

Effects of *Didymosphenia germinata* on American Dipper (*Cinclus mexicanus*) Territory Size

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Abstract:

The American dipper (*Cinclus mexicanus*) is a medium-sized passerine that forages predominantly in fast-moving streams of the western United States. Breeding territories at high elevation are typically found in montane headwaters, especially sections of fast-moving, clear water with cliffs, ledges or bridges that provide suitable nest sites. The American dipper's diet is chiefly comprised of aquatic insects; their larvae; other small macro-invertebrates including Midges (*Chironomidae*), Mayflies (*Ephemeroptera*), Caddisflies (*Trichoptera*), Stoneflies (*Plecoptera*), Mosquitoes (*Culicidae*), Dragonflies (*Anisoptera*); as well as small fish, fish eggs, worms or flying insects (Ormerod, 1985).

Didymosphenia germinata is a stalked, single-celled diatom native to aquatic ecosystems in the cool temperate regions of the northern hemisphere (Kumar et al. 2009). Blooms of *Didymosphenia germinata* create rapid changes and dramatic shifts in the benthic macro-invertebrate community, including fluctuations in the presence, absence and abundance of invertebrate species depending on the size and duration of the blooms (Brothwell et al., 2014). Given didymo's range expansion, increased bloom intensity and the effect large blooms have on the benthic macro-invertebrate community, we hypothesize that the American dipper will undergo effects of changes in territory quality and size related to foraging habits and strategies. 2017 was the first year of this multi-year project, an insufficient sample size was collected to analyze sites without didymo compared to sites with didymo. A total of 4 territories were estimated with enough confidence in the accuracy of the upper and lower boundaries of 8 breeding adult dippers. Of those 4 territories, only 3 were analyzed: North Pole basin (39° 1'58.65 N 107° 4'31.52 W), Copper Creek Bridge (" (38°57'25.32 N 106°59'18.18 W) and Richards rapids (38°57'31.62 N 106°58'59.82 W). The 4th territory was located at Vera Falls on the East river just North of Gothic town site (38°57'38.57 N 106°59'32.75 W) and was not used in the analysis. The average lengths of the territories sampled (N=3) was 764m with the median territory size at 744m and a standard deviation at 94.16m. Due to an inadequate literature search prior to the submission of the Institutional Animal Care and Use Committee protocol at the beginning of the 2017 field season, several methods of capture previously used in Price and Bock (1983) were not utilized hence an insufficient number of birds were banded. Only the female American Dipper in a breeding pair will incubate, by not utilizing the methods of capture using

butterfly nets placed in front of the nest we did not capture any female dippers until July 7. Other concerns addressed this field season include, impractical flushing techniques and inaccurately flagged boundaries early in the season as well as interference with other researchers that halted the banding and territory estimation process.

Introduction:

The American dipper (*Cinclus mexicanus*) (hereafter, dipper) is a medium-sized passerine that forages predominantly in fast-moving streams of the western United States. Breeding territories at high elevation are typically found in montane headwaters. Here, dippers prefer sections of fast moving clear water with cliffs, ledges or bridges that provide suitable nest sites (Anderson, et al. 2008). Some individuals will remain sedentary at lower elevations to initiate nesting earlier and have a greater chance of raising a second brood (Anderson, et al. 2008). Migrants typically obtain higher quality food sources at higher elevations due to enhanced water quality at headwaters, while sedimentation downstream creates a lower quality habitat for the aquatic benthic macro-invertebrates that the dippers feed on (Morrissey, 2004). The American dipper's diet is chiefly comprised of aquatic insects; their larvae; other small macro-invertebrates including Midges (*Chironomidae*), Mayflies (*Ephemeroptera*), Caddisflies (*Trichoptera*), Stoneflies (*Plecoptera*), Mosquitoes (*Culicidae*), Dragonflies (*Anisoptera*); as well as small fish, fish eggs, worms or flying insects (Ormerod, 1985). An analysis of blood isotopes revealed that dippers prefer to feed on larger prey items during the breeding season. This typically manifests as a more piscivorous diet, which provides high caloric value, lipid content, and skeletal calcium essential for the production of eggs. (Morrissey, et al. 2010).

Listed as a species of least concern from the International Union for Conservation of Nature, American dipper populations globally are estimated at 190,000 breeding individuals with about 86% of the population in the US. Though their unique adaptation to forage underwater provides a competitive advantage over other flying insectivores. One study found that fluctuations in dipper populations are directly reflected upon disturbances that influenced prey species abundance (Feck and Hall, 2004). This suggests that low abundances of stream invertebrates do affect dipper populations.

On February 2nd, 2017, the United States Congress passed a joint resolution to block implementation of an Obama administration coal mining regulation known as the Stream Protection Rule (Tabuchi, 2017). A key

feature of the rule was the limitation on dumping waste materials from mining into rivers and streams. This blocking now allows mining companies to dump any waste material from the mining process into any watershed without restriction. This has been shown to cause heavy flows of sedimentation and heavy metal deposition that inhibits the natural processes of benthic macro-invertebrates of the aqueous environment (Clements, et al. 1988). If even 5% of an entire watershed is affected by anthropogenic disturbance or activities, the stream biodiversity as well as water quality suffer (Ferrari et al. 2009). Pollution-intolerant macro-invertebrates, such as Ephemeroptera, Trichoptera, and Plecoptera, are all essential to the dipper diet and hence, it is reasonable to assume dippers will experience habitat loss with the continued degradation of our waterways (Omerod and Tyler, 1987). Other such causes for the degradation of our waterways include invasive vegetation such as *Hydrocotyle ranunculoides*, *Ludwigia grandiflora* and *Myriophyllum aquaticum*, that have all shown significant negative impacts to the freshwater ecosystems (Pejchar, et al. 2009). As our environment changes, some native species do as well and have been documented causing changes in the native freshwater ecosystems (Brothwell, et al. 2014). Algae species such as *Didymosphenia germinata* have recently been documented expanding its native range and has changed its blooming frequency and intensity causing dramatic shifts in the composition and species richness of the macro-invertebrate community (Brothwell, et al. 2014).

Didymosphenia germinata (hereafter, didymo) is a stalked, single-celled diatom native to aquatic ecosystems in the cool temperate regions of the northern hemisphere, including alpine regions of Europe, Asia and parts of North America (Kumar et al. 2009). By attaching itself to the benthic substrate of the aquatic system, didymo creates multi-layered mats of algae through vegetative reproduction that can cover up to 100% of the substrate in a 20cm thick mat (Spaulding et al. 2007). Until recently, didymo was thought to be restricted to nutrient-poor waters and to occur only in small patchy blooms (Gillis, et al. 2010). In the last two decades, it has been increasingly detected in more nutrient rich waters, expanding outside of its native range. Within its native range, areas with previously low concentrations of the diatom now have excessive growth and more frequent and larger blooms (Spaulding et al. 2007). In 2004, Didymo blooms were discovered in New Zealand, introduced there by accident through the soles of fisherman's wading shoes (Kilroy, et al. 2011). By acting as an aquatic hitchhiker, didymo is able to move amongst watersheds, but blooms can only occur with specific conditions. In

a study from 2011, substantial evidence is provided that the proximate cause for didymo blooms in rivers is low concentrations of phosphorus (Brothwell and Kilroy, 2011). From this, four potential causes for low levels of phosphorus entering the fluvial system globally are discussed in Brothwell and Taylor 2014:

(1) Atmospheric deposition of reactive nitrogen (2) climate-induced shifts of snowmelt and growing season timing; (3) N-enrichment of landscapes during agricultural and silvicultural activities; and (4) a decline in marine-derived nutrients, particularly phosphorus (Brothwell, et al. 2014)

It is not likely that a single one of these hypotheses is causing this invasive behavior of this native diatom; rather, they are likely acting synergistically (Brothwell, et al. 2014).

Blooms of didymo create rapid changes and dramatic shifts in the benthic macro-invertebrate community, including fluctuations in the composition and abundance of invertebrate species depending on the size and duration of the bloom (Brothwell et al., 2014). When blooms occur in native form they produce small patches that cover only small sections of the bottom invertebrate densities increased due to increased structural diversity and hence habitat available (Gillis and Chalifour, 2010). In areas or during years where blooms cover the entire benthic region, the macro-invertebrate community goes through a major shift in the species composition (Gillis, et al. 2010). In 2010 at the Matapedia River in Quebec, Canada one study found that sites with didymo have increased proportions of Chironomids that became the dominant taxa in the affected areas and had decreased proportions of Ephemeroptera, Plecoptera and Trichoptera that typically dominate the aquatic invertebrate community in oligotrophic waters (Gillis, et al. 2010).

Given didymo's range expansion, increased bloom intensity and the effect large blooms have on the benthic macro-invertebrate community, we hypothesize that the American dipper will undergo dietary changes as related to benthic macro-invertebrate species composition changes associated with didymo blooms. With the species composition changes dippers will also experience effects of changes in territory quality and size related to foraging habits and strategies. We predict that in territories containing didymo, dippers will 1) spend more time foraging and 2) have larger territories to access sufficient prey.

Methods:

Study site: Data collection occurred on 13 sites at the headwaters of the Gunnison river watershed in the West Elk Range of the Rocky Mountains, in Gunnison national forest, Gunnison County, Colorado, US. These included sites with didymo blooms (Cement creek, Coal creek, West Brush creek, Washington Gulch creek, Oh-Be-Joyful creek and Lower East River) and without (Upper East River, Lower Rock creek, Copper creek, Poverty Gulch creek, upper Slate River, North Pole creek and Rustler's Gulch creek). In the first year of the study (2017), only four sites (Lower East River, Cement Creek, Copper creek and North Pole creek) were monitored from 1 July to 1 August. All four sites are oligotrophic riverine systems with a variety of benthic substrates including igneous, sedimentary and metamorphic rock. The vegetation within the riparian sections of each site include species of subalpine willow (*Salix spp.*), Douglas Fir (*Pseudotsuga menziesii*), Engelmann Spruce (*Picea engelmannii*), Quaking aspen (*Populus tremuloides*), Cinquefoil (*Potentilla*) and herbaceous vegetation consisting of Larkspur (*Delphinium*), Monks Hood (*Aconitum*), Corn lily (*Veratrum californicum*) and Cow Parsnip (*Heracleum*) along with spring ephemerals and many other native wildflower species.

Water and invertebrate sampling: In each watershed invertebrate composition, water quality and turbidity, nutrient content and percent phosphorus, stream flow and width/depth averages of stream were measured following the methodology defined by Feck and Hall (2004). Didymo bloom intensity was also measured in each site.

Bird capture and monitoring: Breeding pairs of American dippers were located at each site. We captured dippers using 6, 9 and 12 m mist nets placed across streams and rivers, often adjacent to bridges or beaver dams to facilitate easier bird extraction. Each individual received four leg bands: three plastic darvic color combination bands and one US Geological Services (USGS) metal band with a unique 9-digit number. Two color bands were applied to one leg and one color band and a USGS metal band was applied to the other leg. Once banded, we measured weight, wing length, tail length, culmen length, tarsus length and cloacal protuberance height and width. Once each individual was processed and released, they were observed to determine behavioral time budgets of territory holding individuals and the estimation of each territory size.

To determine dipper territories, the methods of Feck and Hall (2004) were utilized:

We flushed birds into the mist nets by hazing them. Netted dippers were color banded to estimate the territory boundary. To measure territories, we flushed birds several times to the upper and lower extents of the territory where birds were spotted turning around (Price & Bock, 1983; Archuleta, 1999; Osborn, 1999; Feck & Hall 2004).

If the flushed bird turned around in the same place after several separate flushing attempts we determined that was a territory boundary (Price & Bock, 1983; Archuleta, 1999; Osborn, 1999; Feck & Hall 2004). Audio recordings of local male songs were broadcasted at the far end of what was estimated to be the territory at each site. The adult male of the territory was then observed either flying to the edge of the territory we estimated and singing or flying past the marked boundary to a more accurate estimate of the territory.

For our observations, we first identified a point within the territory of an individual by noting the location of the nest. From that location, we were able to determine the residents in each territory. Observations were conducted from 06:00 hours to 20:00 hours and broken down for analysis by morning (06:00-10:00), afternoon (11:00-14:00) and evening (15:00-20:00). Observations were recorded at a minimum 100-foot distance by watching birds using binoculars or a spotting scope to avoid disruption of the natural behaviors. Possible activities we recorded include: active foraging in water, active foraging on land, preening, sleeping, standing still dipping, male singing, flying silently, flying alarm calling, alarm calling, feeding fledgling, territorial interaction, male-female interaction, male visit nest, female visit nest, as well as recording birds travelling beyond the initially flagged territory boundary or locations of males singing to further indicate a territory boundary (Price and Bock, 1983). Each activity performed by the bird and the duration of each activity was recorded. Additionally, the stage of the nest is recorded (under construction, eggs, nestlings, or fledged) to account for a bias of birds behaving differently in different stages of the nesting cycle. All the locations for nest sites, observations, bird capture, and territory estimations were recorded on a Garmin eTrex legend HCx GPS

Data Analysis: The analysis of the data was performed using Microsoft Excel and Stats Plus to build a line graph that depicts the territory size and time spent feeding at sites without Didymo compared to sites with Didymo. An Akaike test will be performed using the parameters territory size, time budgets of activities, stream turbidity, invertebrate composition, width/depth averages of streams, morphologic measurements, didymo bloom intensity, and stream flow to determine the quality of each model for a better means of model selection.

Results:

The program Stats Plus was used to analyze the data to compare territory sizes and build a descriptive analysis of the territory of the dipper. A total of 4 territories of 8 breeding adult dippers were estimated with consistent accuracy (Figures 1-4). Of those 4 territories only 3 were analyzed. (North Pole Basin (39° 1'58.65 N 107° 4'31.52 W), Copper creek bridge (" (38°57'25.32 N 106°59'18.18 W) and Richard's Rapids (38°57'31.62 N 106°58'59.82 W) (Table 1). The fourth territory was located at Vera Falls on the East River, (38°57'38.57 N 106°59'32.75 W) just north of the Gothic town site, and was not used in the analysis (Figure 4). The average lengths of the territories sampled (N=3) was 764m with the median territory size at 744m and a standard deviation at 94.16m (Table 1). Google Earth Pro was used to download the GPS points recorded as territory boundaries. Maps were built measuring distances of territories by tracing the distance up or down river between the two territory boundaries in meters (Figures 1-4).

Discussion:

As this was the first year of this multi-year project, an insufficient sample size was collected to analyze sites without didymo compared to sites with didymo. The continuance of this paper will cover the finding of the 2017 field season including an analysis of territory size and considerations for replications of this project in future years.

Due to an inadequate literature search prior to the submission of the Institutional Animal Care and Use Committee (IACUC) protocol, several methods of capture previously used in Price and Bock (1983) were not utilized and an insufficient number of birds were banded. Dipper capture and banding did not begin until 1

June; hence, banding and territory estimations consumed the majority of the field season. This occurred at the most difficult time for capture due to high melt waters at peak flow and an overall late start because the breeding season can begin as early as late March. Only the female dipper in a breeding pair will incubate. Thus, by not utilizing the methods of capture via butterfly nets placed in front of the nest we did not capture any female dippers until July 7. Other concerns addressed this field season include impractical flushing techniques; inaccurately flagged boundaries early in the season; and interference with other researchers that halted the banding and territory estimation process. In the coming years corrections to the IACUC protocol as well as earlier starts to the field season will be implemented to facilitate a minimally time-consuming capture process and allow for increased time spent on observations and other monitoring and territory estimation techniques. With the increase in time for capture and monitoring, a larger sample size may be obtained in the coming years allowing for a proper analysis to compare sites with didymo to those without. A further sample size restriction was the loss of the Vera Falls territory data. The Vera Falls territory on the East river was not an accurate representation as the adults utilized Benthette brook, a feeder stream with several beaver ponds that were not sampled (Figure 4). The adults of that territory frequented the beaver ponds throughout the breeding season to forage by flying over land in several different directional paths indicated by the blue lines (Figure 4). Furthermore, when the East river became excessively turbid the dippers of that territory exclusively foraged in Benthette brook and the beaver pond that then biased the territory size on the East river dramatically.

Through this research we can identify if there are life history or dietary changes in the American Dipper due to benthic macro-invertebrate species composition changes associated with didymo blooms. To quantify these changes in the life history of the American dipper it can help us to better identify the changes occurring in the aquatic environment as a whole. By laying the ground work for understanding such ecosystem changes, factual, evidence based legislature can be produced to protect and preserve this fragile aspect of our montane ecosystem.

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Tables & Figures:

	NPB		Copper Creek bridge		Richards Rapids
Territory Size in Meters	867		744		682
Nest location	39° 1'58.65"N 107° 4'31.52"W		38°57'25.32"N 106 59'18.18"W		38°57'31.62"N 106°58'59.82"W
		Average Territory size	Median Territory size	Standard Deviation	
		764.3333333	744	94.16120928	

Table 1.

Average, Median, Standard Deviation and territory sizes in meters of the 3 estimated territories of the 2017 field season.



Figure 1.

Map of North Pole Basin territory, flagged by upper and lower boundaries (867m long). The nest site was located underneath the 2nd large waterfall on the north side (39° 1'58.65"N 107°4'31.52"W).



Figure 2.

Map of Copper creek territory, flagged by upper and lower boundaries (744m long). The nest site was located under the Copper creek bridge ($38^{\circ}57'25.32''\text{N}$ $106^{\circ}59'18.18''\text{W}$).



Figure 3.

Map of Richards rapids territory on Copper creek, flagged with upper and lower boundaries (682m). The nest site was located on the north side at the top of the largest waterfall ($38^{\circ}57'31.62''\text{N}$ $106^{\circ}58'59.82''\text{W}$).



Figure 4.

Map of Vera Falls territory on the East River, flagged by upper and lower boundaries (563m). The nest site was located on Benthette brook behind a waterfall that feeds into the East river just below Vera Falls ($38^{\circ}57'38.57''\text{N}$ $106^{\circ}59'32.75''\text{W}$). The blue lines show the flight paths leading out to the two 90° angles over the beaver ponds they frequent to forage.