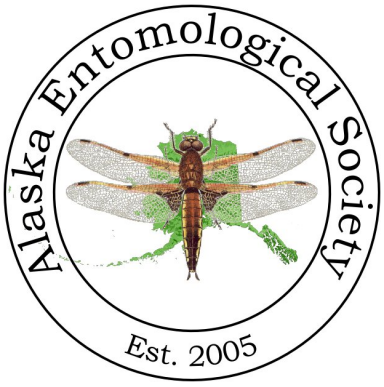

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In this issue:

Miller Creek Watershed aquatic invertebrate inventory

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Abstract

Because benthic macroinvertebrates and zooplankton are susceptible to the pesticide rotenone, surveys of freshwater macroinvertebrates were conducted in the Miller Creek Watershed, Kenai Peninsula, Alaska ahead of a planned rotenone treatment in fall 2021. Currently, 32 of 32 planned samples have been collected in 2021. Another 32 post-treatment invertebrate samples are planned in 2022 to enable comparison of pre- and post-treatment freshwater invertebrate communities.

Introduction

Invasive northern pike (*Esox lucius* Linnaeus, 1758) were discovered in the Miller Creek watershed in 2019 (Kenai National Wildlife Refuge, 2021) and a rotenone treatment to eradicate pike from the drainage was planned for fall 2021.

Benthic macroinvertebrates and zooplankton are susceptible to rotenone (Finlayson et al., 2018), some of them dying at lower rotenone concentrations than fish (Rach et al., 1988). Planktonic crustations decline rapidly after rotenone treatments, generally taking months to years to fully recover (Kiser et al., 1963; Anderson, 1970). In other southcentral Alaska lakes, most pre-treatment plankton species were present in lake water with in one to two years of treatment and plankton abundace returned to pre-treatment levels in the third year (Chlupach, 1977). On the Kenai Peninsula, most invertebrate taxa that were present in lakes before present were again present one year after treatment (Massengill, 2014, 2017).

Finlayson et al. (2018) recommended sampling aquatic invertebrates before and after rotenone treatments to document survival and recovery of these animals.

Methods

We began collecting a small number of invertebrate specimens in the Miller Creek watershed after northern pike and then elodea were discovered in the drainage. A small number of additional samples were collected in 2020.

A more thorough inventory effort began in 2021. We generally followed the methods used in previous aquatic inventories that were performed before and after rotenone applications on the Kenai Peninsula (Massengill, 2014, 2017) with the exception that most identifications are to be obtained by metabarcoding instead of morphological identifications.

Aquatic mollusks were sampled under Alaska Department and Fish and Game Aquatic Resource Permit No. SF2021-134.

The rotenone treatment of these three connected water bodies was completed on October 4–7, 2021.

Study area

The waterbodies surveyed were those in which rotenone will be applied, the same water bodies in which northern pike have been detected. These include North Vogel Lake, Vogel Lake, and Miller Creek. North Vogel Lake (14 ha) and Vogel Lake (49 ha) are separated by about 200 m and connected by a small stream that flows through a wetland. Both lakes are similar in terms of water quality metrics (Meyer, 2021). Miller Creek drains Vogel Lake, running approximately 7 km to Cook Inlet.

Field sampling

We generally followed the methods of Massengill (2014, 2017) for lake sampling aquatic invertebrates. Each of the three waterbodies was to be surveyed once in July and once in August. Sampling was to be conducted in the same temporal windows in 2021 and 2022.

At North Vogel Lake, Vogel Lake, and upper Miller Creek we sampled twice in 2021: first on July 20–23 and

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second on August 28. We sampled at 15 sites using three methods. We failed to make it out to the mouth of Miller Creek in July and August, collecting samples there only on September 13, 2021.

Sample sizes were chosen to be similar to those used by Massengill (2014, 2017). Massengill (2014), to survey invertebrates in 38 ha Scout Lake, collected 3 light trap samples, 5 Ekman samples, 5 D-net samples, and 2 Wisconsin net samples. For 157 ha Stormy Lake, Massengill (2014, 2017) collected 5 Ekman samples, 5 D-net samples, and 3 Wisconsin net sample.

Table 1: Numbers of samples to be collected in the three water bodies by sampling method.

Water body	D-net	Ekman dredge	Wisconsin net	Total
North Vogel Lake	2	2	1	5
Vogel Lake	3	3	2	8
Miller Creek	3	0	0	3
Total	8	5	3	16

At each of two visits per year we collected 3 D-net samples, 3 Ekman dredge samples, and 2 Wisconsin net samples in Vogel Lake; 2 D-net samples, 2 Ekman dredge samples, and 1 Wisconsin net sample in North Vogel Lake; and 3 D-net samples in Miller Creek, a total of 16 invertebrate samples per visit (Table 1), 32 samples per year, and 64 samples over the two year project.

In addition to the 64 samples intended to be processed by metabarcoding, invertebrates were collected opportunistically while the authors were working within the study area.

Field methods generally followed Massengill (2014, 2017). We took vertical plankton tows in the deepest parts of the lakes using an Aquatic Research Instruments Wisconsin net. We sampled littoral areas using with D-nets. We obtained benthic samples using either an AMS Incorporated model 445.11 Ekman dredge or an AMS Incorporated model 445.60 stainless steel dredge. Most benthic samples were sorted using a series of sieves.

Samples were collected into ethanol or SK picglobal 99.9% pure propylene glycol. Samples intended for metabarcoding were collected into propylene glycol or, in the case of two samples, collected into ethanol and then transferred to propylene glycol.

Specimen processing

Specimens were deposited in the Kenai National Wildlife Refuge's entomology and invertebrate collections (international collection coden: KNWR) and managed with Arctos (`https://arctos.database.museum/`).

For the morphologically identified specimens, we used the following references: Borror et al. (1989), Collet (2008), Durfee et al. (2005), Haney et al. (2013), Hatch (1953), Kenner (2009), Marx (1957), Merritt and Cummins (1996), Merritt et al. (2008), Reid (1987), Riley et al. (2002), Roughley (2000), Wallis (1933), and White (1983).

Metabarcoding samples were stored in a -23 °C freezer exept when samples were being processed. Invertebrates were separated from debris by hand under a dissecting microscope. Care was taken to reduce possible cross-contamination of DNA among samples. Samples were shipped out on ice on September 29, 2021, arriving the next day at Molecular Research Laboratory (Shallowater, Texas, `http://www.mrdnalab.com`).

We chose to use the *mlCOIintF/jgHCO2198* (GGWACWGGWTGAACWGTWTAYCCYCC/TAIACYTCIGGRTGICCRAARAAYCA) primer set of Leray et al. (2013) for PCR, targeting a 313 bp region of the COI DNA barcoding region. This primer set has been successfully used for a wide variety of invertebrates, including terrestrial invertebrates (Bowser et al., 2020), freshwater macroinvertebrates (Hajibabaei et al., 2019), freshwater plankton (Yang et al., 2017), and stomach contents of freshwater fish (Bowser and Bowser, 2020).

Analysis

We plan to process raw read data using MetaWorks (Porter and Hajibabaei, 2020), running this on the Yeti supercomputer (USGS Advanced Research Computing, 2021). As in Massengill (2014, 2017), the metric will be simple presence or absence of taxa within the lakes before and after treatment.

Results

Specimen data and images are available via an Arctos project (`https://arctos.database.museum/project/10003613`). Specimen records are available via a search for records from this project (`https://arctos.database.museum/SpecimenResults.cfm?project_id=10003613`). All Arctos records from this project are also published

to the Global Biodiversity Information Facility (GBIF, https://www.gbif.org/) via the VertNet Integrated Publishing Toolkit (http://ipt.vertnet.org/). "Research grade" iNaturalist observations are also published to GBIF.

Georeferenced photo observations have been gathered in a project on iNaturalist.org at https://www.inaturalist.org/projects/miller-creek-invertebrate-inventory.

A corresponding record was also made on ServCat (https://ecos.fws.gov/ServCat/Reference/Profile/139305). Raw metabarcoding data are available from Servcat (https://ecos.fws.gov/ServCat/Reference/Profile/139306).

Table 2: Invertebrates collected.

Identification Kingdom Phylum Class Order Family Subfamily, etc.	**Miller Creek**	**North Vogel Lake**	**Vogel Lake**
Annelida			
Clitellata			
Arhynchobdellida			
Haemopidae sp. BOLD-A15			KNWR:Inv:76
Hirudinea	KNWR:Inv:122		KNWR:Inv:118, KNWR:Inv:125
Rhynchobdellida			
Glossiphoniidae sp. MOBIL9915-19			KNWR:Inv:54
Piscicolidae			
Piscicola			KNWR:Inv:108
Arthropoda			
Arachnida		KNWR:Ento:11429	KNWR:Ento:11419, KNWR:Ento:11448
Branchiopoda			
Diplostraca			
Daphniidae			
Daphnia O. F. Müller, 1785 ?			KNWR:Inv:119
Insecta		KNWR:Ento:11430	KNWR:Ento:11417
Insecta ?			KNWR:Ento:11421, KNWR:Ento:11439
Coleoptera			
Chrysomelidae		KNWR:Ento:11413	
Chrysomelidae ?	KNWR:Ento:11456		
Donacia hirticollis Kirby 1837	KNWR:Ento:11449		
Galerucella nymphaeae (Linnaeus 1758)			KNWR:Ento:11420
Galerucella nymphaeae (Linnaeus 1758) ?	KNWR:Ento:11450, KNWR:Ento:11472, KNWR:Ento:11473, KNWR:Ento:11474		
Dytiscidae			KNWR:Ento:11443
Haliplidae			

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Identification	Miller Creek	North Vogel Lake	Vogel Lake
Haliplus immaculicollis Harris 1828 ?		KNWR:Ento:11426	KNWR:Ento:11446, KNWR:Ento:11461, KNWR:Ento:11462, KNWR:Ento:11463, KNWR:Ento:11464, KNWR:Ento:11466, KNWR:Ento:11479
Haliplus leechi Wallis 1933 ?			KNWR:Ento:11438, KNWR:Ento:11465
Diptera	KNWR:Ento:11395, KNWR:Ento:11451, KNWR:Ento:11475, KNWR:Ento:11476, KNWR:Ento:11477, KNWR:Ento:11478		
Ceratopogonidae			KNWR:Ento:11433
Chironomidae			KNWR:Ento:11434, KNWR:Ento:11442
Tanypodinae		KNWR:Ento:11431	
Tanypodinae ?			KNWR:Ento:11422
Paraboreochlus ?			KNWR:Ento:11416
Parochlus Enderlein, 1912 ?		KNWR:Ento:11425	
Culicidae ?			KNWR:Ento:11459
Empididae ?	KNWR:Ento:11410		
Tabanidae ?		KNWR:Ento:11414	
Ephemeroptera			
Baetidae ?			KNWR:Ento:11447
Caenidae			
Caenis youngi Roemhild 1984			KNWR:Ento:11384
Heptageniidae	KNWR:Ento:11468		
Odonata			
Aeshnidae	KNWR:Ento:11455		
Aeshna eremita Scudder, 1866			KNWR:Ento:11412
Boyeria ?			KNWR:Ento:11423
Remartinia Navás, 1911 ?			KNWR:Ento:11444
Coenagrionidae	KNWR:Ento:11452	KNWR:Ento:11428	KNWR:Ento:11460
Coenagrionidae ?	KNWR:Ento:11453		
Amphiagrion ?			KNWR:Ento:11435
Corduliidae ?			KNWR:Ento:11437
Cordulia Needham, 1901		KNWR:Ento:11424	
Cordulia Needham, 1901 ?			KNWR:Ento:11418
Libellulidae		KNWR:Ento:11411	KNWR:Ento:11445
Libellulidae ?			KNWR:Ento:11436
Plecoptera			
Chloroperlidae ?	KNWR:Ento:11470		
Perlodidae ?	KNWR:Ento:11467		
Trichoptera	KNWR:Ento:11458	KNWR:Ento:11409	
Hydropsychidae	KNWR:Ento:11398		
Hydropsychidae ?	KNWR:Ento:11469		
Leptoceridae ?	KNWR:Ento:11457		
Malacostraca			

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Identification	Miller Creek	North Vogel Lake	Vogel Lake
Amphipoda	KNWR:Ento:11454	KNWR:Ento:11427	KNWR:Ento:11432, KNWR:Ento:11440, KNWR:Ento:11441
Gammaridae			KNWR:Ento:11415
Gammarus lacustris G. O. Sars, 1863			KNWR:Ento:11383
Ostracoda ?			KNWR:Inv:120
Mollusca			
Bivalvia			
Sphaeriidae		KNWR:Inv:68, KNWR:Inv:110	KNWR:Inv:74
Sphaerium Scopoli, 1777			
Sphaerium sp. BOLD:AAG0345		KNWR:Inv:53	
Gastropoda	KNWR:Inv:123	KNWR:Inv:69, KNWR:Inv:114, KNWR:Inv:115	KNWR:Inv:73, KNWR:Inv:111, KNWR:Inv:112, KNWR:Inv:117, KNWR:Inv:126
Basommatophora			
Planorbidae			
Helisoma			KNWR:Inv:116
Helisoma ?		KNWR:Inv:113	
Pelecypoda		KNWR:Inv:66, KNWR:Inv:67	KNWR:Inv:71, KNWR:Inv:72, KNWR:Inv:121
Platyhelminthes			
Cestoda			KNWR:Inv:109
Porifera			
Demospongiae			
Haplosclerida			
Spongillidae			
Spongilla lacustris	KNWR:Inv:124		

Discussion

Donacia hirticollis Kirby, 1837 had not previously been documented from the Kenai National Wildlife Refuge.

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