

Glacier National Park Bumble Bee Survey Report 2023

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Background

Glacier National Park's grasslands provide important contributions to the character and ecology of the park. In 1999-2001, Glacier National Park (hereafter, the park) established 155 permanent vegetation monitoring plots to inventory grassland vegetation communities east of the Continental Divide. In June of 2023, the Blackfeet Nation (Amskapi Piikuni) re-introduced a herd of free roaming bison on adjacent tribal land (the linnii Initiative), and those bison may enter the park. Because grasslands are important areas for bison grazing, and because biological communities have likely changed since the initial inventories, the park's vegetation program began revisiting grassland monitoring plots in 2018. New data will provide insights into how these grasslands, and the wildlife that depend on them, have changed over the past two decades. Data on pollinators in these grasslands, however, are limited. Bison have been shown to influence plant communities, for example, by increasing herbaceous forb diversity and drought resilience (Elson and Hartnett 2017; Ratajczak et al. 2022). These changes directly influence resources available to foraging pollinators. To provide understanding of pollinators prior to bison reintroduction, bumble bee surveying at these grassland plots began in 2020 and has continued through 2023. Target sites for 2020-2022 focused on collecting data at plots where new vegetation surveys were recently completed to match current vegetation data with the bumble bee data.

In 2023, new protocols and site selection processes were introduced to support a collaborative study in the development of using environmental DNA (eDNA) surveys as a detection method for bumble bee species of concern. This collaboration with 6 U.S. Geological Survey Science Centers seeks to develop the infrastructure for using eDNA methods for sampling. Traditional sampling methods used to determine pollinator species distribution (e.g., netting and visual surveys) are time and labor intensive, subject to sampling bias, and require a high level of taxonomic expertise to identify many species. Environmental DNA sampling methods show promise in their ability to minimize sampling bias and to simplify and expedite the data collection process by collecting bumble bee DNA shed onto flowers during floral

visitation. The western bumble bee (*Bombus occidentalis*) is currently under consideration for listing by the U.S. Fish and Wildlife Service under the Endangered Species Act and is still present in Glacier's ecosystems, making it a well-suited target species for the study. Sites with high predicted likelihood or recent detections of western bumble bee presence were chosen for sampling in 2023, primarily in grassland habitat at sites that are part of the overall grassland monitoring program (Figure 1). In addition to species occupancy and distribution information collected from the netting surveys, data collected will inform how detection rates differ between eDNA and traditional surveys, how many eDNA samples are needed for reliable western bumble bee detection, and how weather and habitat characteristics influence eDNA detection rates. To determine most efficient practices for monitoring the broader bumble bee and overall pollinator communities, we conducted extensive flower collections at additional "saturation" sites in the park. Together, this work will inform whether and to what degree eDNA surveys are a more effective means of monitoring western bumble bee, as well as overall pollinator communities, than traditional netting surveys.

Approach & Preliminary Results

Western bumble bee eDNA sites

During each site visit we conducted both traditional (i.e., netting, visual) surveys and eDNA surveys. Traditional surveys were comprised of three 15 min netting surveys for all bumble bees. When a bee was caught, the timer was stopped to remove the bee from the net and place it into a vial. Bees were chilled on ice for about 10 minutes before being photographed. Four key identification regions were photographed on each bee: abdomen, side body with hind legs, top of thorax, and face. These photos will be identified by a bee taxonomist. If the specimen was found on a plant, the plant species was recorded. In 2023, information on the coverage of flowering plants was collected using quadrat sampling along two 60 meter transects running north and south. All blooming flower species within a 1x1 meter quadrat were recorded along the outside edge of the transect at 10-meter intervals. The distance of the transects east and west of the site center differed each visit to avoid resampling, while maintaining 50 meters between transects. Additionally, all blooming flower species present at the site were recorded in a comprehensive site list.

Three 15-minute visual surveys specifically targeting the western bumble bee were also conducted at the site if a western bumble bee was not caught during the initial netting surveys.

Environmental DNA surveys in 2023 were comprised of fist-sized flower collections of the most abundant flower species blooming at the site known to be used by western bumble bee, the flower species that a western bumble bee was observed visiting on the day of the survey (if applicable), and the individual flowers that a western bumble bee was observed visiting on the day of the survey (if applicable). In this way, for every eDNA sample collected we knew whether a western bumble bee was detected at the site at some point during the field season, on the day of sample collection, on the flower species collected at the site on the day of sample collection, and on the individual flower collected on the day of sample collection. All eDNA samples were frozen immediately upon collection. Traditional and eDNA bumble bee surveys were paired with descriptions of blooming flower communities (e.g., species composition and abundance) as well as habitat and weather characteristics (e.g., time since last rain, temperature, cloud cover, etc.).

Seventy-five surveys across 27 sites were conducted for the western bumble bee eDNA project during the 2023 season. This total includes 66 formal, complete surveys across 22 sites, and eight informal scouting or training surveys across six sites, two of which contained both formal and informal surveys. One additional site was surveyed later in the season, but second and third visits were not completed due to a lack of floral resources (Table 1, Figure 1). Thirteen of the 22 formal sites matched sites included in the grassland vegetation monitoring program (Table 2). Ultimately, twenty-two sites and 66 surveys will be included in formal analyses for eDNA project goals. Western bumble bees were caught at 20 of the 22 formal western bumble bee eDNA sites.

Surveys were completed between May 27th and August 28th, 2023 (Figure 2). A total of 1,330 bumble bees were caught during netting surveys, and 71 floral associates were recorded with *Centaurea stoebe*, *Lupinus sericeus*, and *Symphoricarpos albus* being the most frequent (Figure 3). Throughout the entire season, 196 blooming flower species were recorded with *Achillea millefolium* being recorded at the most site visits. Several plants were visited by bumble bees at 100% of sites in which the plant was present, of which *Camassia quamash* and *Senecio triangularis* had the most bees caught on them (Table 3). The highest floral richness at sites was generally recorded in late June to early July with as many as 35 unique blooming species at a site (Figure 4).

We collected 373 eDNA samples during western bumble bee eDNA site visits. Forty-nine of the western bumble bee eDNA samples contained flowers with a known western bumble bee visitation during that site visit, 105 contained flower species that were visited by a western bumble bee during the survey, but did not contain a known visitation flower, and 78 samples were collected from a flower species

present at the site when a western was observed, but no visitations to that flower species were observed (Figure 5). The remaining samples either contained flower species that did not have a western bumble bee observation that day (107 samples) or were from a site where no western bumble bees were caught throughout the summer (34 samples).

A western bumble bee nest was found during surveying at formal site “GRAS-01-045” near the southern border of the park (Figure 1). Temperature data loggers, 2 remotely triggered cameras, and an acoustic monitor were deployed near the nest to characterize nest usage and record the acoustic signature of the western bumble bee. In person observations of bumble bees entering and leaving the nest were also conducted and will be later paired with the acoustic data. Pending park approval, excavation of the nest will provide further information about the colony. To our knowledge, this is only the second western bumble bee nest site that has been discovered.

Broader Pollinator Community Saturation Sites

We conducted extensive flower collections at 3 additional “saturation” sites in the park, including “GRAS-99-033” in the park’s grassland vegetation monitoring program (Table 2). Each site was visited once and flower samples from the top 10 most abundant blooming flower species at each site were collected. Flower collections included fist-sized whirl-pak samples that were immediately frozen upon collection, as well as dandelion head-sized samples that were dried in teabags surrounded by desiccant upon collection, to enable comparison among these two collection approaches. The dried collection method was previously used in surveys in 2022 at a site near the Cutbank trailhead, and sampling in 2021 across Montana. For all flower collections in 2023, surveyors ensured that no more than 10% of blooms for each flower species were collected during a sampling event. A total of 240 eDNA samples were collected from saturation sites (80 samples at each of 3 sites), comprised of 120 whirl-pak samples and 120 tea bag samples.

Table 1. Number of bumble bee sampling sites visited in Glacier National Park throughout field seasons in 2020-2023. Three surveys across three saturation sites are not included in 2023 because no netting or visual bumble bee surveys were conducted at those sites.

Number of Site Visits	2020	2021	2022	2023
Visited once	0	1	2	4
Visited twice	0	1	1	0

Visited three times	16	10	17	21
Visited four times	1	5	0	2
Total unique sites visited	17	17	20	27
Total number of site visits	52	53	55	75

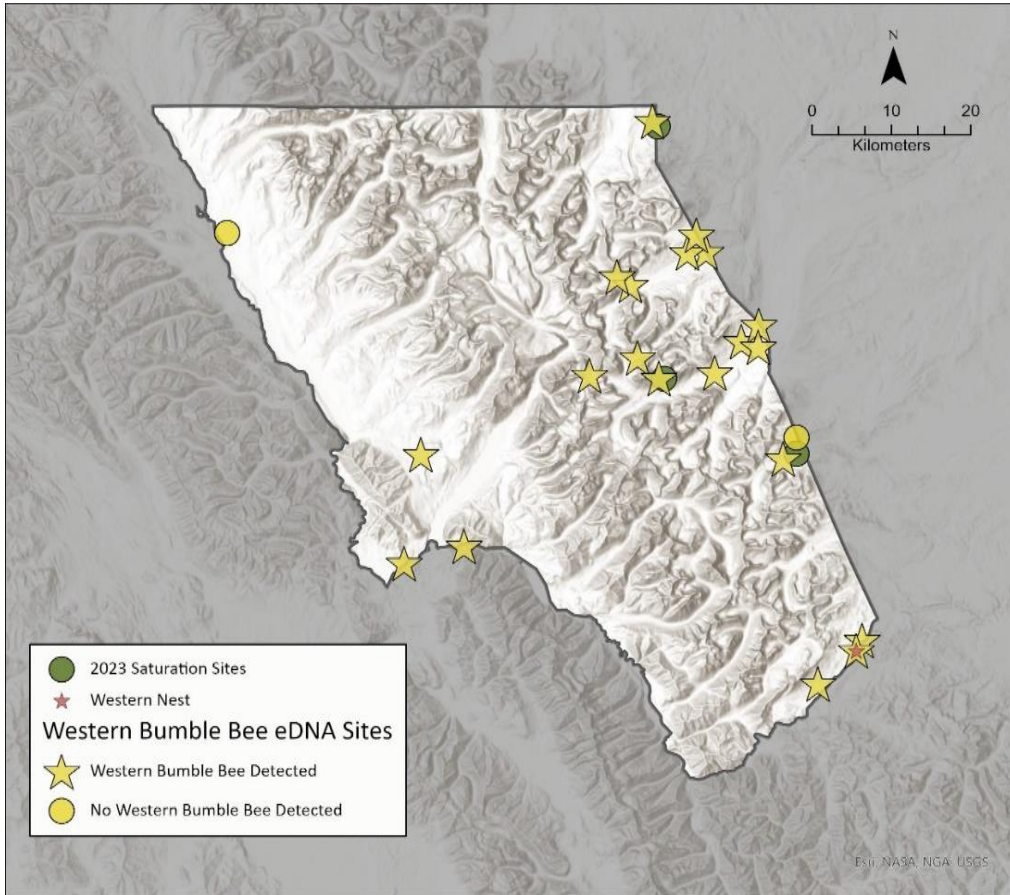


Figure 1. Map of 2023 survey site locations in Glacier National Park. Sites where western bumble bees were observed are denoted with a yellow star. Yellow dots represent sites sampled three times, but western bumble bees were never observed. Green dots represent saturation sites for which no bumble bee surveys were conducted. A western bumble bee nest was found at formal site “GRAS-01-045,” denoted by a red star.

Table 2. Names of grassland monitoring plots visited in 2020, 2021, 2022, and 2023, as named in the database provided by the park. Western bumble bee detected at sites with an * in that year of sampling. The site where only saturation sampling occurred is denoted with +.

2020	2021	2022	2023
GRAS-00-013 NEW *	GRAS-00-003	GRAS-00-002	GRAS-00-013*
GRAS-99-015 NEW	GRAS-00-008	GRAS-00-009	GRAS-00-023*
GRAS-99-017 83 NEW	GRAS-00-025	GRAS-00-014	GRAS-00-039
GRAS-99-018	GRAS-00-026	GRAS-00-016	GRAS-00-048*

GRAS-00-004 NEW	GRAS-00-029	GRAS-00-020	GRAS-01-013*
GRAS-01-013 NEW*	GRAS-00-042	GRAS-00-024	GRAS-01-045*
GRAS-01-039	GRAS-00-048*	GRAS-00-036	GRAS-01-046*
GRAS-01-043 NEW	GRAS-01-003	GRAS-00-038	GRAS-99-005 83*
GRAS-99-038 NEW*	GRAS-01-007	GRAS-00-039*	GRAS-99-025*
GRAS-00-001 NEW	GRAS-01-010	GRAS-01-008	GRAS-99-029*
GRAS-01-001*	GRAS-01-014*	GRAS-01-016	GRAS-99-033*
GRAS-01-002	GRAS-99-027	GRAS-01-020	GRAS-99-044*
GRAS-01-005	GRAS-99-029*	GRAS-01-039	GRAS-99-046*
GRAS-01-021 NEW	GRAS-99-030*	GRAS-01-046*	GRAS-99-048*
GRAS-99-005 83*	GRAS-99-053*	GRAS-01-048	
GRAS-99-024-NEW	GRAS-01-013 NEW	GRAS-99-026	
GRAS-99-025*	GRAS-99-005 83	GRAS-99-033	
		GRAS-99-035	
		GRAS-99-044*	

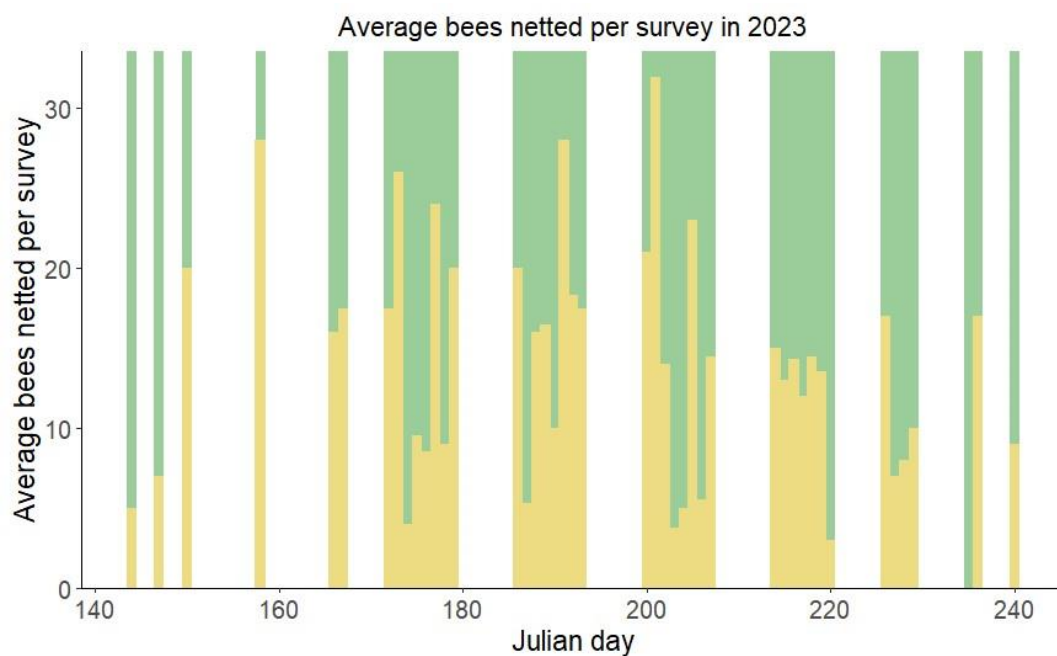


Figure 2. Average number of bees netted per survey throughout the 2023 field season. Days on which surveys were conducted are denoted by green. Yellow columns show average bees netted during surveying.

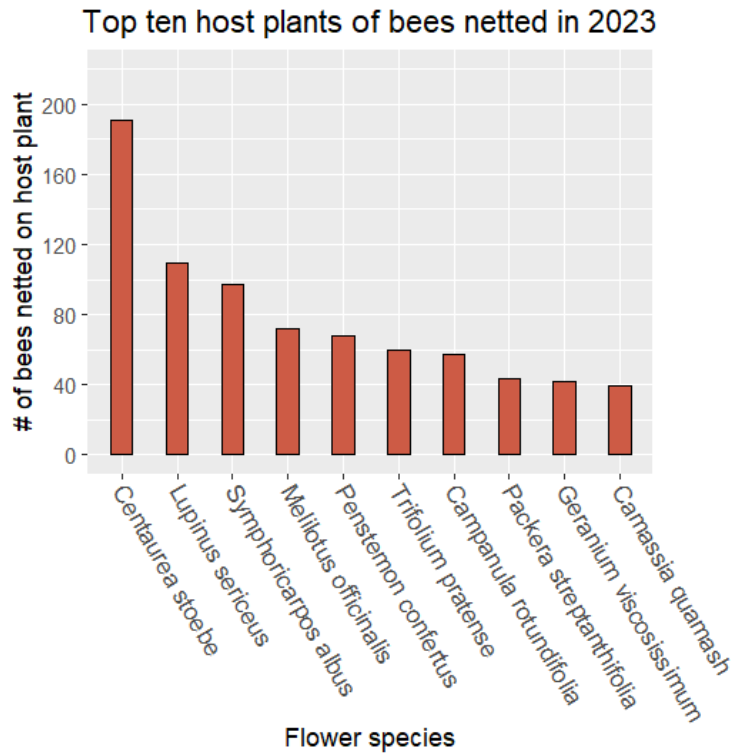


Figure 3. The ten most commonly used flower species by bumble bees during surveying in 2023.

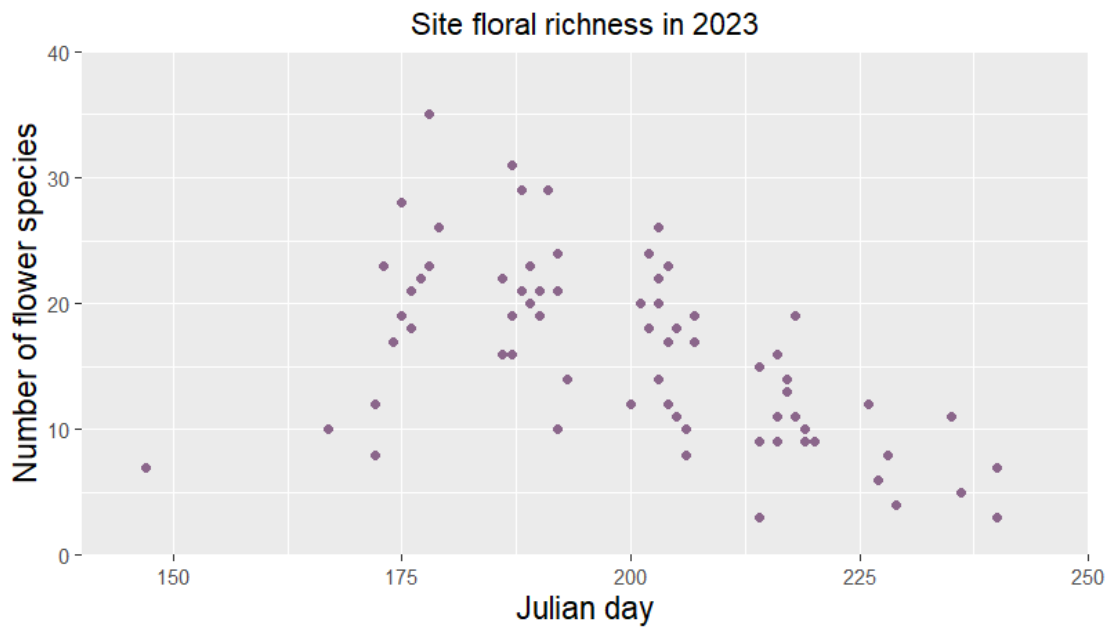


Figure 4. Number of flower species recorded during each survey throughout the 2023 field season.

Table 3. Floral resources with bees captured on them over 50% of the time when present at site. Bees caught shows the total number of bees caught on a specific flower species throughout the season, including instances where more than one bee was captured on the flower during a survey. Survey count shows the number of surveys at which the flower species was present. Percent usage represents the percent of time that one or more bees was captured on a flower species, given that the flower species was recorded during the survey.

Flower species	Bees caught	Survey count	Percent usage
<i>Centaurea stoebe</i>	191	21	57.1
<i>Lupinus sericeus</i>	109	28	53.6
<i>Symphoricarpos albus</i>	97	24	54.2
<i>Melilotus officinalis</i>	72	7	57.1
<i>Penstemon confertus</i>	68	18	55.6
<i>Trifolium pratense</i>	61	15	53.3
<i>Camassia quamash</i>	39	3	100
<i>Senecio triangularis</i>	21	2	100
<i>Primula sp.</i>	14	3	100
<i>Hypericum scouleri</i>	14	2	100
<i>Allium cernuum</i>	13	4	100
<i>Erigeron peregrinus</i>	11	5	60
<i>Cirsium scariosum</i>	7	5	60
<i>Pedicularis contorta</i>	6	2	100
<i>Rhinanthus minor</i>	6	1	100
<i>Symphyotrichum bracteolatum</i>	6	1	100
<i>Apocynum androsaemifolium</i>	4	2	100
<i>Astragalus sp.</i>	4	1	100
<i>Melilotus albus</i>	4	1	100
<i>Hedysarum alpinum</i>	3	1	100
<i>Erythranthe lewisii</i>	3	3	66.7
<i>Prunella vulgaris</i>	3	3	66.7
<i>Pedicularis bracteosa</i>	2	1	100
<i>Phleum pratense</i>	2	1	100
<i>Geum rivale</i>	1	1	100
<i>Spiraea betulifolia</i>	1	1	100

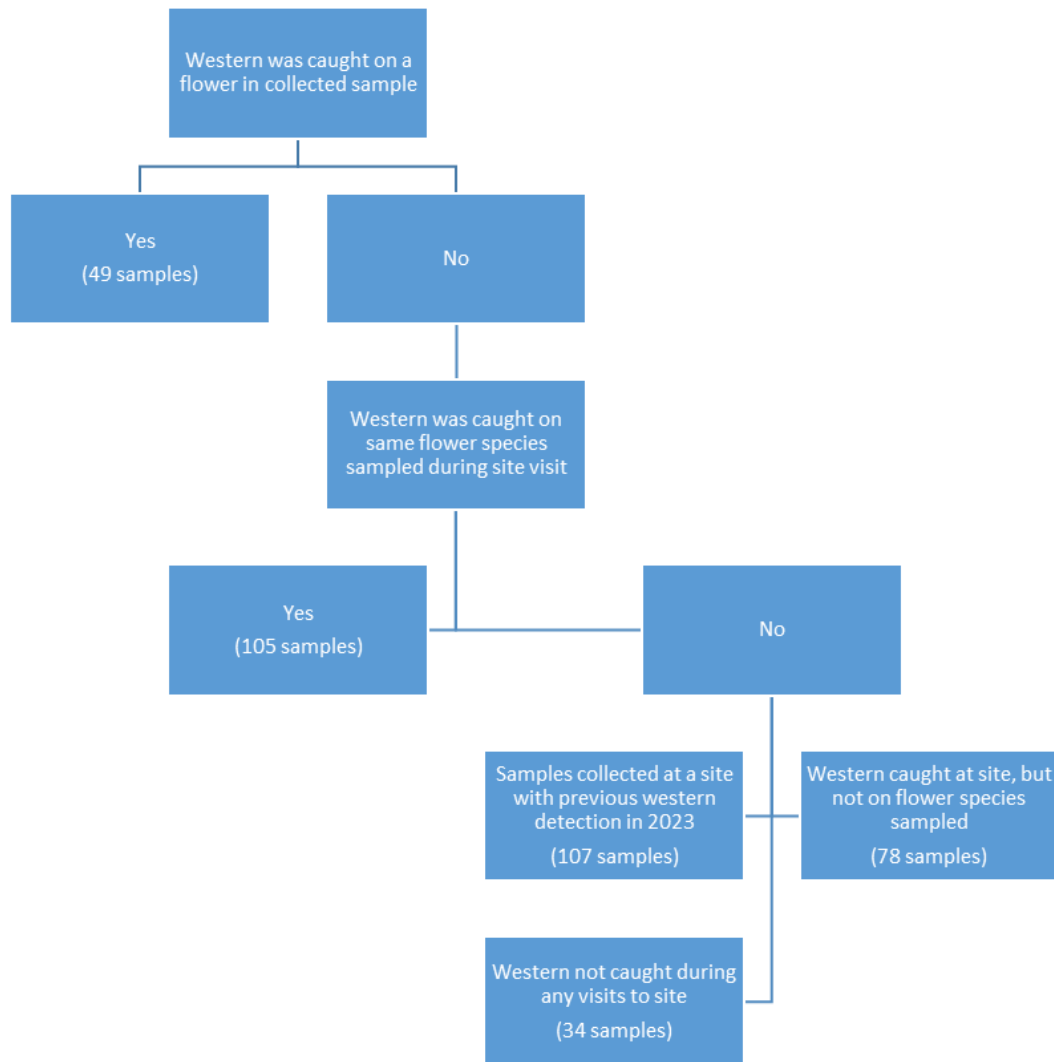


Figure 5. A flowchart showing the number of eDNA samples collected under various conditions at western bumble bee eDNA (non-saturation) sites (n=373).

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