Mosquito Surveillance at Billy Frank Jr. Nisqually National Wildlife Refuge

2019-2023

Climate and Health Program

Washington State Department of Health

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This report summarizes mosquito surveillance data, including West Nile virus surveillance, collected at Billy Frank Jr. Nisqually National Wildlife Refuge from 2019-2023.

We would like to acknowledge and extend our sincere thanks and appreciation to Glynnis Nakai, Project Leader, Nisqually National Wildlife Refuge Complex, for her ongoing collaboration and support for surveillance efforts conducted at the Refuge.

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Introduction

The Washington State Department of Health (DOH), with the permission of the Billy Frank Jr. Nisqually National Wildlife Refuge (NNWR), has conducted mosquito and West Nile virus (WNV) surveillance at the refuge since 2007. Surveillance increases our understanding of the distribution and abundance of vector mosquito populations and allows us to monitor overall mosquito population trends. Additionally, surveillance helps us monitor risk for WNV, other arboviruses, and new invasive mosquito species that might be disease vectors. When appropriate, targeted prevention messages can be released to alert the public and reduce the risk of human infection.

This report focuses on surveillance findings over a five-year time frame, the 2019 through 2023 seasons, with comparisons to earlier seasons to highlight notable observations.

Background and Methods

Established in 1974, the Billy Frank, Jr. NNWR protects the Nisqually River delta and its diversity of fish and wildlife habitats. Located in southwestern Washington, the refuge is nestled near the shores of Puget Sound. Much of the land comprising the refuge was drained and diked for agricultural use in the early twentieth century. After a century of diking off tidal flow, the Brown Farm Dike was removed on November 11, 2009 (Figure 1). Since then, NNWR and close partners, including the Nisqually Indian Tribe and Ducks Unlimited, have restored more than 21 miles of the historic tidal slough systems and re-connected 762 acres of historic floodplains to Puget Sound, increasing potential salt marsh habitat in the southern reach of Puget Sound by 50%.

Figure 1. Actions implemented in 2008-2009 to remove man-made dikes and restore 762 acres of estuary to tides, Billy Frank Jr. Nisqually National Wildlife Refuge.

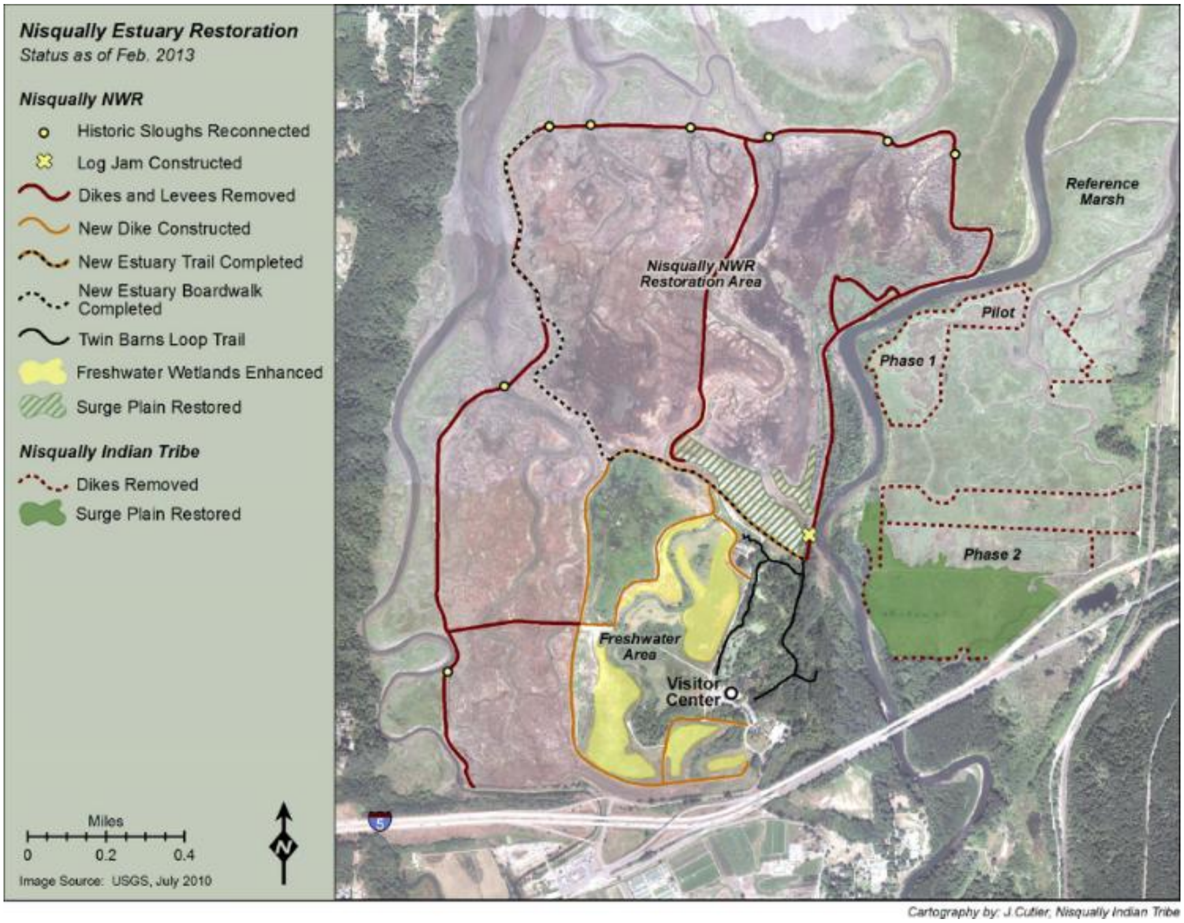


Figure 2. Satellite photographs showing estuary recovery at NNWR before dike removal (2009) and eight years after dike removal (2017).

Mosquitoes were collected from May through September using dry ice baited light traps placed at two sites in the refuge. The Pond site was located by the edge of the pond behind the refuge visitor's center and the Woods site was situated in a more riparian area with alder and big leaf maple overstory (Fig. 3).

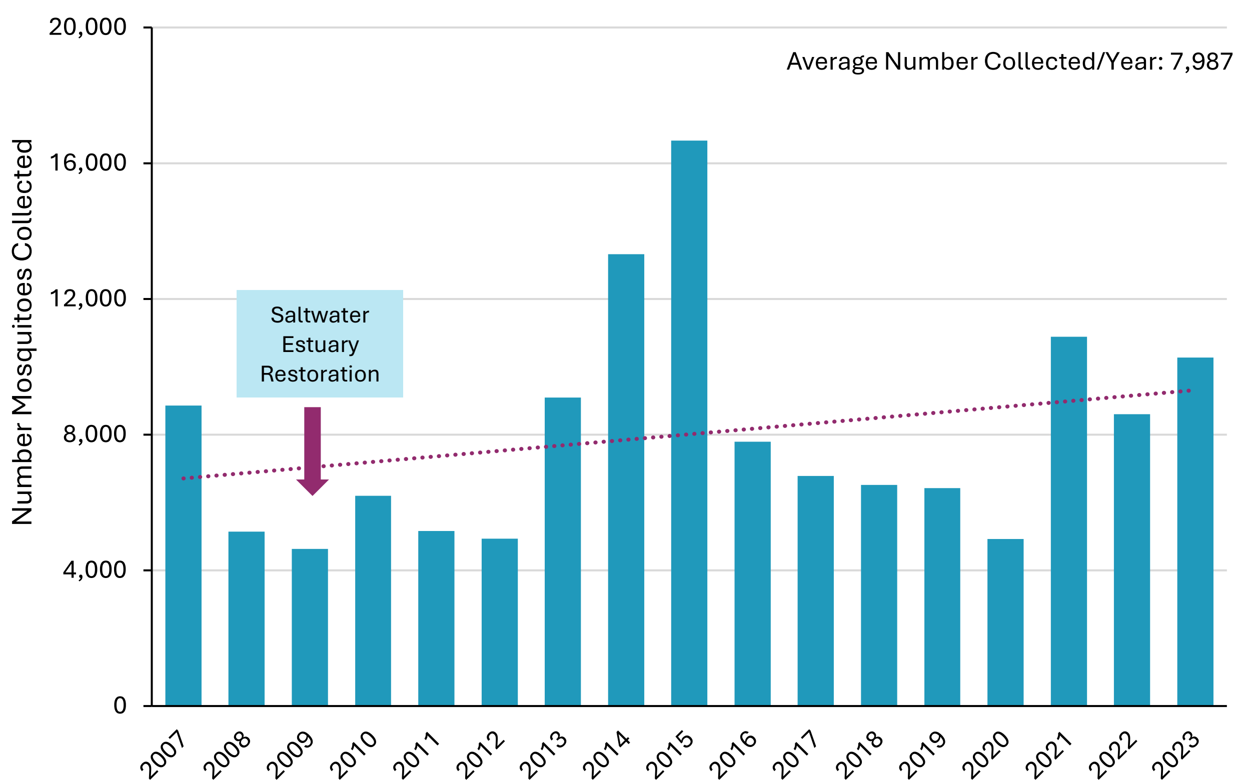
Figure 3. Location of mosquito trap sites NNWR, 2007-2023

Abundance and Diversity

A total of 40,700 mosquitoes representing 17 species was collected at NNWR from 2019-2023 (Appendices 1 and 2). Collections at the Pond site followed historical trends and remained substantially higher than at the Woods site (Fig. 4).

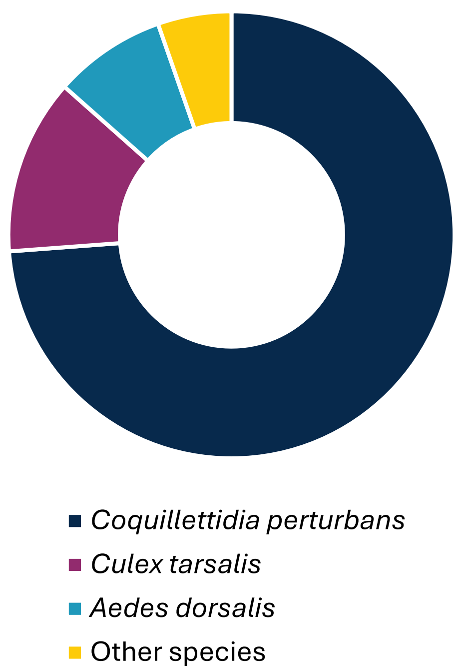
Overall abundance of mosquitoes from 2021 to 2023 was the highest since the 2014 to 2015 seasons (Fig. 4). The 2020 season had lower counts because of a later trapping start date due to staff demands associated with the COVID-19 pandemic response. Annual abundance fluctuates by year, with occasional years of high abundance. Fluctuations are impacted by annual weather patterns and habitat availability. Annual total numbers averaged 7,987 mosquitoes collected per year.

Figure 4. Total mosquitoes collected by year at NNWR, 2007-2023 (n=135,775).



The most common mosquito species at NNWR was *Coquillettidia perturbans* (Fig. 5). *Culex tarsalis*, the encephalitis mosquito, and *Aedes dorsalis*, the salt marsh mosquito were the second and third most abundant species, respectively.

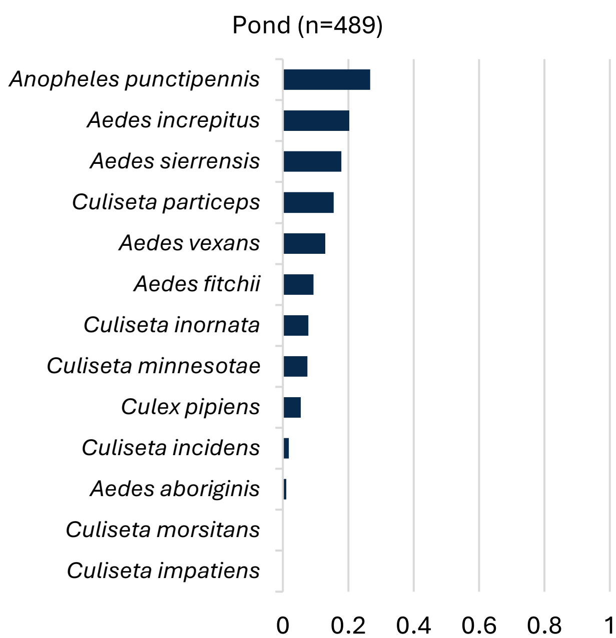
Figure 5. Primary mosquito species abundance, 2019-2023, NNWR (n=40,700).

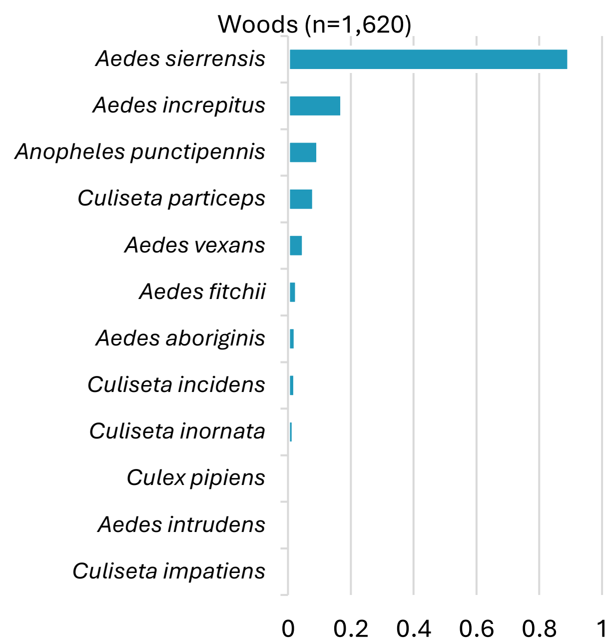


Culex tarsalis is one of Washington's two primary WNV vectors. *Coquillettidia perturbans* is a serious nuisance biter, but is not a known disease vector in the western U.S. *Aedes dorsalis*, while not considered a primary disease vector, can create discomfort from nuisance biting.

Among the secondary species collected at the NNWR, the most abundant at the Pond site was *Anopheles punctipennis*, the woodland malaria mosquito, while *Aedes sierrensis*, the western tree hole mosquito, was the dominant species collected at the Woods site (Fig. 6). Although collections at both sites were comparatively small, total secondary mosquito numbers at the Woods site were triple that of the Pond site. Species richness was essentially the same with 16 species collected at the Pond site compared to 15 species collected at the Woods site.

Figure 6. Secondary mosquito species relative abundance, NNWR, 2019-2023 (n=2,109).





Temporal Distribution Patterns

Temporal distribution of NNWR's mosquito population peaked in July with most mosquitoes collected during June and July. Temporal distributions comparing average monthly (Fig. 7) and weekly (Fig. 8) mosquito collections from 2019-2023 to the initial 12 years of data (2007-2018) show a shift to earlier activity at the Pond site. Average June collection numbers from 2019-2023 at this site increased 74% from 2007-2018, indicating increased mosquito activity earlier in the season.

Figure 7. Average monthly collections of mosquitoes by collection site, NNWR, 2019-2023 (n=40,700) compared to average monthly collections of mosquitoes by collection site from 2007-2018 (n=95,075).

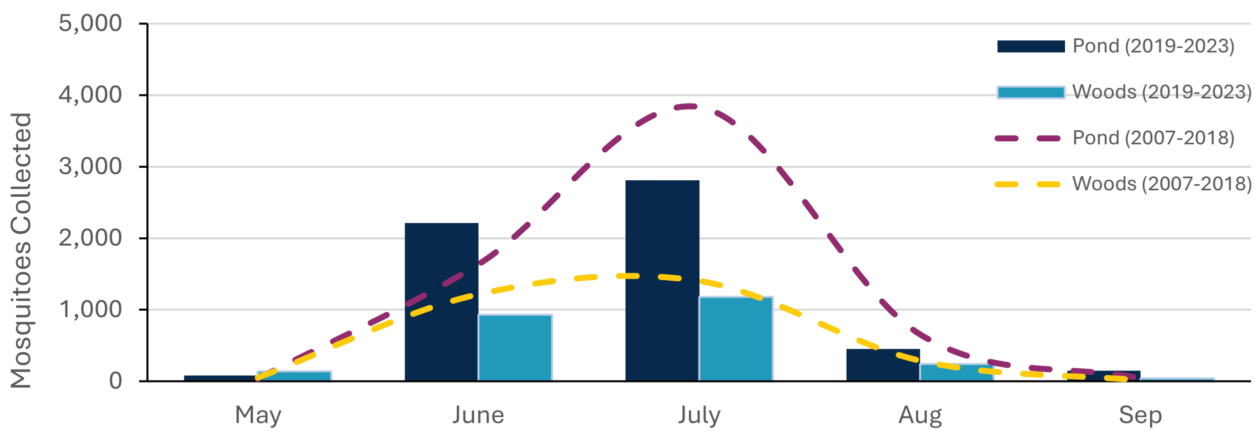
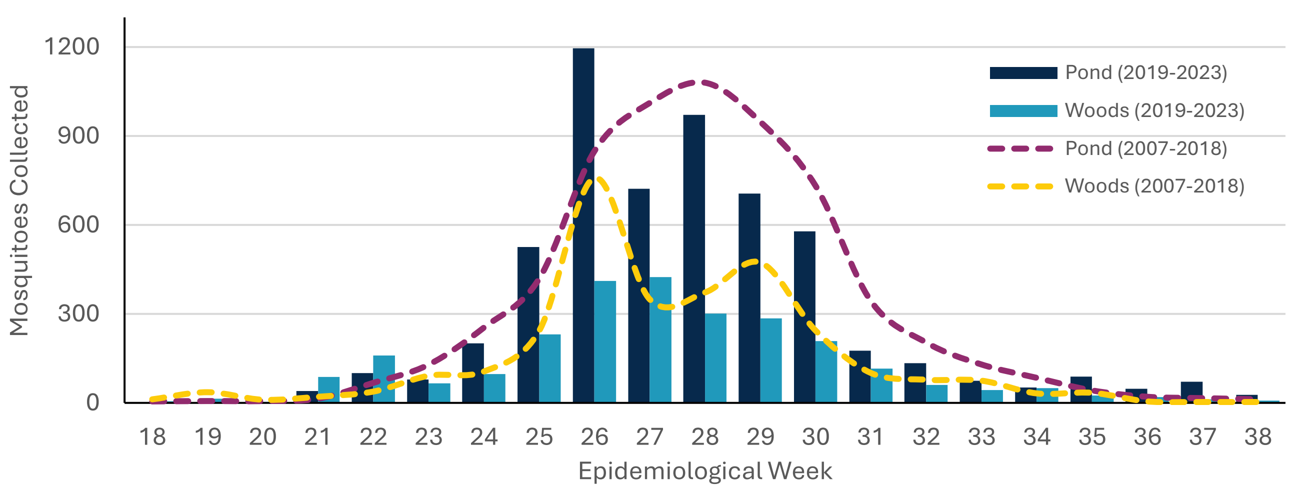


Figure 8. Average weekly collections of mosquitoes by collection site, NNWR, 2019-2023 (n=40,700) compared to average weekly collections of mosquitoes by collection site from 2007-2018 (n=95,075).



Figures 9-11 display the temporal distribution patterns for the three most common species collected at NNWR. *Culex tarsalis* numbers were the first to increase, beginning in Week 21 (early June), peaking during Week 26 (early July), but remaining relatively high through Week 29 before falling off (Fig. 9). By contrast, *Cq. perturbans* numbers rose relatively quickly from Week 25 to peak during Week 26, with numbers remaining high through Week 30 before quickly declining in Weeks 31 and 32 (Fig. 10). Both *Cx. tarsalis* and *Cq. perturbans* populations were most numerous through mid-July. For both species, most were collected at the Pond site.

Figure 9. *Culex tarsalis* average weekly collections by site from 2019-2023 (n=5,174) compared to average weekly collections from both sites from 2007-2018 (n=11,148).

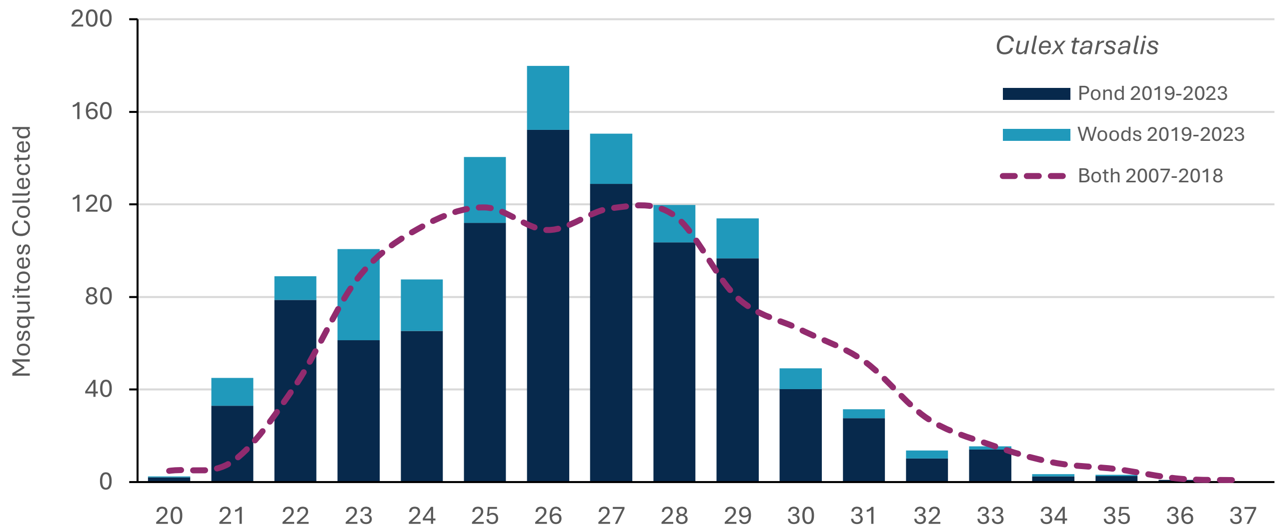
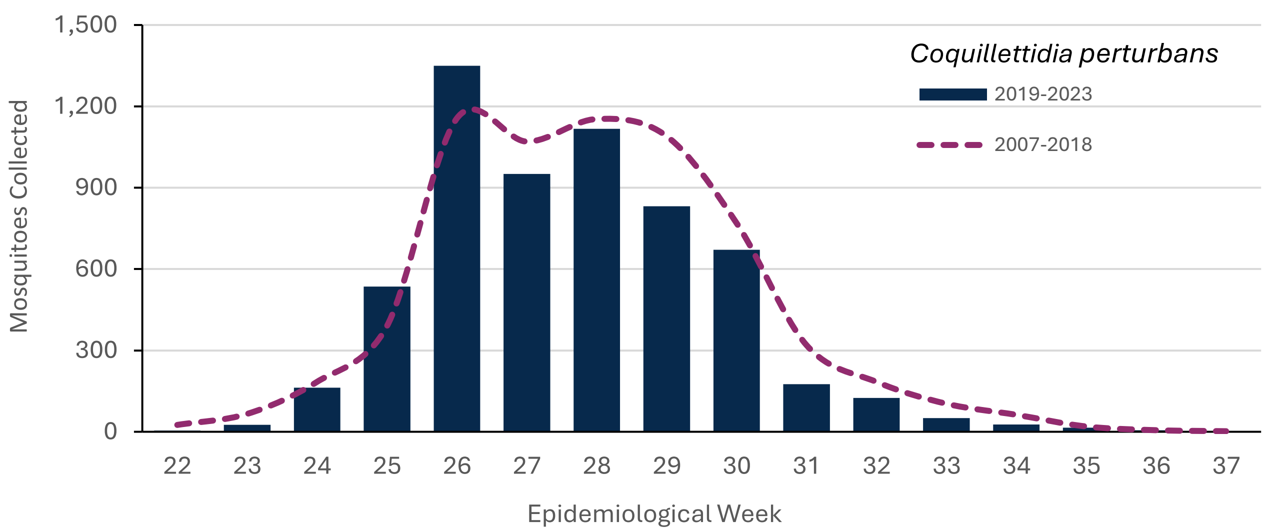


Figure 10. *Coquilletidia perturbans* average weekly collections from 2019-2023 (n=30,044) compared to average monthly collections from 2007-2018 (n=77,569).



While the substantially greater numbers of *Cq. perturbans* make this species more of a nuisance to NNWR visitors, *Cx. tarsalis'* capacity to transmit WNV makes taking preventive measures to reduce bites from this species more important.

Unlike *Cx. tarsalis* and *Cq. perturbans*, *Ae. dorsalis* numbers began increasing in Week 30 (early August) and peaked in Weeks 35-37 (early September) (Fig. 11). Although *Ae. dorsalis* was not as numerous as either of the other two, this species was collected throughout the summer and fall but predominated during the latter part of the season. Removal of the refuge's dikes in 2009 expanded the salt marsh habitat preferred by this species and its population numbers reflect that change (Fig. 12).

Figure 11. *Aedes dorsalis* average weekly collections from 2019-2023 (n=3,308) compared to average weekly collections from 2007-2018 (n=3,120).

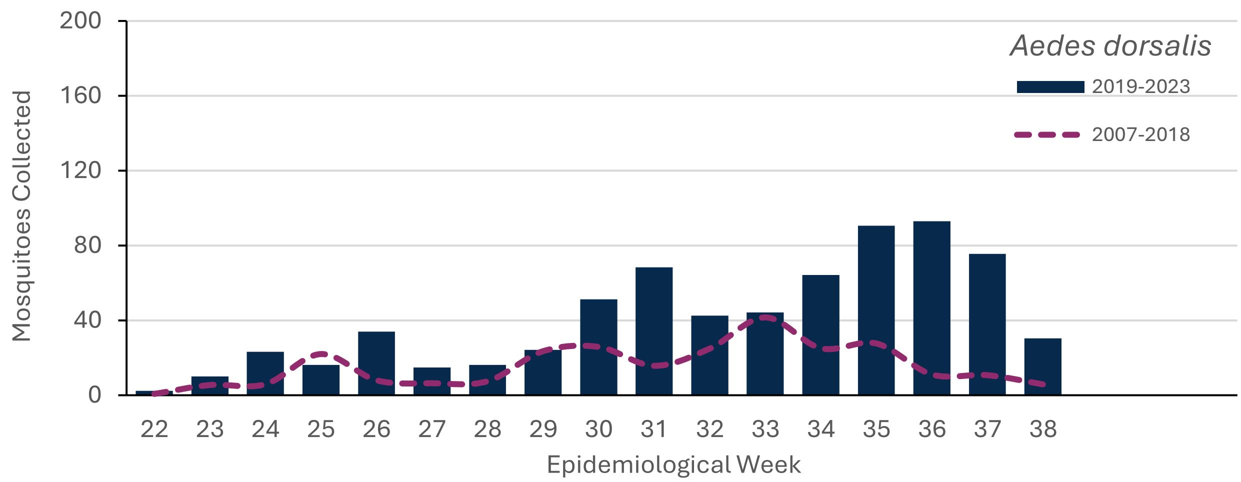
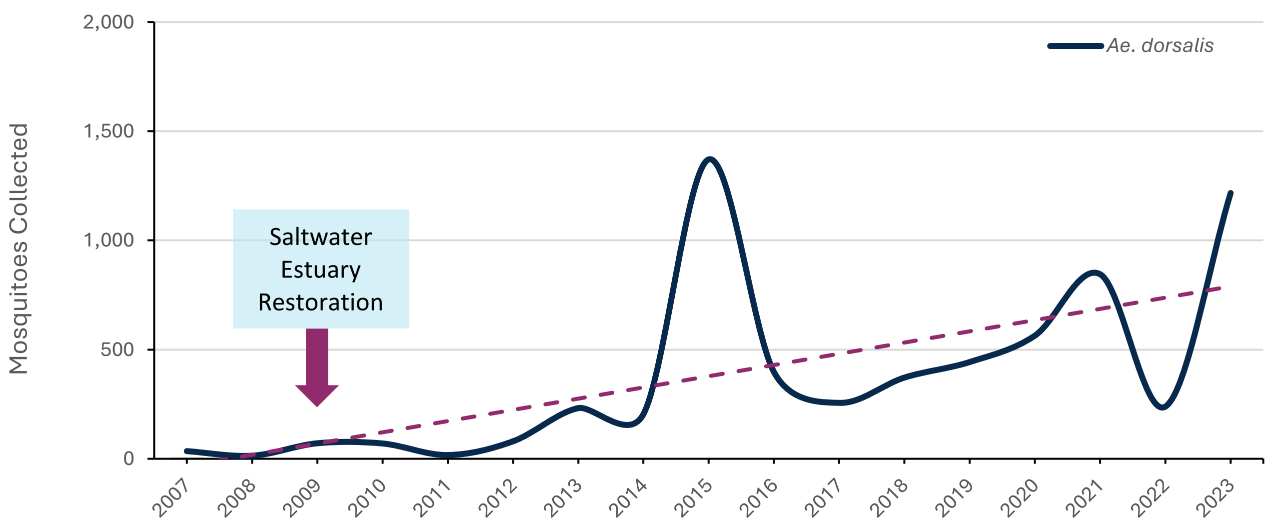


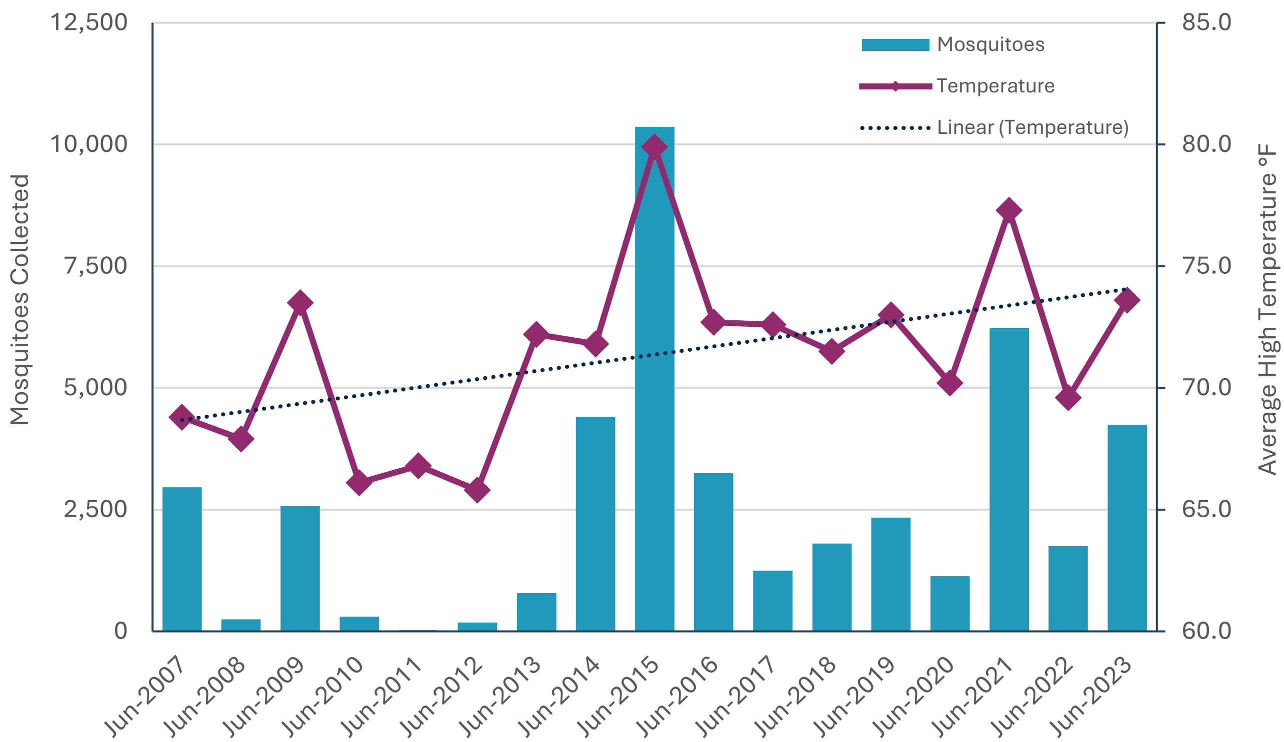
Figure 12. Population trend for *Aedes dorsalis* following 2009 dike removal, NNWR, 2007-2023 (n=6,428).



Climate Impacts

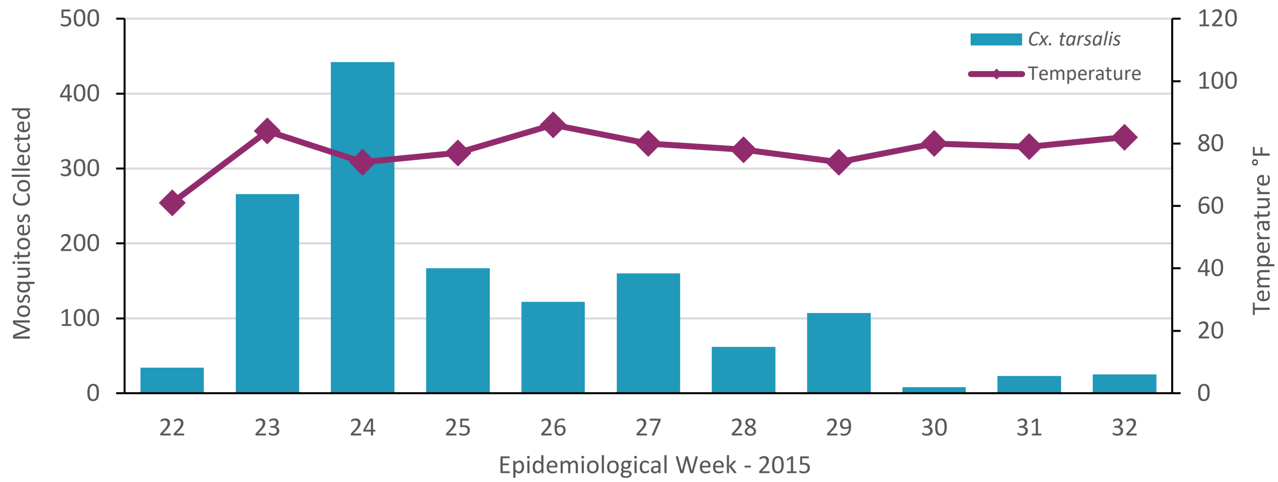
Mosquitoes are known to respond quickly to weather events and the populations at NNWR have demonstrated the impact temperature and precipitation dynamics can have on their activity. Weekly collection data provided a granular illustration of when mosquito population activity began to increase. When plotted against average June temperatures, the shift to earlier seasonal activity provides evidence that mosquito populations responded to corresponding higher temperatures (Fig. 13).

Figure 13. Total mosquito collections from both sites in June compared to average June high temperatures, NNWR 2007-2023 (n=43,838).



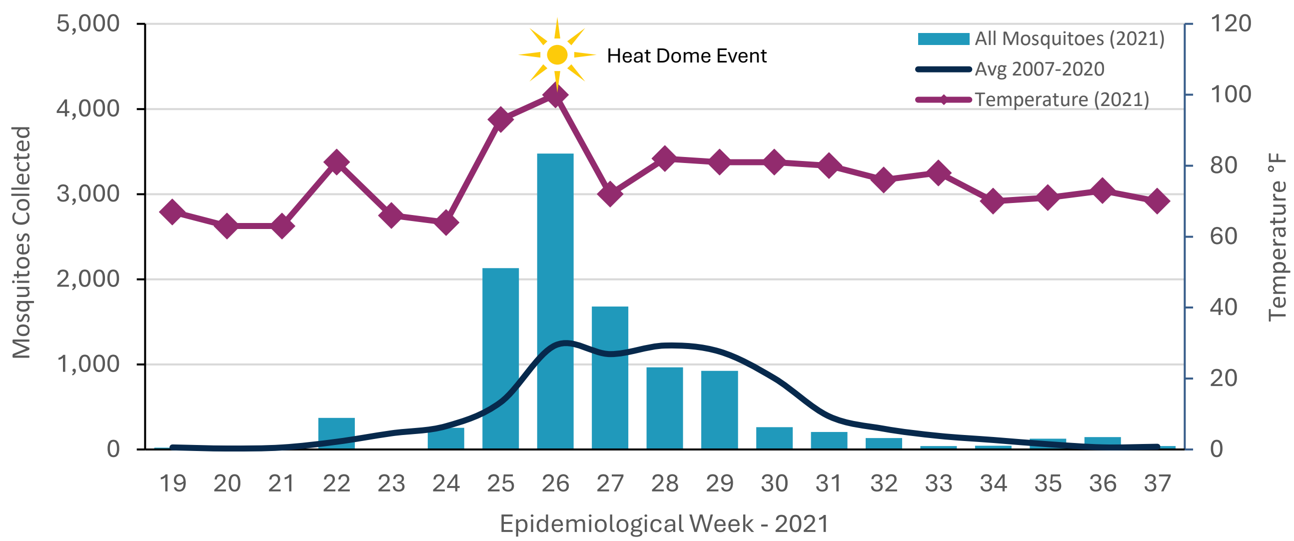
Since 2015, higher average June temperatures have corresponded with increased mosquito activity. Higher than usual temperatures in June 2015 corresponded with *Cx. tarsalis* numbers peaking during that same period in Weeks 23-24 – two weeks earlier than what was observed during the 2019-2023 seasons (Fig. 14) and four weeks earlier than what was observed from 2007-2014. June temperatures in 2015 rose from the 60s°F during Week 22 to the 80s°F by Week 23., creating ideal conditions for *Cx. tarsalis* and resulting in earlier than usual mosquito activity levels.

Figure 14. *Culex tarsalis* weekly collections (n=2,023) and temperature recorded at time of trap set up, 2015, NNWR.



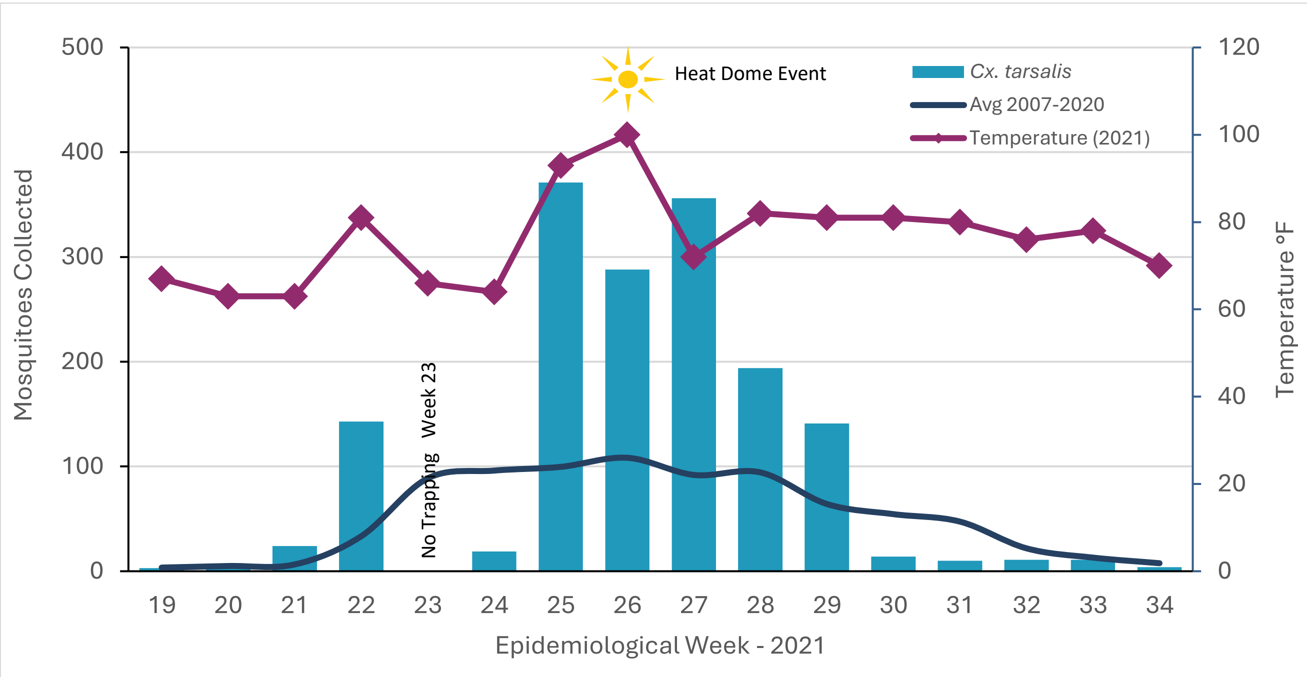
During the summer of 2021, Washington state experienced a "heat dome," where temperatures reached 100°F. The heat dome event occurred over the weekend ending Week 25 and beginning of Week 26. Total mosquito collections, dominated by *Cq. perturbans*, matched the rise and fall of temperatures and peaked during the week immediately following the heat dome event (Fig. 15). Week 26, however, corresponded to historical peak mosquito collections at NNWR. That week approximately three times as many mosquitos were collected compared to the average for years 2007-2020.

Figure 15. Weekly mosquito collections of all species in 2021 and corresponding temperature at time of trap set up compared to average collections from 2007-2020, NNWR (n=10,885).



Culex tarsalis numbers rose dramatically in Week 25 of 2021, when temperatures increased from the mid-60s°F to low 90s°F (Fig. 16). Collections decreased somewhat during the week immediately following the heat dome weekend, suggesting that more of the population was in an immature stage at that time, given that collection numbers bounced back the following week. The few days of extremely high temperatures may have ultimately helped prolong high population levels by boosting reproductive rates.

Figure 16. *Culex tarsalis* weekly collections in 2021 and corresponding temperature at time of trap set up compared to average collections from 2007-2020, NNWR (n=1,854).



The 2015 and 2021 data demonstrate how mosquito vectors like *Cx. tarsalis* respond quickly to temperature changes. Data collected by the state climatologist's office show that, over a 50-year period, average temperatures rose steadily throughout the summer months (Fig. 14). While temperature trends did not change appreciably during the spring months, average precipitation trends rose over the same time period but decreased during the summer months (Fig. 15).

These trends suggest that increased precipitation during the spring months could provide additional aquatic habitat for vector mosquitoes. Coupled with increased summer temperatures, especially during early summer or late spring, the resulting warmer water may allow some species, such as *Cx. tarsalis*, to begin development and maturation to adults earlier in the season. Such a shift in adult activity could also start arboviral amplification sooner, thereby increasing the risk of viral transmission to humans and other animals earlier while potentially extending the length of the arboviral transmission season.

Increasing summer temperatures combined with static precipitation levels suggest that suitable aquatic habitat for some species may be increasingly scarce as summer progresses, resulting in reduced activity by those species affected. The drop in *Ae. dorsalis* numbers in 2022 correspond to fewer collections in September of that year, the only year when there was no precipitation during early September. This suggests that the brackish water favored by *Ae. dorsalis* larvae was negatively impacted by increased salt content, reduced vegetation and organic matter, or a combination of these factors, that resulted from the reduced precipitation.

Figure 17. Average temperature trends, Spring (A) and Summer (B), South Puget Sound, 1973-2022 .

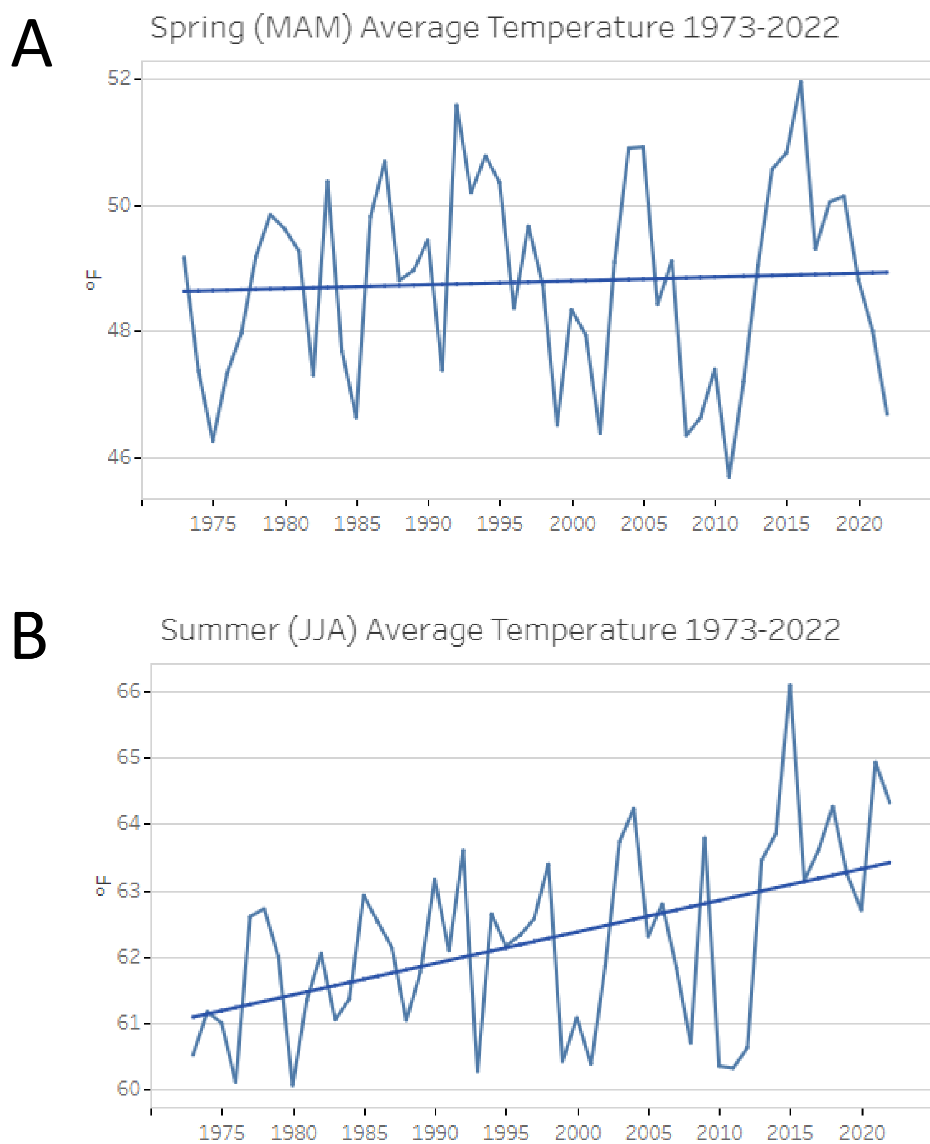
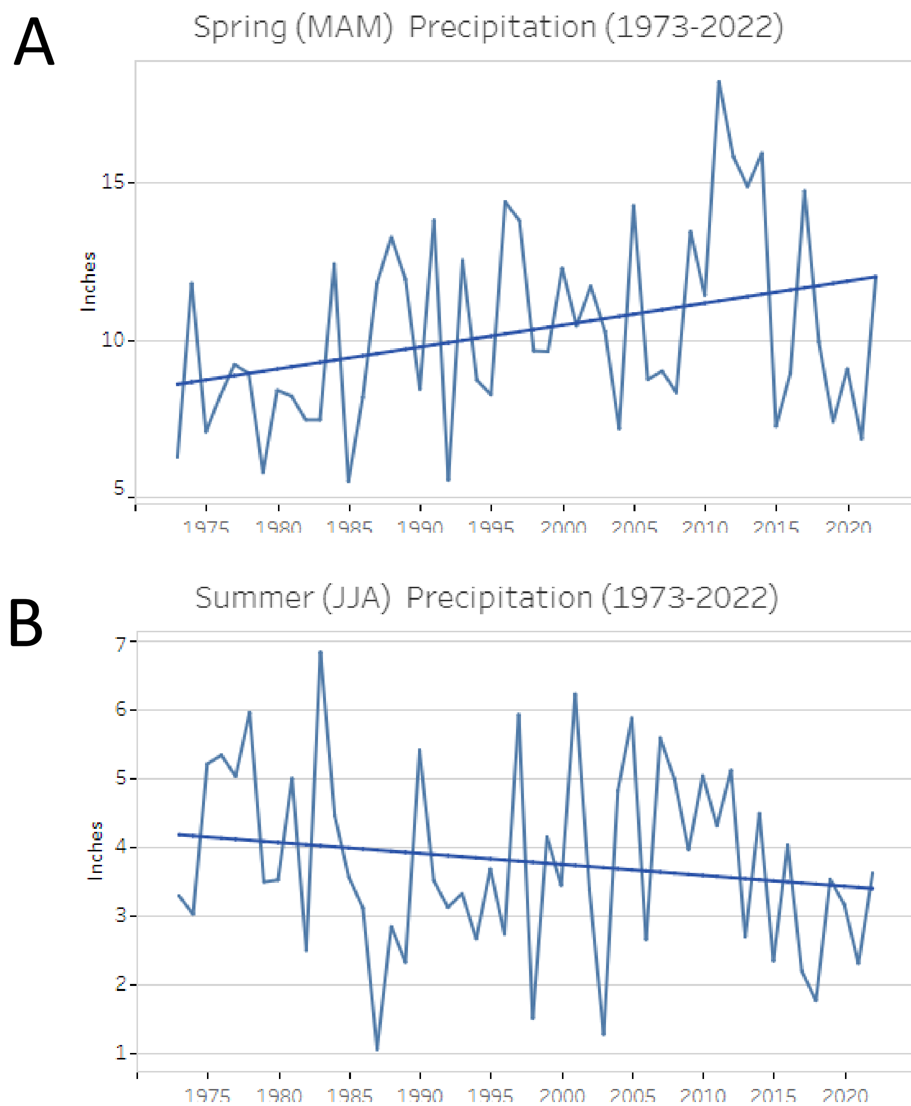


Figure 18. Average precipitation trends, Spring (A) and Summer (B), South Puget Sound, 1973-20221.



Arbovirus Testing

The two primary vectors of WNV in Washington state, *Cx. pipiens* and *Cx. tarsalis*, are both found at NNWR. Both species are tested for the presence of WNV and, when capacity has allowed, for St. Louis encephalitis (SLE) as well (Table 1). Since the dikes were removed in 2009 however, populations of *Cx. pipiens*, the northern house mosquito, have decreased to such an extent that there have not been enough specimens collected per week to allow for testing since 2013.

Arboviral testing was suspended in 2020 due to all available capacity being directed towards the COVID-19 pandemic response. Testing resumed in 2021 and 2022, but did not occur in 2023 due to workforce limitations. From 2019-2023, DOH collected 5,174 *Cx. tarsalis* specimens, testing 1,011 mosquitoes (39 pools) for WNV and SLE. All were negative for both viruses.

Table 1. Arboviral testing results of *Cx. pipiens* and *Cx. tarsalis*, NNWR, 2007-2023.

Year	*Cx. pipiens*		*Cx. tarsalis*		Total Pools Tested
	WNV	SLE	WNV	SLE	
2007	6	0	38	0	44
2008	1	1	4	4	5
2009	2	2	11	11	13
2010	2	2	9	9	11
2011	0	0	6	6	6
2012	1	1	10	10	11
2013	4	0	19	0	23
2014	0	0	15	0	15
2015	0	0	50	0	50
2016	0	0	20	0	20
2017	0	0	20	0	20
2018	0	0	22	0	22
2019	0	0	12	12	24
2020*	N/A	N/A	N/A	N/A	N/A
2021	0	0	12	12	12
2022	0	0	3	3	3
2023*	N/A	N/A	N/A	N/A	N/A
Total	**16**	**6**	**236**	**52**	**264**
*Collections were not tested due to workforce capacity demands.					

Summary

Ongoing surveillance at NNWR by DOH is providing both agencies with important information on mosquito population dynamics at the refuge including the presence of disease vector species and arboviral disease risk as well as how certain mosquito species respond to habitat modifications and climate change. Periodic shifts in temporal distribution patterns indicate that refuge WNV vector populations are responding to warmer temperatures occurring earlier in the season.

In 2018, four pools of *Cx. pipiens*, collected in Pierce County, were found positive for WNV. Pierce County lies directly north of Thurston County, where NNWR is located. These were, and remain as of 2023, the first and only detections of WNV in mosquitoes in western Washington. These detections reinforce the importance of continuing surveillance for vectors and pathogens throughout the western side of the state.

Although WNV has yet to be detected in vector mosquitoes collected at NNWR, a robust population of *Cx. tarsalis* continues to flourish at the refuge and has demonstrated the ability to shift its activity in response to changing weather patterns. This behavioral response to a changing climate increases the potential risk for disease transmission to earlier in the year as well. Continued surveillance will provide ongoing information about this critical species, as well as mosquito population trends and arboviral activity at NNWR.

Resources

Washington State Department of Health

- Mosquitoes: https://www.doh.wa.gov/CommunityandEnvironment/Pests/Mosquitoes
- West Nile virus: https://www.doh.wa.gov/YouandYourFamily/IllnessandDisease/WestNileVirus

Gjullin, C. M. and G. W. Eddy. 1972. *The Mosquitoes of the Northwestern United States*. Agricultural Research Service, United States Department of Agriculture, Technical Bulletin No. 1447, 111 p

Appendix 1 - Mosquito Species Common to Billy Frank Jr. Nisqually NWR

Aedes aboriginis
Aedes aboriginis is common in timbered coastal areas of Washington, as well as at moderate elevations in the Cascade Range and other mountainous areas. Larvae are found in snow and rain pools in wooded and semi-wooded areas. This species is not known to vector any pathogens.

***Aedes dorsalis* – Salt Marsh Mosquito**
Aedes dorsalis is the only species in the Pacific Northwest that breeds naturally in brackish or salt-water marshes. It will also breed in irrigated areas and in flooded grasslands. Females will bite at any time of the day or night but are most active in the evening. It has a recorded flight range of up to 30 miles. Natural infections of WEE and SLE have been isolated from this species.

Aedes fitchii
Aedes fitchii is most prevalent in mountainous regions but is also found near sea level. Larvae develop in flooded meadows or potholes in semi-wooded areas. This species is not known to vector any pathogens.

Aedes increpitus
Aedes increpitus is a relatively common species in western Washington. Larvae are found in open meadows and small pools in semi-wooded country from sea level to an elevation of about 6,000 feet. This species is not known to vector any pathogens.

***Aedes sierrensis* – Western Tree-hole Mosquito**
Aedes sierrensis typically breed in tree holes and in various artificial containers. It may also be found in rock pools. It is a persistent biter but has a restricted flight range. It can experimentally transmit WEE. *Aedes sierrensis* is a known vector of dog heartworm (*Dirofilaria immitis*).

***Aedes vexans* – Inland Floodwater Mosquito**
Aedes vexans is one of the most widely distributed floodwater mosquitoes in the world. This species lays its eggs in moist soil in areas liable to flooding. *Aedes vexans* overwinters as eggs, which can survive desiccation and weather extremes for up to three years. Larvae are found in mainly sunlit habitats including flood pools on prairies and semi-wooded flood plains, ditches, swamps, storm water facilities, drying bogs, and woodland pools. Habitats usually have little aquatic vegetation or algae. It has a recorded flight range of up to 30 miles. *Aedes vexans* can serve as a vector for WNV and has been found naturally infected with WEE. It has also been experimentally infected with SLE.

***Anopheles punctipennis* – Woodland Malaria Mosquito**
Anopheles punctipennis is common throughout western Washington in small open, sunlit freshwater stream pools or margins, flood-plain ground pools and drainage ditches, usually with abundant filamentous green algae (e.g. *Spirogyra*), upon which the larvae rely for food and protection from predators. It is not found in large numbers in NNWR. Larvae are found in clear shaded pools. It is susceptible to infection by malarial parasites and may serve as a vector of malaria.

Coquillettidia perturbans

Coquillettidia perturbans is the most common mosquito found at NNWR. Female *Cq. perturbans* deposit 150–350 eggs in a single raft on or near the leaves of emergent aquatic plants, like sedges, rushes, and cattails. Newly hatched, 1st instar larvae submerge and pierce the submerged roots or stems of these plants with the modified tip of their air tube for respiration. Pupae also attach themselves to plants using modified air tubes. Larval diapause, which can last up to nine months in northern populations, ends with the synchronized adult emergence in early summer. They are strong fliers, often found up to 3 miles from their immature habitats. This species is not considered a vector of disease in the western U.S.A.

***Culex pipiens* - Northern House Mosquito**

Culex pipiens develops in large numbers where human populations provide a favorable environment. Larvae develop in temporary and permanent pools, catch basins, improperly covered cesspools, and artificial containers. Females commonly enter houses and usually bite after dark. Females prefer to feed on birds and play an important role in the natural maintenance of WNV and SLE.

***Culex tarsalis* - Encephalitis Mosquito**

Culex tarsalis is the most important and widespread species in the Pacific Northwest. Larvae develop in many types of permanent and semi-permanent water such as log ponds, ditches, and marshes. Other important breeding places are pools formed by floodwater and irrigation. Adults seek shelter during the daytime, becoming most active after sundown. Preferred hosts are domestic and wild birds, but *Culex tarsalis* will readily bite humans, livestock, and other animal species. Females overwinter in storage cellars, rock piles, under rocks, on rock-covered hillsides, and in abandoned mines or caves. A flight range of 7 miles is typical of this species. *Culex tarsalis* is one of Washington's two primary vectors of WNV and SLE. It is also capable of vectoring WEE and California encephalitis viruses.

Culex territans

Culex territans, while rarely collected at NNWR, is well distributed in the Pacific Northwest. It is found in swampy areas or in other permanent or semi-permanent pools containing considerable aquatic or nonaquatic vegetation. They also occur along the grassy margins of streams. Females prefer to feed on amphibians, such as frogs. They do not bite humans.

Culiseta impatiens

Culiseta impatiens is found in small numbers at NNWR. Larvae can be found in roadside ditches, holes left from fallen or uprooted trees, and small pools with bordering brush or trees. Females will bite humans, but are seldom present in sufficient numbers to be of importance. This species is not known to vector any pathogens.

Culiseta incidens

Culiseta incidens is widely distributed at lower elevations in the Pacific Northwest, predominantly west of the Cascades. Larvae are found in both permanent and semi-permanent pools as well as artificial containers. They are often associated with *Cs. inornata* and *Cx tarsalis*. Females hibernate over the winter. They readily attack humans but occur in small numbers and are not considered important as vectors or nuisance species. This species is not known to vector any pathogens.

Culiseta inornata

Culiseta inornata occurs in largest numbers in poorly drained irrigated areas, but it has been collected in almost every type of semi-permanent and permanent water body. Larvae are often found with those of *An. freeborni* and *Cx. tarsalis*. Larvae can be found in shaded pools in forests at elevations up to 6,000 feet. It is not a serious pest of humans but can become a pest of livestock because of its long breeding season and wide distribution in irrigated areas. Females hibernate and some larvae may overwinter as well since they are very resistant to low temperatures. WEE has been isolated from this species in nature.

Culiseta minnesotae

Culiseta minnesotae is found in small numbers at NNWR. Larvae are found in unshaded pools with scattered grass fed by fresh water. Females do not readily bite humans. This species is not known to vector any pathogens.

Culiseta particeps

Culiseta particeps larvae are found in pools overgrown with vegetation. They are not considered important disease vectors or nuisance pests.

Appendix 2 – Annual Collection of Mosquitoes 2007-2023

Mosquito species most commonly collected at Billy Frank Jr. Nisqually National Wildlife Refuge from 2007-2023*‡.

Species	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	TOTAL
Aedes aboriginis	218	16	40	3	0	4	6	11	17	9	32	7	0	6	3	8	15	**395**
Aedes dorsalis	35	14	71	70	17	78	232	205	1,371	397	256	372	427	564	845	239	1,217	**6,428**
Aedes fitchii	8	0	1	4	2	3	12	4	7	9	2	7	13	6	26	5	19	**128**
Aedes increpitus	185	8	22	5	0	0	2	2	0	12	25	1	0	87	49	103	38	**539**
Aedes sierrensis	199	8	7	5	4	4	128	123	2	7	135	24	0	35	186	447	431	**1,745**
Aedes vexans	3	78	34	19	9	1	5	5	29	16	2	3	0	2	51	16	39	**312**
Anopheles punctipennis	46	8	36	16	17	26	44	37	127	87	32	43	26	31	33	19	105	**733**
Coquillettidia perturbans	6,092	4,726	2,732	5,641	4,898	4,267	7,857	12,149	13,026	6,012	5,243	4,926	4,381	3,887	7,806	7,251	6,719	**107,613**
Culex pipiens	178	48	138	33	6	30	54	21	24	24	17	26	16	6	3	1	9	**634**
Culex tarsalis	1,742	173	1,498	387	199	486	723	731	2,023	1,142	986	1,058	1,452	333	1,854	465	1,539	**16,322**
Culex territans	0	0	0	0	0	0	0	1	0	4	1	1	0	0	0	0	0	**7**
Culiseta impatiens	0	1	3	1	0	0	0	0	1	1	1	0	1	1	0	0	1	**11**
Culiseta incidens	2	15	0	0	0	0	2	1	1	13	1	2	10	1	4	1	17	**70**
Culiseta inornata	19	2	17	3	0	2	5	2	6	8	14	1	9	4	3	8	27	**130**
Culiseta minnesotae	9	0	20	5	0	0	19	19	10	7	0	9	8	2	3	6	10	**127**
Culiseta particeps	32	19	11	2	1	2	2	2	19	35	22	10	14	20	9	25	87	**312**
TOTAL	**8,768**	**5,116**	**4,630**	**6,194**	**5,153**	**4,905**	**9,091**	**13,313**	**16,663**	**7,783**	**6,679**	**6,490**	**5,904**	**4,985**	**10,875**	**8,594**	**10,273**	**135,506**

*Collections of <5 specimens of species not shown in the table included: *Aedes intrudens, Ae. sticticus, Ae. ventrovittis, Anopheles freeborni*, and *Culiseta morsitans*.
‡Mosquito species formerly listed under the genus *Ochlerotatus* are now listed under the genus *Aedes*.