EFFECTS OF TEMPERATURE AND HUMIDITY ON TAYLOR'S CHECKERSPOT BUTTERFLY LIFE STAGE LENGTH AND DEVELOPLMENT: RESEARCH IN COLLABORATION WITH THE SUSTAINABILITY IN PRISONS PROJECT AND INCARCERATED TECHNICIANS

By

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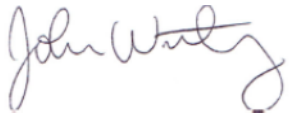

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ABSTRACT:

Effects of Temperature and Humidity on Taylor's Checkerspot Butterfly Life Stage Length and Development: Research in Collaboration with the Sustainability in Prisons Project and Incarcerated Technicians

Raychel E. Dunning

Captive rearing programs are used in conservation research to prevent critically endangered species from becoming extinct and increasing their populations. One additional benefit of captive rearing programs is the ability to collect data and conduct research and analysis on endangered species reared in captivity within a more controlled environment. Environmental conditions are especially important to understand for species' whose internal processes are regulated by external conditions, such as butterflies. The Taylor's checkerspot butterfly (*Euphydryas editha taylori*) is a federally endangered butterfly found in imperiled prairie areas of the Puget Lowlands, Oregon's Willamette Valley, and parts of British Columbia, Canada. In 2012 the Sustainability in Prisons Project's butterfly program was established at Mission Creek Correctional Center for Women (MCCCW). This captive rearing program relies on keeping strictly controlled environmental conditions and meeting temperature and humidity targets to successfully raise the butterfly through its life stages. Conditions in the wild are gradually experiencing hotter summers and warmer, wetter winters under climate change. This may result in additional risks to the butterfly's survival, such as resulting in a phenological mismatch with its host species, *Plantago lanceolata*. In order to determine to what extent increases in temperature and humidity influence the Taylor's checkerspot life stage length and development, an analysis of average temperature and average relative humidity was conducted. Environmental conditions for the 2021 and 2022 years at MCCCW were compared to the length of different instar stages, and additional growing degree day calculations were completed to obtain an idea as to if GDD could be a suitable metric to use in future studies. The influence of average temperature and average relative humidity on different instar stages and periods of development were variable between the two years and greenhouses, but increased temperature typically decreased the time spent within a given life stage, while relative humidity was shown to weakly increase time spent in an instar stage.

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ACRONYMS

TCB – Taylor's checkerspot butterfly

JBLM – Joint base Lewis-McChord

MCCCW – Mission Creek Corrections Center for Women

CCC – Coffee Creek Corrections Center

OR – Oregon

SPP – Sustainability in Prisons Project

WDFW – Washington Department of Fish and Wildlife

DOC – Department of Corrections

Introduction

Prairie and oak-woodland ecosystems were once one of the more dominant ecoregion types in the Pacific Northwest lowlands, maintained through Native American burning and wildfires (Bachelet et al., 2011). Euro-American settlement occurred around the 1840s in the Pacific Northwest, and the process of colonization resulted in dramatic alterations of the prairie-savannah landscape. The genocide and cultural assimilation of Native Americans by Euro-Americans in the Pacific Northwest and governmental emphasis on fire suppression disrupted the natural prairie-savannah burn cycle (Bachelet et al., 2011; Martin & Kronland, 2015). Additionally, Euro-American agricultural practices created habitat loss and fragmentation which drastically shrank the prairie landscape. Prairies are now classified as one of the rarest ecosystems of North America and have been reduced to only 3% of what existed prior to Euro-American settlement (Bachelet et al., 2011; Martin & Kronland, 2015).

With Pacific Northwest prairie-savannahs imperiled, these ecoregions may be especially sensitive to the negative effects of climate change. Current trends predict that the Pacific Northwest will overall become warmer, with increased risk of droughts during the summers and more variable precipitation (USGCRP, 2018). Though prairie-savannas are a fire-dependent ecosystem, an increased risk of wildfires and hotter summers may cause more harm than provide benefits. Climate change is projected to lengthen growing seasons, fire seasons, reduce moisture in the soil, and increase fire severity and area burned (McKenzie et al. 2004; Westerling, 2016; Halofsky et al., 2020). Fire that is too frequent, intense, or severe runs the risk of sterilizing soils, causing native perennials to struggle, and negatively impacting pollinator presence (Adedoja et al., 2019; Potts et al., 2003). The changes of fire regimes within Pacific Northwest prairies in

addition to shifting environmental conditions and growing season length can in turn drastically affect the several endangered species only found within Pacific Northwest prairie ecosystems.

One of the most sensitive groups of organisms to temperature and precipitation effects of climate change are butterflies, which function as indicators of both climate change and overall environmental health (Vickery, 2008). Butterflies have a co-evolutionary relationship with plants and are vital to plant pollination (Janzen, 1980; Vickery, 2008). A greater abundance of butterflies found within an ecosystem is indicative of good ecosystem health, and increased populations of butterflies may indicate increased diversity in plant communities, in addition to the possible presence of other pollinators (Ghazanfar et al., 2016). Butterflies also are a food supply for other organisms and may pass plant chemical properties and toxins onto higher trophic levels. The Taylor's checkerspot butterfly is of special concern to Pacific Northwest researchers and conservationists; this species has been listed as federally endangered since 2013, and declines in their populations foreshadow the possible loss of the entire glacial outwash prairies they inhabit (Bachelet et al., 2011; Hill & Martin, 2019; Murphy & Weiss, 1988).

Research on the influence of environmental conditions on checkerspots is scarce. Ehrlich et al. (1980) studied the effects of the 1977 California drought on Californian *Euphydryas* populations and found that drought stress on plants lead to harsh declines in these populations. Parmesan et al. (2015) found that climate change was driving range shifts for the Quino Checkerspot, particularly temperature. Hill et al. (2017) examined the relationship between weather and prescribed fire fuel conditions and their impact on butterfly communities in the Puget Sound area. Bennett et al. (2014) analyzed the effects of microclimate on Taylor's checkerspot habitat use, larval distribution, and adult butterfly movement *in situ* within Oregon

State. However, the association of environmental conditions and growing degree days to checkerspot development and life stage length has gone generally unexplored.

Due to conservation concerns, captive rearing programs for the butterfly have surfaced over the last two decades. The Oregon Zoo began a captive rearing program in 2003, in which zoo staff developed the first husbandry manual for the butterfly and raised it through its life stages (Leroy, 2012). This in part helped to initiate the establishment of the butterfly program at Mission Creek Correctional Center for Women (MCCCW) in Belfair, Washington, founded by the Sustainability in Prisons Project in collaboration with the Department of Corrections (DOC) and Washington State Department of Fish and Wildlife (WDFW) in the 2011 and 2012 years (Leroy, 2012). A third butterfly program was then initiated at Coffee Creek Correctional Facility (CCCF) in Corvalis, Oregon, in 2017. The establishment of these programs provide an opportunity to study the relationship of temperature, relative humidity, and GDD of different instar stages in a captive rearing setting, providing data and observations that can then be compared with wild populations of the butterfly and controlling for genetics, since butterfly matrilines and their eggs and larvae could be tracked and considered separately.

This thesis research seeks to provide an understanding of temperature and humidity on the length of individual instar stages for the Taylor's checkerspot butterfly using data collected between 2021 and 2022 years from the Sustainability in Prisons Project's (SPP) captive-rearing program at Mission Creek Correctional Facility for Women (MCCCW). Growing degree days (GDD) was also analyzed to help understand whether it could be used successfully as a metric to potentially predict butterfly phenology for the Taylor's checkerspot in future studies. This research may be used to help better inform program decisions made at the butterfly program at

MCCCW and contribute to scientific knowledge surrounding climate change and the effects on pollinator species.

Literature Review

The Taylor's checkerspot butterfly (TCB) is endemic to Pacific Northwest prairies and balds (dry lowland sites dominated by herbaceous plants and dwarf shrubs) within Vancouver Island, Oregon's Willamette Valley, and the Olympic Peninsula (Potter, 2016). Once described to "swarm by the thousands", Euro-American urban development, disruptions to Native American burning, and the introduction of exotic grasses have since resulted in local extinctions of Taylor's checkerspot populations (Severns & Warren, 2008). Several agencies and organizations have undertaken efforts to increase Taylor's checkerspot survival in Washington State, including organizations that focus on captively rearing the butterfly. To better help inform program decisions surrounding captive-rearing, growing degree days (GDD) may be used. GDD has the potential to help predict when the different Taylor's checkerspot life stages will occur and provide information on the effects that accumulated heat have on Taylor's checkerspot development.

For this literature review, since butterflies are typically referred to by their common names, "checkerspot" may be used in place of *Euphydryas*, and the Taylor's checkerspot butterfly will be shortened to its commonly used acronym "TCB". Additionally, there are several common definitions of the "Pacific Northwest," none of which are universally accepted (Richards, 1981). For this review, I define "Pacific Northwest" as the geographic region that encompasses British Columbia, Washington State, and Oregon. This review will begin with a description of GDD and its general uses before moving on to provide an overview of TCB. Effects of environmental conditions on checkerspots, phenological asynchrony of host plants, and current conservation measures will be the primary focus of this literature review.

Growing Degree Days (GDD) – General uses

Growing degree days (GDD) is a unit of measurement describing total annual heat accumulation above a specific temperature threshold (Cayton et al., 2015). GDD has been used in agricultural research since the 1980s to predict the timing of key events within plant and insect lifecycles (Parry & Carter, 1985; as cited in Cayton et al., 2015). Plant and insects have a total heat requirement for each developmental stage, and GDD is used to predict an organism's length of life stage by calculating accumulated degree days (Bonhomme, 2000; Miller et al., 2001). Predicting an organism's development can be useful to know in order to treat for pests and allows for better crop management (Miller et al., 2001). Additionally, exceeding a certain accumulated heat threshold may slow or prevent an organism's development.

Over the years the concept of using GDD in phenological studies has extended from the field of agriculture to ecology, and has been used throughout research as a predictor for butterfly species development, abundance, and distribution (Hellegers et al., 2022). Cayton et al. (2015) found that GDD was a better tool for predicting butterfly phenological changes as opposed to calendar dates. Bristow et al. (2022) found that number of degree-days needed for triggering the hatching and eclosion of the endangered Karner blue butterfly was decreased under warming temperatures, indicating that development was accelerated for these life stages. In other studies, accumulated degree days were used to predict early flight periods for the Michigan butterfly and skipper populations (Perkins 2007), and growing degree days had greater ability to predict butterfly dispersal in boreal ecosystems than other variables (Luoto et al., 2006).

Although GDD has been used in butterfly research and the Taylor's checkerspot butterfly is a federally endangered species, GDD has not been studied within Taylor's checkerspot butterfly populations. Understanding how total heat accumulation relates to checkerspot life

stage length and development in captivity can provide several benefits to agencies, researchers, and other organizations supporting checkerspot conservation efforts and prairie ecosystems. The potential for predicting the timing of key life stages may be used to help guide husbandry methods and program management decisions, such as preparing for larval releases or accommodating care for various instar stages. GDD may also help to inform how the butterfly is enduring climatic changes, and perhaps be compared to GDD research on its primary host plant, *Plantago lanceolata,* to identify phenological asynchrony.

Taylor's Checkerspot Butterfly – General Overview

Euphydryas editha

The Taylor's checkerspot butterfly is a subspecies to *Euphydryas editha*. *Euphydryas editha* is a univoltine (producing one generation in a season), non-migratory species of butterfly occurring in populations or metapopulations throughout the Pacific Northwest United States and Canada (Bennett et al., 2015). The flight season of *E. editha* lasts anywhere from three to five weeks ranging from March through July, and eggs generally hatch after two weeks of being laid (Bennett et al., 2015). New larvae generally stay near where they hatch and generally begin feeding on the plant they hatch from, therefore, mother butterflies finding a suitable host plant in ideal habitat is important (Bennett et al., 2015). The plant families Plantaginaceae and Orobanchaceae offer suitable candidates as hosts, with *Plantago, Castilleja,* and *Collinsia* being the most popular choices (Bennett et al., 2015; Buckingham et al., 2016; Dunwiddie et al., 2016).

Larval development and behavior

A significant portion of checkerspot research was undertaken by Paul Ehrlich, who studied *Euphydryas editha taylorii* extensively throughout his career. He published *On the Wings of Checkerspots: A Model System for Population Biology* with researcher Ilkka Hanski in 2004.

Their work has greatly informed scientific understanding of *E. editha taylorii*, including larval development and behavior. As described within this book, Taylor checkerspots lay their eggs in clusters of anywhere between 5-500 eggs during late springtime, though at the butterfly program at Mission Creek Correctional Facility some butterflies have been observed to lay as many as 800 eggs (personal observation). This is a unique characteristic to the species since 90-95% of members in the Lepidoptera order lay eggs singularly. Laying their eggs in clusters may put checkerspots at greater risk for extinction since egg clusters are more prone to population fluctuations (Ehrlich & Hanski, 2004).

When checkerspots hatch, the larvae live in groups and molt through five instar stages prior to entering diapause together, which is an immobile period of suspended development that occurs extreme temperatures, usually during the fifth instar stage (Ehrlich & Hanski, 2004). Taylor's checkerspot larvae consume their host plants as a food source, which is mostly either *Plantago lanceolata* or *Castilleja spp*. After diapause larvae molt into a sixth instar stage, and then eventually pupate (Ehrlich & Hanski, 2004). Checkerspot caterpillars are only able to move short distances from their original host plants and spin webs, which may provide them with further protection during diapause (Buckingham et al., 2016; Ehrlich & Hanski, 2004; Stinson, 2005). A proportion of larvae within a population may re-enter diapause multiple times and remain in the larval life stage for years if conditions are unfavorable (Ehrlich & Hanski, 2004).

Beginning in the third instar stage, larvae are black and have brightly orange-colored dots along their backs. Larvae consume and sequester iridoid glycosides (plant chemical compounds) from their host plants, which provide them with a chemical defense against bird prey and arthropods such as spiders, making then unpalatable to these predators both in their larvae and adult life stages (Ehrlich & Hanski, 2004). However, the extent of this unpalatability has been

observed to be variable in populations. Though checkerspots have developmental benefits that aid to their survival—such as multi-diapausing and sequestering iridoid glycosides—other environmental factors have resulted in their endangerment.

History and Endangerment Status

The Puget lowland prairie landscape was formed by a 3,000-foot glacier known as the Puget lobe of the Cordilleran Ice Sheet entering the area roughly 17,600 years ago, melting approximately 2,700 years later (Williams, 2016). This glacier deposited softer sediments as it made its way through the lowland area, creating areas with practically no hard rock. Currents of water beneath the Puget lobe as it melted, in addition to the ice movement, sculpted this sediment layer, and both these glacial sediments and the currents formed the topography of the land (Williams, 2016). Glacial deposits of these sediment types within Taylor's checkerspot habitat have resulted in loamy and well-draining prairie soils with high organic content. In addition to this glacier and its currents carving the landscape and depositing sediments, after the Puget lobe melted, the unique vegetation of prairie ecosystems in the Puget Sound was influenced by Indigenous "fire economies" within the region, until the introduction of Euro-American fire suppression (Boyd & Lake, 2021).

European settlers and Native tribes in the Pacific Northwest made contact as early as 1774, with most early European colonization activities beginning in 1812 when fur trading was established (University of Washington, n.d.). Early European explorers described the prairies of the Willamette Valley in Oregon and areas in Victoria that Indigenous people shaped with fire as being "open", and because of this openness was selected as the central site for the Hudson Bay Colony in 1845 (Boyd & Lake, 2021). Indigenous use of fire in the Pacific Northwest was primarily used to help source food commonly found on the same prairies home to the Taylor's

checkerspot butterfly, such as camas and wild berries. Within the Nisqually region specifically, which is within the area of Joint Base Lewis-McChord—where the butterfly survives today—fires were noted to have been very patterned. Burns seemed to fall between August 13 and September 12, with some sporadic fires prior to this date (Boyd & Lake, 2021). Suppression of Indigenous burning by Euro-Americans began around the early 1900s, and eventually led to the suppression of fire entirely (Bachelet et al., 2011; Boyd & Lake, 2021). Fires from Indigenous burning were critical to Taylor's checkerspot habitat, preventing the encroachment of conifers and nourishing native nectar and host plants used by the butterfly (Hill et al., 2017; Hill & Martin, 2019).

Due to urban expansion being more convenient on prairies than mountainous landscapes, the Puget lowlands has become one of the most densely populated areas of Washington State. Prairie grassland vegetation has been reduced by 90% of its original landscape, and assessments conducted from 1994-1995 showed significant degradation and loss of prairie habitat on Joint Base Lewis-McChord due to both military use and urban development (Crawford & Hall, 1997). Due to fire suppression and urban expansion, checkerspot habitat fragmented and their populations decreased over time. If species are unable to move within fragmented habitats, their populations become isolated and are less resilient to disasters, unfavorable conditions, or drastic changes to their environments (Bennett et al., 2013). It is common for butterflies to exist as metapopulations (collections of subpopulations), and the Taylor's checkerspot butterfly specifically does not disperse long distances, making it especially difficult to recover from local extinction events (Stinson, 2005).

Another influencer to Taylor's checkerspot habitat fragmentation was the advent of European agricultural practices and invasion of exotic grasses, which drastically degraded

Pacific Northwest prairies, further reducing the resilience of these metapopulations (Bachelet et al., 2011; Bennett et al., 2013; Buckingham et al., 2016). Exotic grasses quickly take over the native prairies of Oregon's Willamette Valley and Joint Base Lewis-McChord, which are also occupied by the butterfly (Poulos & Roy, 2015; Severns & Warren, 2008). The TCB primary host plant *Plantago lanceolata* is quickly crowded out by other taller invasive grasses, and flowers of *Fragaria virginiana*, a primary nectar source for *E. taylori*, also become outcompeted (Severns, 2008). Anthropogenic climate change provides invasive species that are already outcompeting checkerspot resources with further advantages, which could be devastating to checkerspot metapopulations. Hotter summers caused by climate change will increase the frequency and intensity of wildfires, and in turn these fires perpetuate the spread of taller invasives, which usually grow more quickly after burns (Poulos & Roy, 2015). This results in a grass-fire cycle, wherein invasive grasses create favorable, low-intensity fire conditions that further benefit their seeding and quick establishment (D'Antonio & Vitousek, 1992).

Phenological asynchrony and mismatch

The timing of key events during plant lifecycles (phenology) has been disrupted due to climatic changes (Reed et al., 2019). These changes can result in negative outcomes for pollinator species. Temperature is a major control of phenology, triggering the budding of new leaves and earlier production of fruits and seeds when it is warmer (Moore, Lauenroth, Bell, & Schlaepfer, 2015; Rathcke & Lacey, 1985; as mentioned in Reed et al., 2019). Warming temperatures have also been found to be more impactful on plant phenology within Pacific Northwest prairie habitats than either soil moisture or precipitation (Reed et al., 2019). An increase in temperature has been demonstrated to delay the timing of wet seasons and either prolong or initiate drought within prairie systems, which in turn has advanced flowering dates

(Reed et al., 2019). As this trend continues, phenological asynchrony may be at increased risk of being amplified for the Taylor's checkerspot butterfly and its host plants, since this species is found within prairies and grasslands.

Checkerspot butterflies already have a natural disadvantage in the face of climate change because of their natural asynchrony with its host plants. The Bay checkerspot butterfly, another subspecies within *E. editha* and native to California, has been shown to experience severe losses of its larvae due to starvation, with 90% larval mortality during the late springtime (Weiss et al., 1988; Singer, 1972; as mentioned in Parmesan et al., 2015; Singer & Parmesan, 2010). These heavy losses are a result of the early senescence (dying) of its host plant *Plantago erecta* prior to the larvae being healthy enough to enter diapause for the hotter summer season (Harrison et al., 1988).

This asynchrony between *E. bayensis* and *Plantago* seem to have existed before changing climatic conditions, indicating that larval mortality may be an adaptive tradeoff for adult fecundity (maximum reproductive potential) (Weiss et al., 1988; Singer, 1972; as mentioned in Parmesan et al., 2015; Singer et al, 2010). As seasonal warming trends increase and key events within *Plantago*'s life cycle happen earlier and earlier, the asynchrony that already exists between *E. bayensis* and *Plantago* is likely to be exacerbated far beyond the ability for the Bay checkerspot to adapt. In other words, the Bay checkerspot butterfly is already considered to be at its ecological threshold for its ability to tolerate this asynchrony, and further increases in asynchrony may result in a phenological mismatch (Renner & Zohner, 2018; Singer & Parmesan, 2010) with negative consequences for the Bay checkerspot.

In *E. editha* species including the Taylor's checkerspot, pre-diapause larvae that come from females who fly the earliest tend to have higher survivorship than those descended from

later flying females (Weiss et al., 1988). This is because female butterflies that fly the earliest lay their eggs on north-facing slopes, which tend to be cooler and grow plants that senesce later, so these larvae can feed on these plants for longer (Weiss et al., 1988). However, the early-flying female butterflies that these pre-diapause larvae descend from develop as larvae on south-facing slopes, which have higher temperatures and higher starvation rates for pre-diapause larvae, and therefore fewer survivors (Weiss et al., 1988). Under climate change, this dichotomy is likely to be further disrupted by increasing temperatures. This is because temperatures on both the cooler north-facing and south-facing slopes will rise from global warming, so host plants on the cooler north-facing slopes would senesce earlier, thereby resulting in less food availability for checkerspot larvae.

Phenological changes in butterflies under climate change

In addition to asynchrony with host plants, the effects of climatic changes have resulted in changes within butterfly development. As ectotherms (a term that describes organisms which obtain heat from their environments), butterfly development is heavily dependent upon temperature (Forrest, 2016). The rate at which a butterfly develops through a given life stage—including checkerspot butterflies—increases when temperatures are warmer. Warmer spring times have been observed to result in earlier eclosion (emergence from chrysalis) and flight periods for butterflies (Forrest, 2016; Roy et al., 2015).

Male Taylor's checkerspot butterflies also tend to eclose earlier than females, and research seems to be limited on whether increased temperatures may affect sex bias (greater ratio of one sex in comparison to the other) of the organism in the field during the flight period. Additionally, some butterfly species have also shown a shift in previously being a univoltine

(producing a single brood of offspring per year) to becoming multivoltine under warming temperatures, with multivoltine species producing more generations per year (Altermatt, 2010).

In contrast, increased precipitation has been demonstrated to lengthen the life stage of pupation and delay eclosion, but too much rainfall can also create a delay for post-diapause larval development (Lagerquist, 2019). This may result in a shorter flight period for adult females and a smaller window for them to oviposit prior to host plant senescence (Lagerquist, 2019). Additionally, cooler temperatures and greater humidity during the egg period seem to indicate a longer duration of time spent in the egg stage (Severns & Grosboll, 2011). Precipitation patterns have been predicted to change within the Pacific Northwest region based on global projections, and under climate change increased temperatures and greater or more sporadic rainfall may disrupt flight periods for Taylor's checkerspot butterflies, result in shorter developmental windows for life stages, and potentially result in heavy sex bias during flight season (USGCRP, 2018).

Current Conservation Measures

Institute for Applied Ecology and Oregon Zoo efforts

The first captively-reared Taylor's checkerspot butterfly within the Pacific Northwest started accidentally in 2003, after a Washington Department Fish and Wildlife voucher specimen originating from Clallam County began to oviposit on a species of *Castilleja* (Schultz et al., 2011). Following their establishment of Oregon silverspot butterfly (*Argynnis zerene hippolyta*) captive rearing in 1999, the Oregon Zoo initiated a captive rearing program for the Taylor's checkerspot butterfly in 2004, collecting wild eggs from field locations and raising them to pre-diapause larvae wherein they were released back out into the field. The collection of eggs was limited to 1-2% of the butterfly's estimated population, which translated to no more than 600

eggs (Shultz et al., 2011). Much of the groundwork science in Taylor's checkerspot captive rearing began under the care of Oregon zoo staff, with guidance for the development of protocols created in collaboration with Dr. Gordon Pratt (Shultz et al., 2011).

Beginning 2008 the Oregon Zoo successfully began to rear larvae through to the adult stage and initiated captive breeding. Wild adult females were also collected and brought into the zoo in 2008 to allow for morphometric data to be gathered and compared between captively bred and wild-caught populations (Shultz et al., 2011). This proved to be an ideal time, since the following year the population of Taylor's checkerspot butterflies at the field collection site sharply declined, and out of concern for the strain placed on the population biologists refrained from collecting wild eggs and adults. This same year, the zoo published a captive rearing husbandry manual in 2009 for the butterfly, further helping to inform captive rearing practices and techniques (Barclay et al., 2009; Shultz et al., 2011).

Despite the 2009 plunge in population numbers, captively raised butterflies manage to produce over 10,000 estimated eggs and 8500 larvae the following year, far exceeding the zoo's capacity, with these larvae being released at several different site locations at the pre-diapause stage. Ideal spring weather conditions from spring 2009 to the following spring in 2010 allowed for a greater flight period and successful copulations among adults released at these sites (Shultz et al., 2011). In the year of 2020, the Oregon zoo stopped its captive rearing program at the onset of the COVID-19 pandemic (Ronda Naseth, personal communication).

In 2016 the Institute for Applied Ecology created a 9 year action plan for the Taylor's checkerspot butterfly in Oregon. The nonprofit's goal was to restore habitat and improve habitat quality for the butterfly, increase butterfly population numbers, and assist in providing information for other checkerspot recovery plans (Menke & Kaye, 2016). Restoring and

maintaining suitable habitat, increasing TCB numbers and populations and creating a metapopulation structure, and contribute to TCB recovery planning were goals established and still presently undertaken to support checkerspot conservation work under this plan (Menke & Kaye, 2016).

Sustainability in Prisons Project and MCCCW

The Sustainability in Prisons Project (SPP) was formed in 2004 as a partnership between the Department of Corrections and The Evergreen State College with the goal of bringing science education into prisons and reducing the economic, environmental, and human cost of prisons through sustainable initiatives. Initially beginning as a lecture series, SPP inspired several sustainability projects at Cedar Creek Correctional Center, and the success of these projects lead to other sustainability programs establishing within other Washington State prisons (Aubrey, 2013).

From 2011-2012, ten incarcerated women were hired to be the first butterfly technicians for the first year of SPP's butterfly program. The program initially relied heavily upon the butterfly husbandry manual developed by the Oregon Zoo, and training in addition to weekly support was provided by an Evergreen State College graduate student enrolled in the Master of Environmental Studies (MES) program, and employed as SPP's butterfly program coordinator. Biologists from the Oregon Zoo also occasionally visited the butterfly program to provide guidance. The painted lady butterfly (*Vanessa cardui*) was used as the original training candidate for butterfly technicians and the butterfly coordinator to practice husbandry rearing techniques prior to working with the federally endangered Taylor's checkerspot butterfly.

In March 2012, 755 Taylor's checkerspot larvae were provided to the SPP butterfly program by staff at the Oregon Zoo. These larvae were raised successfully, with 600 of them

being released onto the JBLM prairie, and the rest retained for captive breeding. Another 3395 larvae were raised as offspring from the original 155 larvae retained for breeding (Leroy et al., 2012). During these first two captive rearing years, MES graduate student Dennis Buckingham (previously Dennis Aubrey) conducted a thesis research project examining Taylor's checkerspot host plant preference, which would go on to later be published (Aubrey, 2013; Leroy et al., 2012).

Today, SPP is either directly or indirectly involved in the establishment of sustainable programs within every Washington State prison, including captive rearing and other conservation efforts for the Taylor's checkerspot. Native and rare plant propagation operations were started at Stafford Creek Corrections Center (SCCC) and Washington Corrections Center for Women (WCCW), which produce native plant species to be planted in Taylor's checkerspot sites on JBLM to help restore butterfly habitat. In 2012 a new SPP program guided by the Washington State Department of Fish and Wildlife began at Mission Creek Correctional Center for Women (MCCCW) in Belfair, wherein incarcerated women directly captively raised Taylor's checkerspots to be released at these locations.

The SPP butterfly program has also been a catalyst in supporting Master of Environmental Studies (MES) graduate student thesis research on the butterfly. Dennis Buckingham was the first butterfly program coordinator for SPP, and the first graduate student to conduct research on the butterfly in SPP's captive rearing program in 2013 (Leroy, 2012). His research focused on Taylor's checkerspot oviposition host plant preference and found that the butterfly preferred *Castilleja levisecta* and *Castilleja hispida* over *Plantago lanceolata* (Aubrey, 2013). Wendy Lagerquist in 2019 focused her research on winter temperature, humidity, and precipitation impacts on peak abundance of the butterfly across three different sites, finding

weak associations with winter climate variables on some of these sites while discovering that peak abundance increased across these years, potentially due to checkerspot reintroduction efforts (Lagerquist, 2019). Keegan Curry analyzed ten years' worth of reproductive data for the butterfly at MCCCW and CCCF captive rearing facilities, finding that the Taylor's checkerspot produces less offspring than other *Euphydryas editha* subspecies (Curry, 2019). Carly Boyd examined how often environmental targets at MCCCW were achieved over seven captive-rearing years, and whether falling outside of these environmental targets impacted larval survival, hatch rate, fecundity, and other metrics. Boyd found that meeting these conditions was highly variable over the years, and that achieving environmental targets did not necessarily impact developmental milestones and captive rearing program goals for the butterfly (Boyd, 2021). Ultimately, MES students have studied different metrics and influences on both captively reared and wild butterflies, with several of these individuals utilizing SPP butterfly program captive rearing research.

Coffee Creek Correctional Facility and Oregon Fish & Wildlife

A Taylor's checkerspot captive-rearing program was launched in 2017 at Coffee Creek Correctional Center Facility (CCCF), a women's prison in Oregon State. This butterfly program was the first butterfly conservation program to be introduced into a medium security facility, and modelled the SPP butterfly program at MCCCW, receiving funding from U.S. Fish and Wildlife Service (USFWS) and the Oregon Zoo (Oregon Zoo, 2019). Chad Naugle from Oregon Department of Corrections advocated for the program to be implemented due to wanting a meaningful work and educational opportunity to be available to adults serving longer sentences, and initially establishing the program was challenging due to the vast majority of conservation programs existing within minimum security facilities (Ronda Naseth, personal communication).

Ronda Naseth, a zookeeper at the Oregon Zoo, became employed as CCCF's program manager, and her two years of butterfly husbandry training for the zoo in addition to the Oregon Zoo's butterfly husbandry manual was relied upon at the program's inception. Naseth modified the Oregon Zoo's procedures to allow butterfly technicians at the Coffee Creek program to work more independently, collaborating with Oregon Zoo staff to supplement visits to the facility and receive answers to any inquiries (Ronda Naseth, personal communication). While for the most part butterfly technicians work independently, Ronda plays a central and important role in supporting the CCCF butterfly team and providing them with guidance.

Unlike the SPP butterfly program area, which consists of two greenhouses and a shed outside of the prison perimeter fence, CCCF's butterfly lab exists within the medium security facility and is connected to one of the secure housing units. The room that is now the butterfly lab previously was used as a classroom and for overflow housing, and additions had to be made to the infrastructure for the lab's creation, including plumbing, filtration, a sink, and an HVAC unit. Longer counters and shelving units were also built within the room to allow for better workstations and storage. Two windows overlook the yard, and the CCCF's diapause shed rests outside underneath one of these windows (personal communication, Ronda Naseth). While there were several modifications needed to be made both in the infrastructure and how work processes were done in order to meet security standards, ultimately the program has been a success in allowing for both incarcerated women to receive essential work skills within the sciences and a butterfly conservation lab to be tailored to captively rearing Oregon Taylor's checkerspots.

CCCF's butterfly lab has also invested important knowledge and scientific observations pertaining to mortality causes of the Taylor's checkerspot during the wake-up stage, since this butterfly lab has experienced increased mortality numbers in its animals during this time. The lab

had its plantain quality assessed and ruled out as a cause for these mortality trends, and the butterfly technicians under the guidance of Ronda Naseth conducted a light study and introduced a limited amount of UV lighting into the lab. While this study is still ongoing, the team hypothesizes that UV light plays a significant factor in larval survivorship.

The butterfly program at CCCF also had a surplus of larvae during the 2021-2022 captive rearing season, which resulted in the creation of a backup lab for 2000 of these larvae and the CCCF butterfly technicians and butterfly program manager observing a potential correlation with length of time spent as pre-diapause larvae and survival at wake up when temperatures in the back up lab decreased lower than CCCF's original butterfly lab. Naseth observed larvae in the back up lab spending an average of 2 days longer in the 4th instar life stage duration, and these larvae had higher survival than CCCF larvae post-diapause. During the 2022-2023 season, CCCF attempted to replicate these conditions at 4^{th} instar using air conditioning, improving larvae survivability by 10% more than their previous best season (Ronda Naseth, personal communication).

As of the 2023 year, the Coffee Creek Correctional Center's butterfly program has successfully captively reared and released a total of 9,089 animals since its conception, and are retaining multi-diapause larvae for the first time this year, with the intention of these animals being released to a newly established Hood River Taylor's checkerspot conservation facility when it opens. While CCCC's butterfly program does not breed Taylor's checkerspot butterflies, the butterfly technicians observed the eclosion of a butterfly for the first time in 2023 when one was accidentally left behind during a pupae release and returned to the prison. Since the program's beginning, roughly 17 butterfly technicians have had experience working in the lab,

with 8 of these individuals having had been a part of the butterfly program for the past three years.

Conclusion

Under climate change, landscape changes due to shifts in fire regimes and phenological asynchrony introduce new challenges to endangered species conservation. Growing degree days has been widely used in agriculture and implemented in butterfly research, demonstrating promise to be a useful tool for predicting checkerspot phenology. Some research exists which analyzes the effects of environmental conditions on Taylor's checkerspot, but there is no current research that analyzes temperature, relative humidity, and growing degree days as statistical variables with potential influences on checkerspot life stage length. This study uses datasets collected for captively-reared Taylor's checkerspot butterflies from the Sustainability in Prisons Project to ask how environmental conditions affect butterfly life stage length and development, with a focus on growing degree days (GDD).

Methods

All research was conducted at Mission Creek Correctional Center for Women (MCCCW), a minimum-security women's prison located in Belfair, Washington within the Tehuya State Forest. Captive-rearing procedures for MCCCW were developed by the Sustainability in Prisons Project (SPP), a partnership between the Department of Corrections and The Evergreen State College, and these procedures sometimes were adapted from husbandry practices conducted by Oregon Zoo staff. Checkerspot developmental data and dates were collected by a team of incarcerated butterfly technicians from the 2021-2022 and 2022-2023 captive rearing years, overseen by an Evergreen State College graduate student employed as the butterfly program coordinator by SPP.

Figure 1. Evergreen State College graduate students and SPP coordinators Raychel Dunning and Jen Bass assisting butterfly technicians in butterfly husbandry care.

Site

The captive-rearing life stages for the butterfly occurred within two greenhouses at MCCCW built and designed for the purpose of Taylor's checkerspot captive-rearing, and these greenhouses are located just outside the prison fence. "Raven" is the oldest greenhouse, built in 2011, and is a 24ft x 10ft glass greenhouse with UV transmitting panels. This greenhouse is partitioned into two rooms by a glass door, with the main room 16ft x 10ft and the smaller room 8ft x 10ft. The newer greenhouse, "Turtle", is constructed with a similar design, larger than the Raven greenhouse. When temperatures exceed 86°F, exhaust fans, roof windows, and motorized dampers auto-function to help regulate environmental conditions. Technicians also cover both greenhouse roofs with 50% reflective aluminet shade cloths during mid-spring and throughout the summer to help mitigate extreme heat. Both greenhouses also contain heating systems which allow for heat to be adjusted both manually and automatically during colder periods.

For the diapause life stage, checkerspot larvae were kept inside an 8' x 10' x 8' wooden shed with two 2' x 2' windows. Both the shed and the greenhouses were built with the intention of exposing butterflies, eggs, and larvae to ambient environmental conditions but not extreme weather events. There are eight 4' x 10' grille vents for the shed, with six of these vents built into the bottom of three walls and two near the ceiling on the walls across from the other six vents (Curry, 2019). During the diapause stage, butterfly technicians do not regulate temperature or humidity.

Figure 2. Mission Creek Correctional Center for Women's Taylor's checkerspot butterfly program area.

Figure 3. Image of greenhouses used for Taylor's checkerspot butterfly captive-rearing and breeding. Left is Raven greenhouse; right is Turtle greenhouse. Plantain beds near the greenhouses are covered in cloches during the colder, winter months.

Figure 4. The two greenhouses and the shed are located directly next to the prison's perimeter fence.

Environmental Data

HOBO loggers are programmed prior to deployment to capture minimum relative humidity, maximum relative humidity, minimum temperature, maximum temperature, and average temperature every hour for the captive-rearing year. There is an option for light intensity data to also be captured, however this data is rarely used. The loggers are changed out generally every 1-2 weeks during the active rearing year with 3-8 new separate loggers of the same brand to avoid battery depletion, and the new loggers are pre-set to begin capturing data at a set time in order to ensure that environmental data is captured continuously. During diapause, loggers are

changed out approximately once a month due to less need for the use of environmental data for reporting during the winter time. The placement of the loggers attempts to emulate the current environment of the Checkerspot's life stage (i.e., if larvae are being kept in a plexi cup with a folded paper towel and plantain leaves which is then placed in a shoe bin misted for humidity, so are these loggers). The loggers are always replaced after each life stage of development is complete. Data from the loggers are uploaded every time loggers are replaced.

The number of loggers capturing environmental data and how frequently they are rotated out and replaced with new loggers is dependent upon the life stage. Stricter guidelines to this process were developed and implemented in 2022, in which it was decided that at least three HOBO loggers per greenhouse would be placed on top and mid-shelf of wire racks where oviposition chambers are placed during the active season. HOBO loggers are kept in their own oviposition chambers without a butterfly to attempt to capture exact conditions during oviposition. As butterflies die and the eggs produced increase, HOBO loggers are then removed from oviposition chambers and placed with the eggs on the egg shelves—two per rack, on one high and one low shelf. During the third instar to diapause life stage, there continues to be two loggers per metal rack in each greenhouse, one recording conditions on the highest shelf and one on the bottom shelf of each rack. Depending on the number of larvae, this could mean that anywhere from 6-8 loggers are capturing environmental data during this life stage.

After being exchanged with new loggers, the loggers previously capturing environmental data are taken back to The Evergreen State College where their data is read out using HOBOware software. HOBO logger data is then saved as a CSV file, and then copied and saved as an Excel file with any data collected outside of the program area (such as during transportation to be uploaded), deleted. The raw HOBO logger data (uploaded as a .hobo

filetype), CSV files, and Excel files are then uploaded and saved to SPP's digital library where they are then used for annual reporting purposes.

Collection of Wild Females

Female butterflies are collected by WDFW field biologists from mid-April to early May, depending on field weather conditions. During the 2022 season, wild Taylor's checkerspot eggs were also collected from the field and brought to MCCCW as an emergency conservation effort due to the concern of low butterfly numbers observed by WDFW, Oregon Department of Fish & Wildlife (ODFW), and JBLM Fish & Wildlife butterfly surveys. These wild eggs were left on the host plants they were found on, with the sections of the host plant containing the eggs carefully cut away and placed inside ventilated glass specimen jars. Butterflies are typically collected from Range 76 on JBLM, and anywhere from 40–50 butterflies are taken from the field. During the 2022 year, butterflies were collected from Range 53 and wild eggs were collected from the Scatter Creek South area**.** Adult butterflies are transported to MCCCW also within ventilated glass specimen containers, then placed inside coolers. Once brought into the greenhouses, these butterflies and any wild eggs are then processed.

Figure 5. Wild gravid females are collected in glass containers and placed within coolers for transportation from the field to the prison.

Butterfly technicians divide the total number of butterflies and wild eggs between each greenhouse and assign each specimen an ID following SPP *Oviposition* procedures. This ID contains an abbreviation of the site the butterflies were collected from (such as “FL” for “Fort Lewis), the year they were collected, and a number based upon the order that they were being processed.

Procedures and life stages

Butterfly technicians follow procedures for each given life stage, which were originally based upon protocols previously developed and husbandry techniques practiced by Oregon Zoo staff within their checkerspot rearing program. As MCCCW's butterfly program became more established over the years, captive-rearing procedures were modified SPP and undergoing heavy edits each year to help better suit captive-rearing techniques practiced by MCCCW relative to the infrastructure. Procedures for the 2020–2022 years separately provide guidelines for the oviposition, eggs to third instar, third instar to diapause, diapause, and wake-up life stages. Prior to 2020, previous breeding procedures for post-diapause, pupation, eclosion, adult care, and captive breeding life stages were also practiced. Captive breeding ended after the 2019 year and was intended to permanently no longer be a function of the butterfly program, but this portion of the program is being brought back in 2023 due to conservation concerns.

Oviposition Experimental Setup and Daily Care

Upon being processed into each greenhouse, adult female butterflies are placed into oviposition chambers one butterfly at a time containing a robust plantain plant, a water sponge saturated with filtered water, and a cotton ball soaked in a 1:3 honey solution placed into a bottle cap. The oviposition chambers are covered with soft tule netting. The chambers are then labelled with each female's unique matriline ID. Butterfly technicians check for eggs daily by first carefully removing the female butterfly using a Q-tip soaked in 1:3 honey solution and then placing her underneath an upside down large (16 oz) clear deli cup with this Q-tip inside. As the butterfly feeds, butterfly technicians search her oviposition plant for eggs. The technicians remove any eggs found by carefully cutting off portions of the leaf they are laid on using

scissors, estimate how many eggs are in the cluster, and then place these eggs inside a lidded and prepared small (5.5 oz) deli cup with a cone-shaped paper towel inside.

Technicians label these small egg cups with their matriline ID, the date they were collected, and assign a new egg cup number based upon the date and order in which they were collected. Matrilines are kept separately to help preserve genetic diversity and better inform WDFW reporting. Eggs develop over 10-14 days and gradually change color over time from bright yellow, to reddish orange, to dark purple, and their percent development is tracked on the 7^{th} day based what percentage of eggs within each cup are changing color. Estimated number of eggs each mother butterfly has laid per day, date eggs are laid, the egg cup number that each of these egg clusters are placed in, egg percent development, hatch date, and 2^{nd} instar date are all recorded on a Female to Third Instar form per each matriline. Daily and grand total egg estimates produced from each female butterfly are also tracked separately on an Egg Tally form. Female butterflies are fed with the 1:3 honey solution and their water sponges are changed daily, and they continue producing eggs until they die of natural causes.

Following the current oviposition procedures as produced by the Sustainability in Prisons Project (Bush, 2022a), six HOBO loggers (three per greenhouse) are kept in a butterfly-free oviposition chamber set up as pictured in figures 4 & 5, mimicking the environmental conditions of the adult butterflies. For this study, the end of the oviposition life stage for each given matriline is considered to be completed after a female butterfly has laid her last egg, and this information can be found on both the egg tally sheets and Female to Third Instar forms documented by the butterfly technicians. Oviposition as a life stage is considered to be completed when all butterflies in both greenhouses have died.

Figure 6. Oviposition chamber with honey dome and water sponge. Image by the Sustainability in Prisons Project.

Figure 7. Oviposition chamber enclosure with a honey dome and soaked sponge to nourish an adult Taylor's checkerspot butterfly. Two HOBO loggers per greenhouse rack are placed in replicate butterfly-free chambers. Image by the Sustainability in Prisons Project.

Egg to third instar daily care and experimental set-up

The beginning of the egg to third instar life stage is considered to have begun once the first eggs have been laid, therefore there is some ongoing overlap with the oviposition life stage. The date in which all larvae in a small egg cup hatch form their eggs is recorded on the Female to Third Instar form, as is the date in which all larvae within a 5.5oz cup enter into 2nd instar. 2nd instar larvae are observed to be slightly larger than 1st instar larvae. Egg to third instar larvae are continued to be kept inside their 5.5oz deli cups, generally undisturbed except to provide them with a fresh *Plantago* leaf daily. Up to eight of these deli cups are placed inside plastic shoe bins with blue shop towels placed in the bottom of these bins. The blue shop towels are saturated daily to help meet humidity environmental targets.

Figure 8. Image by SPP, from SPP's Eggs to Third Instar procedures. Eggs are cut from leaf and placed into a 5.5oz cup, where they are then stored inside plastic shoe bins with a saturated blue paper towel.

Butterfly technicians record the dates on the Female to Third Instar form for the following: the percent of egg development after seven days, the date that the first larvae in a cup has hatched, the date in which the first larvae in a cup have entered into second instar, and the date in which the first third instar molt is noticed within a cup. When all larvae within a 5.5oz egg cup are observed to have developed into the third instar stage, the entire cup is then counted

and transferred into 16oz deli cups of ~15 larvae, following the *Third Instar to Diapause* procedures.

If a 5.5oz cup is observed to become too overcrowded because of the presence of too many larger third instar larvae being present within the small deli cup, but not all larvae within this cup have molted into third instar, then an exception can be made. These third instar larvae can be counted and transferred into larger 16oz cups following the *Third Instar to Diapause* procedures, while any other larvae not yet molted into the third instar stage within the 5.5oz deli cup remain until they, too, reach third instar and can be counted separately into bigger 16oz cups.

During this life stage, at least three HOBO loggers per each greenhouse are positioned to mimic the conditions in which the eggs are kept following the current *Eggs to Third Instar Larvae* procedures as written by SPP (Bush, 2022b). Since the HOBO loggers are larger than the 5.5oz deli cup, they are instead placed inside a 16oz cup with a paper towel and a lid. This life stage is considered to have ended when each deli cup has been observed to have completely developed into larger third instar larvae.

Figure 9. Photo by SPP, as found in their Eggs to Third Instar procedures.

Third Instar to Diapause

When all larvae within a 5.5oz egg cup are observed to have molted into 3rd instar, larvae are transferred from these 5.5oz cups into 16oz cups one matriline at a time. Each 16oz cup contains batches of approximately 15 larvae per matriline, with larvae from different matrilines never being mixed together into these 16oz cups. Any 2nd instar larvae are left in 5.5oz cups until they reach third instar and are not transferred into 16oz cups due to their fragility. The new 16oz cups are labelled with the matriline ID, the date in which the larvae are transferred into 16oz cups, their original hatch date, total larvae within the 16oz cup, and a unique cup ID that incorporates the ID number on each original egg cup from a single matriline and a letter, used as an identifier for each 16oz cup. A singularly folded paper towel is placed into 16oz cups.

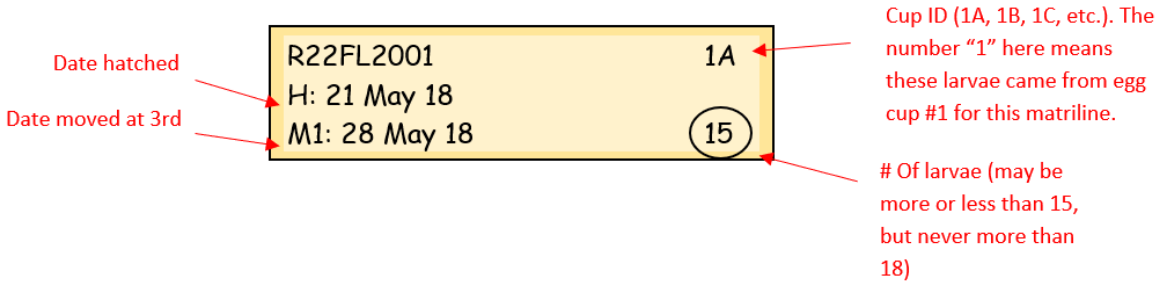


Figure 10. Example of the 16oz cup label created when larvae are transferred from 5.5oz egg cups into 16oz third instar cups. Image credit Sustainability in Prisons Project.

Butterfly technicians perform daily care for 3rd-5th instar larvae following the *Third Instar to Diapause* procedures, which follow the same guidelines for daily care for the *Eggs to Third Instar* procedures. Larvae are provided anywhere from 4-6 leaves daily with old leaves removed from the cups every day. Larvae are visually assessed for good general health and mortality, and paper towel liners in cups are changed when 25% of the towel liner has been soiled by frass or if mold is observed. Old paper towel liners and old leaves are placed into a separate bin, then

double checked at the end of the day for any larvae that may have been missing. Any stray larvae found in these bins or within the lab space are placed into a "QC" (quality control) 16oz cup since their matriline is unknown. Like previous life stages, 16oz cups of larvae are kept inside plastic shoe bins lined with a saturated blue shop towel, with three cups each fitting into these bins. Bin lids are kept 3/4ths of the way closed.

When all larvae within a cup have molted into 5th instar, their cup is marked with a small colored sticker dot with the date in which it was observed the cup had fully entered into 5th. Technicians discern when larvae enter into 5th instar based on molts found within cups and based upon the larvae's slower and more inactive activity, such as frassing less, moving less, and eating less leaves. Two weeks after the last cup of larvae within the program area has entered into 5th instar, all larvae are allocated into diapause cups following the *Diapause* procedures.

Butterfly technicians record the following data on the Third Instar to Diapause form: Matriline, number of larvae counted into 16oz cups at third instar, date counted into 16oz cups, date in which the first larvae is observed to have molted into 4th instar, and date in which the first larvae in each individual cup has molted into 5th instar. For this study, the 4th instar life stage is not analyzed or used as an indicator due to the difficulty in adequately discerning the 4th instar stage, resulting in large disparities in the data recorded. The beginning of the third instar stage is considered to have started when larvae in a 5.5oz cup are able to be transferred into their 16oz cups. For this research, the end of this life stage is recorded based on the date in which each individual cup has been observed to have molted into 5th instar.

HOBO loggers for this stage are managed in the same way as for the *Egg to Third Instar* life stage. Loggers are replaced at minimum once every 2-3 weeks during this life stage. HOBO loggers are kept on the same shelves that third to fifth instar larvae are kept, within their own

container and in an empty shoe bin with a blue saturated shop towel along with the Min/Max readers that butterfly technicians use to assess the environmental conditions in the greenhouse.

Diapause

Following the Sustainability in Prison's Project's *Diapause* procedures (Bush, 2021), larvae are allocated into 16oz deli cups of approximately 50 larvae each after larvae have been confirmed to have molted into 5th instar and show signs of diapause. Larvae are kept in their greenhouses for two more weeks prior to being moved into the outside shed and placed underneath terracotta pots. Three HOBO loggers are moved into their own 16oz deli cups. They are then placed inside the shed and underneath tree separate terracotta pots, each on one rack, without larvae underneath these pots. Technicians monitor larval movement during the diapause period, conducting one movement check a month from September–December, every other week for January, and then once a week for February. Larvae are considered to have "moved" if they are observed to be outside their paper towel liners, counted, and then placed back in between their two paper towels. Once larval movement reaches 15% or greater for two consecutive movement checks after February 1st and WDFW has confirmed conditions out in the field to be deemed fit for larval survival, larvae are brought back into their previously assigned greenhouses for approximately two weeks, in which the end date for the diapause life stage and beginning of the wake-up life stage is recorded. This stage was not included in the analysis for this thesis research.

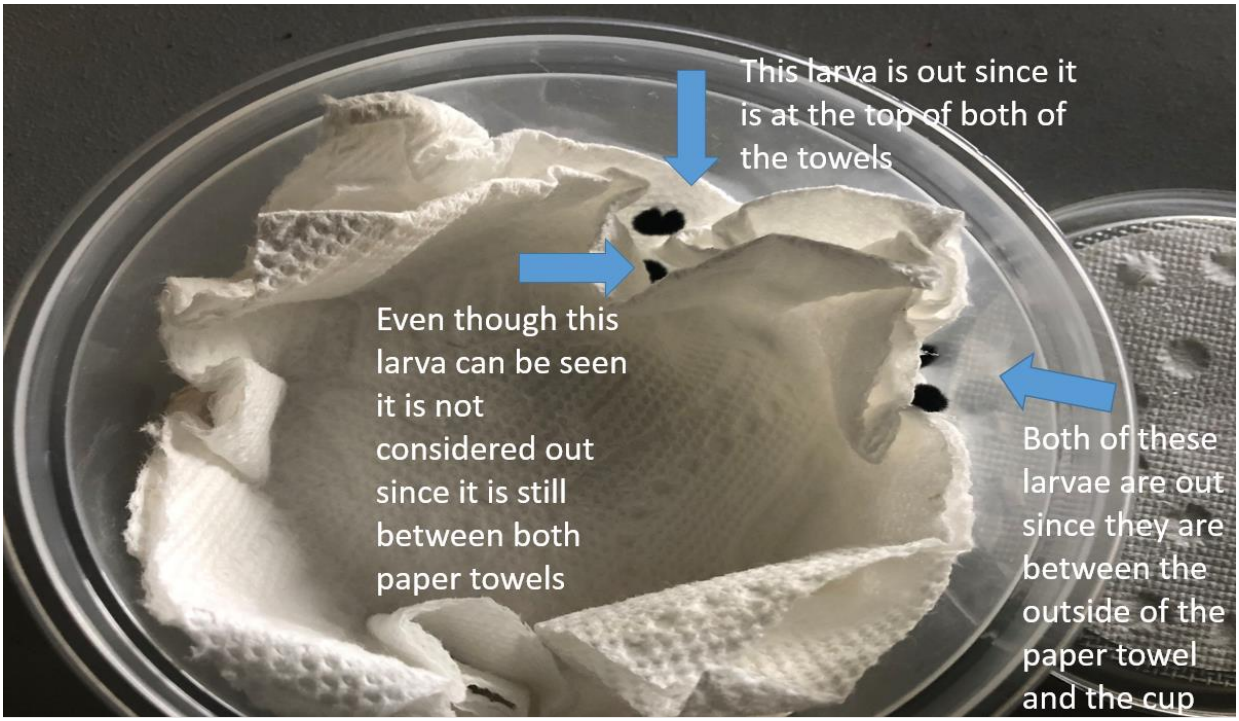


Figure 11. Image from SPP Diapause procedures demonstrating which larvae are considered "moved" during movement checks. Image credit: Sustainability in Prisons Project.

Figure 12. HOBO loggers are placed under terracotta pots in the outside shed during diapause to mimic the conditions of the larvae. Image by the Sustainability in Prisons Project.

Figure 13. Larvae in diapause are placed under terracotta pots. Image credit to the Sustainability in Prisons Project.

Data Analysis

Life stage length and development

At MCCCW, data is collected per matriline and larvae cup ID, with every matriline having its own dedicated form per life stage. Butterfly technicians fill out these forms by hand, and these forms are then stored in binders within the greenhouse in which the relative matriline is kept until they are turned in to an SPP staff member at the end of the captive-rearing year. This data is used by both SPP and WDFW to complete annual reporting and helps to inform program decision making, measure captive rearing success, and compare with data taken from the field. The dates of specific developmental milestones as recorded by technicians were transcribed from these physical data forms to an Excel workbook, and then used to calculate the length of each life stage length. The beginning and end of each life stage was analyzed per the dates written down for each individual cup of larvae.

For oviposition, the life stage was considered to begin the day that each individual butterfly laid its first eggs, and the life stage was considered to have ended the last day that each butterfly laid eggs. The entire life stage of oviposition itself was considered "ended" after the last butterfly within the entire program area stopped producing eggs.

For the egg to third instar life stage, this was considered to begin the given day that a cluster of eggs per matriline was observed to have been laid by an adult female butterfly, and then collected by the technicians and placed into its egg cup. Technicians collect eggs daily, so "date collected" is approximately the same day the butterfly has laid eggs. Larvae then hatch (and this date indicates the beginning of the 1st instar stage), develop into second instar, and then third instar. This life stage was complete for an individual cup once larvae within an egg cup reached third instar and were transferred to larger cups of approximately 15 third instar larvae. Thus, the date that third instar larvae from a given cup were transferred to 16oz cups is used as the end date for the egg to third instar life stage, and varied for each egg cup within each matriline. Since the small egg cups sometimes had hundreds of eggs, if third instar larvae were present in the cup and it was deemed to be too crowded, sometimes these larger third instar larvae were transferred to the larger 16oz cups prior to the entire egg cup developing into 3rd instar larvae.

The third instar to diapause life stage also begins the date larvae were transferred from their egg cups into their 16 oz cups. Therefore, the end date of the egg to third instar life stage is the start date of the third instar to diapause life stage. The date that the first larvae within a given cup was observed to have molted into 5th instar marked the first day of diapause, and this was used as the end date for this life stage.

The days between the start date and the end dates for each of these life stages, per each of these cups, were then calculated using the =DAYS( ) formula in Excel.

Environmental Data

After collecting data, HOBO loggers were read out following the SPP HOBO Logger Data Upload and Clean Up procedures provided to butterfly coordinators. Uploaded data contained the minimum, maximum, and average temperature, relative humidity, and light data per hour for every day. In addition to this hourly data, separate columns of the average temperature and humidity data for each 24-hour period was also recorded.

For the 2021 year, environmental data was collected for both greenhouses from May 2, 2021 (a few days prior to the processing of adult butterflies) until May 1, 2022. For the 2022 year, data was collected from May 6th, 2022 until February 27th, 2023. However, since this research did not encompass the cold diapause and wake up life stages, only data through September 15th for the 2021 year and August 26th of the 2022 year was considered.

Environmental data were stored in an Excel table which included the greenhouse, year, average temperature, and average humidity for each day in addition to logger number, life stage, and rack placement (if this info was provided). Since there were multiple loggers per greenhouse, the overall average temperature and humidity for each day of the rearing year across all loggers with each greenhouse was calculated.

Using these daily averages, for a given individual cup in a specific greenhouse the average temperature and relative humidity were calculated across the duration of the given life stage based on its start and end date.

Growing Degree Days

GDD baseline of 50°F (10°C, respectively) was used. GDD values per each given day were first calculated within Excel, with separate columns created for each GDD baseline, by subtracting the minimum baseline value from the given average temperature in the table for each date and per greenhouse. Using an excel formula, GDD values were set to return an output value of 0 if the given average temperature exceeded the baseline.

Similar to how the average temperature for each individual cup's life stage was calculated, growing degree days for each individual cup's life stage was determined by taking the sum of all GDD values for the total duration of each individual cup's specific life stage, based upon provided start and end dates. GDD values were not calculated for the collected to 3rd and collected to 5th life stages due to these life stages being analyzed per matriline as opposed to per larvae cup.

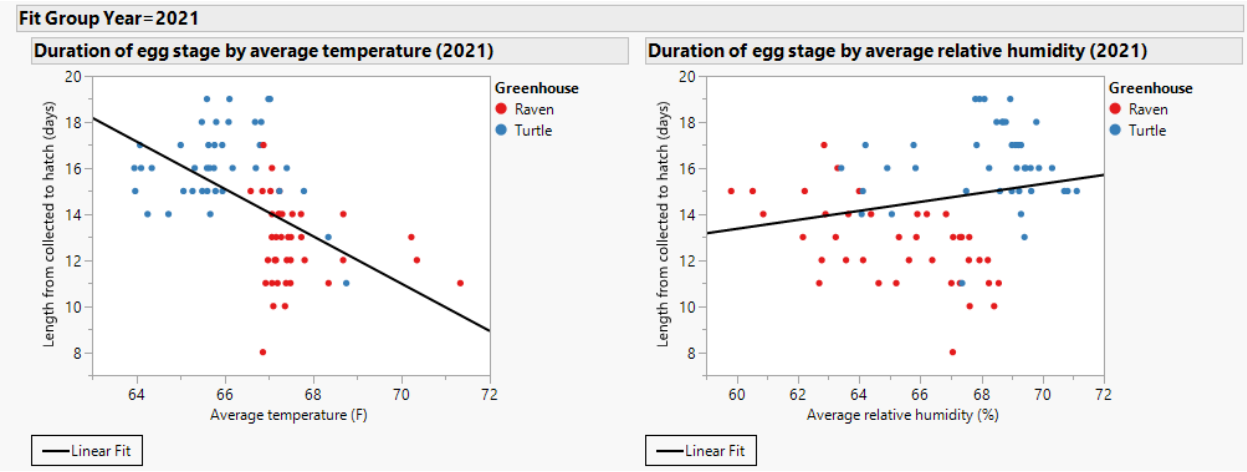
JMP

Simple linear regression was used for each given life stage to analyze relationships between life stage duration and both average temperature and average relative humidity (across the duration of that life stage) for individual egg and larvae cups per each year. Matrilines or cup IDs with missing data, such as unrecorded dates, were excluded from analysis. Analyses for each given life stage were for 2021 and 2022 years were then compared. For the collected to third and collected to 5th life stages, average duration were calculated for each individual matriline, across all of her egg and larvae cups. JMP was used to create boxplots for GDD, and 50F (10C) was used as a baseline.

Results

Collected to hatch (egg stage)

Both 2021 and 2022 show a relationship between increased average temperatures with a shorter duration spent in the egg stage (Figure 14). For the 2021 year, for every degree Fahrenheit increase the duration spent in the egg stage decreased by 1.06 days, and for the 2022 year for every degree Fahrenheit increase the duration spent in the egg stage decreased by 0.59 days. There was an association with increased average relative humidity and a longer duration spent in the egg stage for the 2021 year, in which for every percent increase in average relative humidity the duration spent in the egg life stage increased by 0.17 days. However, no statistical meaningful relationship was found between increased average relative humidity and egg life stage duration for the 2022 year. The median accumulated GDD across the egg stage for 2021 was 255 and 80% of the values were between 221 and 287 GDD (Figure 15). The median accumulated GDD across the egg stage for 2022 was 237, and 80% of the values were between 194 and 271 GDD



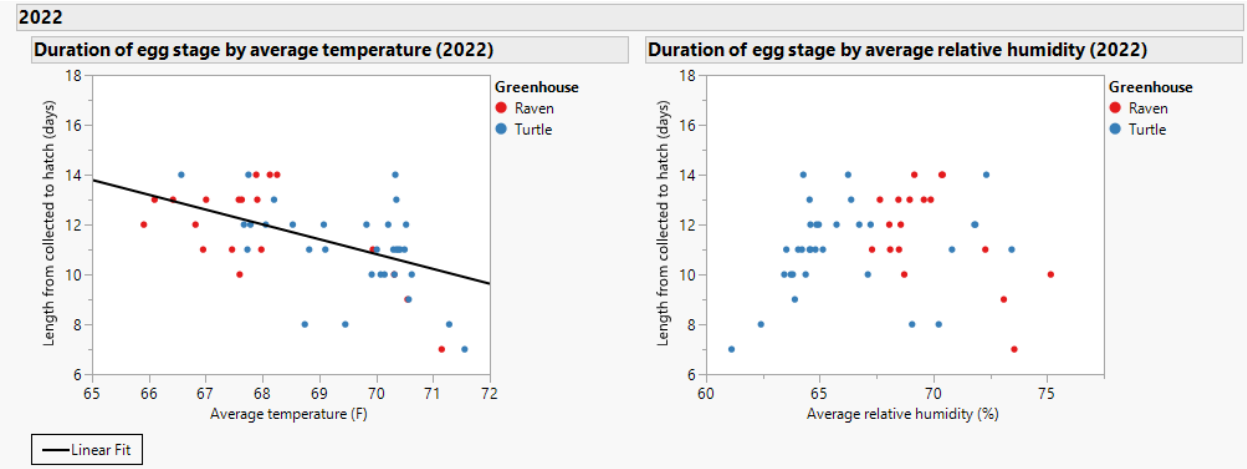


Figure 14. Linear regression output of the average temperature and average relative humidity versus life stage length for the egg life stage. Egg cups in Raven greenhouse in red, egg cups for Turtle greenhouse in blue (n=253 individual egg cups). In 2021 and 2022, higher average temperatures for egg stage were associated with shorter life stage duration (for 2021, $F_{1,169} = 96.6$, $p < 0.001$, $R^2 = 0.36$. For 2022, $F_{1,80} = 35.3$, $p < 0.0001$, $R^2 = 0.31$). For 2021 higher average relative humidity for egg stage was associated with longer life stage duration, for 2022 no relationship between average relative humidity and life stage duration was found (for 2021, $F_{1,169} = 9.82$, $p < 0.0020$, $R^2 = 0.05$. For 2022, $F_{1,80} = 0.81$, $p = 0.37$, $R^2 = 0.01$).

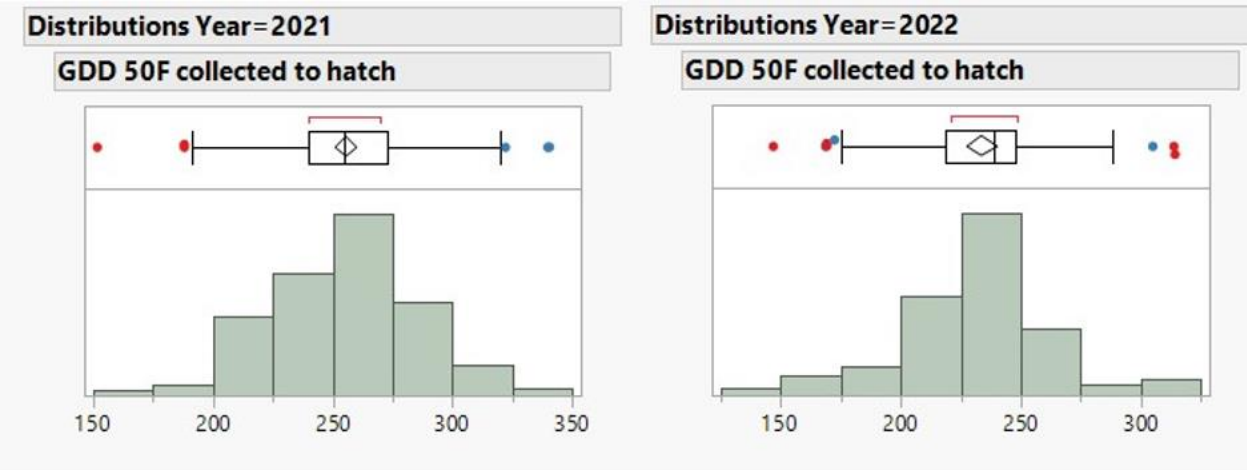
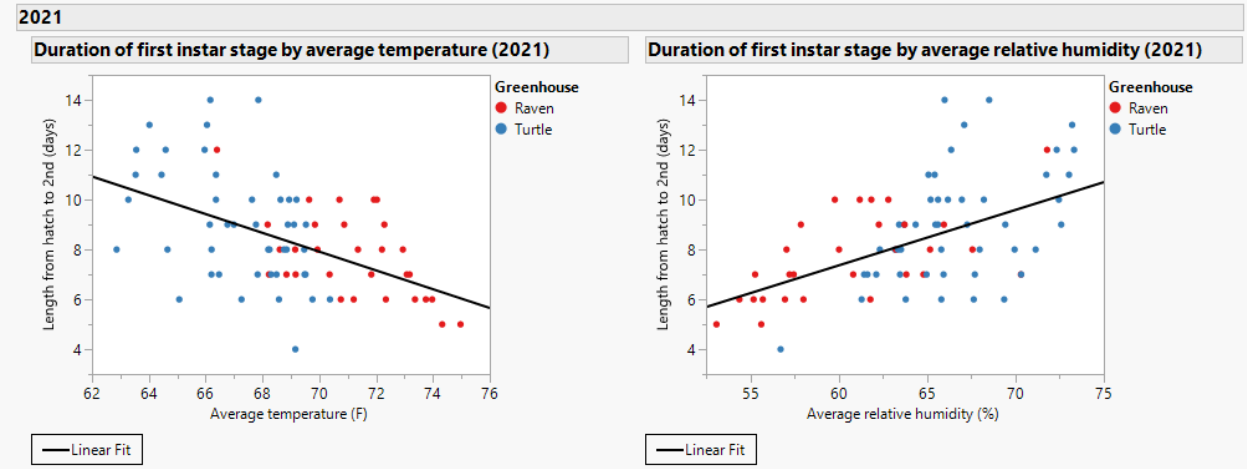


Figure 15. Boxplot and histogram for GDD egg life stage. In 2021, accumulated GDD min = 151, median = 255, max = 340. In 2022, accumulated GDD min = 147, median = 239, max = 314.

Hatch to 2nd Instar (1st instar stage)

The 2021 year shows a relationship of increased average temperatures with a shorter duration spent in the 1st instar stage (Figure 16), but no statistical meaningful relationship was found between average increased temperature and 1st instar duration for the 2022 year. In the

2021 year, for every degree Fahrenheit increase, the duration spent in 1st instar decreased by 0.37 days. There was an association with increased average relative humidity and a longer duration spent in 1st instar for both the 2021 and 2022 years. In 2021, for every percent increase in average relative humidity, the duration spent in 1st instar increased by 0.22 days, and for 2022 for every percent increase in average relative humidity the duration spent in 1st instar increased by 0.30 days. The median accumulated GDD across the 1st instar stage for 2021 was 175 and 80% of the values were between 140 and 209 GDD (Figure 17). The median accumulated GDD across the 1st instar stage for 2022 was 198, and 80% of the values were between 104 and 299 GDD.



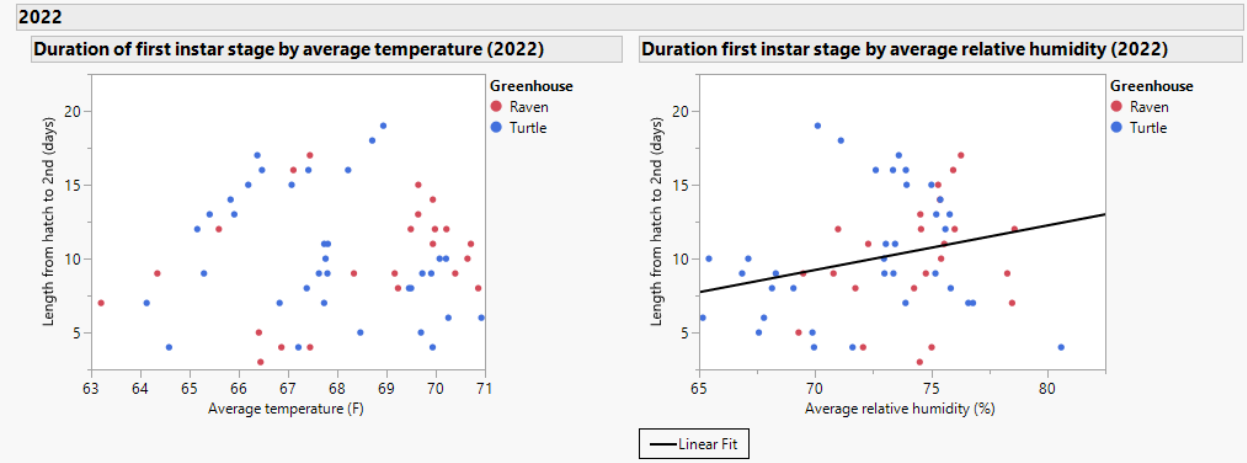


Figure 16. Linear regression output of the average temperature and average relative humidity versus life stage length for the 1st instar stage. Larvae cups in Raven greenhouse in red, larvae cups for Turtle greenhouse in blue (n=267 individual larval cups). In 2021, higher average temperatures for 1st instar stage were associated with shorter life stage duration, but in 2022 no statistical meaningful relationship for these variables was found (for 2021, $F_{1,173} = 72.6$, $p < 0.001$, $R^2 = 0.30$. For 2022, $F_{1,86} = 0.60$, $p < 0.4376$, $R^2 = 0.07$). For 2021 and 2022, higher average relative humidity for egg stage was associated with longer life stage duration (for 2021, $F_{1,173} = 84.5$, $p < 0.0001$, $R^2 = 0.33$. For 2022, $F_{1,86} = 6.12$, $p < 0.0154$, $R^2 = 0.06$).

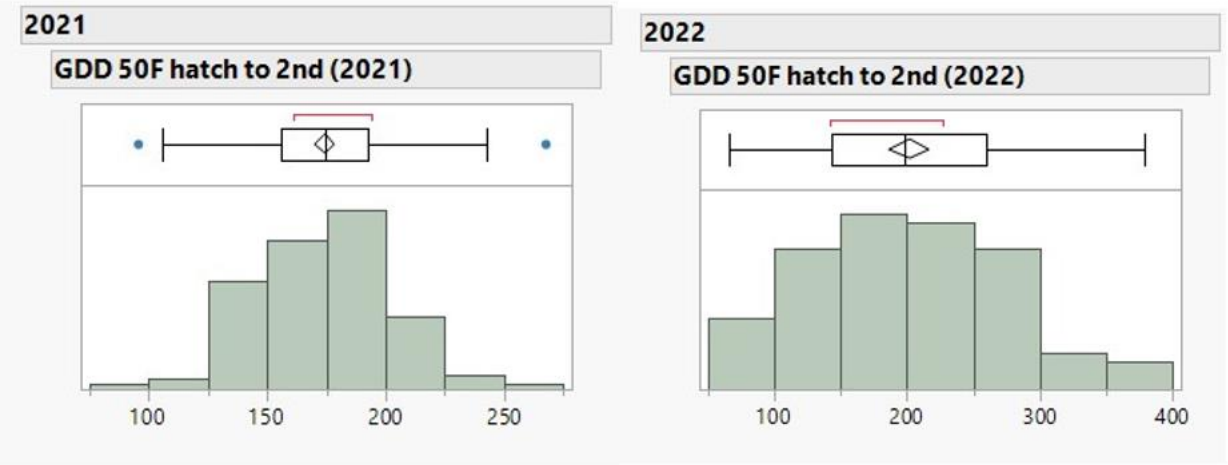


Figure 17., Boxplot and histogram for GDD 1st instar life stage. In 2021, accumulated GDD min = 96, median = 175, max = 268. In 2022, accumulated GDD min = 66, median = 198, max = 379.

2nd to 3rd Instar (2nd instar stage)

In 2021 there was an association with increased average temperatures and a shorter duration spent in the 2nd instar stage (Figure 18), but no statistical meaningful relationship was found between average increased temperature and 2nd instar duration for the 2022 year. In the

2021 year, for every degree Fahrenheit increase, the duration spent in 2nd instar decreased by 0.34 days. There was an association with increased average relative humidity and a longer duration spent in 2nd instar for the 2021 year, but no association found in 2022. In 2021, for every percent increase in average relative humidity, the duration spent in 2nd instar increased by 0.26 days. The median accumulated GDD across the 2nd instar stage for 2021 was 22 and 80% of the values were between 13 and 137 GDD (Figure 19). The median accumulated GDD across the 2nd instar stage for 2022 was 128, and 80% of the values were between 37 and 244 GDD.

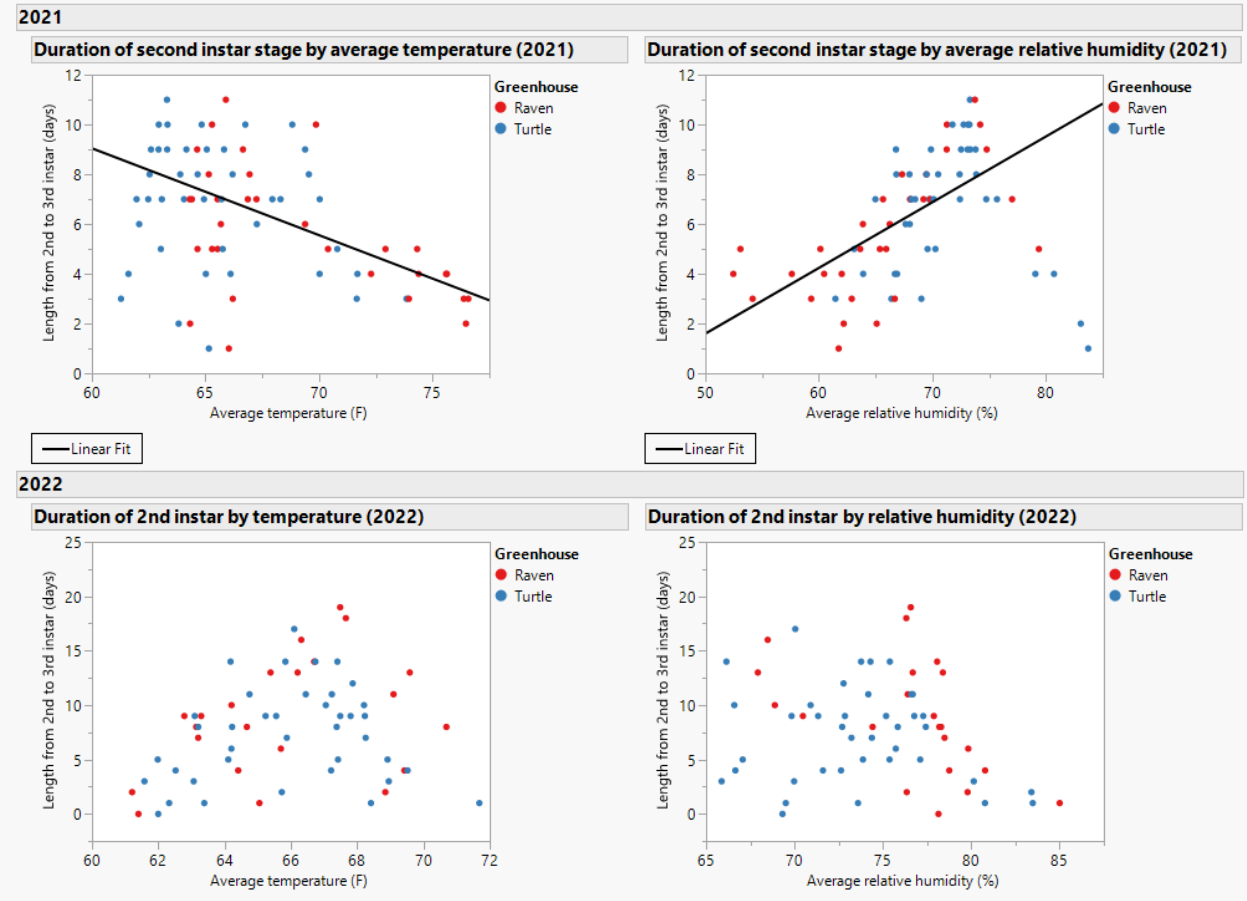


Figure 18. Linear regression output of the average temperature and average relative humidity versus life stage length for the 2nd instar life stage. Larvae cups in Raven greenhouse in red, larvae cups for Turtle greenhouse in blue (n=267 individual larval cups). In 2021, higher average temperatures for 2nd instar stage were associated with shorter life stage duration, but in 2022 no statistical meaningful relationship was found (for 2021, $F_{1,172} = 123.2$, $p < 0.0001$, $R^2 = 0.42$, for 2022, $F_{1,85} = 1.64$, $p < 0.2029$, $R^2 = 0.02$). For 2021, increase in percent average relative humidity was associated with a longer 2nd instar duration, but in 2022 no statistical meaningful relationship was found (for 2021, $F_{1,172} = 114.7$, $p < 0.0001$, $R^2 = 0.40$. For 2022, $F_{1,85} = 2.74$, $p < 0.1016$, $R^2 = 0.03$).

Figure 19., Boxplot and histogram for GDD 2nd instar life stage. In 2021, accumulated GDD min = 11, median = 22, max = 218. In 2022, accumulated GDD min = 11, median = 128, max = 350.

Hatch to 3rd Instar

In 2021 and 2022 there was an association with increased average temperatures and a shorter average length of time between 1st to 3rd instar stage (Figure 20). In the 2021 year, for every degree Fahrenheit increase, the duration from 1st to 3rd instar decreased by 0.9 days. In the 2022 year, for every degree Fahrenheit increase, the duration from 1st to 3rd instar decreased by 1.2 days. Additionally, there was an association with increased average relative humidity and a longer 1st to 3rd instar duration for both 2021 and 2022 years. In 2021, for every percent increase in average relative humidity, the duration from 1st to 3rd instar increased by 0.55 days. In comparison, in the 2022 year for every percent increase in average relative humidity, the length from 1st to 3rd instar increased by 0.62 days. The median accumulated GDD across the hatch to 3rd instar period for 2021 was 274 and 80% of the values were between 251 and 309 GDD (Figure 21). The median accumulated GDD across the hatch to 3rd instar period for 2022 was 324, and 80% of the values were between 246 and 401 GDD.

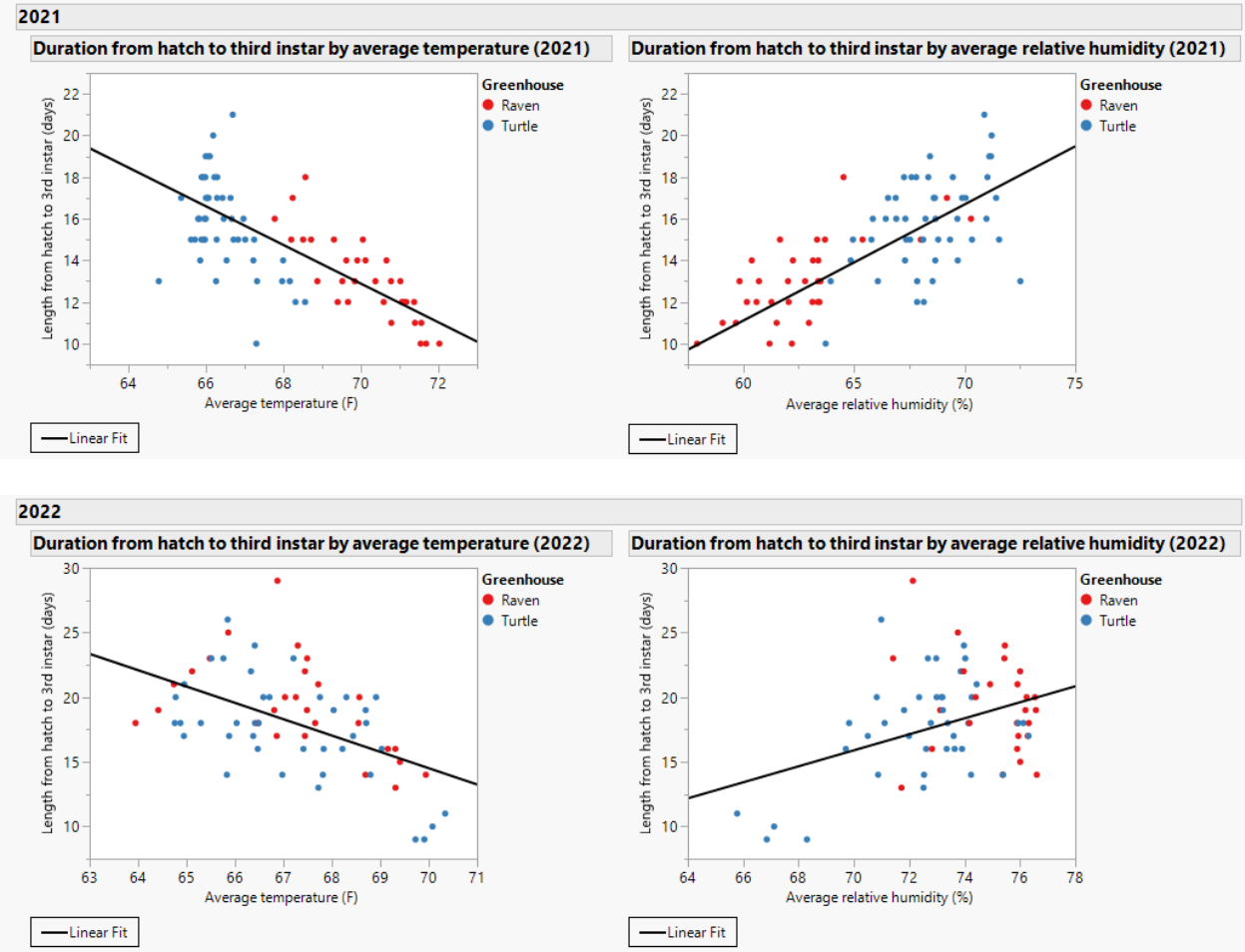


Figure 20. Linear regression output of the average temperature and average relative humidity versus life stage length for 1st instar to 3rd instar duration. Larvae cups in Raven greenhouse in red, larvae cups for Turtle greenhouse in blue (n=267 individual larval cups). In 2021 and 2022, higher average temperatures from hatch to 3rd instar stage were associated with shorter life stage duration (for 2021, $F_{1,171} = 296.4$, $p < 0.0001$, $R^2 = 0.64$. For 2022, $F_{1,84} = 30.3$, $p < 0.0001$, $R^2 = 0.27$). For 2021 and 2022, increase in percent average relative humidity was associated with longer hatch to 3rd instar duration (for 2021, $F_{1,171} = 254.1$, $p < 0.0001$, $R^2 = 0.60$. For 2022, $F_{1,84} = 16.6$, $p < 0.0001$, $R^2 = 0.17$).

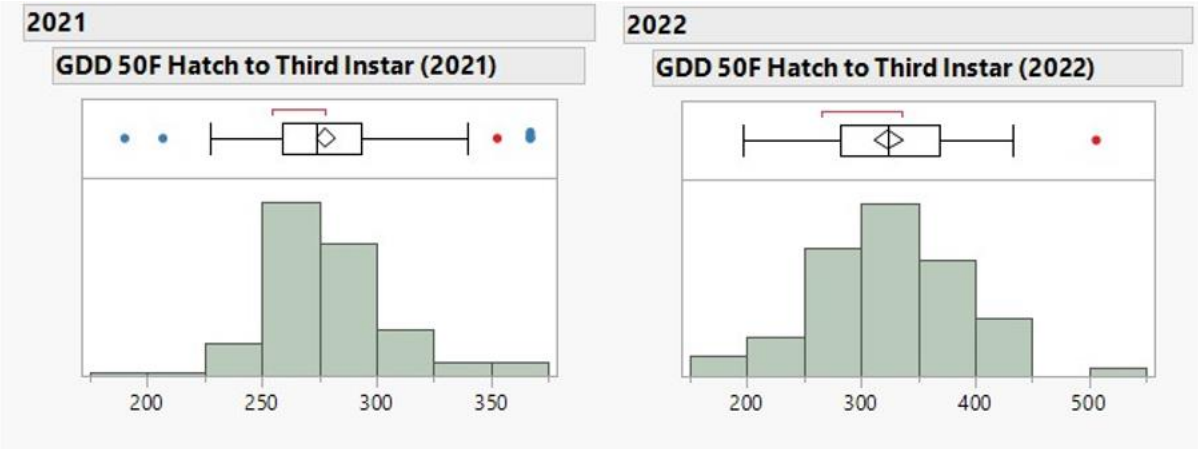


Figure 21. Boxplot and histogram for GDD hatch to 3rd instar life stage. In 2021, accumulated GDD min = 190, median = 274, max =367. In 2022, accumulated GDD min =197, median 324, max = 506.

Collected to Third Instar

In 2021 and 2022 there was an association with increased average temperatures and a shorter average length of time between egg to 3rd instar stage (Figure 22). In the 2021 year, for every degree Fahrenheit increase, the duration from egg to 3rd instar decreased by 2.1 days. In the 2022 year, for every degree Fahrenheit increase, the duration from egg to 3rd instar decreased by 3.1 days. Additionally, there was an association with increased average relative humidity and a longer egg to 3rd instar duration for both 2021 and 2022 years. In 2021, for every percent increase in average relative humidity, the duration from egg to 3rd instar increased by 1 day. In comparison, in the 2022 year for every percent increase in average relative humidity, the length from egg to 3rd instar increased by 1.2 days.

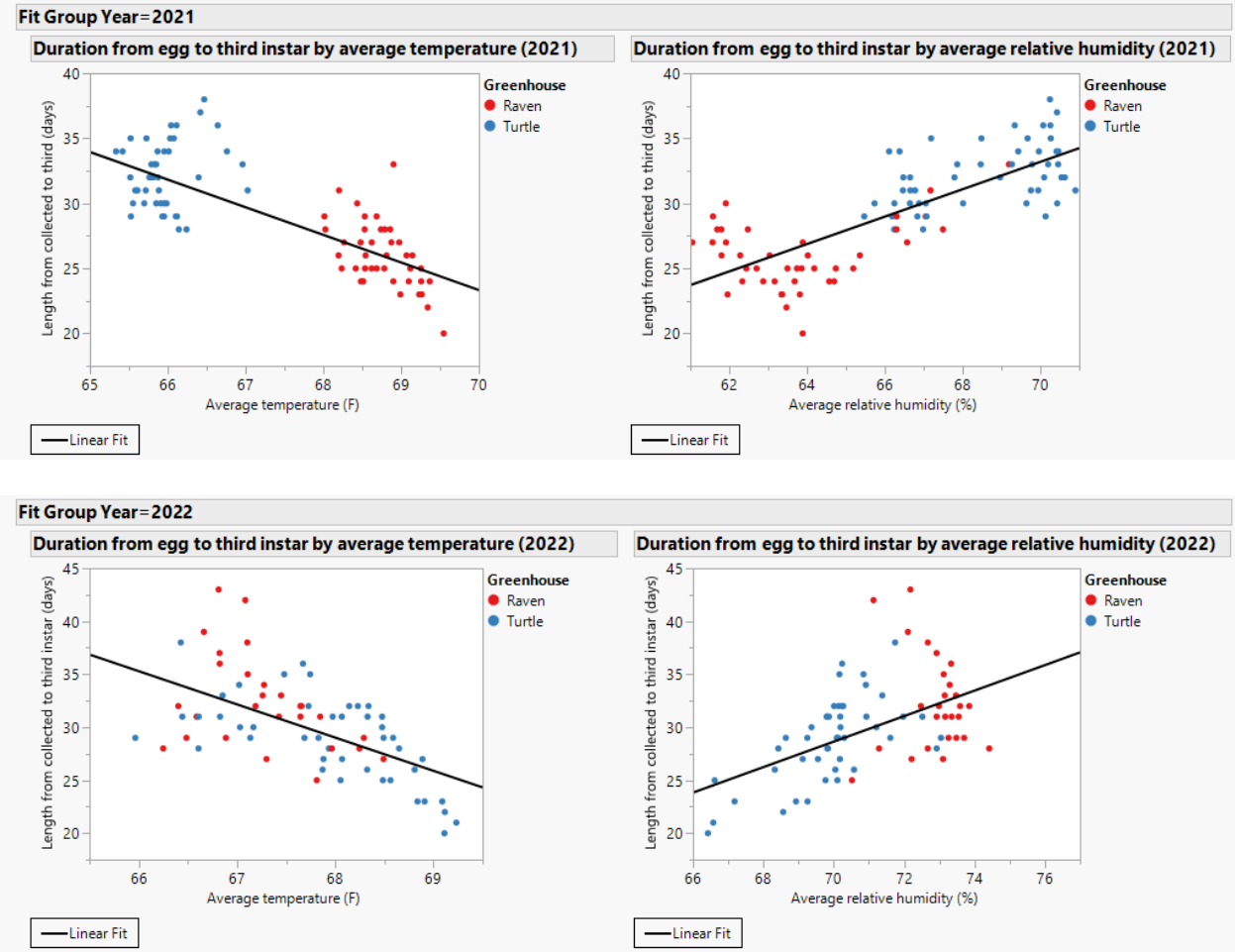
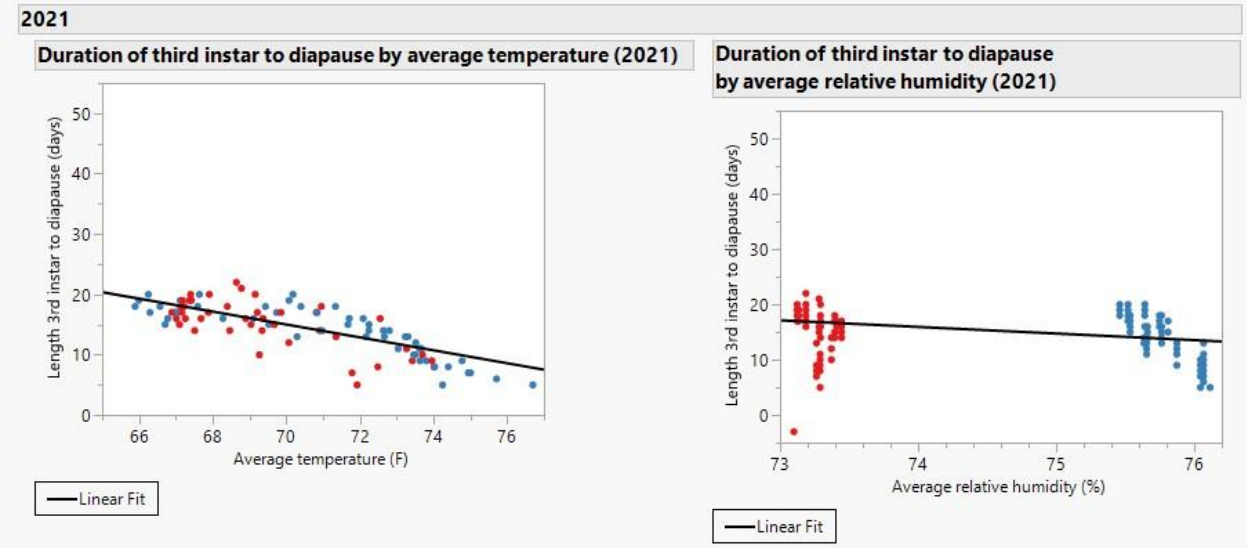


Figure 22. Linear regression output of the average temperature and average relative humidity versus life stage length for the egg to 3rd instar duration. Larvae cups in Raven greenhouse in red, larvae cups for Turtle greenhouse in blue (n=267 individual larval cups). In 2021 and 2022, higher average temperatures from egg to 3rd instar stage were associated with shorter life stage duration (For 2021, $F_{1,173} = 366.3$, $p < 0.0001$, $R^2 = 0.68$. For 2022, $F_{1,87} = 46.9$, $p < 0.0001$, $R^2 = 0.36$). For 2021 and 2022, increase in percent average relative humidity was associated with longer egg to 3rd instar duration (for 2021, $F_{1,173} = 350.3$, $p < 0.0001$, $R^2 = 0.67$. For 2022, $F_{1,87} = 36.8$, $p < 0.0001$, $R^2 = 0.30$).

Third to Fifth Instar Life Stage

In 2021 and 2022 there was an association with increased average temperatures and a shorter average length of time between 3rd to diapause (Figure 23). In the 2021 year, for every degree Fahrenheit increase, the duration from 3rd instar to diapause decreased by 1 day. In the 2022 year, for every degree Fahrenheit increase, the duration from 3rd instar to diapause decreased by 0.95 days. Additionally, there was an association with increased average relative

humidity and a shorter 3rd to diapause duration for 2021, and an association with increased average relative humidity and longer 3rd instar to diapause duration for 2022. In 2021, for every percent increase in average relative humidity, the duration from 3rd instar to diapause decreased by 1.2 days. In comparison, in the 2022 year for every percent increase in average relative humidity, the length from 3rd instar to diapause increased by 0.64 days. The median accumulated GDD across the 3rd to 5th instar stage for 2021 was 315 and 80% of the values were between 258 and 370 GDD (Figure 24). The median accumulated GDD across the 3rd to 5th instar stage for 2022 was 389, and 80% of the values were between 333 and 468.



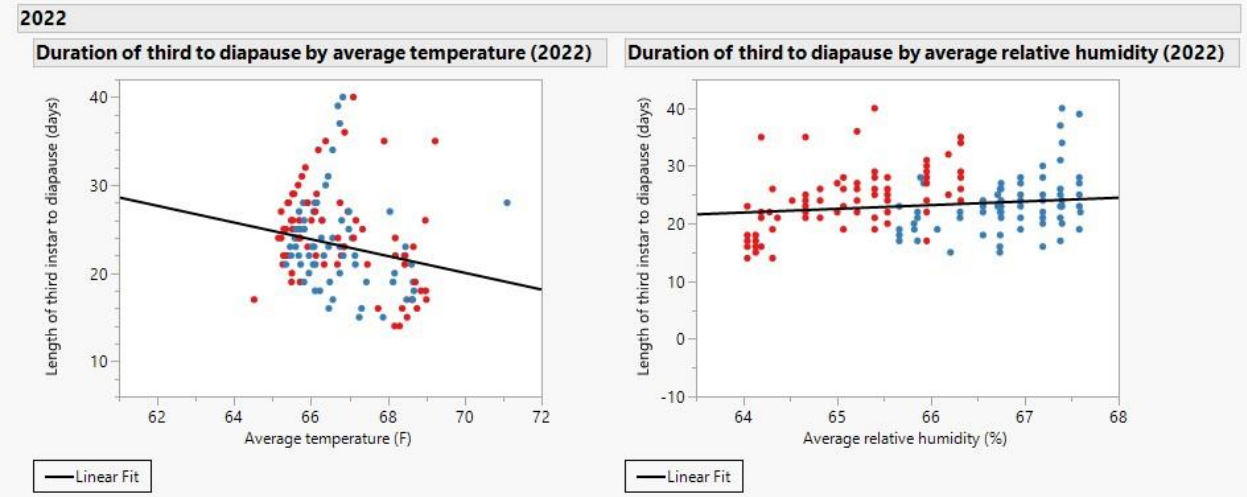


Figure 23. Linear regression output of the average temperature and average relative humidity versus life stage length for the 3rd instar to diapause duration. Larvae cups in Raven greenhouse in red, larvae cups for Turtle greenhouse in blue (n=745 individual larval cups). In 2021 and 2022, higher average temperatures from 3rd to 5th instar stage were associated with shorter life stage duration (for 2021, $F_{1,458} = 1219$, $p < 0.0001$, $R^2 = 0.72$. For 2022, $F_{1,283} = 15.7$, $p < 0.0001$, $R^2 = 0.53$). For 2021 and 2022, increase in percent average relative humidity was associated with longer 3rd to 5th instar duration (for 2021, $F_{1,459} = 96.4$, $p < 0.0001$, $R^2 = 0.17$. For 2022, $F_{1,283} = 6.8$, $p < 0.0097$, $R^2 = 0.02$).

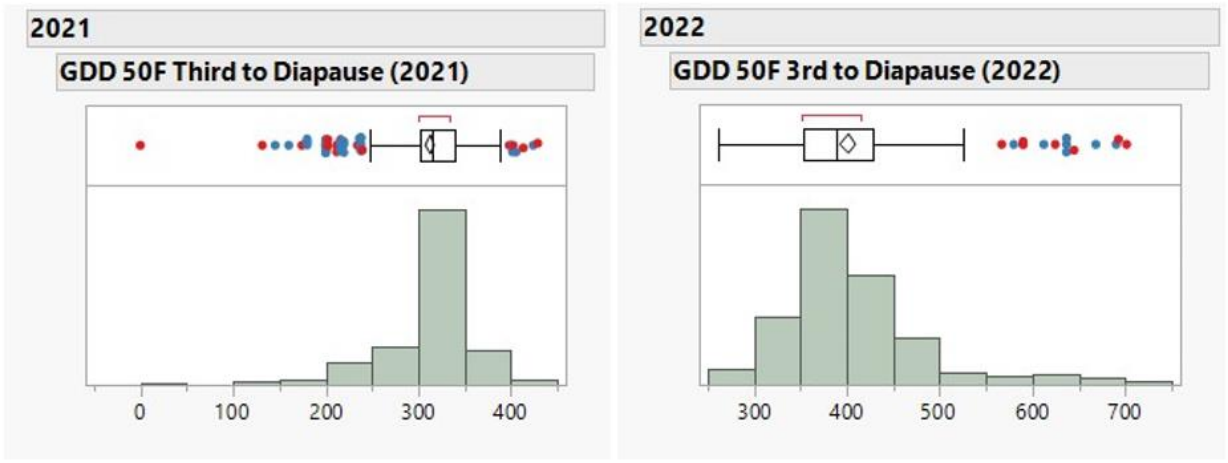


Figure 24. Boxplot and histogram for GDD third to 5th instar life stage. In 2021, accumulated GDD min = 0, median = 315, max = 428. In 2022, accumulated GDD min = 261, median = 389, max = 701.

Collected to 5th instar (diapause):

In 2021 there was an association with increased average temperatures and a shorter average length of time between egg to diapause stage, but not for 2022 (Figure 25). In the 2021

year, for every degree Fahrenheit increase, the duration from egg to diapause decreased by 3.1 days. Additionally, there was an association of increased average relative humidity and a longer egg to diapause duration for 2021. In 2021, for every percent increase in average relative humidity, the duration from egg to diapause increased by 0.56 days. In 2022, the relationship between egg to diapause duration and average relative humidity was curvilinear (Figure 25). Shorter durations were associated with both low average RH (<68.5) and high average RH (>70).

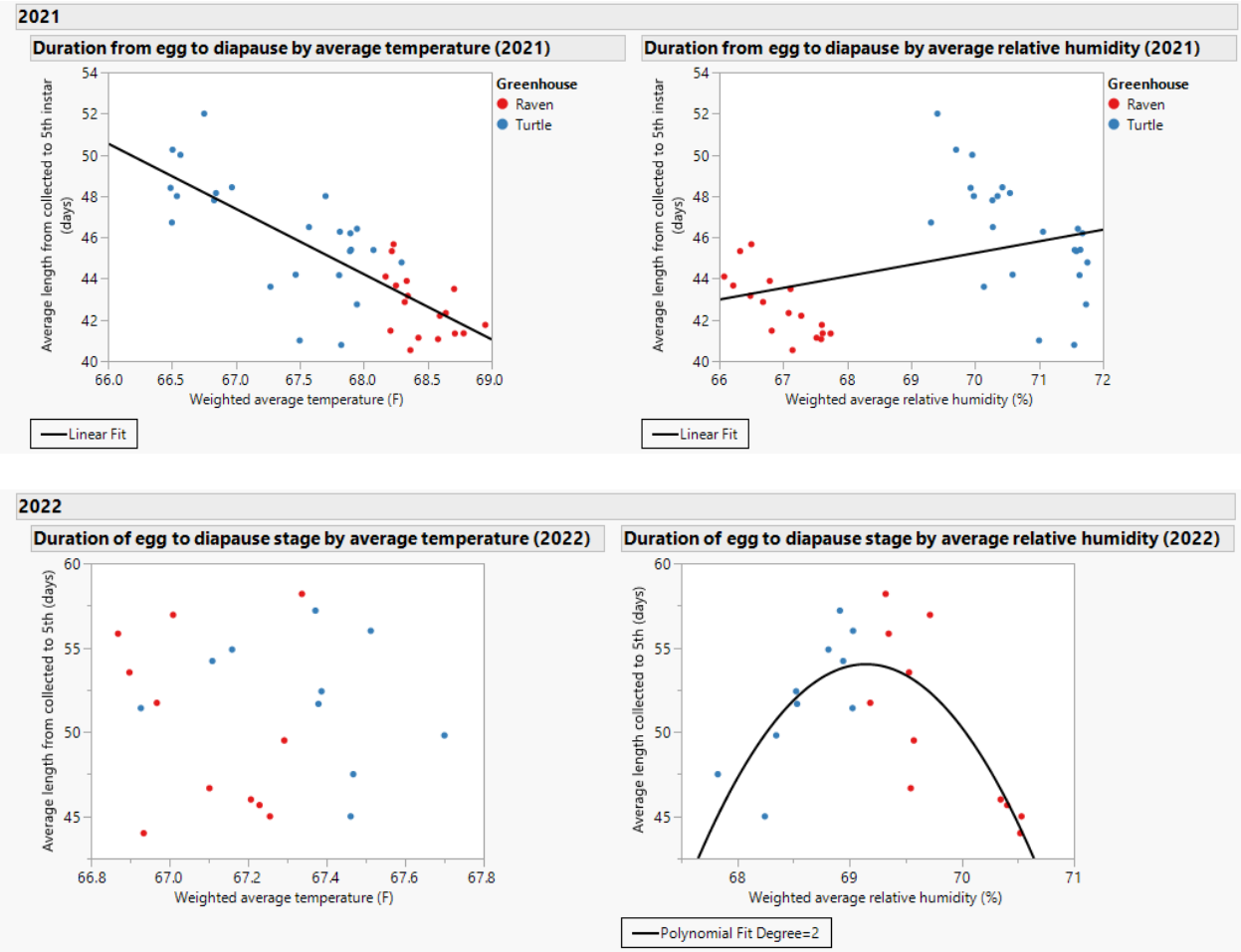


Figure 25. Linear regression output of the average temperature and average relative humidity versus life stage length for the egg to diapause duration. Larvae cups in Raven greenhouse in red, larvae cups for Turtle greenhouse in blue (n=63 individual larval cups). In 2021, higher average temperatures from egg to diapause instar stage were associated with shorter life stage duration, but no statistically meaningful relationship was found for 2022 (for 2021, $F_{1,39} = 63.5$, $p < 0.0001$, $R^2 = 0.62$. For 2022, $F_{1,19} = 1.4$, $p < 0.2505$, $R^2 = 0.07$). For 2021 and 2022, increase in percent average relative humidity was associated with longer egg to diapause instar duration, for 2022 a polynomial curve fit this relationship better than a line (for 2021, $F_{1,39} = 6.9$, $p < 0.0001$, $R^2 = 0.12$. For 2022, $F_{1,19} = 8.9$, $p < 0.0019$, $R^2 = 0.48$).

Table 1. Parameter estimates (effect size) for average temperature and average relative humidity by development period per year. All reported effect sizes with a standard error (SE) are statistically significant ($p < 0.05$). NS = no significant relationship.

	2021	2022	2021	2022
Life Stage	Avg T (SE)	Avg T (SE)	Avg RH	Avg RH
Egg	-1.06 (0.11)	-0.59 (0.10)	0.17	NS
1st Instar	-0.37 (0.04)	NS	0.22	0.3
2nd Instar	-0.34 (0.03)	NS	0.26	NS
Hatch to 3rd	-0.9 (0.05)	-1.2 (0.23)	0.55	0.62
3rd to 5th	-1.0 (0.03)	-0.95 (0.23)	-1.20	0.64
Collected to 3rd	-2.1 (0.11)	-3.1 (0.45)	1.00	1.2
Collected to 5th	-3.1 (0.39)	NS	0.56	n/a

Table 2. Hatch rate, egg estimates, and larvae counted at 3rd instar by period per year.

Year	Egg estimate	Larvae at 3rd instar	Hatch rate
2021	7114	6433	0.90
2022	3319	3216	0.97

Table 3. Accumulated GDD values by life stage period and quartiles per year.

	2021			2022		
Life stage	10th %	Median	90th %	10	Median	90
Egg	220	225	287	147	239	314
1st instar	140	175	208	66	198	299
2nd instar	11	21	218	11	128	350
3rd – 5th instar	0	315	429	261	389	701
1st – 3rd instar	190	274	367	197	324	506

Discussion

The duration of life stages based on average temperature and relative humidity were highly variable across different instars stages. All statistically significant periods of development showed an association with increased average temperature and a shorter amount of time spent in each instar between the two years. Comparatively, both single instar stages and aggregated instar stages (such as collected to 5th, hatch to 3rd, etc.) showed an association between a longer time spent in each life stage and increased relative humidity, apart from the 3rd to 5th duration for the 2021 year. For this period of development, every percent increase in average relative humidity the time spent in these life stages decreased by 1.2 days (Table 1.). In some years and life stages there were notable differences in the average temperatures by greenhouse (Raven vs. Turtle). The Raven greenhouse generally experiences increased temperatures throughout all life stages due to its placement and smaller infrastructure, which likely explains this difference (Kelli Bush and Mary Linders, personal communication).

The aggregated instar stages from third to 5th for the 2021 year showed the tightest association with temperature (*Figure 23*, R^2=0.72) and a shorter length of time spent in this life stage. For every 1 degree Fahrenheit increase, the total duration spent across this life stage decreased by 1 day in 2021. Additionally, the collected to 3rd and collected to 5th periods of development showed the greatest effect sizes (Table 1). For example, in 2021 for the egg to 3rd instar period, for every degree Fahrenheit increase, the duration of this period of development decreased by 2.1 days, and in 2022 for every degree increase in temperature, the duration of this developmental period decreased by 3.1 days.

One reason behind why these developmental stages might show such a dramatic decrease in developmental duration is because they encompass the 4th instar life stages. This individual life stage was not considered in this study due to a significant amount of data collection errors between both years, but other studies have observed fourth instar larvae to be more mobile (Haan et al., 2018), and it is broadly understood that increased temperatures are related to more activity and movement in checkerspots. The fourth instar stage normally occurs in the summer months and takes place directly before larvae enter into diapause, so increased temperatures could mean more ability for the larvae to find and consume food necessary for nutrient uptake, speculated to be necessary to survive the diapause and post-diapause periods.

Coffee Creek Correctional Facility struggles with higher post-diapause mortality, and while introducing more UV lighting has found to help alleviate some of this mortality, the duration that larvae spend within their instar stages is speculated to be another factor in post-diapause death, possibly because larvae—especially in the fourth instar stage—rely on adequate nutrient uptake to survive through the diapause and wake up periods (Ronda Naseth, personal communication). Although this study did not place focus on the fourth instar, diapause, or post-diapause stages, the life stages that were analyzed in this research provide an indication that there is an association with increased temperature and a shorter life stage duration, and this may influence post-diapause mortality.

In a previous thesis study, Boyd (2021) found that environmental temperature and humidity targets were not consistently met 100% of the time in past years, with some life stages for some years meeting environmental target conditions 0% of the time. However, this study also found that the percentage of success of different life stages, such as the fecundity of butterflies or larval survival, was not generally associated with whether these environmental targets were met

(Boyd, 2021). While meeting environmental targets may not influence metrics such as fecundity, hatch rate, and larval survival within a captive rearing setting, this study provides support that environmental factors might play a more significant role out in the field. As temperatures continue to increase under climate change, based on this captive rearing data it is reasonable to assume that the duration of larvae life stages will be shortened. This could result in a phenological mismatch with the butterfly's host plant.

Based on research conducted by Nate Haan and observations made at Oregon's Coffee Creek Correctional Center by butterfly technicians and butterfly husbandry expert Ronda Naseth, the length of the fourth instar stage in particular may be crucial for larvae post-diapause survival, and this key life stage occurs during the hotter parts of the year, from June to July (Hann et al., 2018; Ronda Naseth, personal communication). It is currently unknown as to whether there is an association with an increase in temperatures and 4th instar duration. Ultimately, a shorter duration in previous life stages would affect when larvae enter into 4th instar and whether this developmental period aligns beneficially with plantain abundancy and prior to host plant senescence. More research in this area and particularly for these later instar stages is needed. Additionally, more research on temperature effects on checkerspot host plants such as *Plantago lanceolata* and *Castilleja hispida* may help to predict future trends for butterfly and host-plant phenological changes.

For the life stages and years that showed a statistically significant relationship, increased relative humidity in this study was weakly associated with longer life stages, except for the 3rd to 5th instar stage for the 2021 year in which the duration of the life stage was decreased by 1.2 days (Table 1.). Precipitation cues at this stage may be an indication to the organism to prepare for seasonal dormancy more quickly since fall and winter within its native habitat in the Pacific

Northwest tends to experience greater rainfall. It is possible that for the other life stages, more humid conditions are an indicator to the organism that there would be a greater abundance of a food source for a longer period of time since food plants would be theoretically obtaining more water. However, this is speculative—more studies are needed to explore the relationship between the influence of humidity on the organism.

While accumulated GDD is expected to have an effect on life stage length, for this study, it was more intuitive to analyze potential associations with average temperature and average relative humidity and length of life stage, since GDD is typically used in research as an independent variable when considering the timing of key life stages (hatching, emergence, etc.) in a species across multiple years. In this study, even though the range of accumulated GDD across all individual egg cups or larvae cups was generally broad, most values of accumulated GDD fell within a narrow range. For example, for the 3rd to 5th life stage for the 2021 year, 80% of accumulated GDD values fell between 258 and 370 GDD (Figure 24). Research by Cayton et al. (2015), has identified GDD to be used as a better predictor than calendar date when it comes to assessing different butterfly species and variables such as emergence and abundance across several years. Cayton et al. (2015) state that for species that respond to GDD, ecological responses can be predicted under global warming as opposed to just described. Based on that most values of accumulated GDD fell within a narrow range in this study in addition to Cayton et al. (2015)'s findings, it is possible that GDD may be used effectively as a variable TCB phenology in the wild across multiple years.

Ultimately, it is worth noting that this study could benefit from being repeated with the control for other variables. Food quality and quantity of leaves placed within each larval cup is not currently recorded or tracked by butterfly technicians. It is unknown whether other variables,

such as iridoid glycoside concentrations within the larvae's food source for example, may be influencing checkerspot life stage durations within at least some of these results. Iridoid glycoside concentrations within individual host plants are understood to potentially influence oviposition selection (Aubrey, 2013), and may also influence the triggering of key events for other life stages, such as diapause and the percent of post-diapause larvae that return to diapause rather than pupate.

Limitations

There were several limitations to this research. A portion of the data for the 2022 year was excluded due to inconsistencies in being recorded, and this was ultimately why the duration of the 4th instar life stage was not analyzed as a variable in this study. The 4th instar life stage is particularly tricky for butterfly technicians and coordinators to correctly assess, which is likely why much of this data went unrecorded. This was also true for a large portion of the 2nd instar stage for the 2022 year. These larvae sometimes get incorrectly judged as being larger 1st instar larvae due to technicians being unable to observe visible 2nd instar molts.

Collecting data within a prison environment also has its challenges. Butterfly technicians are sometimes called inside or are otherwise have limited time in the greenhouse lab spaces, which could cause technicians to prioritize husbandry care such as quickly feeding larvae and result in data being not recorded or not recorded accurately. Communication between Sustainability in Prisons Project coordinators and butterfly technicians is also difficult, since remote electronic technology for communication such as cell phones disallowed for incarcerated populations, and messages sent through prison staff are sometimes delayed.

Butterfly technicians and coordinators both have to read and internalize specific details within scientific protocols for the captive rearing of the butterfly, and butterfly technicians sometimes have to juggle several procedures during the active seasons since life stages overlap. This sometimes results in details being confused or forgotten. A complete revision of all procedures was done during the diapause stage of the 2022 year after the data for this thesis was recorded, with additional details and corrections being implemented into the procedures.

Finally, the 2022 year was especially challenging for the butterfly program. The prison facility the butterfly program is located in was still under COVID-19 restrictions for most of the year, transitioning out of these restrictions towards the winter. Butterfly technicians from the two different units were expected to socially distance and keep separated from one another, with members of one unit per each greenhouse. These were also the prison's guidelines for the 2021 year. At the beginning of the 2022 year, all experienced butterfly technicians had transitioned out of the program, in addition to the butterfly coordinator for this year being new to the job, resulting in a team more inexperienced than previous years.

Future research

The captive rearing program for Taylor's Checkerspots could benefit from additional research. Analyzing how temperature and humidity affect the fourth instar, diapause, and post-diapause instar life stage durations could be used to obtain a better understanding of how the length of these life stages influence post-diapause mortality rates. Other metrics drawing from these life stages that are collected by butterfly technicians and coordinators, such as percent of larvae into diapause and percent of larvae released, could also then be analyzed and compared with previous studies. The 2021 and 2022 years of the butterfly program did not do captive breeding of Taylor's checkerspots as was done in years prior. Captive breeding was restarted

during the 2023 year, and so analyzing life stages and variables pertaining to the pupae, eclosion, and breeding stages could provide insight as to how life stage duration affects butterfly emergence and copulation success. Furthermore, analyzing more than two years of data could help paint a broader picture of how temperature and humidity influence larval life stage length and development.

Finally, studies that focus on the influence of plantain quality, plantain abundance provided to larvae in cups, and iridoid glycoside concentrations of SPP butterfly program plantain plants could help butterfly technicians, butterfly coordinators, and program partners such as WDFW better understand how food quantity and quality impacts larval development and life stage duration.

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