Proposal to rear and assess populations for the Hudsonian emerald (*Somatochlora hudsonica*), Mountain emerald (Somatochlora semicircularis), and American emerald (Cordulia shurtleffi) on multiple Boulder County Parks and Open Space sites.

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Abstract

In 2017, the Butterfly Pavilion (BP) started a dragonfly conservation program. In our work targeting the Hudsonian emerald dragonfly (*Somatochlora hudsonica*, Hagen), we found the need to expand our efforts to support the American Emerald (*Cordulia shurtleffi)* and the Mountain Emerald (*Somatochlora semicircularis*). In the past years (2022 and 2023), efforts to find *S. hudsonica* and *S. shurtleffi* females at Delonde Ponds Boulder, CO and Duck Lake Ward, CO have been unsuccessful. Our goals are to develop a population abundance index to permit annual monitoring of the three species of emerald dragonflies of interest: *S. hudsonica, C. shurtleffi*, and *S. semicircularis* and to collect eggs of *S. hudsonica, C. shurtleffi*, and *S. semicircularis* to rear under human care to enable use of the larvae in a head start program for subsequent reintroduction at Delonde Ponds as well as identify other sites that host *S. hudsonica* and *S. shurtleffi*. In 2023 we collected and marked 32 males of *S. semicircularis* and 4 female which oviposited 12,221,1 and 66 eggs, respectively. To date (12/7/23), 24 larvae have hatched, and we are rearing these eggs the same way we did in 2022. We, however, tested the use of Methylene blue as an antifungal, increase water changes and diversify their diet. Larval survival was statistically not different from last year (2022). Overall, due to not finding any S. *hudsonica* or *C. shurtleffii* at identified sites, contrary to increases seen in populations of *S. semicirularis,* we recommend genetic analysis to identify if population fluctuation is due to genetic drift, interspecies competition, or climate impact. Also, to further understand this we will start genetic analyses in the future as well as testing other changes in environmental factors to increase larval survival.

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,* just like other species of dragonflies, are both vulnerable to population decline due to threats such as climate change, forestry practices, grazing practices, and other impacts to water quality (McCauley et al., 2018). 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.

Odonates are readily collected in the field; however, laboratory rearing, and experimentation allows researchers to regulate environmental variables that are difficult to control in the field. Laboratory based experimental designs require large sample sizes of genetically related organisms at the same developmental stages. Controlled methods of large-scale rearing are needed for these designs. We therefore want to develop a population abundance index to permit annual monitoring of the three species of emerald dragonflies of interest: *S. hudsonica, C. shurtleffi,* and *S. semicircularis* and to collect eggs of *S. hudsonica, C. shurtleffi,* and *S. semicircularis* to rear under human care to enable use of the larvae in a head start program for subsequent reintroduction at Delonde Ponds as well as identify other sites that host *S. hudsonica* and *S. shurtleffi*.

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.

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*.

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.

Methods

Field Collection

Specimen collection was performed from June-August 2023 at Delonde Ponds on Caribou Ranch Open Spaceand Duck Lake (Figs 1 and 2) in Boulder County, CO under a research permit from Boulder County Parks and Open Space allowing for the collection of *S. semicircularis* and *S. hudsonica*. We followed previously approved procedures for capturing adult female *S. semicircularis.* Adult *S. semicircularis* were captured with a soft aerial net in marsh surrounding the ponds identified as historical oviposition sites for emerald species by researchers at Butterfly Pavilion (Voss and Loewy, 2017, Stevens and Reading, 2021, Stevens and Reading, 2022). All specimens were held gently by their wings folded behind the carapace to examine the last abdominal section for sexing.

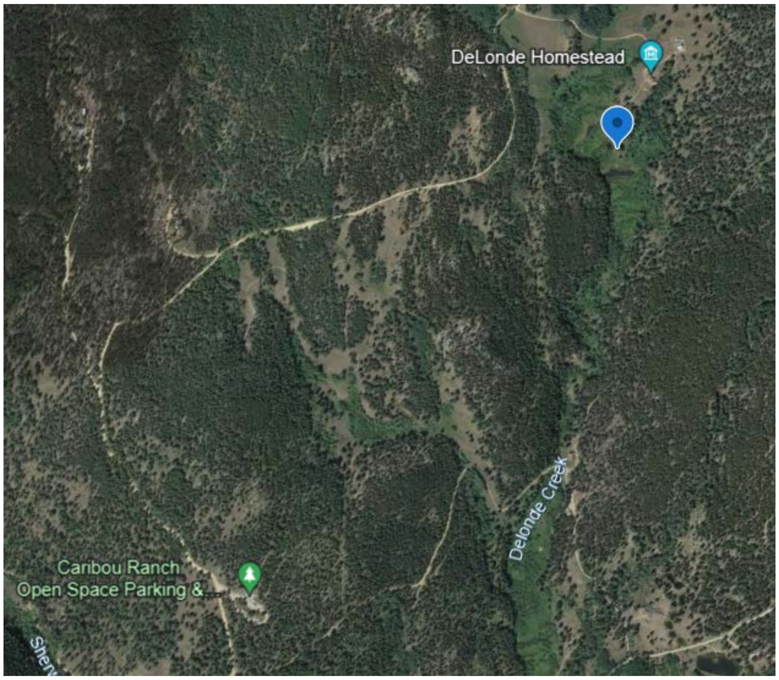

Figure 1. Location of the Delonde Ponds in the Caribou Ranch Open Space, Boulder County. **39**°59’23” N, 105°31’48” W

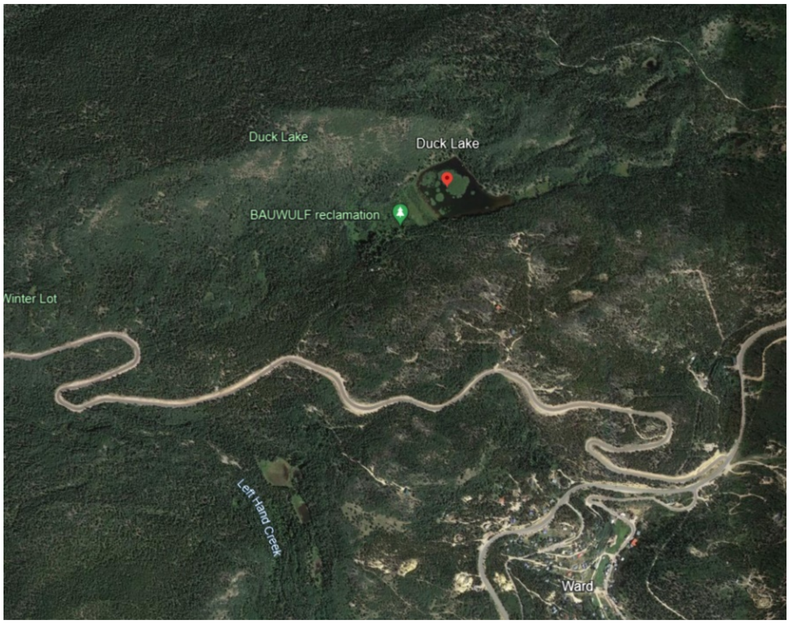

Figure 2. Location of Duck Lake in Ward, CO in Boulder County. **40**°05’00” N, 105°30’48” W

For females, 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 prepared RO/DI water re-mineralized with Equilibrium (Seachem, USA) to induce oviposition. Before release, 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 and ensure individuals weren't counted twice.

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 set up.

The rearing setup to support dragonfly eggs and larvae has successfully reared *S. semicircularis* from eggs to general 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 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. The system is connected to a chiller (JBJ Artica; TransWorld Aquatic Enterprises Inc., USA) to maintain temperature. The setup receives ample daylight from large windows and maintains a natural photoperiod outside of facility operating hours from 7am-5pm, during which fluorescent room lighting is on. Eggs were slowly raised from 62-63°F to 65.8°F and observed every other day for hatching.

Once hatched, larvae were separated into individual 20ml glass vials to avoid risk of cannibalism and allow for independent monitoring. The vials are 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 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.

We used RO water that was re-mineralized using Seachem's Equilibrium at ¼ tablespoon per 5 gallons to reach approximately 0.3 KH and pH 7. Due to the small volume of each vial, the water changed from each condition was collected into a cup and tested collectively by trial condition each week to monitor water quality. In different to last years, water changes were performed 3 times a week to every other day, with as close to 100% volume changed as possible without leaving larvae dry.

The vials of larvae and eggs are maintained in the same system. The larvae were fed from cultured prey items (Table 1). Once a week, we performed water tests (Red Sea Fish Pharm Ltd., United Kingdom) to ensure that ammonia levels were maintained below detectable levels. In winter we lowered temperatures to 38 °F mimicking seasonal temperature fluctuations and induced natural diapause, temperatures were slowly lowered from their peak at 65.8°F to 38°F by lowering 1°F every other day after feeding. After reaching 38°F in December, both larvae and eggs will slowly be raised back up to around 65°F in mid to late June to match normal habitat cycles. At around 50 days post-hatching (mean 48.2) and significant enough growth to easily visualize larvae, a 1inch sprig of Java moss (*Vesicularia dubyana*) was added to each vial to reduce stress and hopefully aid in molting.

This methodology differs from that used by Butterfly Pavilion to raise *S. semicircularis* in 2022 by increasing water changes and water quality monitoring, diversifying prey items, and cooler maximum temperatures. Additionally, 4 of 6 vials of eggs of the divided clutch of 210 laid by Female 2 (n=133) received diluted methylene blue for treatment of an apparent fungal outbreak.

Use of blue methylene to avoid fungal growth

We decided to test two new treatments to increase larval survival. In past trials fungal growth on the eggs was identified as a potential inhibitor to successful hatching. In 2023, we decided to test dosing methylene blue (Fritz ProAquatic, USA) on the dragonfly eggs due to its antifungal properties (Ansari et. al, 2016) (Fig. 1). We therefore diluted 0.5mL of methylene blue (Fritz ProAquatic, USA) in 1 liter of clean re-mineralized water. Each 20cc vial was filled with 10mL of prepared water and received 0.5mL of dilute methylene blue solution. Eggs were kept in solution for approximately 60 days and placed in clean water before cooling for the winter season. While the spread of white fuzz across the eggs anecdotally stopped, no eggs from this clutch hatched in 2023, and eggs retained blue coloration. Effects on egg viability remain to be seen. As seen in 2022 collections, viable eggs may undergo diapause and hatch in spring of the following year (Stevens and Reading, 2022). Anecdotally, more eggs appear to have split in methylene blue treatment conditions, however this has not yet been quantified.

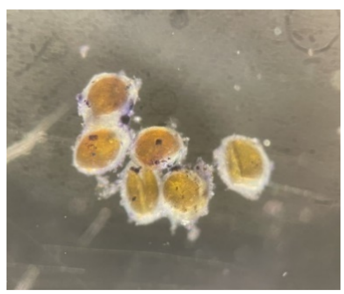

Fig. 1. *S. semicircularis* eggs treated with dilute methylene blue solution after 3 to 5 days.

Results

Field collection

In total, 32 unique male and 4 female *S. semicircularis* were caught and a total of 300 eggs were collected. No *S. hudsonica* were seen at any field site this season, despite field observations beginning on June 15th, 2023, prior to any observable odonate activity. A greater number of *S. semicircularis* were observed this season than either previous field season (Table 2).

Females laid 12, 221, 1, and 66 eggs, respectively. All eggs were split into 20cc glass vials (Qwork Tools, United Kingdom) of no more than 40 eggs per vial to prevent poor oxygenation and potential mold growth. All other eggs were kept with their initial clutch and vials were partially submerged in a hydroponics tray (0.6m x1.2m x11.4cm) (Chlorophyll, USA) using a pond basket (Pond Boss, USA). Larvae began to hatch approximately 6 weeks after collection.

Fertility was determined by examining eggs under a dissecting microscope (40x) at least 48 hours post-oviposition to visualize development. Fertilization rates were consistent with previous findings at 94.8% (Table 3) (Voss and Loewy, 2017, Stevens and Reading, 2021, Stevens and Reading, 2022).

Only 27 of 66 eggs from Female 4 collected hatched in the 2023 season (41.5%).

Survival rates during the first 100 days post-hatching are still to be analyzed and compared to eggs collected in 2021 and 2022. Data collection for week 15 post-oviposition ends December 30th Though data is preliminary, currently there is no significant difference between survivorship in 2022 vs. 2023 cohorts (p=0.45).

Discussion

Cohort 2022

We adjusted our methods to incorporate a late summer period with eggs being kept at room temperature 72 °F. 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. We also addressed water quality issues by 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.

Cohort 2023

We adjusted our methods to try to increase larval survival by using methylene blue on the egg clutches, diversifying the diet, and doing full water changes of each vial. We ran *T* test for each of these changes and found that none of these changes were significantly different to the survival from last year, which suggests that larval mortality might be related to a genetic bottle neck or some other environmental factors that we have not tried yet. We, however, are still successful at rearing a good number of eggs to adulthood which is still a very good result compared to the wild populations. For future trials, we want to try using filamentous algae for larva to use as substrate and be able to grasp on. Past studies have shown that transparent containers work well because sunlight promotes algal growth, and emerging adults are easily seen. The presence of algae in rearing containers appeared to decrease mortality, and they showed that most mortality occurred in containers with little to no filamentous algae. Therefore, we aim to trial this and show a significance in survivability of the larvae.

This season we found the most emerald dragonflies that we have ever sampled in previous years. We captured 36 individual *S. semicircularis*, 32 males and 4 females. We, however, did not find any *S. hudsonica* or *C. shurtleffii* during field collection trips. A possible explanation of these changes was that this year was a particularly cold and wet year which could have influenced the change of dragonfly populations.

In July we collected eggs from *S. semicircularis* early in the season and observed the first hatching within 21 days of oviposition, incubating at 72 °F. In Cohort 2023, 24 individuals hatched since collection. Per previous trails with rearing *S. semicircularis* we 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%.

Attempts were made to survey *Somatochlora* species at Duck and Barron Lakes twice in August of 2023. We ran into barriers accessing the lakes using the existing access easement used by Boulder County Parks and Open Spaces.

Overall, due to not finding any S. *hudsonica* or *C. shurtleffii* at identified sites, contrary to increases seen in populations of *S. semicirularis,* we recommend genetic analysis to identify if population fluctuation is due to genetic drift, interspecies competition, or climate impact.

S. hudsonica typically inhabits elevations above 1500 m in still water (lentic) habitats. The lentic habitats have been described as sedge-bordered, boggy lakes, ponds and streams with nearby or adjacent forest for foraging and mating (Voss and Loewy, 2017). Increasing the amount of preferred habitat may support recruitment or survivability of *S. hudsonica* along Delonde Creek. In addition, previous mitochondrial sequencing of *S. hudsonica* in the literature provides a solid foundation for looking at genetic diversity and estimation of population size through sequencing.

We are also aiming to increase reintroduction releases of late-stage instars to increase wild populations in Delonde Ponds or surrounding areas.

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Prey item	Size of prey	Source	Appropriate size of Odonata larva	Amount per feeding
Paramecium multimicronucleatum	50-300µm	CBS	Hatchling (≤0.5mm) to 1mm	In excess, 0.5mL at density of >20 per field at 40x magnification
Philodina sp.	Up to 500µm	CBS	Hatchling (≤0.5mm) to 1mm	In excess, 0.5mL at density of >20 per field at 40x magnification
Artemia sp. (nauplii)	400- 570µm	CBS	1-2mm	6-10 individuals
Moina sp.	<400 – 1,000µm	CBS	1-2mm	6+ individuals, depending on size
Daphnia sp.	220µm (nauplii) to 5mm (adult)	Wild- caught	1.5-10mm	4-6+, depending on size
Cyclops sp.	78 - 245µm (nauplii) 0.5-5mm (adult)	CBS and wild-caught	1-2mm	6-10 individuals
Drosophila melanogaster (larvae)	2-20mm	CBS	3-10mm	1-2 individuals

Table 1. Prey items used to feed larval *S. semicircularis*, quantities listed per each dragonfly larvae. CBS stands for Carolina Biological Supplies, cultured.

	S. semicircularis		*S. hudsonica*	
	Male	Female	Male	Female
2021	0	1	1	0
2022	16	1	4	0
2023	32	4	0	0

Table 2. Specimens collected at Delonde Ponds field site.

Date collected	Vial ID	Fertilized Eggs	Unfertilized Eggs	Total eggs	Fertilization Rate
7/26/2023	23.1	12	0	12	1
7/26/2023	23.2	200	10	210	0.952380952
8/8/2023	23.3	1	0	1	1
8/10/2023	23.4	65	5	70	0.928571429
Total		278	15	293	**0.948805461**

Table 3. Fertilization rate across all eggs collected during 2023 season. Total fertilization is determined by the total number of fertilized eggs over total number of unfertilized eggs, rather than an average of rates between clutches.