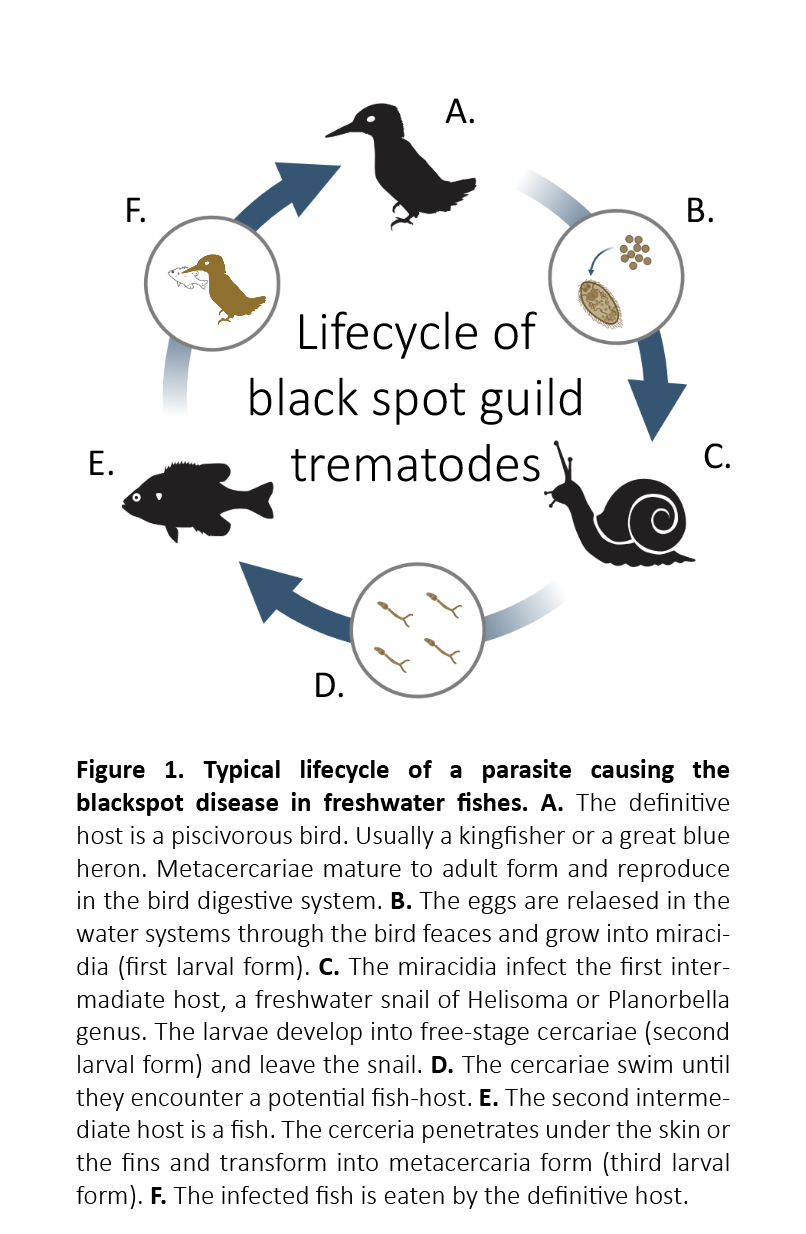
# 2 | METHODS

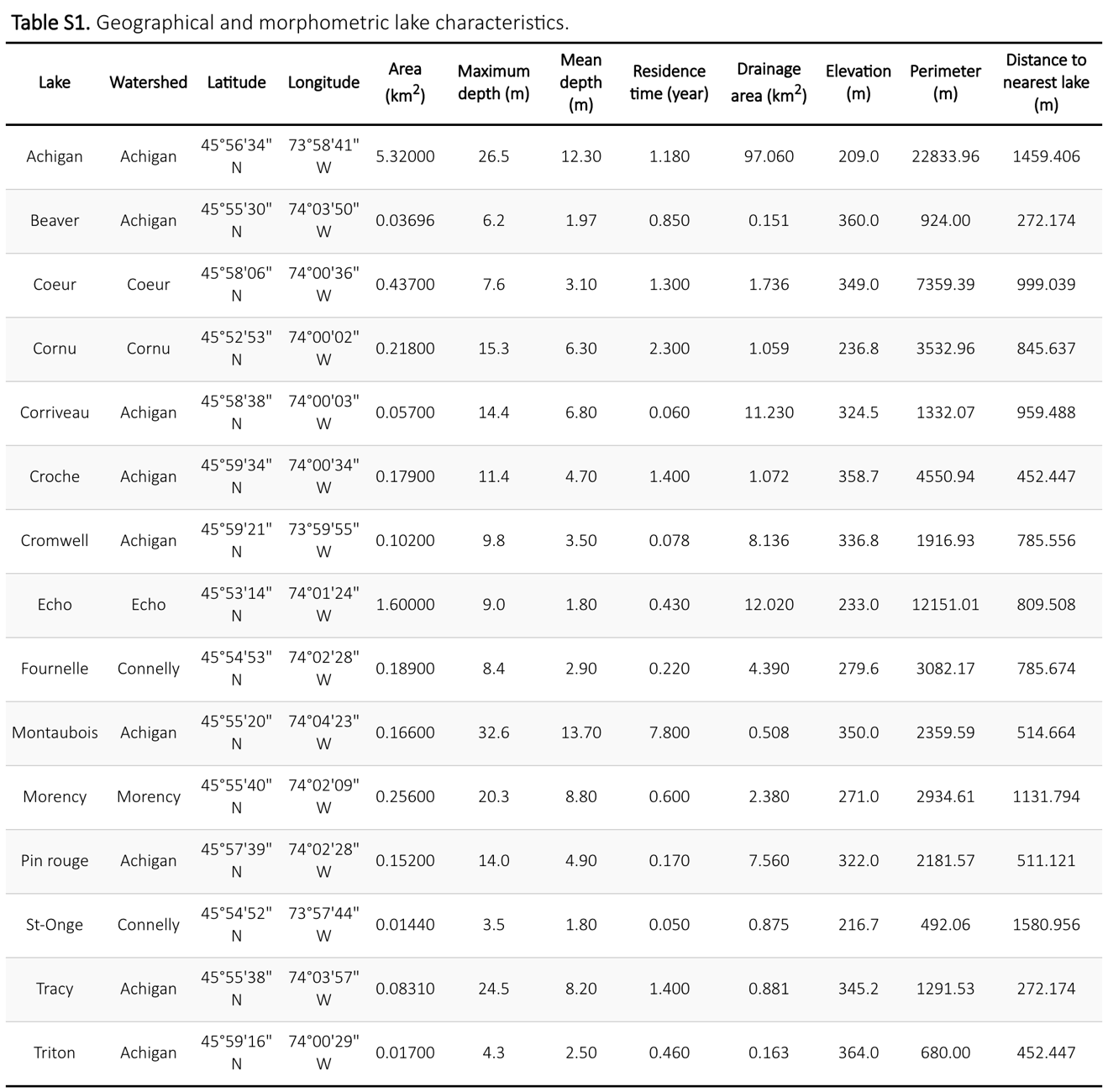
## 2.1. Host-parasite system

The black spot disease is a common infection in marine and freshwater fishes caused by some Digenean trematodes (flukes) (Kurochkin & Biserova, 1996). Some of these parasites (*e.g., Posthodiplostomum cuticula, Uvulifer ambloplitis*, *Crassiphiala bulboglossa* and *Apophallus brevis*) have been studied for a long time and yet, spatiotemporal infection patterns and their relation to environmental conditions are elusive. Black spot causing trematodes all have a similar complex life cycle requiring a snail, a fish and a piscivorous bird as hosts (Figure X). The fish is the second intermediate host of the parasite where it encysts under the skin, in the fins or the muscles (Hoffman, 1956; Krull, 1932, 1934). Because the parasite is dormant in its fish-host, it can survive many years while it waits to be eating by a piscivorous bird (Hoffman & Putz, 1965) leading to increased parasite load with fish age (Lemly & Esch, 1984). The distinctive black cyst results of the melanin pigment stimulation by the larva penetration in the fish body (Davis, 1967; Lemly & Esch, 1984). Because they have similar requirements and cause the same symptoms, we will refer to black spot trematodes as a guild from now on. Akin to other parasitic diseases, infection parameters can vary across time, space, and species. For example, black spot disease prevalence in Bluegill sunfish (*Lepomis macrochirus*) living in a pond reaches its minimum around April/May and its maximum around September/October (Lemly & Esch, 1984). Because of the snail first intermediate host requirements such as macrophyte and shallow waters, infection levels in fish-host are usually higher is lentic rather lotic systems and in the littoral zone rather than pelagic zone because it maximize the encounter window (Ondrackova et al., 2004). Because black spot infection is caused by a parasite guild (more than one trematode species), a large range of fish can show symptoms of this infection. However, within a system, some species tend to be more heavily infected than others. For instance, Lemly & Esch (1984) found that Bluegills were more heavily infected than Largemouth bass (*Micropterus salmoides*).

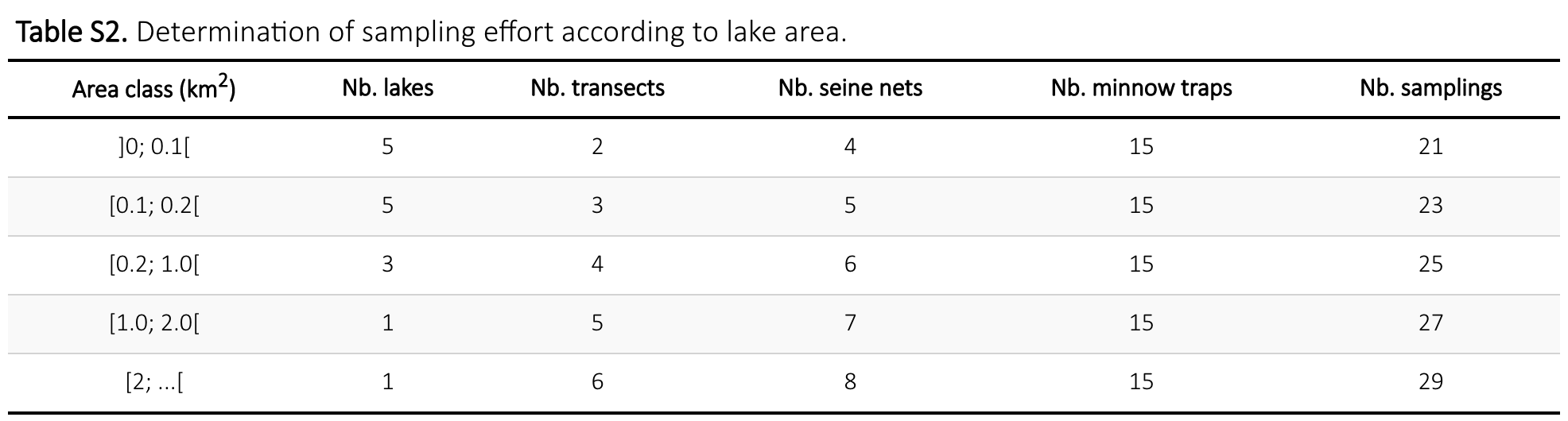


## 2.2. Study area and sampling design (Data collection)

We sampled 15 lakes from six watersheds in Saint-Hippolyte, QC, Canada (Figure X). Corriveau, Croche, Cromwell and Triton lakes are in the protected area of the biology station of the Université de Montréal while the other lakes are surrounding by private properties. Considering their geographical proximity, we assume that all lakes are exposed to the same climatic conditions. The lakes were selected nonrandomly according to accessibility and availability of morphometric data (see Appendix S1 – Table S1). Field work was restricted to unrainy days to avoid sampling bias due to meteorological effects.



The number of samplings within lakes was determined according to lake area except for minnow traps (Appendix S1 – Table S2). As it needed less time and manipulations, all the 15 minnow traps were set in each lake to maximize the number of captures.

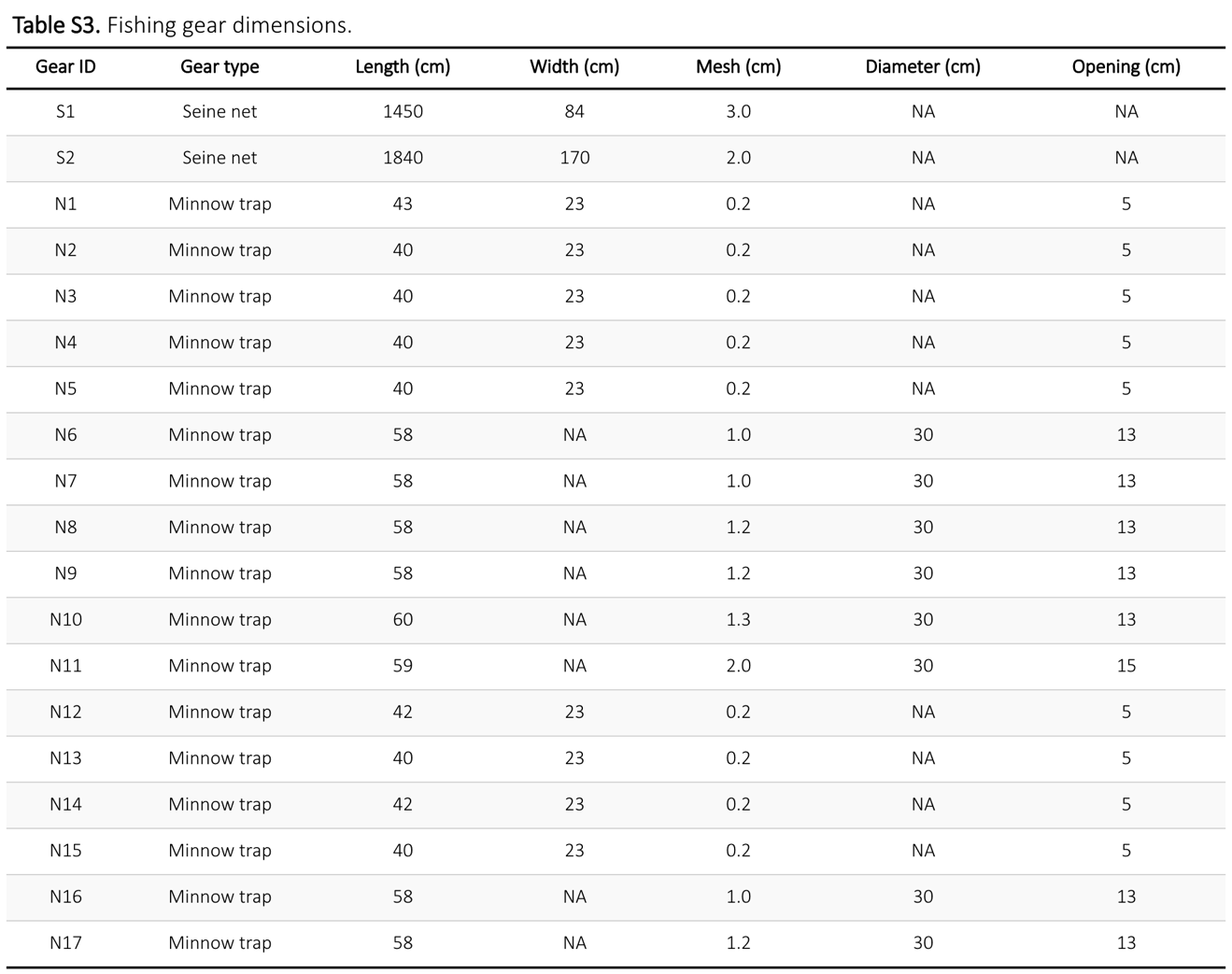


Animal handling was approved by the Université de Montréal’s animal care committee (protocol number 22-025) and scientific fishing permit was delivered by the Ministère des Forêts, de la Faune et des Parcs (MFFP) of Québec (2022-05-16-1971-15-S-P).

Three sampling methods were used to assess prevalence infection and maximize chances of catching species richness. Fishing sampling took place from mid-June to end of July 2022. A seine net was used for daytime sampling and two size of minnow traps were set for dusk sampling (see Appendix S1 – Table S3 for fishing gear dimensions). Minnow traps were set at approximatively equal distance along the shore, from 4PM to 8PM, to target species that are most active at dusk. Half of the minnow traps were baited (3 large and 5 small traps) to sample diverse feeding preferences and habits. All fishing gear was cleaned between lakes following MFFP recommendations to prevent exotic species contamination.

All fishes were counted, identified to species level, and measured (estimation of the total length to the nearest centimeter) directly after capture and released afterwards. Northern redbelly dace (*Chrosomus eos*) and Finescale dace (*Chrosomus neogaeus*) individuals were grouped into *Chrosomus spp*. naming as the two species hybrid in our system and cannot be distinguished based on morphology in the field (Leung et al., 2017). Both juveniles and adults were considered in this study as all individuals are vulnerable to black spot infection.

50 meters snorkeling transects along the lakes shore were made to assess black spot infection in the fish community in August 2022. Due to time constraints, no transects were made in lake Beaver, Montaubois, Tracy and St-Onge were excluded of transect sampling because of null infection level or low fish abundance in fishing sampling. For the site selection, we considered emplacements that were approximately between 0.5 and 3 meters deep, not fully covered by macrophytes, not obstructed by docks and preferably with some vegetal, rock or trunk refuges for the fishes. The sites were first chosen and flagged at every 10 meters in end-May. Transects were done by two observers at the time covering each a field of view of 1 meter radius and moving forward at a pace of 3 minutes by 10 meters for a total time of 15 minutes by transect. The fishes coming in the field of view from behind the observer were not counted. All fishes were identified to the lower taxonomic level possible and black spot infection was noted. All cyprinids were grouped into the same category as they are more difficult to identify to species level and usually move faster. Following the prevalence assessment, the description of habitat characteristics was made for every transect. For each 10 meters, the percentage of substrate category (silt, sand, rock and metric block), the coverage of macrophyte, the number of trunk (or large branch), and the mean depth was estimated by each observer. Temperature, dissolved oxygen, conductivity and pH were measured at mid-depth at the beginning of every transect with a YSI ProDSS Multi-Parameter Water Quality Meter. 1L of unfiltered water was taken in the field at mid-depth in previously acid-washed HDPE bottle for carbon and nutrients content analysis. Samples were placed in a dark cooler until brought back to the laboratory where the 1L sample was separated in previously acid-washed 40 mL vials for total organic carbon (TOC), and 500 mL HDPE bottle for total nitrogen (TN) and total phosphorus (TP). TOC samples were placed in 4°C refrigerator until analyze within the week while TN and TP samples were kept in -20°C freezer until processing. TOC samples contents were measured on … autosampler. TN samples were oxidized with persulfate (O’Dell 1996a; O’Dell 1996b; USEPA-353.2, USEPA-350.1) and then measured on a Lachat QuikChem 8000 Injection Analyzer. TP samples were previously oxidized with persulfate and analyszed on a Astoria 2 Segmented Flow Analyzer (O’Dell 1993; USEPA-365.1; USEPA-365.3).



General patterns and host specificity

A total of 11 295 fishes were recorded - 4324 fishes, belonging to 15 species were sampled through fishing methods (minnow traps and seine net) and 6964 fishes, belonging to 5 taxonomic groups (4 species and 1 family) were observed in snorkeling transects (see Table X et X in annexe X for more details). The mean length of all fish captured was 5.59 ± 2.96 cm (see Table X in annexe X for more details).

The main host for the black spot disease in our system (all lakes – regional scale) is *Lepomis gibbosus* (0.73 : 0.36) followed by *Ambloplites ruspestris* (0.47 : 0.46), *Perca flavescens* (0.39 : 0.41), *Micropterus dolomieu* (0.24 : 0.31), *Semotilus atromaculatus* (0.13 : 0.31), *Pimephales notatus* (0.06 : 0.12)and *Pimephales promelas* (0.01 : 0.01). (Annexe prevalence).

in the historical indigenous lands of Anishinabewaki ᐊᓂᔑᓈᐯᐗᑭ and Omàmìwininìwag (Algonquin) \*\*https://native-land.ca/\*.