Rearing and assessing populations for the Hudsonian emerald (Somatochlora hudsonica), Mountain emerald (Somatochlora semicircularis), and American emerald (Cordulia shurtleffii) at Delonde Ponds, Boulder County, Colorado.

2022 Report

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BUTTERFLY PAVILION

Introduction

In 2017 Butterfly Pavilion (BP) embarked on a multiyear investment in dragonfly conservation with support from Boulder County Parks and Open Space (BCPOS) and Regis University. Dragonflies remain poorly studied despite their outsized impact. Top-level invertebrate predators, dragonflies serve as indicators species of water quality and vegetation change as larvae, as keystone species that exert top-down pressures on their prey, and as habitat connectivity vectors as adults (Bried & Samways, 2015). And increasingly, fascinated odophiles (dragonfly-lovers) go dragonfly watching all over the world (Bried & Samways, 2015; Corbet, 1999; Paulson, 2009). Yet, we know surprisingly little about these animals. During a worldwide evaluation for 1,500 randomly chosen species, only 35% had adequate information to determine their level of threat (Clausnitzer et al., 2009). Of 453 species of North American odonata (dragonflies and damselflies), fewer than 20 have fully recorded life cycles (Tennessen, 2016). The Colorado Natural Heritage Program and Colorado Parks and Wildlife published a list of sensitive dragonfly species in an addendum to their Wildlife Action Plan (2015). For most dragonfly species in the plan, the state listed lack of information as a threat to their survival. Lack of knowledge certainly characterizes the Hudsonian emerald (*Somatochlora hudsonica*), a dragonfly found in Boulder County and listed as Tier 2 Species of Greatest Conservation Need by Colorado Parks and Wildlife (CPW) and a sensitive species the United States Department of Agriculture Forest Service (Colorado Natural Heritage Program, 2015; Packauskas, 2005). Boulder County lists this as a Species of Special Concern in the Environmental Resource Element of the Boulder County Comprehensive Plan (Boulder County, 2020). In October 2020, BCPOS wrote a Draft Species Conservation and Recovery Plan to map action needed to protect populations of *S. hudsonica.*

To support the conservation and recovery of *S. hudsonica*, our goal is to target areas of research still needed in the BCPOS Species Conservation and Recovery Plan . Our first step to successfully measure the effectiveness of management strategies is to track the existing population sizes of *S. hudsonica.* In addition to *S. hudsonica* we propose studying the population sizes of two additional members of the Corduliid family found to use the same habitats as *S. hudsonica*. The American Emerald, *Cordulia shurtleffii*, a Boulder County wildlife Species of Special Concern (#96) due to dependence on a restricted or isolated habitat, and the more common Mountain emerald, *Somatochlora semicircularis,* are both vulnerable to population decline due to threats such as climate change, forestry practices, grazing practices, and other impacts to water quality. All three species occur at the Delonde ponds at Caribou Ranch Open Space during the same flight season, July to August, allowing us to measure all three species with minimal additional effort. This will provide a more complete understanding of how environmental stressors affect the populations of species within this rarer family as a whole and if population trends vary by species or not.

The life history of *S. hudsonica* remains unknown, including the number of years for larvae to reach adulthood and if eggs overwinter. However, based on traits of congeners, Walker estimated that the larval phase of Hudsonian emeralds lasts two full seasons and eggs overwinter. He also estimated that adults live 1.5-2 months (Walker, 1925). Packauskas (2005) found all adult specimens in the region in July; the dragonflies probably started emerging in mid-June. During our work at BP (i.e., under human care), we found that it took the closely related mountain emerald 3 years for some larvae to emerge as adults, with the remaining individuals emerging in year four. Some larvae emerged from eggs prior to overwintering, while others overwintered for a season prior to emerging. We are currently summarizing the life history information we have for the mountain emerald.

In the wild, the years spent as larvae are the most dangerous. In shorter lived genera fewer than 10% of larvae survive to adulthood. Other corduliids have shown an even lower percentage of survivorship with 99.8% mortality over the five years spent as juveniles (Boulder County, 2020). Mortality in juvenile dragonflies most often results from predation pressure, so removing this factor can significantly increase

survivorship. Rearing juveniles under human care (i.e., in a captive setting) to give them a head start may result in quicker population recovery by increasing the number of individuals able to emerge as adults and possibly re-establishing locally extirpated populations.

In August 2021, we collected eggs from the mountain emerald after failing to obtain eggs of the Hudsonian emerald (or even finding a female; although we did find a male) during 3 trips to the mountains to search for them. The mountain emerald eggs developed overwinter and hatched in April 2022. We found that maturation of mountain emeralds took a minimum of three years, thus reintroduction efforts from the current cohort could not occur until 2024 or 2025. This timing provides us with the opportunity to monitor existing populations of mountain emeralds, Hudsonian emeralds, and American emeralds to assess the need for "head starting" (rearing young under human care through their most sensitive stages) of larvae. In addition, during 2022 and 2023 we will redouble our efforts to collect Hudsonian emerald and American emerald eggs to permit us to conduct life history studies and head start programs for both species, permitting reintroduction as early as July 2025 or 2026.

Due to the time needed to reach sexual maturity for all three species, the impact of reintroductions using head starting programs will take several years. This latency makes it imperative to begin recovery efforts now and collect yearly cohorts to ensure the future of these populations over the next several decades. Climate change may well force all the emerald dragonfly species into higher altitude habitat over the next several decades. Refining head starting programs now could prove crucial if assisted dispersal of these species becomes necessary.

We worked in 2021 to try to briefly capture adult female *S. hudsonica* from Boulder County Open Space properties, inducing those females to lay some eggs prior to releasing them back at their capture location (a process that takes just a few minutes), rearing the hatching larvae to their last instar or adulthood at Butterfly Pavilion, and then releasing those individuals back into the wild. Our plan was to maintain detailed records on the life history of *S. hudsonica*, information vital in case "head-starting" is warranted to help conserve the species and expand its range.

In 2022, researchers performed field collection of gravid females, again only coming across *S. semicircularis.* We did find more *S. hudsonica* in 2022 than 2021, though the number of field trips was increased to ten across the flying season to better understand utilization of habitat and seasonality of *S. hudsonica, S. semicircularis,* and *C. shurtleffii*.

Methods

Field Collection

We obtained a Boulder County Parks & Open Space Research Permit through Boulder County to collect *S. hudsonica, S. semicircularis, and C. schurtleffi* eggs.

Upon arrival to site (the Delonde Ponds; Figure 1), we followed previously approved procedures for capturing adult female *S. semicircularis*. Researchers identified oviposition sites of *S. hudsonica* using previously collected data from 2017. During our work, we used sterilized waders to capture adult females while minimizing risk to aquatic habitat. We captured adult females near the water using soft aerial nets to reduce risk of injury. Once captured, we induced ovipositing of eggs into plastic vials (20 mL Clear Polystyrene Plastic Vials with White Caps; Freund Container and Supply, Lisle, IL, USA) of pond water by repeatedly tapping the dragonfly abdomens into water until oviposition occurred. We marked captured females from which we collected eggs unobtrusively on the carapace to help avoid additional collection. In addition, any males captured of *S. hudsonica* were marked with Sharpie, silver on the ventral side of the abdomen to reduce impact on survivability. We counted eggs using a microscope at X40

magnification (OMAX) and then transferred them to plastic vials ¾ full of reverse osmosis, deionized water treated with Equilibrium (Seachem, Madison, GA, USA) in groups of no more than 50 eggs per vial. We are maintaining labeled vials with eggs in submerged water on temperature and photoperiod-controlled larva shelves that we check bi-weekly.

Rearing *S. hudsonica, S. semicircularis, and C. shurtleffii*

The rearing setup to support dragonfly eggs and larvae has successfully reared *S. semicircularis* from eggs to teneral emergence in three to four years. The rearing system to support eggs through emergence is built on a metal shelving unit. Three central shelves hold hydroponics trays (0.6m by 1.2m by 11.4 cm), Chlorophyll, Denver, CO, USA). A bottom shelf holds a sump tank that contains a Eflux DC Flow pump (Current, Vista, CA, USA) in addition to the intake pump/hose and outtake hose for a ¼ HP chiller (JBJ Arctica; TransWorld Aquatic Enterprises Inc., Inglewood, CA, USA). PVC pipes connect the trays to

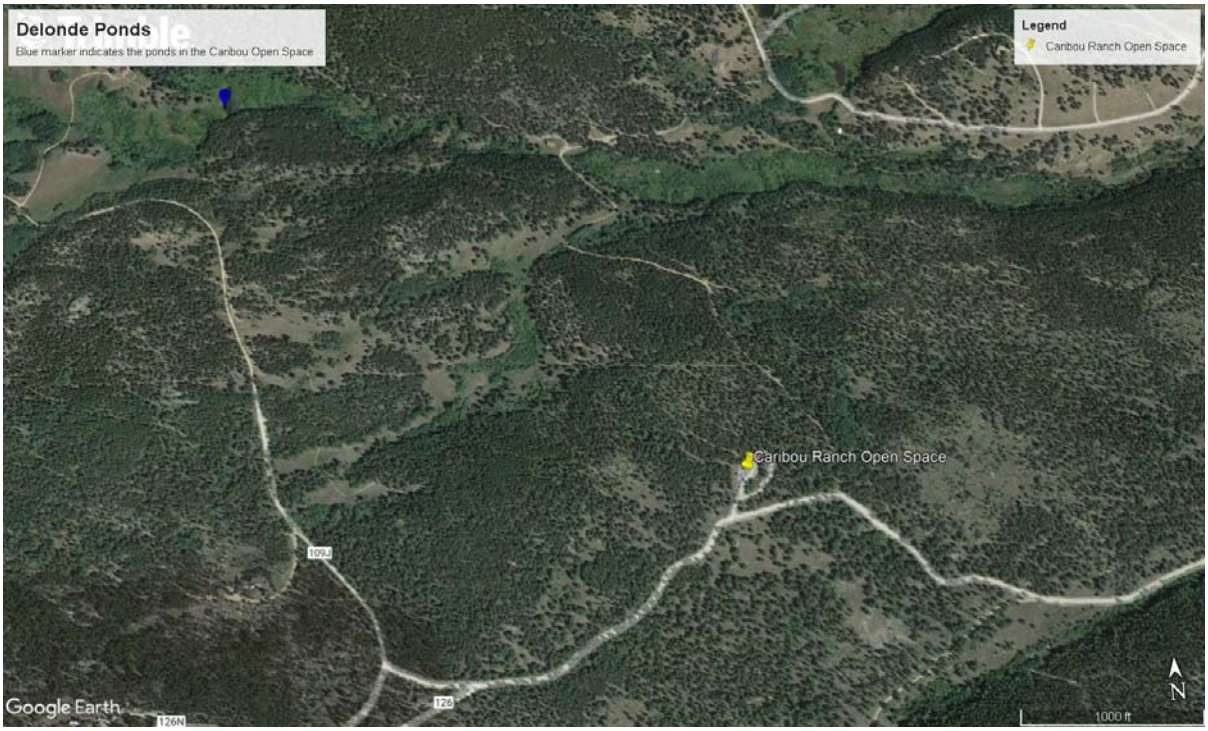

Figure 1. Location of the Delonde Ponds in the Caribou Ranch Open Space, Boulder County.

each other and to the pump. Three 91.4 cm Trulumen Pro LED strips 12000 K (Current, Vista, CA, USA) on photoperiod timers light the three central shelves. We update timers periodically to reflect sunrise and sunset times in Colorado for accurate simulation of photoperiod.

Once the eggs hatch, hatchlings were separated into vials with 1-3 siblings and set in 0.15 L plastic cups. The cups nest securely into trimmed cup bases affixed with silicon into 10 in (25.4 cm) plastic underwater planter baskets (Pond Boss, West Palm Beach, FL, USA). The planter baskets will sit, partially submerged, in the trays. This permits temperature-controlled water to circulate around the cups without water exchange and therefore without the risk of losing a larva into the larger system or of exposing hatchlings to the scent of larger larvae.

Cohort 2021 was fed seined zooplankton from a protected water sources on Butterfly Pavilion grounds containing a mixture of copepods, ostracods, and amphipods. The 2022 Cohort were fed *Paramecium aurelia, Paramecium multimicronucleatum,* and *Moina spp* (Carolina Biological. All larvae were fed

three times a week and perform 30%-80% water changes tri-weekly to maintain water quality. In winter temperatures were lowered to 38°F mimicking seasonal temperature fluctuations and natural diapause.

Results & Discussion

Population Assessment

We traveled with 2-3 researchers to Delonde Ponds from July 9th to August 12th making ten total trips to search for gravid female of *S. hudsonica, S. semicircularis,* and *C. shurtleffii*. All target species captured were marked and released within a 60 min window to identify the number captured within a set time. Fifteen individual S.semicircularis were captured, fourteen males and one female. A total of four S.hudsonica were captured and marked, four males and no females. No C. shurtleffii were captured or noted during field collection trips. On 8/3/22 researchers collected between 5-6:30pm to identify if there were temporal factors associated with the lack of female S. hudsonica. No emerald dragonflies were found on this sampling trip.

Date	*Somatochlora semicircularis*		*Somatochlora hudsonica*		*Cordulia shurtleffii*	
	Male	Female	Male	Female	Male	Female
7/9/22	5	0	0	0	0	0
7/14/22	5	1	2	0	0	0
7/20/22	0	0	1	0	0	0
7/29/22	2	0	1	0	0	0
7/30/22	0	0	0	0	0	0
8/3/22	0	0	0	0	0	0
8/4/22	4	0	0	0	0	0
8/6/22	0	0	0	0	0	0
8/9/22	0	0	0	0	0	0
8/12/22	0	0	0	0	0	0

Table 1 Number of individuals by species captured and marked at Delonde ponds per site visit.

Rearing

Upon arrival at Butterfly Pavilion we counted the eggs, which totaled 165. Eggs collected during the 2022 field season were identified as Cohort 2022, while eggs collected in 2021 will be referred to as Cohort 2021.

Cohort 2021

Eggs collected in August 2021were immediately placed in chilled water which triggered overwintering. While eggs showed signs of development (Figure 1-10) no larvae eclosed in 2021. As temperatures were raised in spring 2022 the eggs began hatching on 4/7/22 at 48.7°F. Newly hatched larvae were fed sein-collected zooplankton containing a mix of ostracods, copepods, and amphipods. Cohort 2021 experienced survival rates comparable to wild populations (2.68%) with most mortalities occurring at less than 45 days post-hatch.

Given the results, care was adjusted to increase survivability focusing on incubation temperatures, diet, and water quality.

Figure 1 Somatochlora semicircularis larva one day post hatch 8/3/22

Cohort 2022

We adjusted our methods to incorporate a late summer period with eggs being kept at room temperature 72°. Adjustments included starting eggs at room temperature to mimic late summer temperatures and allow for a fall hatching group to the hatch group in 2017. In addition, we moved to lab-cultured food with *Paramecium aurelia* and *Paramecium multimicronucleatum* for instars 1-3, and copepods and *Moina spp* for subsequent instars. Water quality issues were also addressed increasing water changes occurring at feeding from 30% total water volume to 50-80% total water volume, dependent on detritus build up. These three changes increased survivorship in early instar development from 2.68% to 38.46% with mortality dropping to zero after 45 days post hatch. In addition, based on previous rearing in 2017, viable eggs may still hatch in spring 2023.

Conclusions and Next Steps

This season we found more emerald dragonflies than we have ever sampled at Delonde Ponds with the majority of specimen captured at the lower two ponds. A total of fifteen individual *S.semicircularis* were captured, fourteen males and one female. A total of four *S.hudsonica* were captured and marked, four males and no females. No *C. shurtleffii* were captured or noted during field collection trips. On 8/3/22 researchers collected between 5-6:30pm to identify if there were temporal factors associated with the lack of female *S. hudsonica*. No emerald dragonflies were found on this sampling trip.

In July we were able to collect eggs from *S. semicircularis* early in the season and saw the first hatching within 21 days of oviposition, incubating at 72°F. In Cohort 2022, 78 individuals hatched since collection, with 30 individuals surviving past the 45 day mark. Per previous trails with rearing *S. semicircularis* we have found that survivability increases significantly after day 100 post-hatch. It seems water quality is a critical component to ensuring survivability in early instars. In addition, early instar larvae do seem to show preferences in food choices with ostracods proving an unviable food source. *Paramecium* and *Moina* have proven the most reliable food sources for *Somatochlora* larvae. With these changes we increased survivability by 35.78%.

Overall, due to limitations of accessing Delonde ponds before July 1st we recommend identifying additional field sites to see if females are more prevalent earlier before July 1st. There is merit to continuing to utilize Delonde Ponds as a site for headstarting as it is clearly viable habitat for *S. semicircularis* and *S. hudsonica*.

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