfly Program Coordinator at the end of the life stage. This joining of qualitative and quantitative data could inform annual reporting to understand how the environmental conditions impacted productivity. The same could be done for males if breeding were to return; qualitative notetaking was extensively carried out before breeding ceased, and incorporating this portion back in could greatly assist in improving program communication. This quantitative and qualitative data could be used to determine if a negative correlation does exist between the environmental targets set for female productivity, and over time could be us to incrementally adjust targets if necessary. Lastly, to improve data tracking in the program, I recommend reinstating the use of original Egg and Larvae Data Sheets and reintroducing morphometric data collection.

Current data forms would need further updates to address larval count discrepancies occurring since the application of these new data forms. Egg & Larvae Data Sheets track important information that could be imperative to further research, including detailed development times. These forms could be used in conjunction with current forms and cross-referenced as needed to understand larval count discrepancies. Morphometric data is commonly used to catch and understand divergencies between wild and captive populations (Lewis and Thomas, 2001; Dzurisin, 2005; Crone, Pickering and Schultz, 2007). Introducing egg measurements and adult body area measurements as well are reinstating pupal weight, adult weight, and wing measurements would help better understand the program, and population, success at MCCCW and within other facilities.

CONCLUSION

This study shows that the current environmental targets for different life stages in captivity are often not met, perhaps fortunately with no clear indication of a negative effect on *E. e. taylori* survival and productivity. With the methods employed in this study, the % of time targets are met is not an exceptional indicator of program success. Outcomes do show an area of the program that could be expanded upon; since the Technicians are the front line for daily care and management, providing clear absolute targets and more focused tasks for how to achieve desired results of productivity and survival. Continuing the research of preferred *E. e. taylori* environmental conditions by looking at current field environmental conditions could change how these targets are utilized, becoming a more reliable indication of success by making them much more precise and accurate.

Further research into the impacts of environmental conditions on development times and morphometric data from seasons could help illuminate the fitness. However, the overall success of this program reveals the importance of rearing wildlife in ambient conditions—even if there is not a complete picture of the species' wild habitat requirements—especially when the subjects are ectotherms that require sunlight at almost all life stages. In addition to this captive rearing facility preventing the loss of an endangered species and advancing *E. e. taylori* conservation objectives, the opportunity for collaboration, education, and research continues to broaden the knowledge available on *E. e. taylori.*

APPENDIX A – Additional Tables

Table 15. Captive rearing productivity and survival targets for seasons 2014-2015 to 2018-2019.

Seasons	Eggs	Larvae	Retained Larvae
2014-2015	5000	2500	300
2015-2016	5000	2500	300
2016-2017	5000	2500	300
2017-2018	5000	2500	300
2018-2019	5000	1800	300

Table 16. Example of a daily minimum, maximum, and average temperature and humidity table experiences duirng captive rearing. To the right it the comparison of the absolute targets to the minimum and maximum temperature.

Date	Min Temp	Max Temp	Avg Temp	Min Rh	Max Rh	Avg Rh	<50º	>90º
4/25/20	64	72	66	52	54	52	FALSE	FALSE
4/26/20	59	71	64	41	70	55	FALSE	FALSE
4/27/20	61	78	67	54	75	63	FALSE	FALSE
4/28/20	61	72	65	53	67	61	FALSE	FALSE
4/29/20	61	77	67	53	79	67	FALSE	FALSE
4/30/20	59	73	64	62	82	72	FALSE	FALSE
5/1/20	57	76	65	68	82	73	FALSE	FALSE
5/2/20	57	71	62	75	89	83	FALSE	FALSE
5/3/20	54	69	61	71	83	78	FALSE	FALSE
5/4/20	51	74	62	69	82	75	FALSE	FALSE
5/5/20	57	77	66	56	84	76	FALSE	FALSE
5/6/20	59	81	67	60	81	72	FALSE	FALSE
5/7/20	55	83	67	58	79	69	FALSE	FALSE
5/8/20	62	88	71	54	75	64	FALSE	FALSE
5/9/20	63	94	73	55	78	68	FALSE	Yes
5/10/20	65	83	72	44	83	64	FALSE	FALSE
5/11/20	61	80	67	69	84	78	FALSE	FALSE
5/12/20	59	84	67	59	88	78	FALSE	FALSE
5/13/20	60	93	70	45	83	69	FALSE	Yes
5/14/20	59	91	68	46	82	71	FALSE	Yes
5/15/20	56	104	73	34	84	64	FALSE	Yes
5/16/20	59	76	64	70	88	78	FALSE	FALSE
Min	51	69	61	34	54	52	0	4
Max	65	104	73	75	89	83		
Avg	59	80	67	57	80	70		

Table 17. Percent relative humidity averages of the minimum, maximum, and average percent relative humidity for life stages pupation and males for seasons 2013-2014 to 2018-2019.

Season	Pupation			Males		
	Min	Max	Avg	Min	Max	Avg
2013-14	41	81	62	28	71	47
2014-15	33	94	68	39	83	65
2015-16	26	97	66	18	100	59
2016-17	23	100	61	21	87	62
2017-18	34	100	77	30	97	65
2018-19	27	100	78	26	98	63
Average	31	95	69	27	89	60

Table 18. Percent relative humidity averages of the minimum, maximum, and average for life stages females and eggs & prediapause larvae for seasons 2013-2014 to 2018-2019 and 2020-2021.

Season	Females & Oviposition			Eggs & Prediapause		
	Min	Max	Avg	Min	Max	Avg
2013-14	27	68	51	27	68	51
2014-15	39	83	68	50	99	77
2015-16	18	100	60	26	89	63
2016-17	19	91	59	21	94	66
2017-18	31	98	67	34	97	64
2018-19	22	86	59	19	95	64
2020-21	34	89	70	24	93	65
Average	27	88	62	29	91	64

Table 19. Percent relative humidity averages of the minimum, maximum, and average for the life stages warm diapause, cold diapause, and postdiapause.

Season	Warm Diapause			Cold Diapause			Postdiapause		
	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg
2013-14	28	70	49	29	90	73	36	86	64
2014-15	24	100	70	41	100	92	24	94	68
2015-16	23	92	61	57	99	90	26	98	68
2016-17	28	97	71	42	98	86	31	93	70
2017-18	26	94	61	31	100	88	22	98	68
2018-19	21	87	60	37	100	86	19	100	68
2020-21	43	97	80	41	99	88	46	91	67
Average	28	91	65	40	98	86	29	94	68

Table 20. Temperature averages of the minimum, maximum, and average for the life stages males and pupation.

Season	Males			Pupation		
	Min	Max	Avg	Min	Max	Avg
2013-14	31	102	60	43	79	60
2014-15	52	81	64	47	92	63
2015-16	46	87	63	37	80	55
2016-17	49	95	66	52	99	65
2017-18	52	99	66	49	91	63
2018-19	49	89	64	53	97	65
Average	47	92	64	47	90	62

Table 21. Overall temperature averages of the minimum, maximum, and average temperature for life stages females & oviposition and eggs & prediapause.

Season	Females & Oviposition			Eggs & Prediapause		
	Min	Max	Avg	Min	Max	Avg
2013-14	31	101	62	31	105	65
2014-15	52	81	64	53	91	66
2015-16	46	87	64	55	95	67
2016-17	41	100	64	48	101	65
2017-18	55	91	66	55	93	70
2018-19	49	95	67	47	96	67
2020-21	51	104	67	47	97	67
Average	46	94	65	48	97	67

Table 22. Overall temperature averages of the minimum, maximum, and average temperature for life stages warm diapause, cold diapause, and postdiapause.

Season	Warm Diapause			Cold Diapause			Postdiapause		
	Min	Max	Avg	Min	Max	Avg	Min	Max	Avg
2013-14	46	104	70	13	78	44	35	79	57
2014-15	51	100	70	21	80	44	39	86	58
2015-16	51	99	71	22	87	46	37	85	56
2016-17	52	89	67	17	82	45	44	83	56
2017-18	58	93	73	17	88	43	32	91	58
2018-19	51	97	70	14	84	44	47	90	63
2020-21	53	99	68	26	86	45	43	82	56
Average	52	97	70	19	84	44	40	85	58

Table 23. Number of eggs and postdiapause larvae per season. Note, prediapause release occurred for captive populations, and 20-21 season totals include larvae in the second greenhouse at MCCCW.

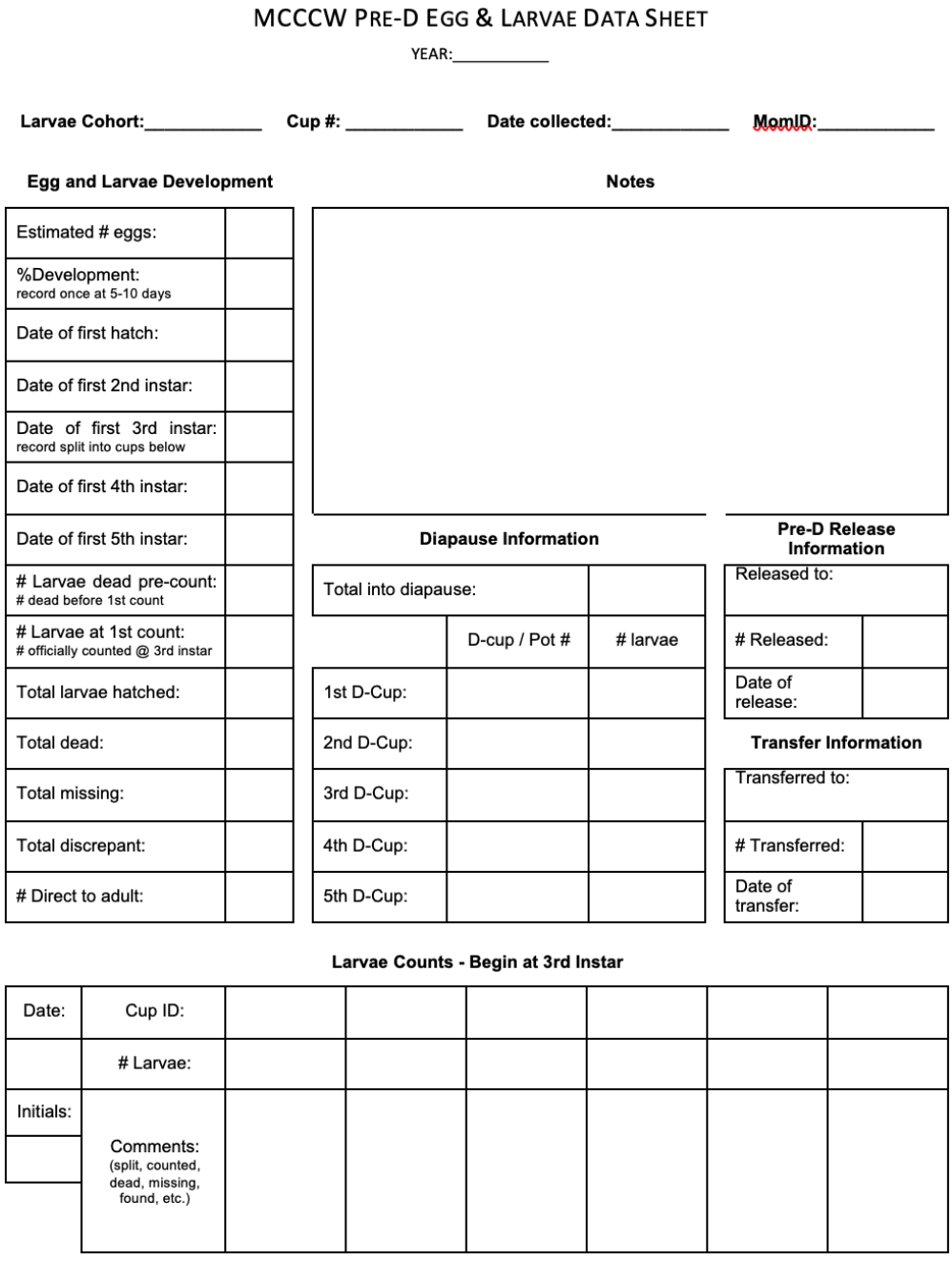
Season	Wild		Captive		Total	
	# Eggs	# Out of Diapause	# Eggs	# Out of Diapause	# Eggs	# Out of Diapause
2013-14	1373	1324	2378	1456	3751	2780
2014-15	2327	1909	2437	685	4764	2594
2015-16	2772	2111	2205	624	4977	2735
2016-17	1261	1426	1121	541	2382	1967
2017-18	2938	3016	609	146	3547	3162
2018-19	2944	2888	4965	1659	7909	4547
2020-21	2414	2279	--	--	4696	3813

Table 24. Example of morphometric data summary table.

Units of Analysis	Descriptive Statistic	Adult Weight(g)	Wing Area (cm2)
Captive Males	n	125	72
	mean	0.0924	0.9631
	sd	0.018	0.1027
	var	0.0003	0.0105
	median	0.09	0.9595
	range	0.0630-0.1820	0.6980-1.2330
Captive Females	n	94	86
	mean	0.1583	1.2631
	sd	0.0211	0.1382
	var	0.0004	0.0191
	median	0.158	1.2495
	range	0.1180-0.2170	0.9220-1.5700
Wild Females	n	24	21
	mean	0.1241	1.2948
	sd	0.0351	0.2097
	var	0.0012	0.044
	median	0.124	1.317
	range	0.0580-0.1820	0.9050-1.6720

APPENDIX B – ADDITIONAL FIGURES

Figure 10. Example of MCCCW data collection form used during captive rearing.



MCCCW PRE-D EGG & LARVAE DATA SHEET

YEAR:_____

Larvae Cohort:_____ **Cup #:** _____ **Date collected:**_____ **MomID:**_____

Egg and Larvae Development

Estimated # eggs:	
%Development: record once at 5-10 days	
Date of first hatch:	
Date of first 2nd instar:	
Date of first 3rd instar: record split into cups below	
Date of first 4th instar:	
Date of first 5th instar:	
# Larvae dead pre-count: # dead before 1st count	
# Larvae at 1st count: # officially counted @ 3rd instar	
Total larvae hatched:	
Total dead:	
Total missing:	
Total discrepant:	
# Direct to adult:	

Notes

Diapause Information

Total into diapause:		
	D-cup / Pot #	# larvae
1st D-Cup:		
2nd D-Cup:		
3rd D-Cup:		
4th D-Cup:		
5th D-Cup:		

Pre-D Release Information

Released to:	
# Released:	
Date of release:	

Transfer Information

Transferred to:	
# Transferred:	
Date of transfer:	

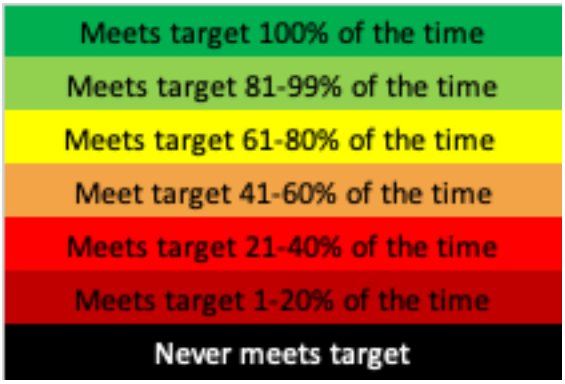
Larvae Counts - Begin at 3rd Instar

Date:	Cup ID:						
	# Larvae:						
Initials:	Comments: (split, counted, dead, missing, found, etc.)						

Figure 11. Example snapshot of the hour environmental data analysis into the percentages of time environmental targets were met.

Eggs & Pre-Diapause 2013-2014									
Hourly Temp - 28 of 68 days (41%) b/w 50-90 for 24 hr/day					Hourly %RH - 65 of 68 days (96%) >/=50% for 24 hr/day				
Date	Time	Temp	50-90	28	Date	Time	%RH	</=65	65
5/1/13	1:00 AM	40	FALSE		5/1/13	1:00 AM	49	Yes	
5/1/13	3:00 AM	36	FALSE		5/1/13	3:00 AM	49	Yes	
5/1/13	5:00 AM	33	FALSE		5/1/13	5:00 AM	49	Yes	
5/1/13	7:00 AM	31	FALSE		5/1/13	7:00 AM	49	Yes	
5/1/13	9:00 AM	40	FALSE		5/1/13	9:00 AM	53	Yes	
5/1/13	11:00 AM	61	Yes	0	5/1/13	11:00 AM	53	Yes	1
5/1/13	1:00 PM	72	Yes		5/1/13	1:00 PM	51	Yes	
5/1/13	3:00 PM	76	Yes		5/1/13	3:00 PM	49	Yes	
5/1/13	5:00 PM	77	Yes		5/1/13	5:00 PM	46	Yes	
5/1/13	7:00 PM	71	Yes		5/1/13	7:00 PM	43	Yes	
5/1/13	9:00 PM	58	Yes		5/1/13	9:00 PM	43	Yes	
5/1/13	11:00 PM	49	FALSE		5/1/13	11:00 PM	43	Yes	
5/2/13	1:00 AM	44	FALSE		5/2/13	1:00 AM	43	Yes	
5/2/13	3:00 AM	40	FALSE		5/2/13	3:00 AM	43	Yes	
5/2/13	5:00 AM	38	FALSE		5/2/13	5:00 AM	43	Yes	
5/2/13	7:00 AM	37	FALSE		5/2/13	7:00 AM	44	Yes	
5/2/13	9:00 AM	47	FALSE		5/2/13	9:00 AM	47	Yes	
5/2/13	11:00 AM	69	Yes	0	5/2/13	11:00 AM	47	Yes	1
5/2/13	1:00 PM	81	Yes		5/2/13	1:00 PM	45	Yes	
5/2/13	3:00 PM	80	Yes		5/2/13	3:00 PM	43	Yes	
5/2/13	5:00 PM	74	Yes		5/2/13	5:00 PM	41	Yes	
5/2/13	7:00 PM	70	Yes		5/2/13	7:00 PM	40	Yes	
5/2/13	9:00 PM	60	Yes		5/2/13	9:00 PM	39	Yes	
5/2/13	11:00 PM	53	Yes		5/2/13	11:00 PM	40	Yes	
5/3/13	1:00 AM	51	Yes		5/3/13	1:00 AM	41	Yes	
5/3/13	3:00 AM	46	FALSE		5/3/13	3:00 AM	41	Yes	
5/3/13	5:00 AM	42	FALSE		5/3/13	5:00 AM	41	Yes	
5/3/13	7:00 AM	40	FALSE		5/3/13	7:00 AM	42	Yes	
5/3/13	9:00 AM	49	FALSE		5/3/13	9:00 AM	45	Yes	
5/3/13	11:00 AM	71	Yes	0	5/3/13	11:00 AM	45	Yes	1
5/3/13	1:00 PM	80	Yes		5/3/13	1:00 PM	44	Yes	
5/3/13	3:00 PM	86	Yes		5/3/13	3:00 PM	42	Yes	
5/3/13	5:00 PM	86	Yes		5/3/13	5:00 PM	40	Yes	
5/3/13	7:00 PM	79	Yes		5/3/13	7:00 PM	38	Yes	
5/3/13	9:00 PM	66	Yes		5/3/13	9:00 PM	37	Yes	
5/3/13	11:00 PM	57	Yes		5/3/13	11:00 PM	38	Yes	
5/4/13	1:00 AM	51	Yes		5/4/13	1:00 AM	39	Yes	
5/4/13	3:00 AM	47	FALSE		5/4/13	3:00 AM	40	Yes	
5/4/13	5:00 AM	45	FALSE		5/4/13	5:00 AM	40	Yes	
5/4/13	7:00 AM	43	FALSE		5/4/13	7:00 AM	42	Yes	
5/4/13	9:00 AM	54	Yes		5/4/13	9:00 AM	45	Yes	
5/4/13	11:00 AM	75	Yes	0	5/4/13	11:00 AM	45	Yes	1
5/4/13	1:00 PM	85	Yes		5/4/13	1:00 PM	42	Yes	
5/4/13	3:00 PM	88	Yes		5/4/13	3:00 PM	39	Yes	
5/4/13	5:00 PM	87	Yes		5/4/13	5:00 PM	35	Yes	
5/4/13	7:00 PM	80	Yes		5/4/13	7:00 PM	32	Yes	
5/4/13	9:00 PM	70	Yes		5/4/13	9:00 PM	31	Yes	
5/4/13	11:00 PM	63	Yes		5/4/13	11:00 PM	31	Yes	

Figure 12. Legend for the percent of time the environmental targets are met.



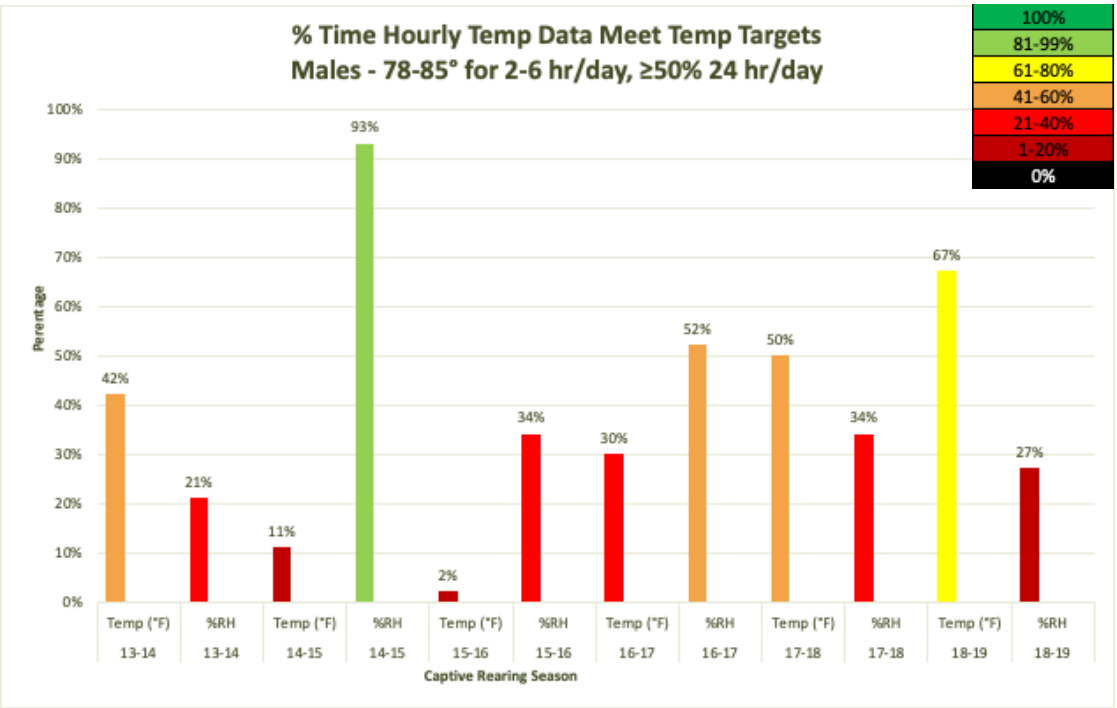


Figure 13. Percent of time environmental targets were met during males for all seasons.

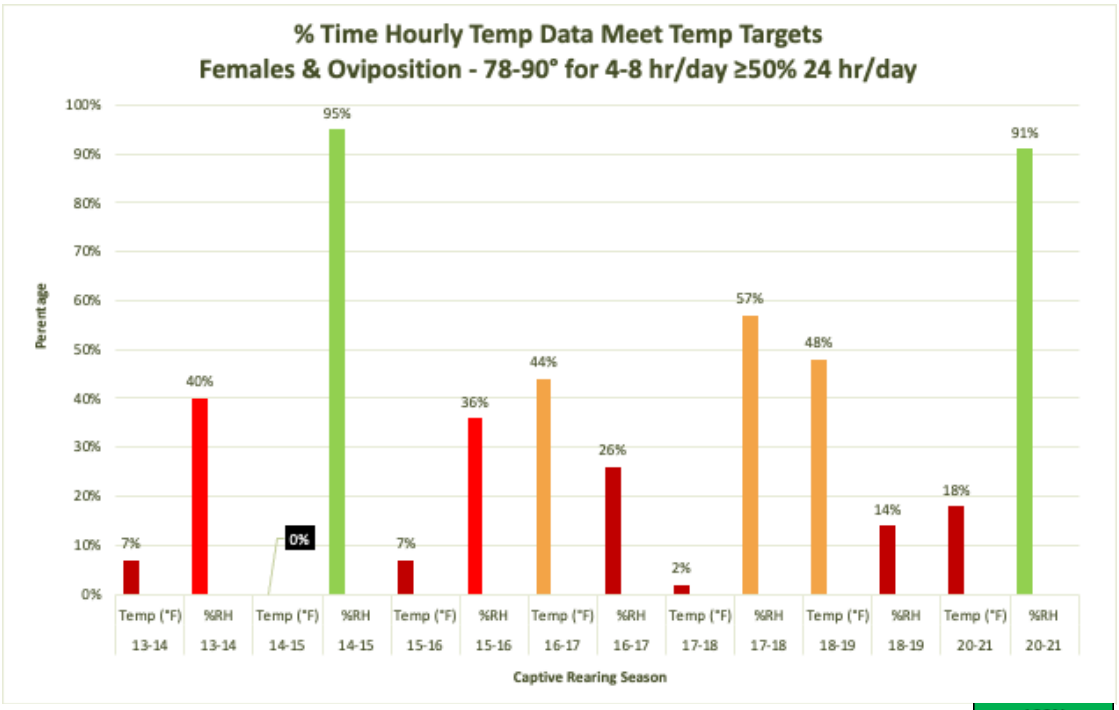
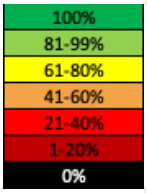


Figure 14. Percent of time females & oviposition environmental targets were met for all seasons.



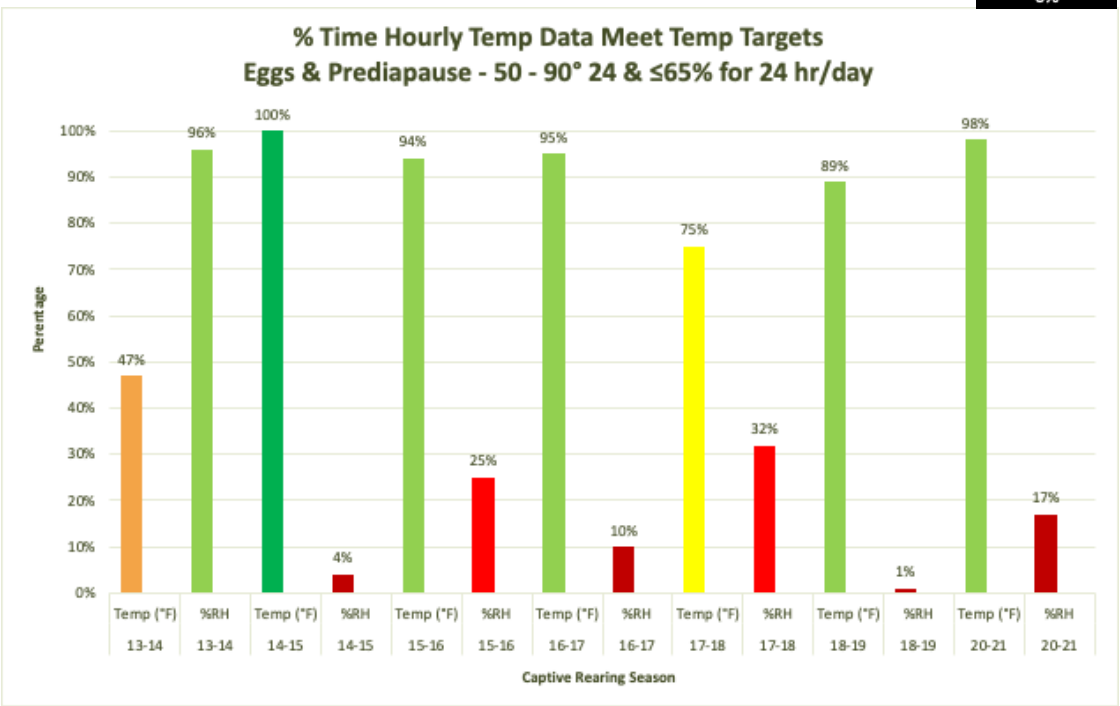


Figure 15. Percent of time eggs & prediapause larvae environmental targets were met during for all seasons.

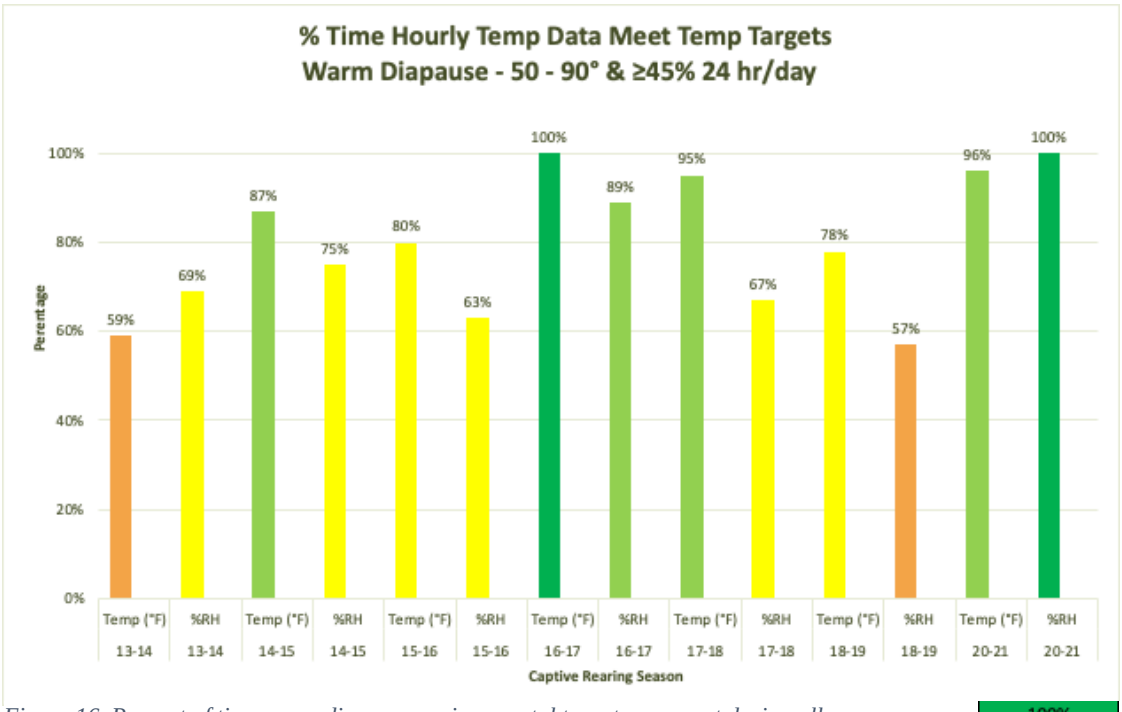
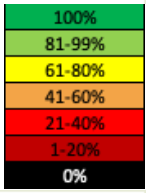


Figure 16. Percent of time warm diapause environmental targets were met during all seasons.



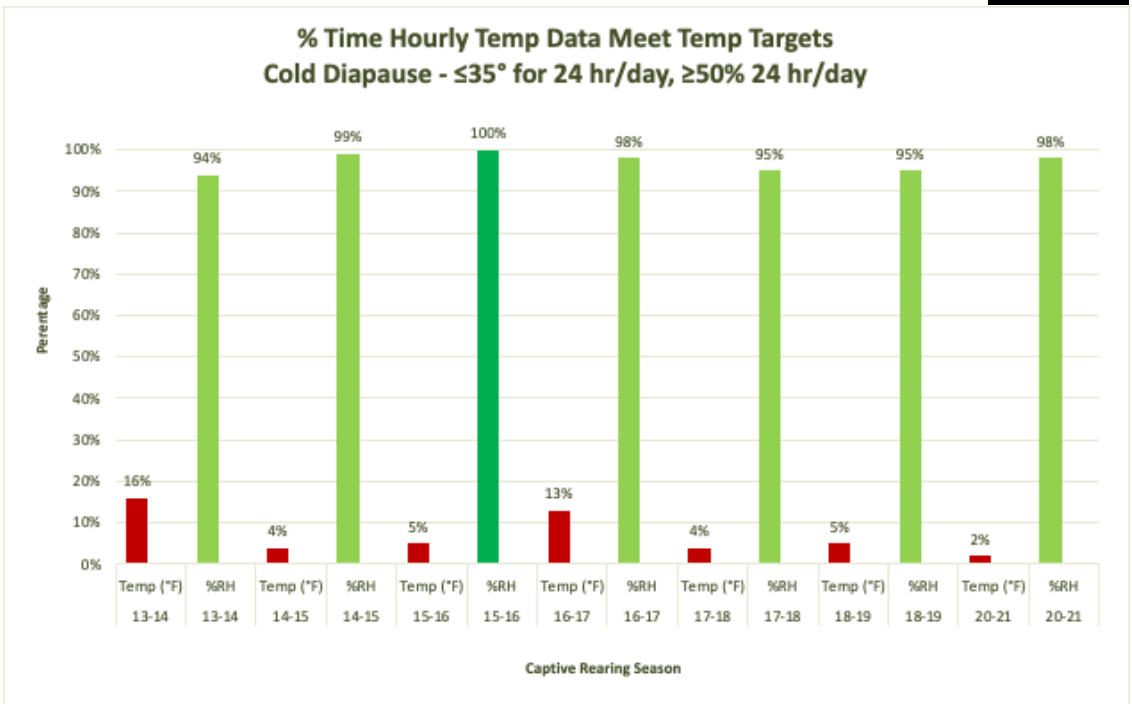


Figure 17. Percent of time cold diapause environmental targets were met for all seasons.

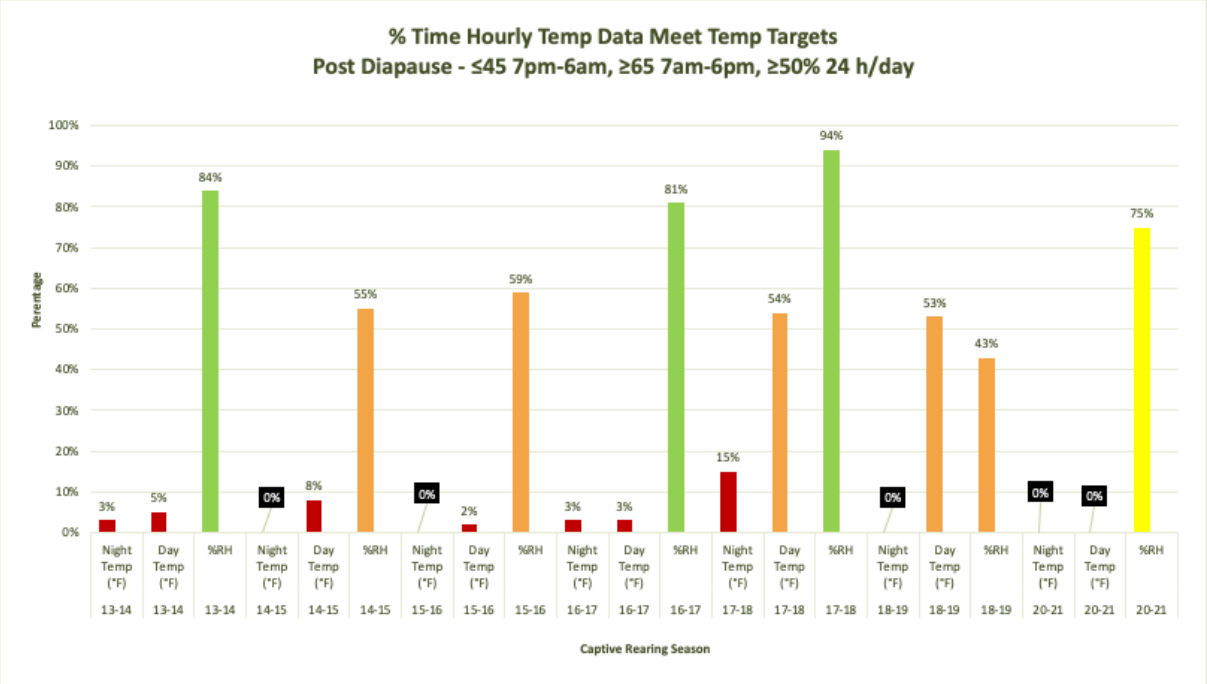


Figure 18. Percent of time postdiapause environmental targets were met for all seasons.

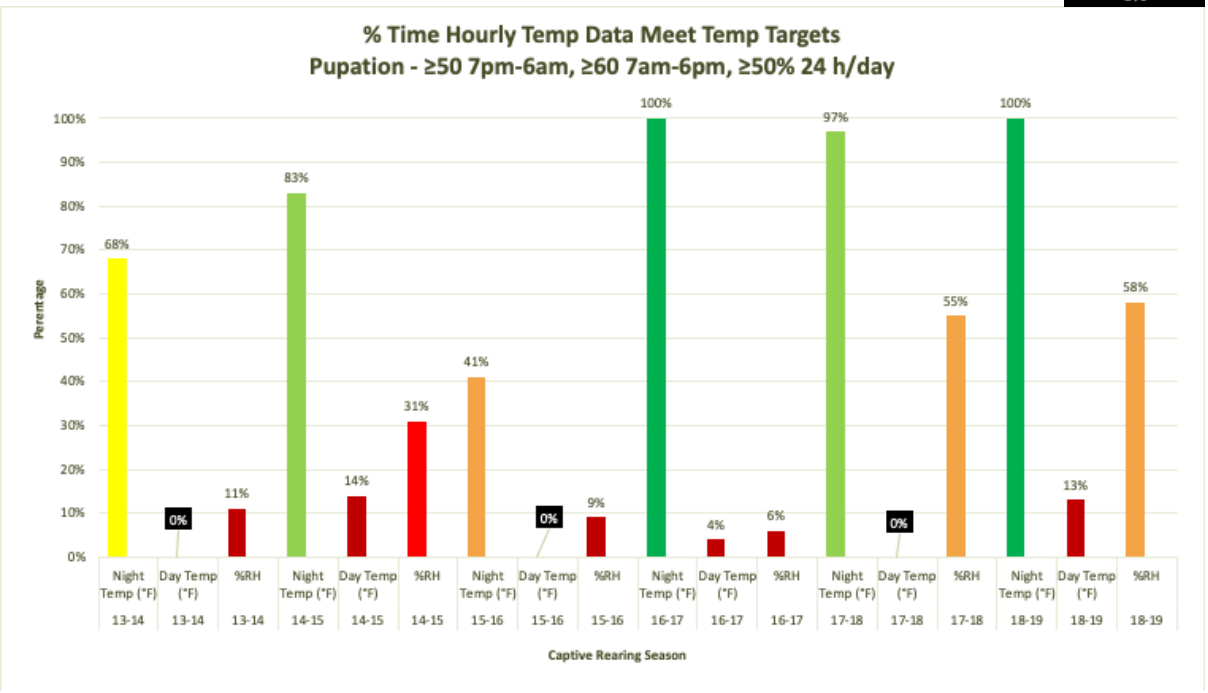
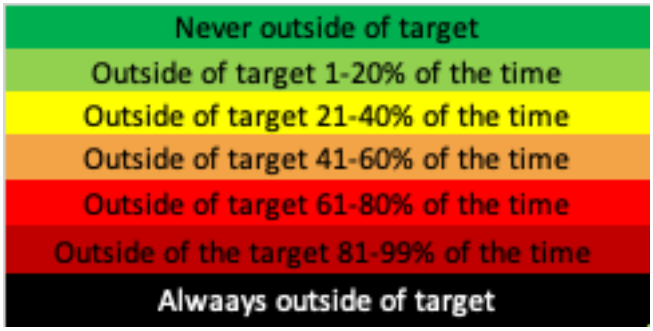


Figure 19. Percent of time pupation environmental targets were met for all seasons.

Figure 20. Legend for the Percent of days the temperature during captive rearing is outside of the temperature targets.



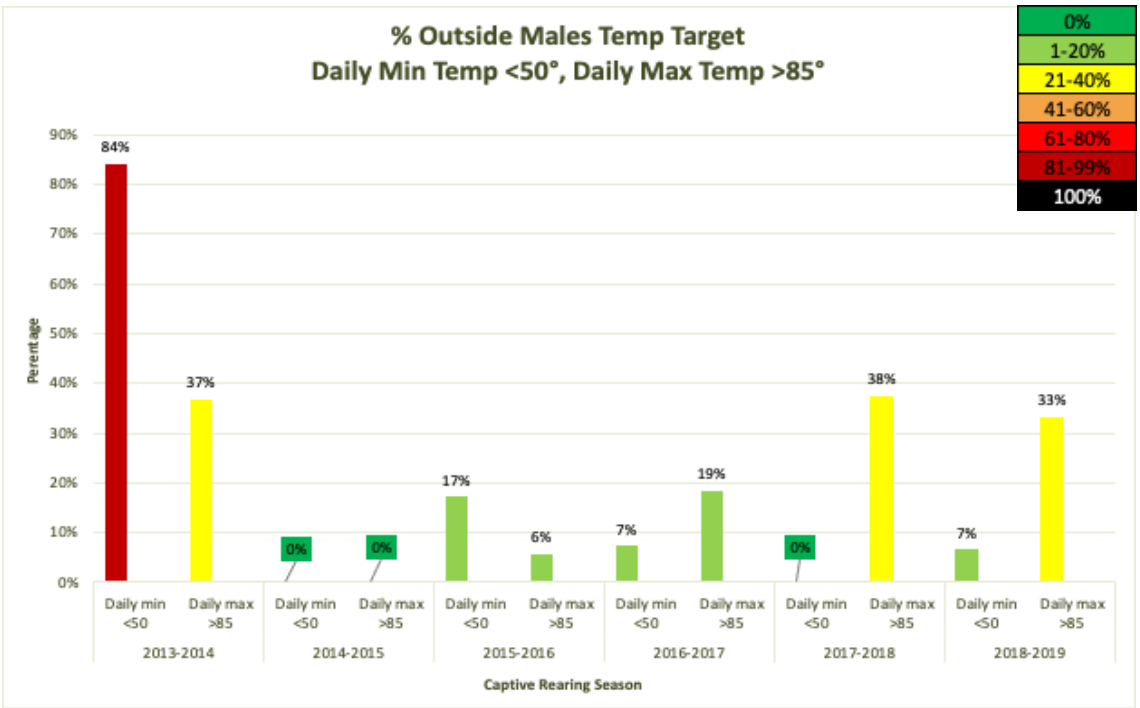


Figure 21. Percent of days the minimum and maximum daily temperature is outside the male temperature target.

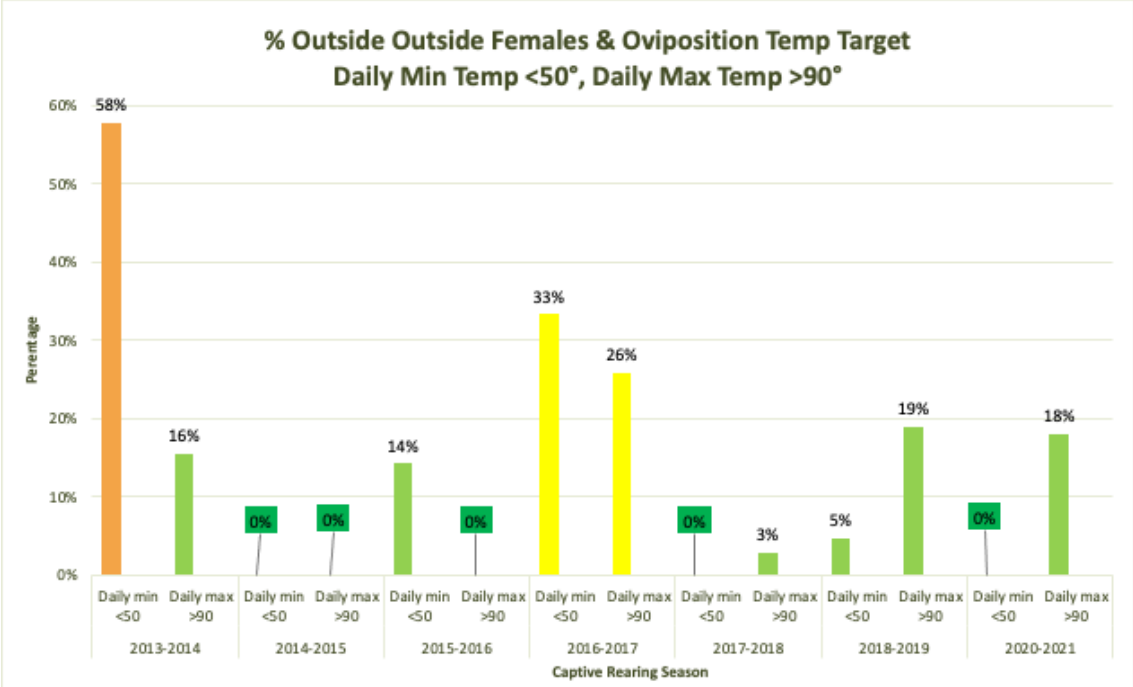
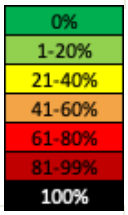


Figure 22. Percent of days the minimum and maximum daily temperature is outside the females & oviposition temperature target.



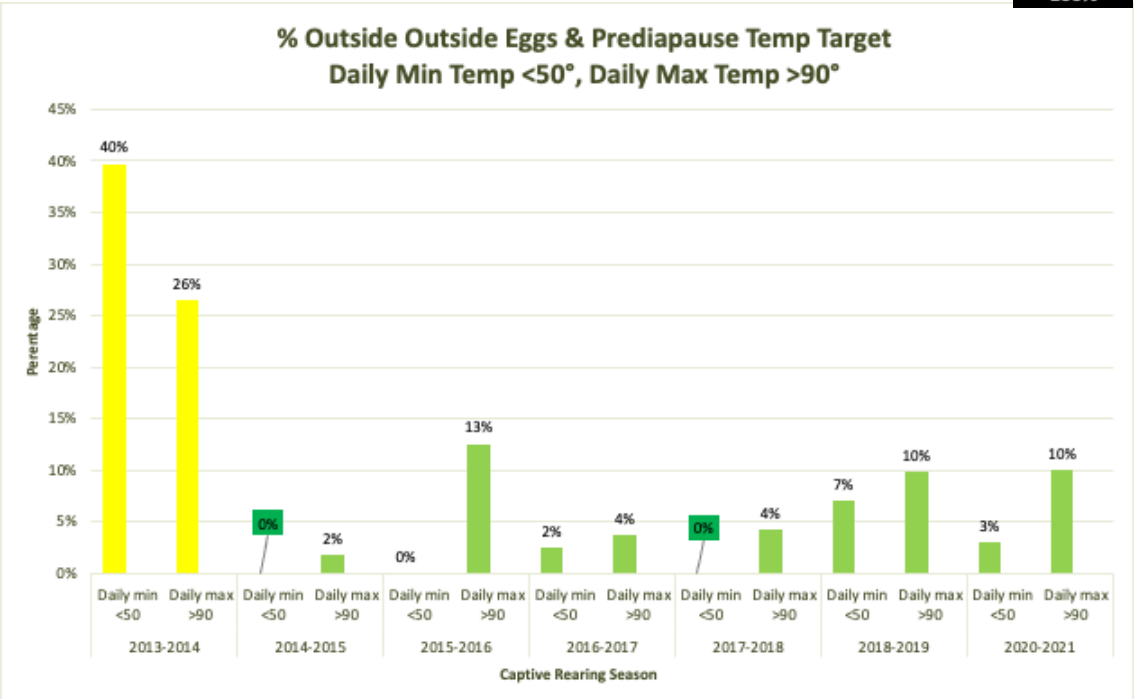


Figure 23. Percent of days the minimum and maximum daily temperature is outside the eggs & prediapause larvae temperature target.

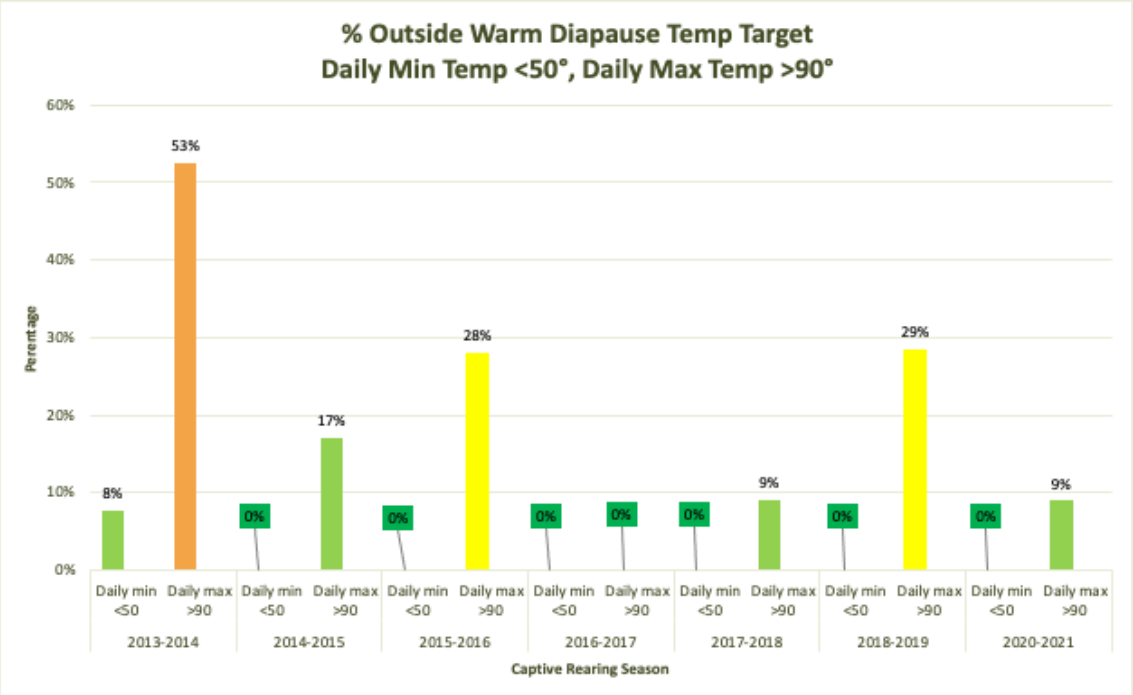
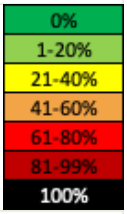


Figure 24. Percent of days the minimum and maximum daily temperature is outside the warm diapause temperature target.



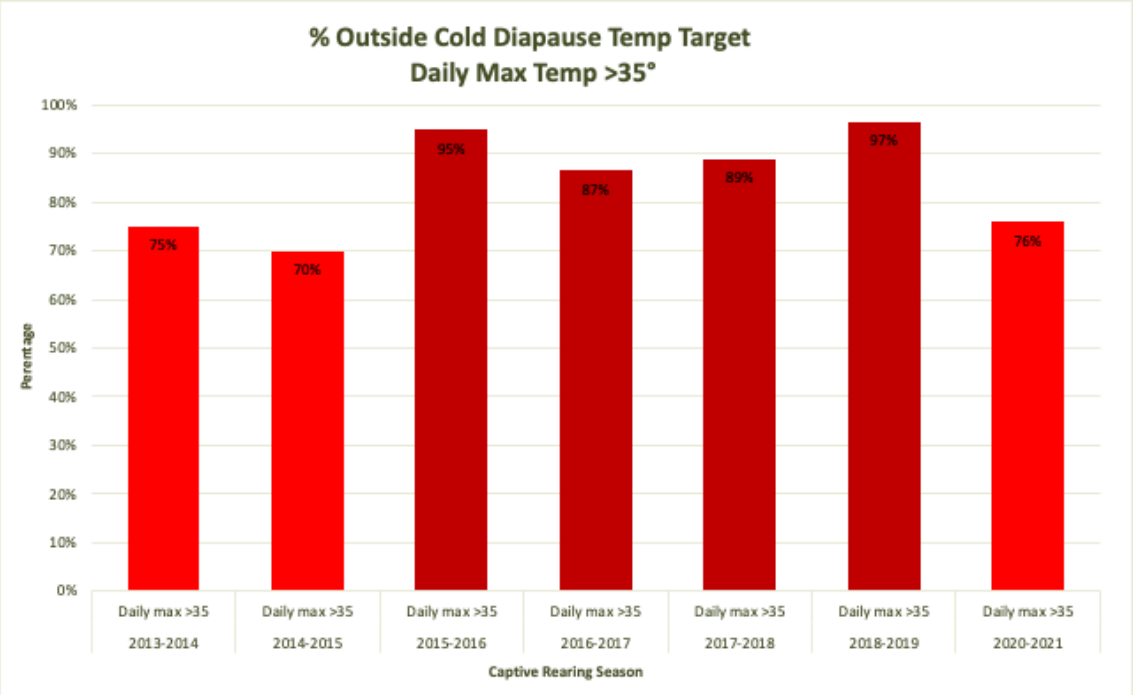


Figure 25. Percent of days the maximum daily temperature is outside the cold diapause temperature target.

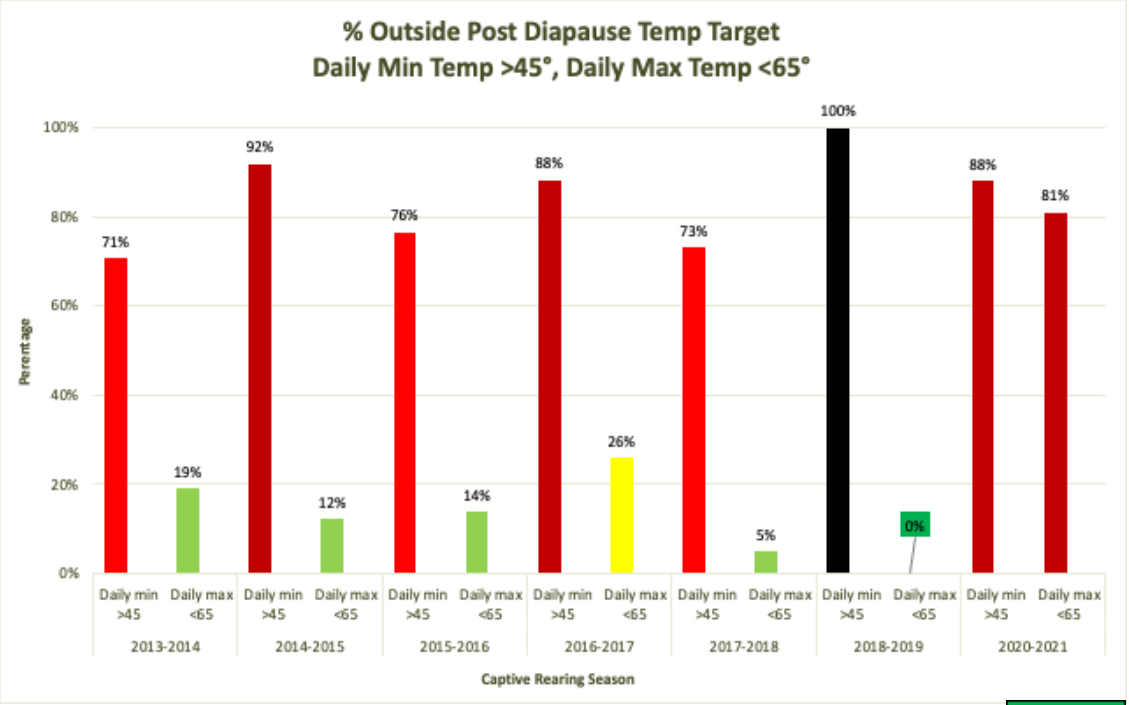
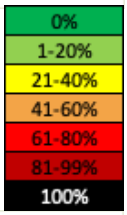


Figure 26. Percent of days the minimum and maximum daily temperature is outside the postdiapause temperature target.



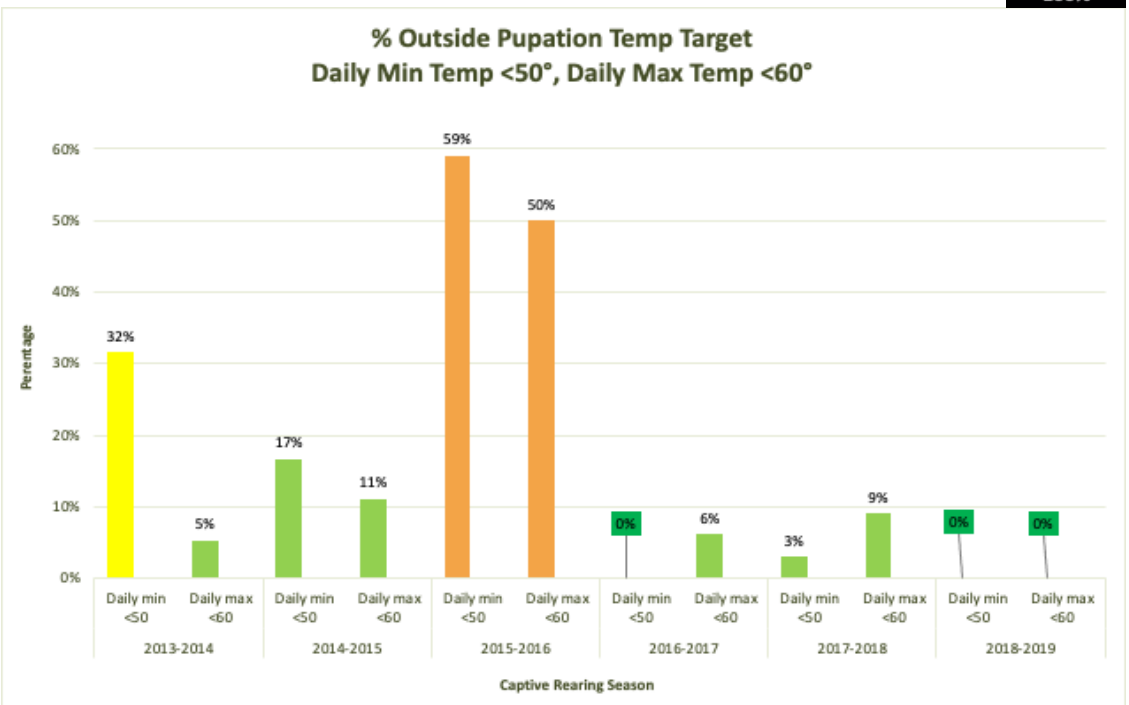


Figure 27. Percent of days the minimum and maximum daily temperature is outside the pupation temperature target.

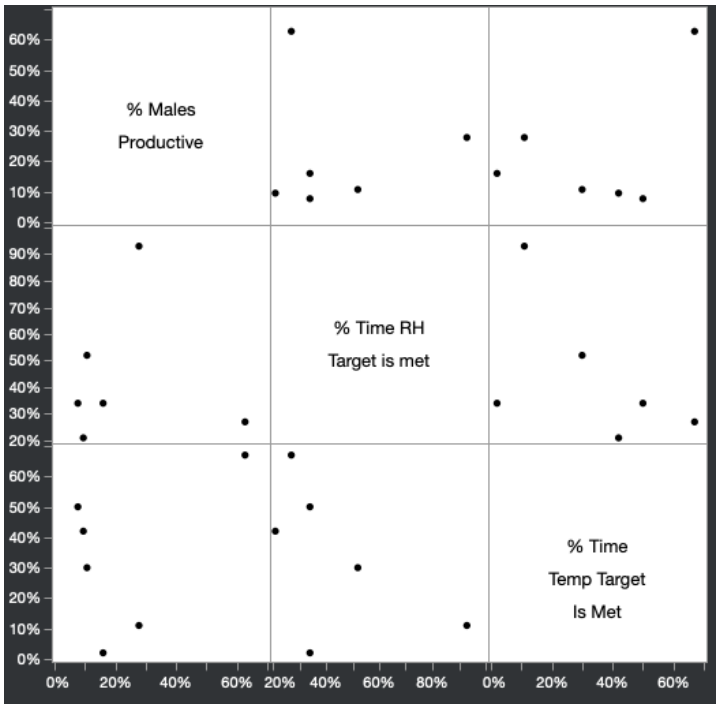


Figure 28. Spearman's rho scatterplot matrix for percent males productive versus percent of time environmental targets were met.

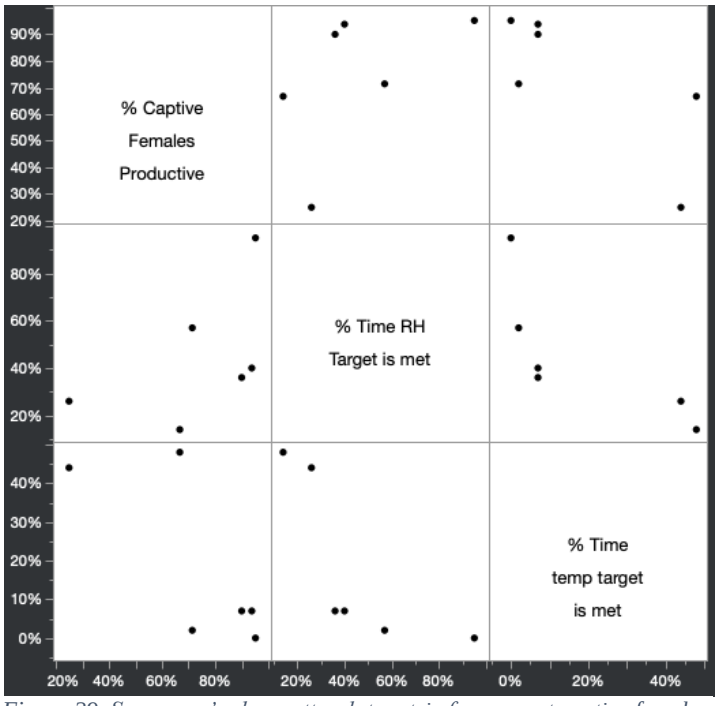


Figure 29. Spearman's rho scatterplot matrix for percent captive females productive versus percent of time environmental targets were met.

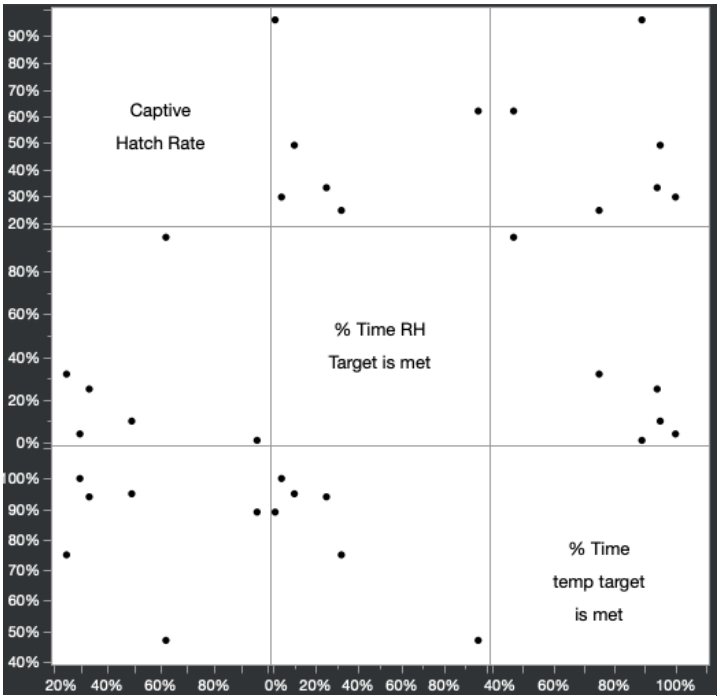


Figure 30. Spearman's rho scatterplot matrix for the percent of captive prediapause larvae versus percent of time environmental targets were met.

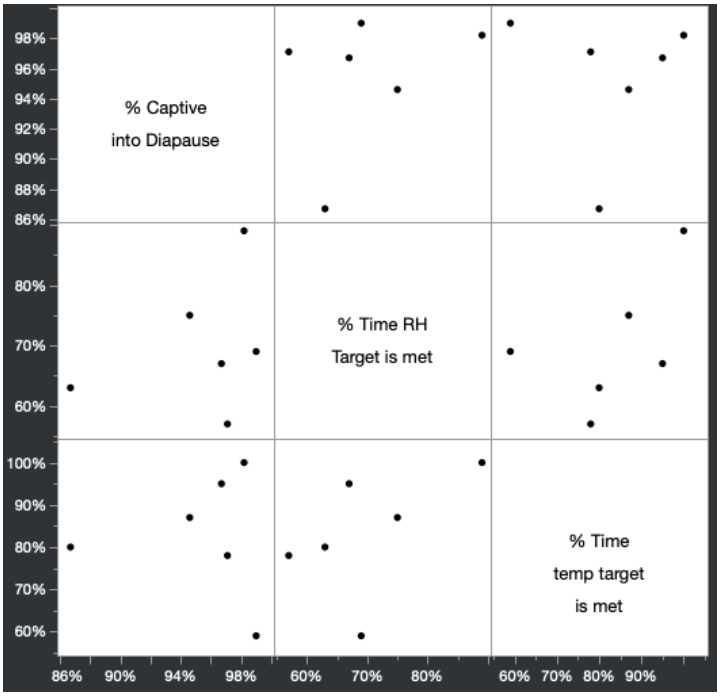


Figure 31. Spearman's rho scatterplot matrix for the percent of captive larvae into diapause versus percent of time environmental targets were met.

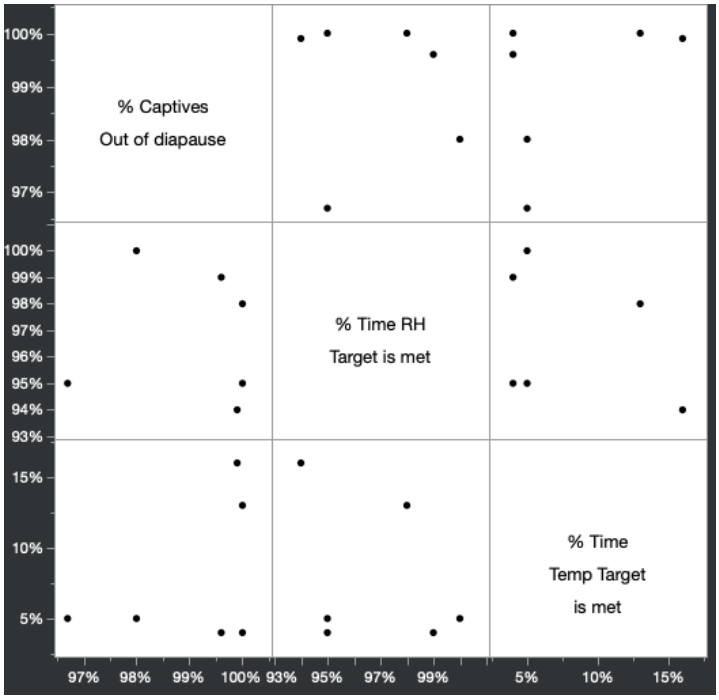


Figure 32. Spearman's rho scatterplot matrix for the percent of captive larvae out of diapause versus the percent of time environmental targets were met.

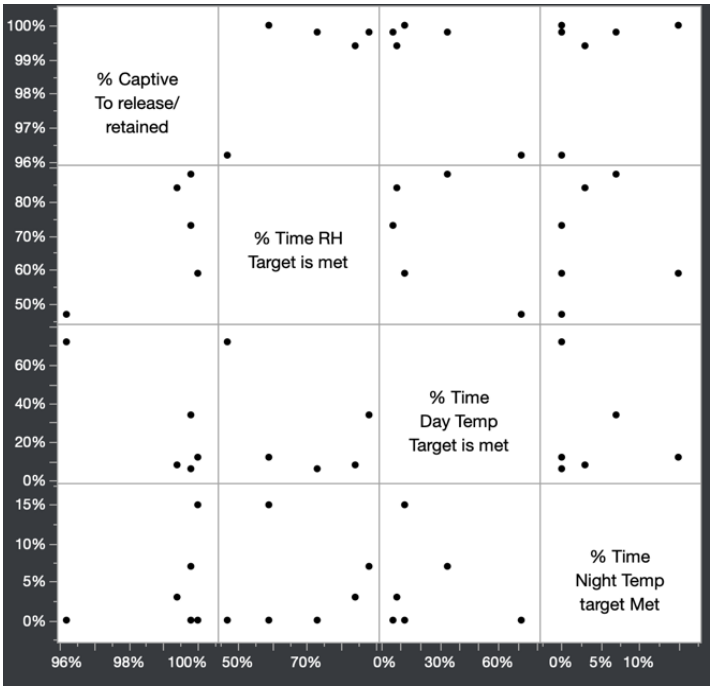


Figure 33. Spearman's rho scatterplot matrix for the percent of captive larvae to release versus percent of time environmental targets were met.

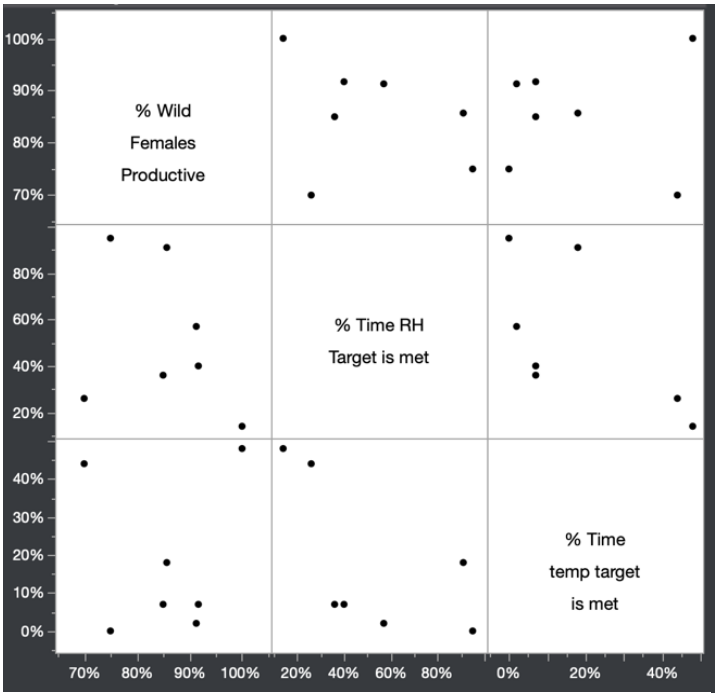


Figure 34. Spearman's rho scatterplot matrix for the percent of wild females productive versus percent of time environmental targets were met.

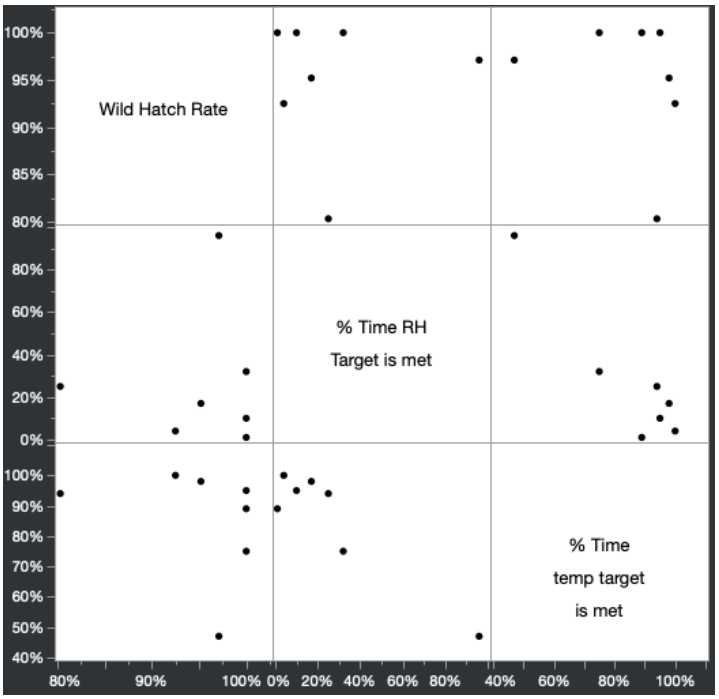


Figure 35. Spearman's rho scatterplot matrix for the percent of the wild prediapause larvae percent of time the environmental targets were met.

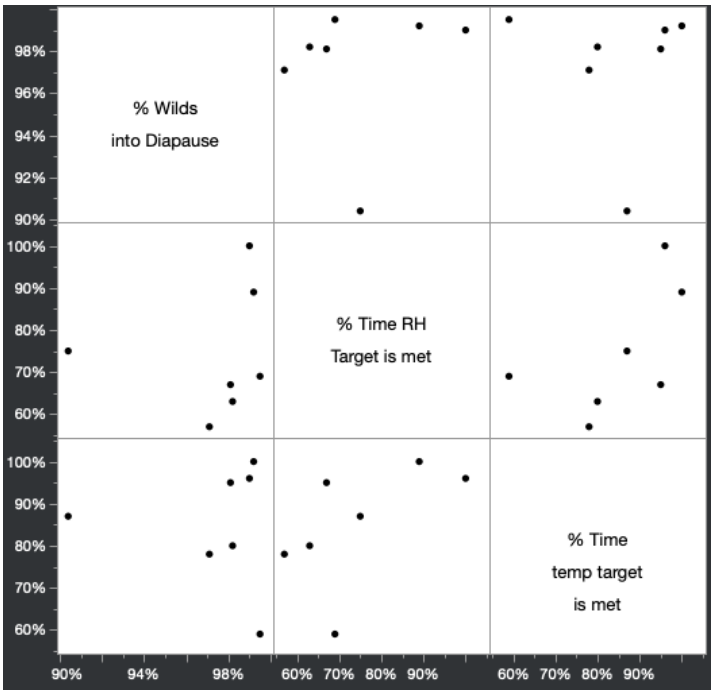


Figure 36. Spearman's rho scatterplot matrix for the percent of wild larvae into diapause versus percent of time environmental targets were met.

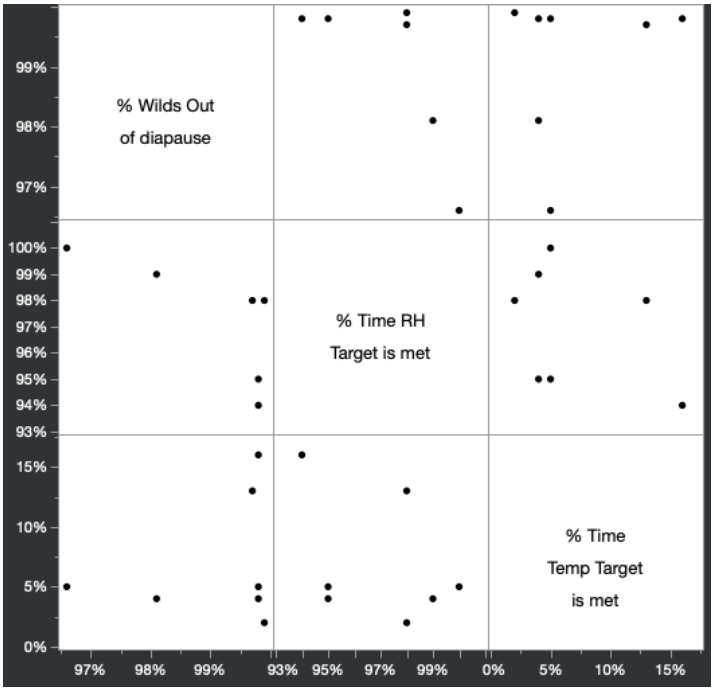


Figure 37. Spearman's rho scatterplot matrix for the percent of wild larvae out of diapause versus percent of time environmental targets were met.

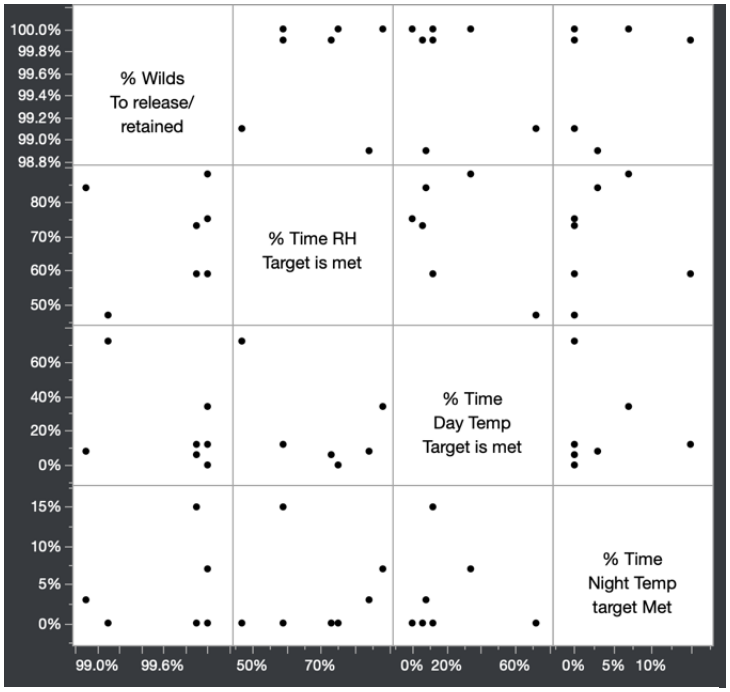


Figure 38. Spearman's rho scatterplot matrix for the percent of wild larvae to release versus the percent of time environmental targets were met.

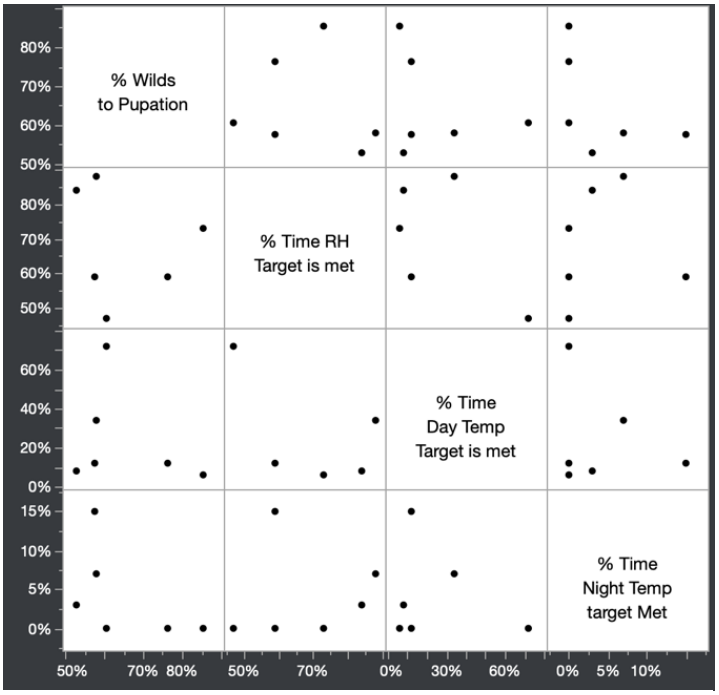


Figure 39. Spearman's rho scatterplot matrix for the percent of wild larvae to pupation (minus the percent of larvae that entered 2nd diapause) versus the percent of time environmental targets were met.

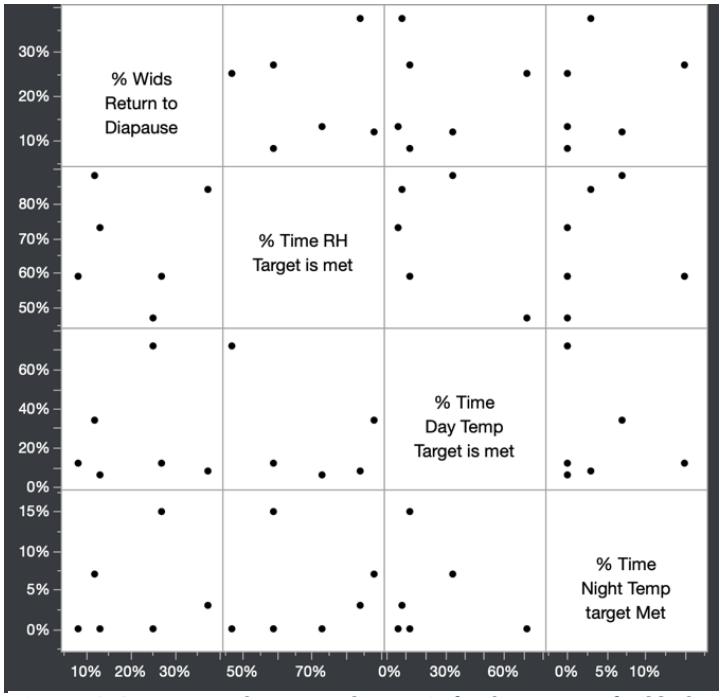


Figure 40. Spearman's rho scatterplot matric for the percent of wilds that entered 2nd diapause (minus the percent of larvae that pupated) versus the percent of time environmental targets were met.

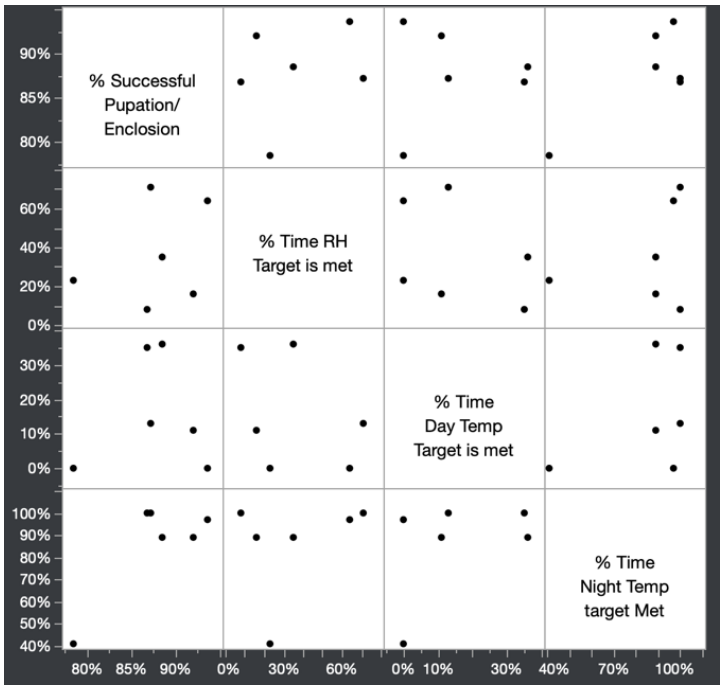


Figure 41. Spearman's rho scatterplot matrix for the percent of pupae the successfully eclosed versus the percent of time environmental targets were met.

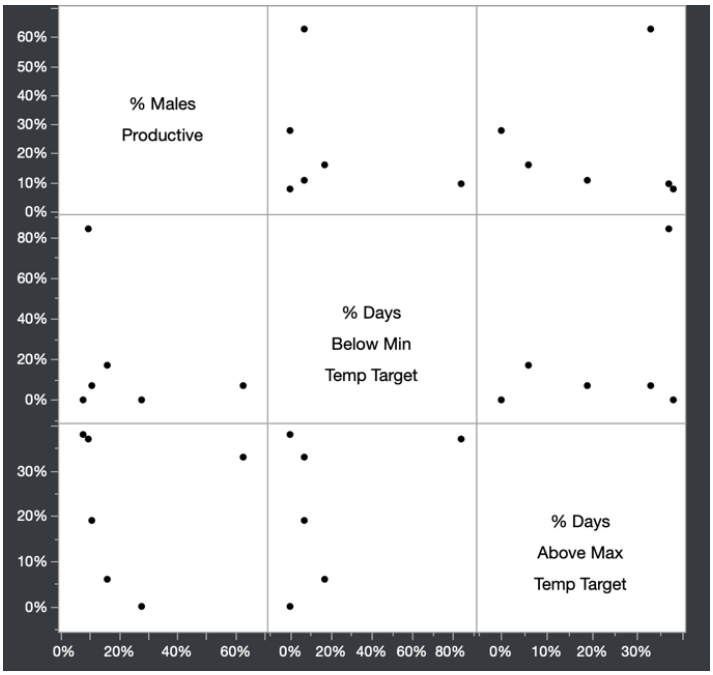


Figure 42. Spearman's rho scatterplot matrix of the percent of males productive versus the percent days outside environmental targets.

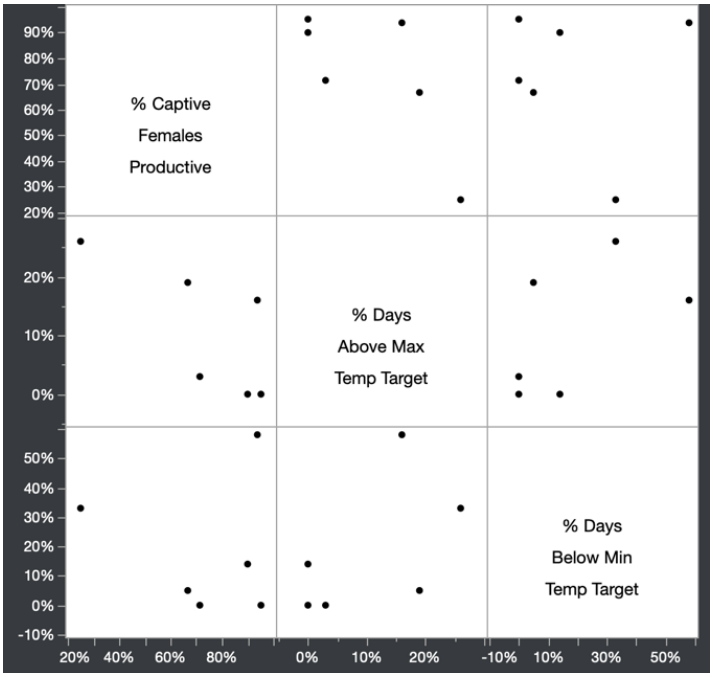


Figure 43. Spearman's rho scatterplot matrix of the percent of captive females productive versus the percent days outside environmental targets.

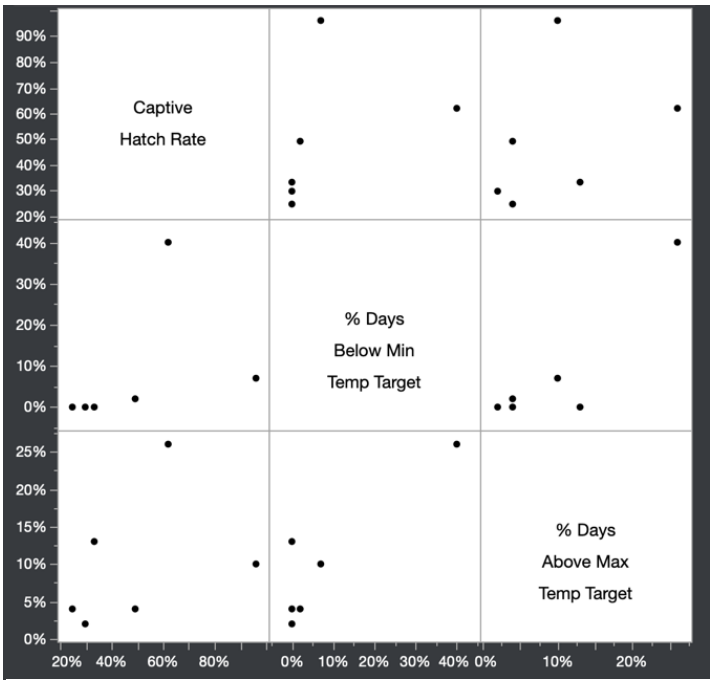


Figure 44. Spearman's rho scatterplot matrix of the percent of captive prediapause larvae versus the percent days outside environmental targets.

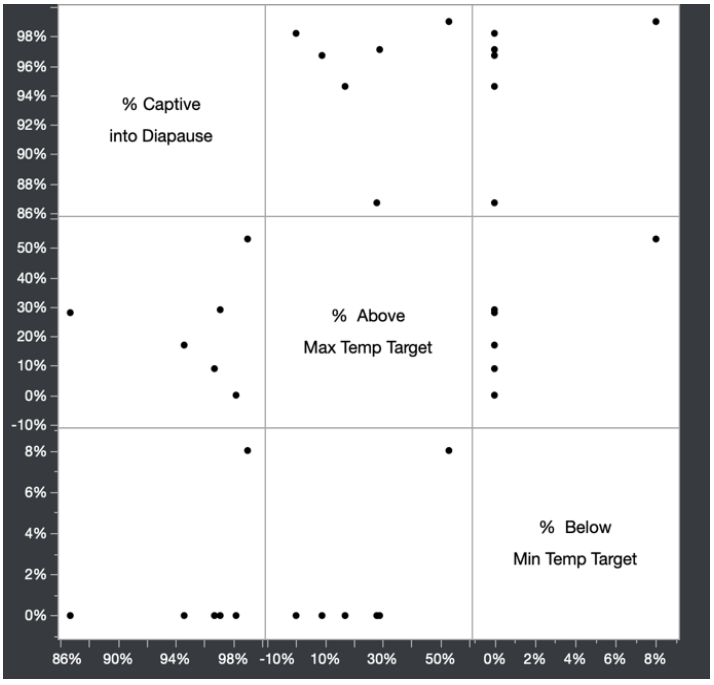


Figure 45. Spearman's rho scatterplot matrix of the percent captive larvae into diapause versus the percent days outside environmental targets.

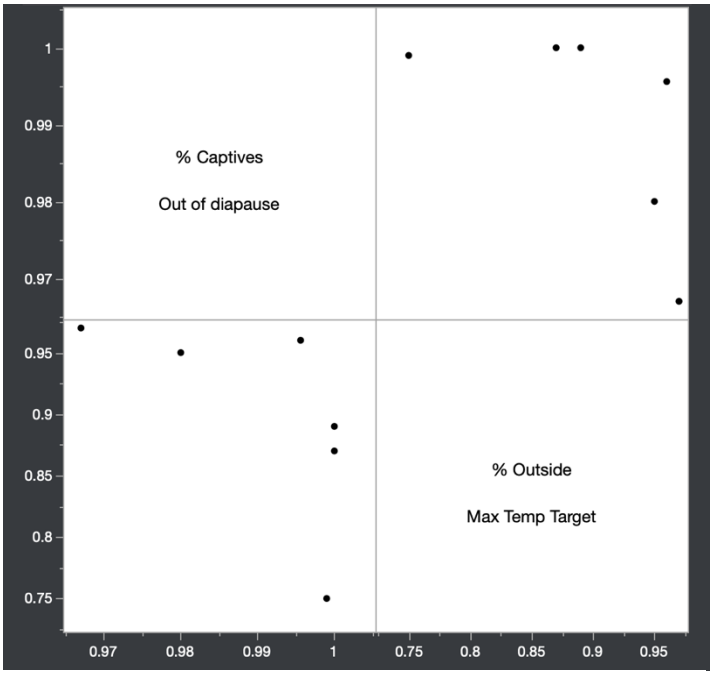


Figure 46. Spearman's rho scatterplot matrix of the percent of captive larvae out of diapause versus the percent days above environmental target.

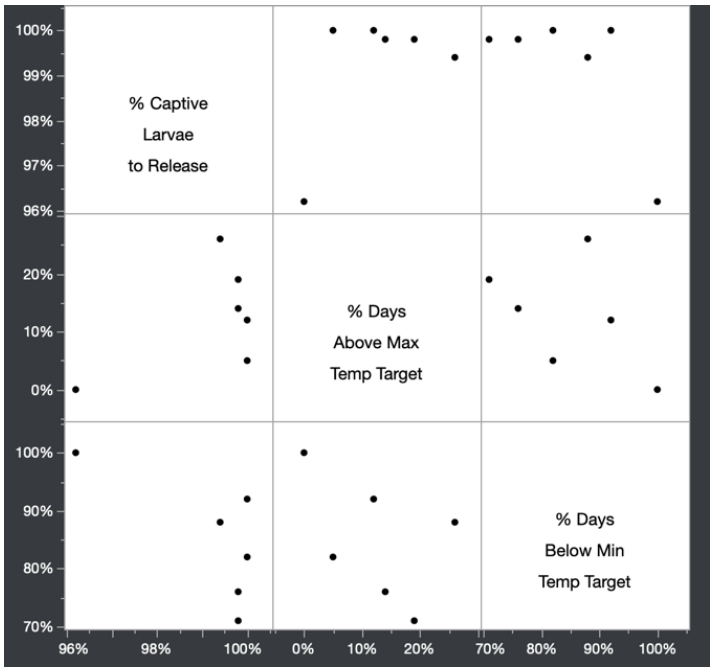


Figure 47. Spearman's rho scatterplot matrix of the percent of captive larvae to release versus the percent days outside environmental targets.

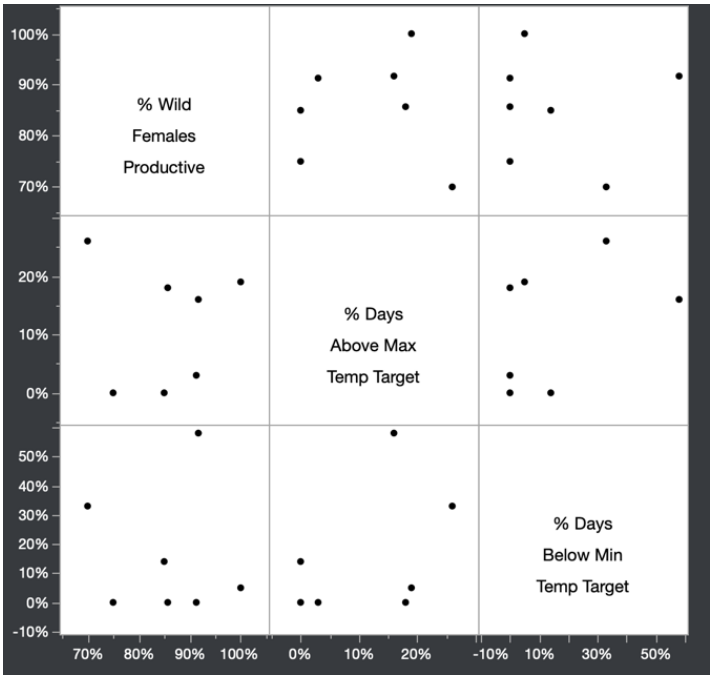


Figure 48. Spearman's rho scatterplot matrix of the percent of wild females productive versus the percent days outside environmental targets.

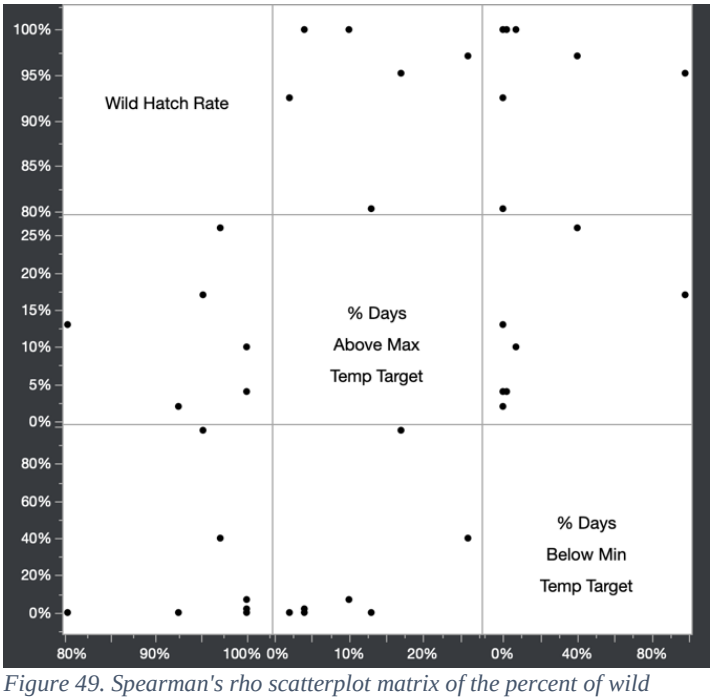


Figure 49. Spearman's rho scatterplot matrix of the percent of wild prediapause larvae versus the percent days outside environmental targets.

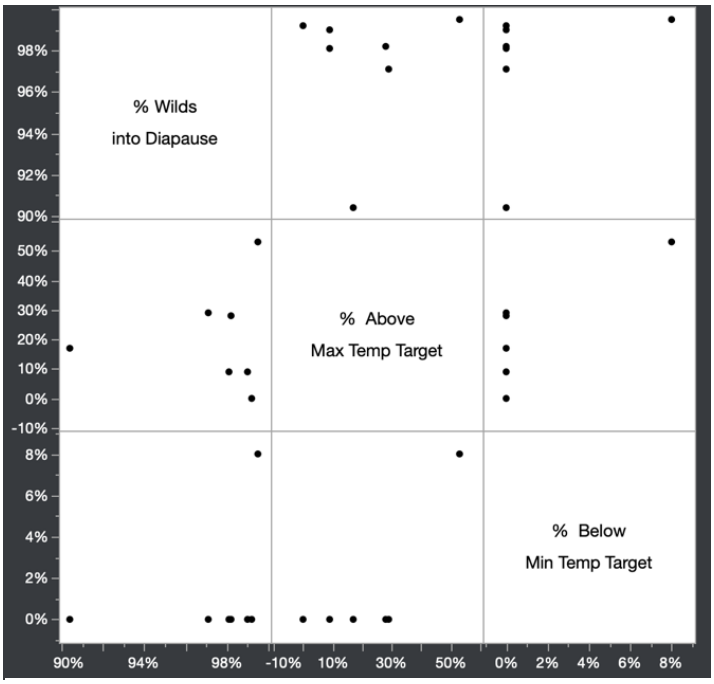


Figure 50. Spearman's rho scatterplot matrix of the percent of wild larvae into diapause versus the percent days outside environmental targets.

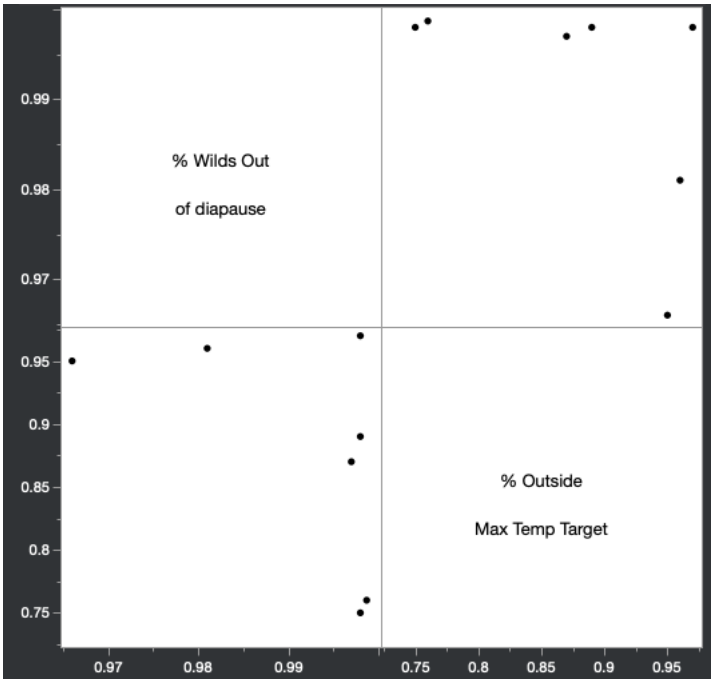


Figure 51. Spearman's rho scatterplot matrix of the percent of wild larvae out of diapause versus the percent days above environmental target.

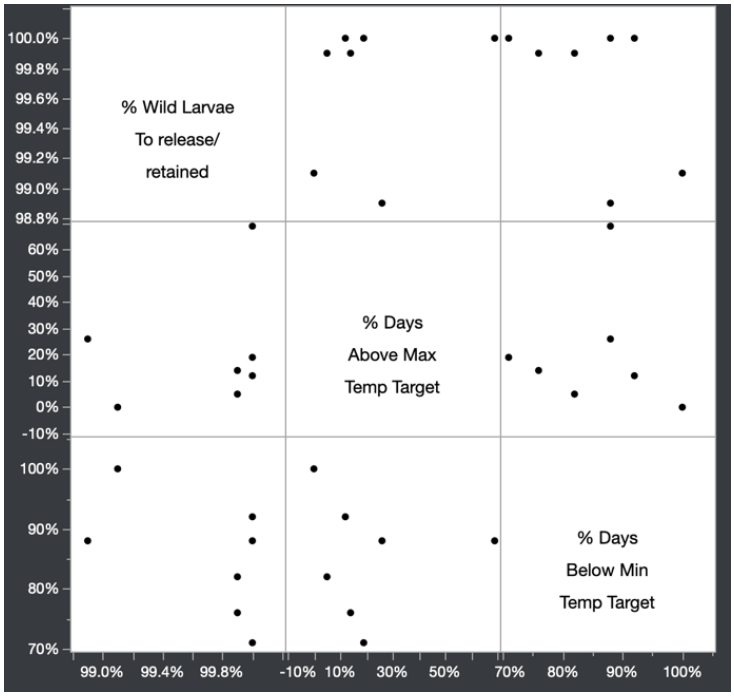


Figure 52. Spearman's rho scatterplot matrix of the percent of wild larvae released/retained versus the percent days outside environmental targets.

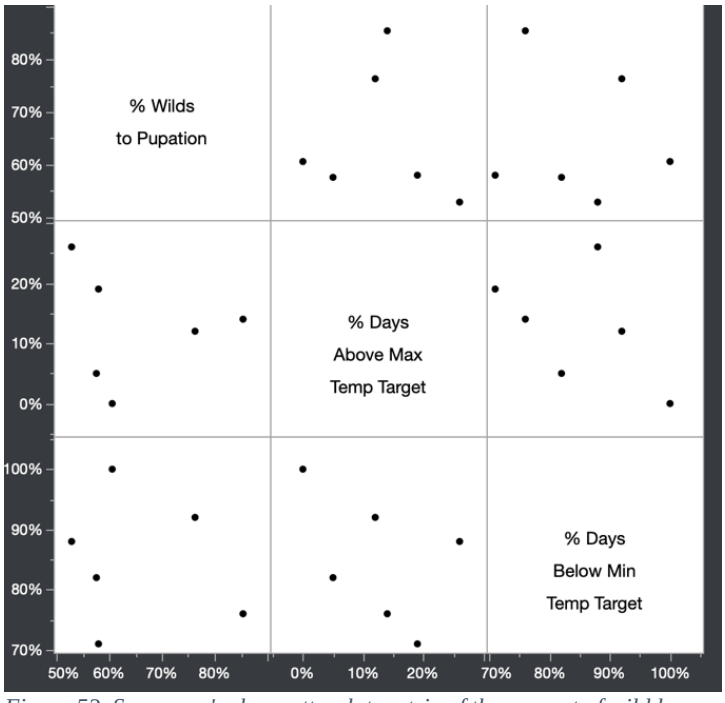


Figure 53. Spearman's rho scatterplot matrix of the percent of wild larvae pupated versus the percent days outside environmental targets.

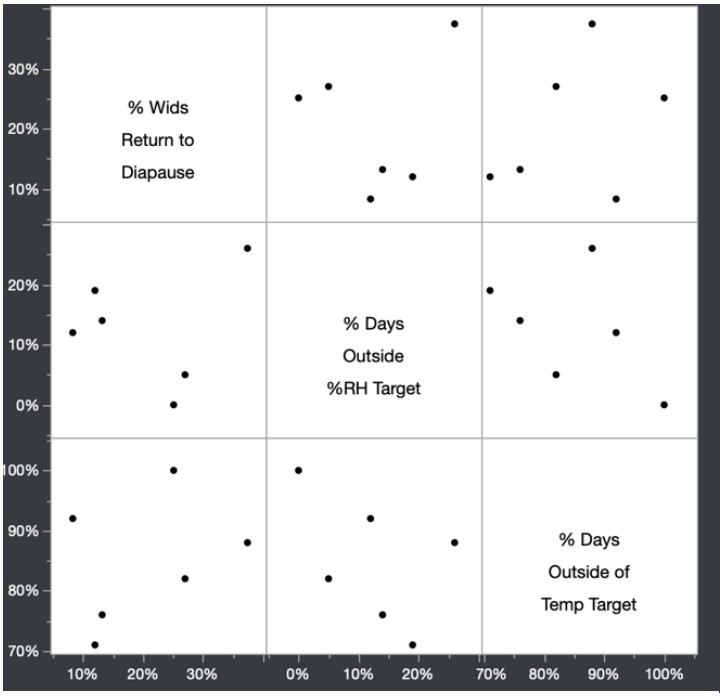


Figure 54. Spearman's rho scatterplot matrix of the percent of wild larvae return to diapause versus the percent days outside environmental targets.

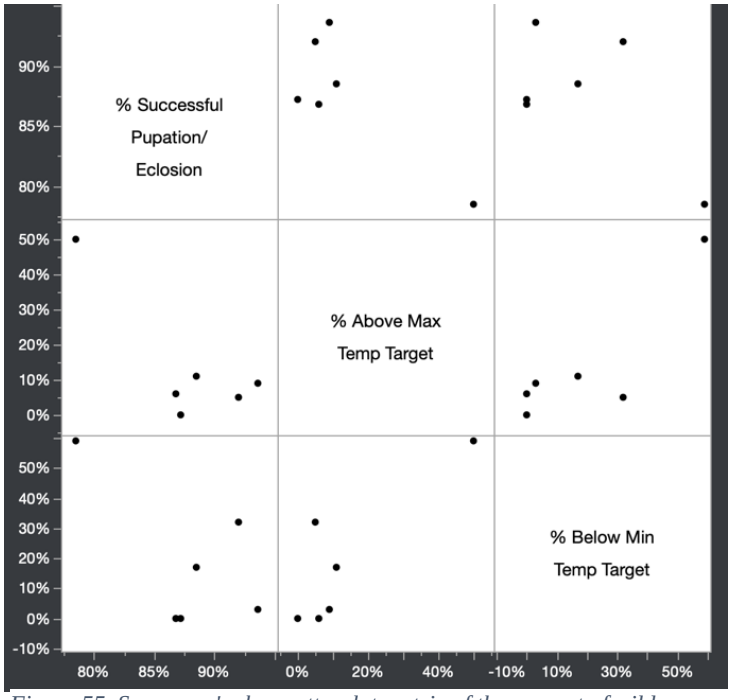


Figure 55. Spearman's rho scatterplot matrix of the percent of wild pupae successfully eclose versus the percent days outside environmental targets.

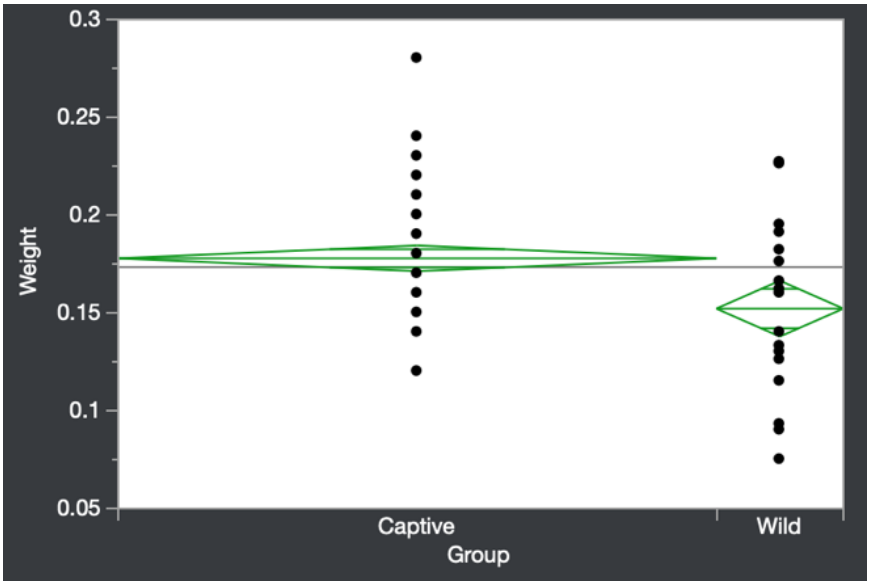


Figure 56. Adult female butterfly weights (captive and wild) in the 2013-2014 season (p=0.03, Wilcoxon rank sum test). The horizontal line inside each diamond is the group mean.

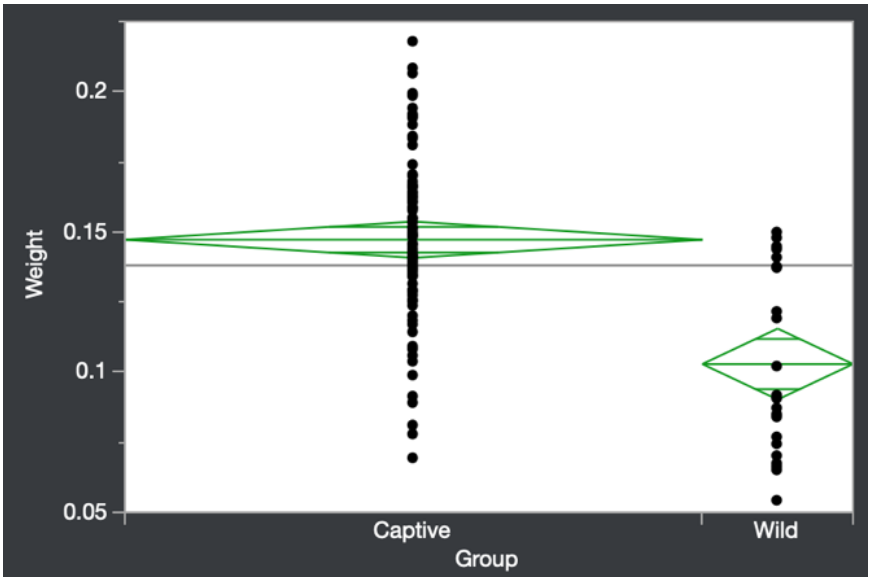


Figure 57. Adult female butterfly weights (captive and wild) in the 2014-2015 season (p<0.0001, Wilcoxon rank sum test. The horizontal line inside each diamond is the group mean.

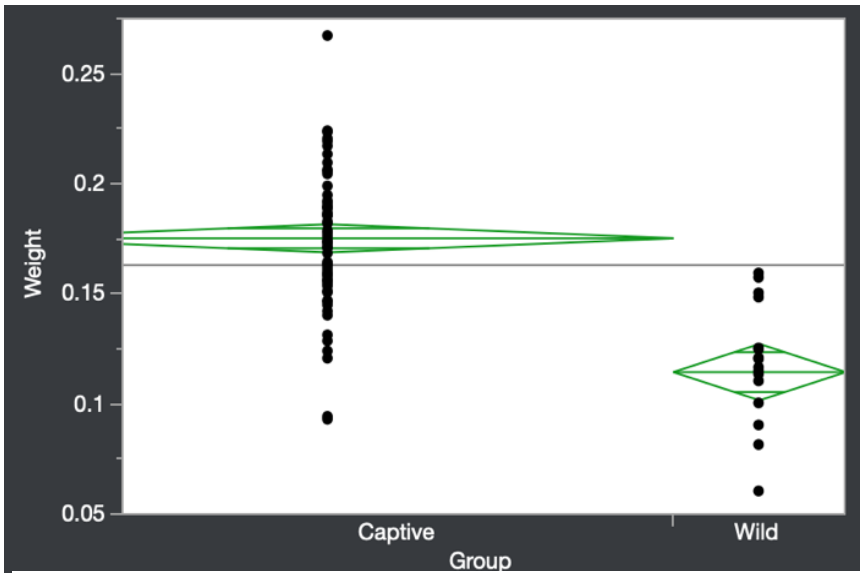


Figure 58. Adult female butterfly weights (captive and wild) in the 2015-2016 season (p<0.0001, Wilcoxon rank sum test. The horizontal line inside each diamond is the group mean.

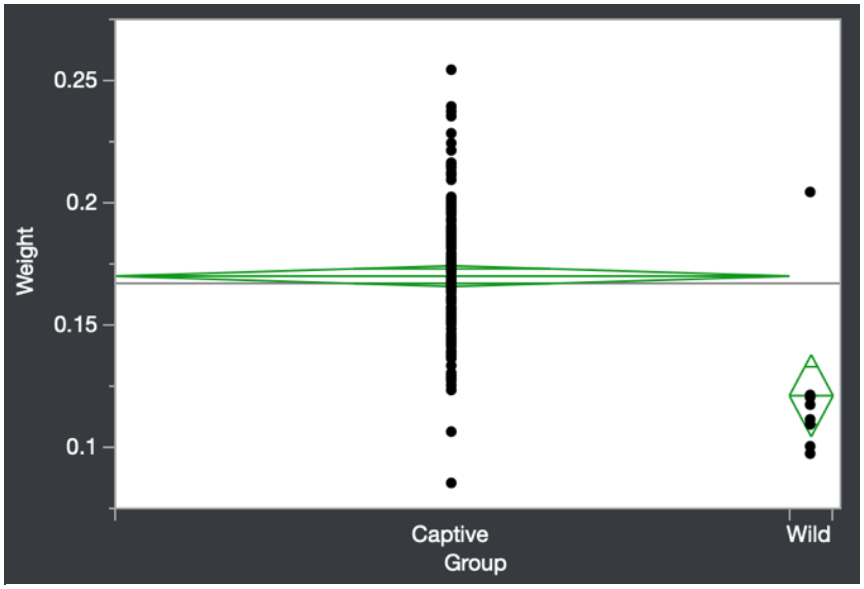


Figure 59. Adult female butterfly weights (captive and wild) in the 2016-2017 season (p<0.0001, Wilcoxon rank sum test. The horizontal line inside each diamond is the group mean.

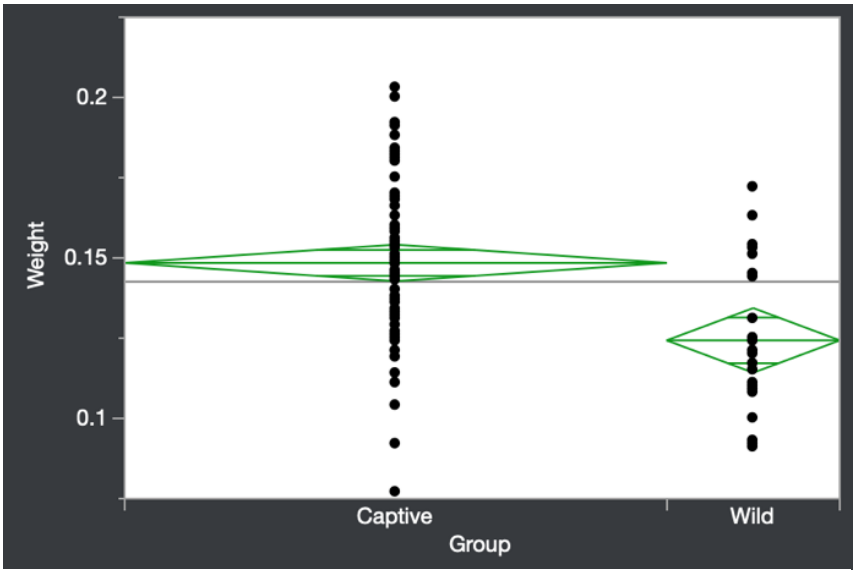


Figure 60. Adult female butterfly weights (captive and wild) in the 2017-2018 season ($p<0.0001$, Wilcoxon rank sum test. The horizontal line inside each diamond is the group mean.

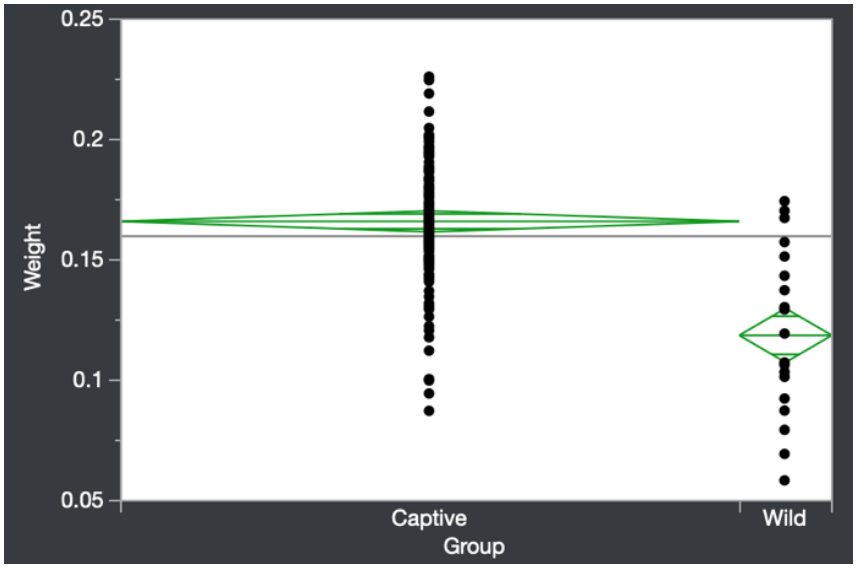


Figure 61. Adult female butterfly weights (captive and wild) in the 2018-2019 season ($p<0.0001$, Wilcoxon rank sum test. The horizontal line inside each diamond is the group mean.

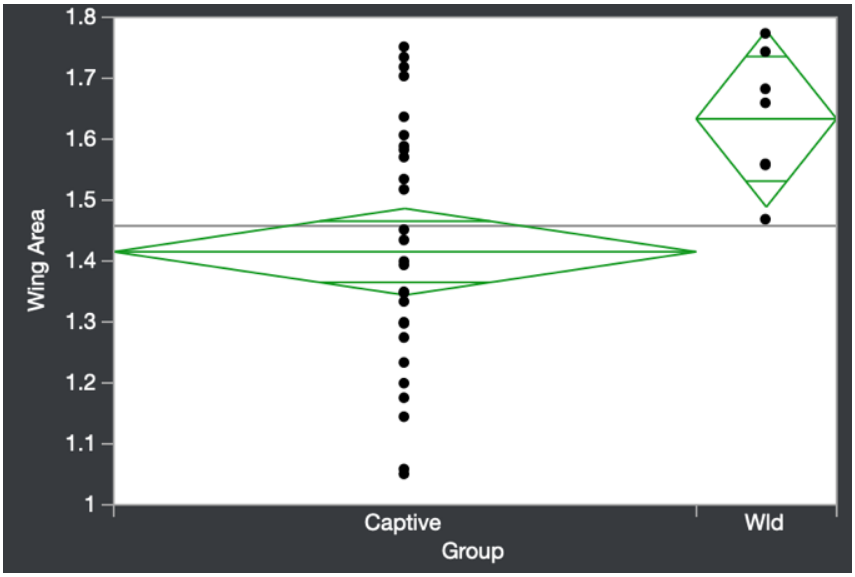


Figure 62. Adult female butterfly wing area (captive and wild) in the 2014-2015 season (p=0.01, Wilcoxon rank sum test). The horizontal line inside each diamond is the group mean.

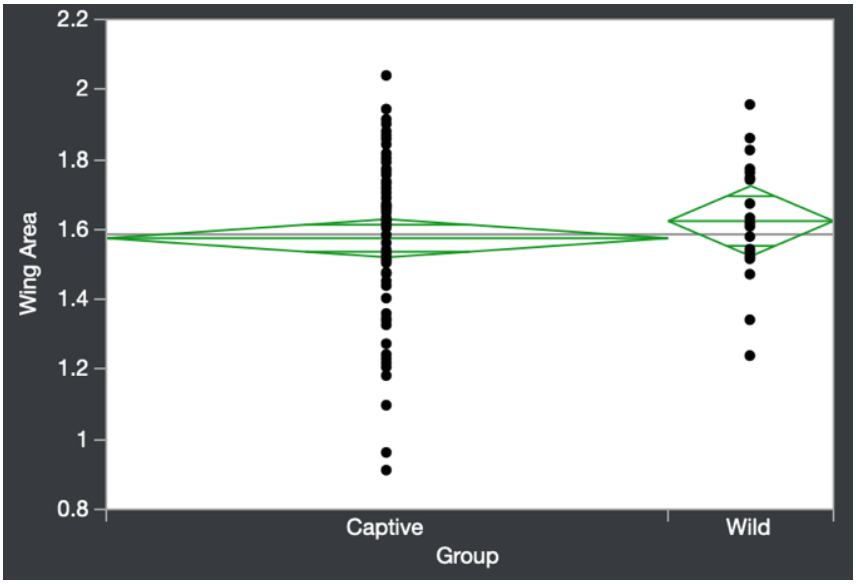


Figure 63. Adult female butterfly wing area (captive and wild) in the 2015-2016 season (p=0.55, Wilcoxon rank sum test). The horizontal line inside each diamond is the group mean.

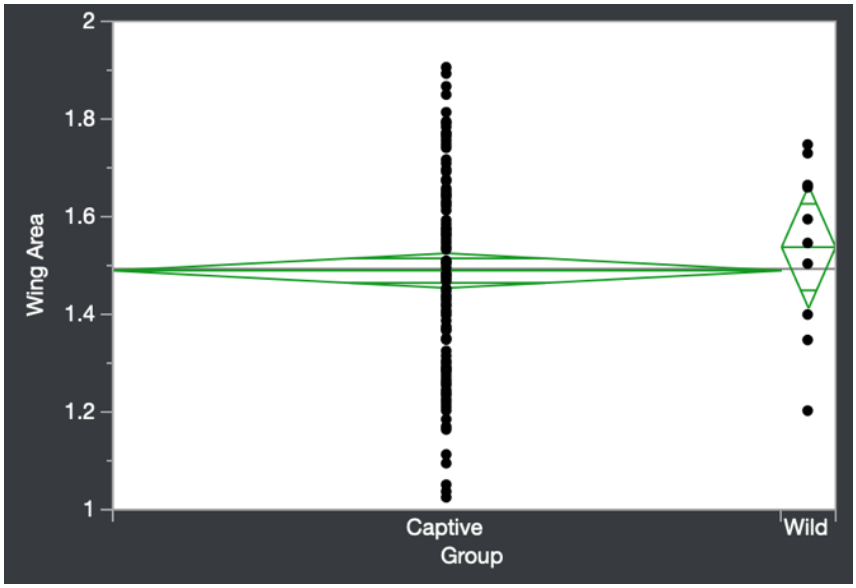


Figure 64. Adult female butterfly wing area (captive and wild) in the 2016-2017 season (p=0.46, Wilcoxon rank sum test). The horizontal line inside each diamond is the group mean.

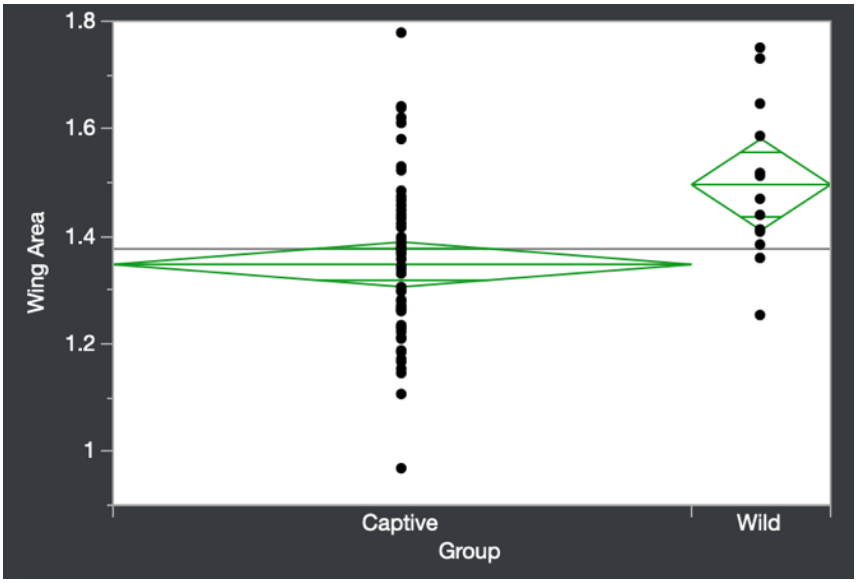


Figure 65. Adult female butterfly wing area (captive and wild) in the 2017-2018 season (p=0.004, Wilcoxon rank sum test). The horizontal line inside each diamond is the group mean.

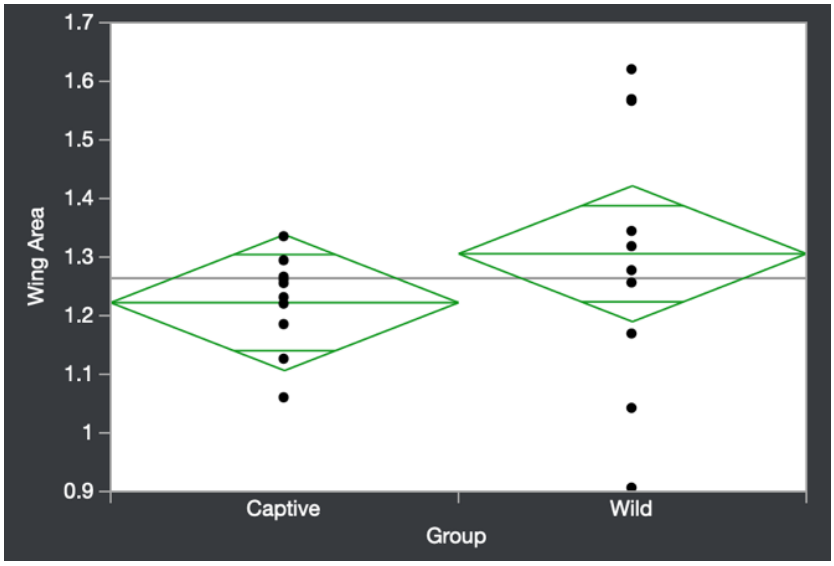


Figure 66. Adult female butterfly wing area (captive and wild) in the 2014-2015 season (p=0.27, Wilcoxon rank sum test). The horizontal line inside each diamond is the group mean.

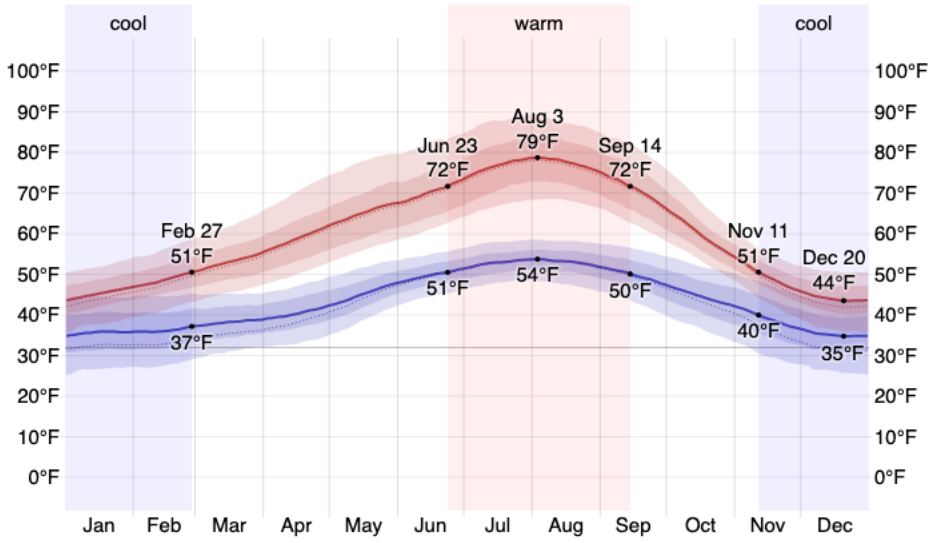


Figure 67. Average temperature over 2021 in Belfair, WA.

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