STANDARDIZED FIELD SAMPLING METHOD FOR MONITORING THE OCCURRENCE AND RELATIVE ABUNDANCE OF THE ROCKY MOUNTAIN SCULPIN (COTTUS SP.) IN CANADA

Camille J. Macnaughton, Tyana Rudolfsen, Doug A. Watkinson, and Eva C. Enders

Fisheries and Oceans Canada
Ecosystems and Oceans Science
Central and Arctic Region
Freshwater Institute
Winnipeg, MB
R3T 2N6

2019

Canadian Technical Report of Fisheries and Aquatic Sciences 3313

Fisheries and Oceans Canada Pêches et Océans Canada

Canada

Canadian Technical Report of Fisheries and Aquatic Sciences

Technical reports contain scientific and technical information that contributes to existing knowledge but which is not normally appropriate for primary literature. Technical reports are directed primarily toward a worldwide audience and have an international distribution. No restriction is placed on subject matter and the series reflects the broad interests and policies of Fisheries and Oceans Canada, namely, fisheries and aquatic sciences.

Technical reports may be cited as full publications. The correct citation appears above the abstract of each report. Each report is abstracted in the data base *Aquatic Sciences and Fisheries Abstracts*.

Technical reports are produced regionally but are numbered nationally. Requests for individual reports will be filled by the issuing establishment listed on the front cover and title page.

Numbers 1-456 in this series were issued as Technical Reports of the Fisheries Research Board of Canada. Numbers 457-714 were issued as Department of the Environment, Fisheries and Marine Service, Research and Development Directorate Technical Reports. Numbers 715-924 were issued as Department of Fisheries and Environment, Fisheries and Marine Service Technical Reports. The current series name was changed with report number 925.

Rapport technique canadien des sciences halieutiques et aquatiques

Les rapports techniques contiennent des renseignements scientifiques et techniques qui constituent une contribution aux connaissances actuelles, mais qui ne sont pas normalement appropriés pour la publication dans un journal scientifique. Les rapports techniques sont destinés essentiellement à un public international et ils sont distribués à cet échelon. II n'y a aucune restriction quant au sujet; de fait, la série reflète la vaste gamme des intérêts et des politiques de Pêches et Océans Canada, c'est-à-dire les sciences halieutiques et aquatiques.

Les rapports techniques peuvent être cités comme des publications à part entière. Le titre exact figure au-dessus du résumé de chaque rapport. Les rapports techniques sont résumés dans la base de données *Résumés des sciences aquatiques et halieutiques.*

Les rapports techniques sont produits à l'échelon régional, mais numérotés à l'échelon national. Les demandes de rapports seront satisfaites par l'établissement auteur dont le nom figure sur la couverture et la page du titre.

Les numéros 1 à 456 de cette série ont été publiés à titre de Rapports techniques de l'Office des recherches sur les pêcheries du Canada. Les numéros 457 à 714 sont parus à titre de Rapports techniques de la Direction générale de la recherche et du développement, Service des pêches et de la mer, ministère de l'Environnement. Les numéros 715 à 924 ont été publiés à titre de Rapports techniques du Service des pêches et de la mer, ministère des Pêches et de l'Environnement. Le nom actuel de la série a été établi lors de la parution du numéro 925.

Canadian Technical Report of
Fisheries and Aquatic Sciences No. 3313

2019

STANDARDIZED FIELD SAMPLING METHOD FOR MONITORING THE OCCURRENCE AND RELATIVE ABUNDANCE OF THE ROCKY MOUNTAIN SCULPIN (*COTTUS* SP.) IN CANADA

by

Camille J. Macnaughton, Tyana Rudolfsen, Doug A. Watkinson, and Eva C. Enders

Fisheries and Oceans Canada
Ecosystems and Oceans Science
Central and Arctic Region
Freshwater Institute
501 University Crescent
Winnipeg, MB
R3T 2N6

Cat. No. Fs97-6/3313E-PDF ISBN 978-0-660-31036-7 ISSN 1488-5379

Correct citation for this publication:

C.J. Macnaughton, T. Rudolfsen, D.A. Watkinson, and E.C. Enders 2019. Standardized field sampling method for monitoring the occurrence and relative abundance of the Rocky Mountain Sculpin (*Cottus* sp.) in Canada. Can. Tech. Rep. Fish. Aquat. Sci. 3313: x + 54 p.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

C.J. Macnaughton, T. Rudolfsen, D.A. Watkinson, and E.C. Enders 2019. Standardized field sampling method for monitoring the occurrence and relative abundance of the Rocky Mountain Sculpin (*Cottus* sp.) in Canada. Can. Tech. Rep. Fish. Aquat. Sci. 3313: x + 54 p.

The Species at Risk (SAR) Program's objective for Rocky Mountain Sculpin (*Cottus* sp.) is to **"***Develop an appropriate monitoring protocol to track abundance, distribution and habitat use for the Rocky Mountain Sculpin."* In an effort to provide science information for SAR Program objectives, this report aims outlines a standardized sampling method and survey design that will document changes in the distribution and relative abundance of the Rocky Mountain Sculpin in the St. Mary and Milk river systems. We also propose propose guidelines for surveying the species in the Flathead River system. This report details (1) the sampling gear, (2) sampling effort required and timing, and (3) sampling sites for Rocky Mountain Sculpin abundance and range extension monitoring. This standardized sampling protocol may improve the monitoring of the species throughout its Canadian range and the assessment of population trends, allowing for a better informed management of the species over time.

RÉSUMÉ

C.J. Macnaughton, T. Rudolfsen, D.A. Watkinson, and E.C. Enders 2019. Standardized field sampling method for monitoring the occurrence and relative abundance of the Rocky Mountain Sculpin (*Cottus* sp.) in Canada. Can. Tech. Rep. Fish. Aquat. Sci. 3313 : x + 54 p.

Une des mesures de gestion provenant de la Loi sur les Espèces en Péril (LEP) pour la conservation du chabot des montagnes Rocheuse (*Cottus* sp.) consiste à élaborer un plan de surveillance suffisamment solide afin de quantifier l'abondance, la distribution et l'habitat du poisson utilisé par l'espèce. Dans le cadre d'établir des cibles quantitatives pour le chabot des montagnes Rocheuse en vue d'assurer sa protection et son rétablissement, ce rapport sert à définir un protocole et un design d'échantillonnage qui serviront à faire l'inventaire des populations de chabot des montagnes Rocheuse dans les bassins versants des rivières St Mary et Milk en Alberta, ainsi que dans le bassin versant de la rivière Flathead en Colombie Britannique. Ce rapport vise à décrire (1) l'engin de pêche recommandé, (2) l'effort et le moment de l'année idéal pour l'échantillonnage, et (3) la localisation des sites d'échantillonnage qui se retrouvent dans l'ensemble de l'aire de répartition de l'espèce, ainsi qu'à l'extérieur de cette zone pour faire le suivi de l'abondance à long-terme. Ce rapport contribue directement à la conservation de l'espèce en mettant en œuvre un plan de surveillance dans les cours d'eau canadiennes pour assurer la viabilité à long-terme de l'espèce.

ACKNOWLEDGEMENTS

Fisheries and Oceans Canada (DFO) wishes to acknowledge the contributions of those who collected field samples and provided data for the technical report: C. Charles, S. Backhouse, A, Batty, D. Boguski, M. Bryski, J. Cooper, T. Clayton, W. Donaldson, J. Eastman, W. Franzin, L. Fraser, C. Kovachik, S. Kjartanson, M. Labelle, D. Leroux, M. Lowdon, M. Martens, E. Macdonald, N. Mochnacz, K. Munroe, L. Queen, K. Munroe, M. Labelle, and J. Zieler.

1.0 INTRODUCTION

The purpose of the *Species at Risk Act* (SARA) is to protect wildlife species at risk of becoming extinct or extirpated in Canada, help with the recovery of extirpated, endangered, and threatened species, and ensure that species of special concern do not become extirpated or threatened as a result of human activity. Under provisions in the *Act*, wildlife species, designatable units (DUs) thereof, and their critical habitats receive protection. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) is an independent body of experts tasked with identifying and assessing the status of wildlife species at risk. Once a species' outcome (i.e., designation) has been decided by COSEWIC and subsequent listing pursuant to SARA, assessments on the distribution and relative abundance of the species concerned are necessary for determining population trends and the effectiveness of recovery strategies. COSEWIC assessments determine the status of a species on a ten year cycle, setting the timeline for when the information is required to update a species' status and to ensure the species' recovery is on the anticipated trajectory. The difficulty in deriving these assessments lies in a) achieving consistent and current population trends, species distribution, b) establishing a frequency of sampling events that fit within COSEWIC timelines, and c) agreeing on surveying methods. This report will address these challenges by establishing a standardized sampling protocol for Rocky Mountain Sculpin.

The Rocky Mountain Sculpin (*Cottus* sp.) is a cryptically-coloured, small-bodied, benthic fish that is found in only two river basins in southern Alberta and one river basin in British Columbia, Canada. The Canada's *Species at Risk Act* (Fisheries and Oceans Canada 2012) designated the Westslope DU of the Rocky Mountain Sculpin as *Special Concern* and the Eastslope DUs as *Threatened* (Fisheries and Oceans Canada 2012). This sedentary species exhibits a restricted distribution, which make it susceptible to anthropogenic impacts (Bailey 1952; Ruppert *et al.* 2017), including land-use change (e.g., road construction and sediment run-off), flow augmentation (e.g., irrigation, dam construction and operation), water extraction, and climate variability (e.g., drought) (COSEWIC 2005; COSEWIC 2010). Various field sampling methods for quantifying the occurrence and relative abundance of small-bodied freshwater fishes in wadeable streams are currently in use. However, different field methods often yield different information, leading to complementary and/or incomplete data records for a given species. Inconsistent sampling effort and survey designs may, therefore, preclude pooling data from different sources for obtaining reliable estimates (e.g., distribution and relative abundance) of target species. No consistent approach has been developed for monitoring Rocky Mountain Sculpin populations and their habitats in Canada.

In an effort to provide science information to meet the Species at Risk Program objectives of monitoring population trends, this report aims to provide a consistent sampling method and survey design that may accurately document changes in the distribution and relative abundance of the

Rocky Mountain Sculpin throughout its Canadian range (Sec.6.2.2 in the federal recovery strategy). This report details (1) the sampling gear, (2) sampling effort required and timing, and (3) sampling sites for Rocky Mountain Sculpin abundance range extension monitoring. The report also provides advice on Catch per Unit Effort (CPUE) required to effectively sample Rocky Mountain Sculpin throughout its current range based on existing field sampling data records for the species and an analysis of survey effort. In addition, specific recommendations on a standardized sampling protocol that also includes the frequency of sampling events for monitoring the Rocky Mountain Sculpin populations.

2.0 ROCKY MOUNTAIN SCULPIN

2.1 MORPHOLOGY

The Rocky Mountain Sculpin morphology reflects the species' and family's bottom-dwelling nature: large-headed with a body that tapers posteriorly and lacks an air bladder (Peden 2000; 2001). Both dorsal and pelvic fins have protective spines (Scott and Crossman 1973). The maximum fork length (FL) recorded for the Rocky Mountain Sculpin in Alberta is 114 mm from the Milk River (R.L. & L. 2002). They have large heads, with head length (HL) up to 3.1-4.4 times into the standard length (SL), the mouth width from 4.2-6.0 times into SL, and the caudal peduncle depth from 12.7-15.0 times into SL. There are two median chin pores and usually a single postmaxillary pore. The first and second dorsal fins are usually weakly conjoined, with 8 or 9 spines in the first dorsal fin and 17-19 rays in the second dorsal fin. There are 12-14 (usually 13 or 14) anal rays and 13-15 (usually 14) pectoral rays. Pelvic fins have 1 spine and 4 rays. The lateral line is incomplete and has 20-25 pores. The pectoral axial is usually without prickles but occasionally there are 1, or rarely 2, axial prickles. Palatine teeth are present but are not connected to the vomerine tooth patch. The occipital region usually is covered with small, fleshy papillae (nubbles) (COSEWIC 2010). The colouration is variable, usually dark (brown or olive) dorsally with slightly darker, indistinct saddles under the soft dorsal fin, and pale lower flanks (Figure 1). In breeding males, the first dorsal fin is black with a yellow or orange edge and the body often is black. In non-breeding adults, the first dorsal fin has two dark spots (one anterior and one posterior) that usually are partially coalesced (COSEWIC draft in review).

Figure 1. Rocky Mountain Sculpin (image from the COSEWIC draft in review)

Rocky Mountain Sculpin are morphologically similar to Mottled Sculpin (*C. bairdii*) and the Shorthead Sculpin (*C. confusus*), and are difficult to differentiate from one another as there is significant morphological variation within the species throughout their range. Rudolfsen *et al.* (2018) found that Rocky Mountain Sculpin varied significantly in body shape, pore counts, and number of fin rays across the North Milk, St. Mary, and Flathead rivers in Canada. Rocky Mountain Sculpin can usually be identified using the following features: (1) no prickles covering the entire body (i.e., only found behind the pectoral fin); (2) well-developed pelvic fin rays; (3) vomerine and palatine teeth; (4) 11-15 anal fin rays and 13-16 pectoral fin rays; and (5) an upper preopercular spine not strongly hooked (summarized in Peden 2001). Likewise, the Rocky Mountain Sculpin is distinguished from the Columbia Sculpin (*Cottus hubbsi*) based on several morphological features (Troffe 1999; Peden 2000). The Columbia Sculpin has a complete lateral line with an average of 29 ± 3 pores, and prickles are present behind the pectoral fin. In contrast, the lateral line of specimens from the Flathead and St. Mary rivers is not complete, with an average of 22 ± 3 pores, and prickles behind the pectoral fin are absent (Troffe 1999; Peden 2000).

2.2 BIOLOGY

<u>Life Cycle and Reproduction</u>

Rocky Mountain Sculpin were believed to reach maturity at 23 months (Roberts 1988) and spawn in the spring like other North American freshwater sculpins. However, recent studies suggest that Rocky Mountain Sculpin mature at three years of age, on average, with a maximum reported age of eight years (Young and Koops 2013). The youngest age at maturity for a female *C. confusus* in British Columbia is 2 years, with the smallest SL recorded at 42 mm (Peden 2001). The only mature two-year-old female collected from the Flathead River measured 71.4 mm SL (Hughes and

Peden 1984). The smallest mature female examined from the Milk and St. Mary rivers was 52.3 mm TL, but age was not estimated (Roberts 1988).

In British Columbia, the exact time of spawning is unknown; however, in the West Gallatin River in eastern Montana (Eastslope), the spawning season spanned all of June (Bailey 1952) and some males were ripe (producing milt) as early as March 25, where water temperatures ranged from 7.8-12.7 °C. For sites on the Flathead River 60 km upstream from the Canada/US border, the minimum daily average temperature for the month of June 2008 was 2.3 °C, the maximum was reached on June 30, 2008 (9.8 °C), and prior to June 21, the average daily temperature did not exceed 6.0 ºC (Clint Muhlfeld pers. comm.). At approximately the same location (50-60 km upstream from the Canadian/US border), water temperatures in 2015 exceeded 6.0 °C by May 25 and ranged from 5.3-13.7 °C in June (Rudolfsen, unpublished data). Both 2008 and 2015 temporal trends suggest that Westslope Rocky Mountain Sculpin (DU1) likely spawn in mid- to late June, one month later than Eastslope populations (DU2 and DU3; see Section 2.3). It is suggested that temperature may drive spawning, with a threshold between 7.5-15 °C (Roberts 1988).

Bailey (1952) described spawning sites in the West Gallatin River as holes under rocks, where surface velocities over nests ranged from 0.0 to 1.4 m s^{-1} and water depths were >40 cm. Typically, males excavate a nest cavity under rocks (ranged in diameter from 13-38 cm), woody debris, or vegetation and court females. The courtship is complex and involves rapid changes in male colour, as well as acoustical and behavioural courtship signals (Savage 1963; Whang and Jannsen 1994). Usually, males spawn with several females. In the West Gallatin River, Bailey (1952) estimated up to five females deposited eggs in a single nest. Eggs are usually attached to rocks and occasionally on aquatic vegetation and woody debris (Bailey 1952). Eggs are a pale yellow or orange-yellow colour and 2.5 mm in diameter (The Alberta Rocky Mountain Sculpin Recovery Team 2013). Males fan and guard the eggs, ensuring they do not accumulate silt until they hatch. For DU2 and DU3, average annual fecundity at sexual maturity was 64 eggs, and increased to as high as 518 eggs by 8 years of age (Young and Koops 2013). Average fecundity for sexually mature females of 4 years of age is ~184 eggs, typically below 400 eggs (The Alberta Rocky Mountain Sculpin Recovery Team 2013). The incubation period is dependent on temperature, where eggs take about 3-4 weeks to hatch at 7.8-17.2 ºC (Bailey 1952). Rocky Mountain Sculpin larvae are likely similar to other sculpin species and burrow into the gravel after hatching (at ~6-8 mm) and remain there for ~2 weeks before they emerge as miniature (~10 mm total length) versions of the adults (McPhail 2007).

Age Structure

As part of a recovery potential analysis, 134 Rocky Mountain Sculpin from both DU2 and DU3 were measured for length and aged using otoliths (Young and Koops 2013). At the end of the first year, Rocky Mountain Sculpin measured 32 mm in length and 54 mm at two years of age (age at maturity). Rocky Mountain Sculpin were as old as eight years of age, for an average length of 99 mm. Analysis of the otoliths suggest that winter growth is absent (Bailey 1952). In the Flathead

River, young-of-the-year were on average 37.0 mm SL by late summer (Hughes and Peden 1984), one-year-old males were on average 64.4 mm SL, and one-year-old females were 48.6 mm SL by October (Hughes and Peden 1984). Generation time is estimated to be 4.1 years (Young and Koops 2013).

Physiology and Adaptability

Although there is little information on the physiology of Rocky Mountain Sculpin, their distribution on the east and west sides of the Continental Divide suggests that the species is sensitive to temperature. Willock (1969) postulated that water temperature was the single most important factor affecting sculpin distribution, where populations are only present in the warmer parts of the Flathead River system in DU1, although competition with Slimy Sculpin may further contribute to this distribution pattern. East of the Rocky Mountains, they extend farther out onto the Great Plains than most "cool" water species, however, competition from other fish species may drive their distribution. Water level (combination of water depth and flow) may also be an important factor, where inadequate water flow resulting from drought conditions and impoundments, diversions, and water removal may have changed the distribution since the 1960s (Paetz 1993; R.L. & L. 2002). Populations in the Milk River may have been reduced as a result of inadequate water flow (Water Survey of Canada gauge 11AA025).

Changes to the flow and thermal regimes stemming from anthropogenic factors such as dams are known to further reduce the distribution of the species downstream of reservoirs. The Rocky Mountain Sculpin have likely been extirpated in the St. Mary River as a result of the species' intolerance to higher water temperatures and reduced flows originating from dam construction and colder water temperatures resulting from altered flows (i.e., bottom-draw dam) (Terry Clayton, pers. comm. 2004). Altered flows downstream from dams, irrigation canals, and stochastic flow events may also drive Rocky Mountain Sculpin to quickly adapt to changing flow regimes over short periods of time. However, evidence is lacking for increased sustained swimming ability or station-holding performance with maximum flows (Veillard *et al.* 2017). In addition, Rocky Mountain Sculpin were unable to reach maximum velocity prior to experimentation after a 30 min rest period, suggesting a rapid switch to anaerobic respiration. Overall, there is little evidence to indicate that Rocky Mountain Sculpin are capable of adapting to quickly or drastically changing water flows, and juveniles (ages 0-2 years) are especially sensitive to such perturbations (Young and Koops 2013). Habitat fragmentation within river systems may also drive local extirpations through reduced gene flow and rescue from other river systems.

Dispersal and Migration

Rocky Mountain Sculpin are recognized as a sedentary species. In a mark-recapture study in Prickly Pear Creek, a small tributary of the upper Missouri River, Montana, 21 of 75 marked fish (28%) were recovered, most of them within the first three months of the year-long study (Bailey 1952). Of those recaptured, 15 were <50 m from the point of first capture and the greatest distance moved was 145 m. Similar outcomes were found for another mark-recapture study conducted in Trout Creek, a tributary of the East Gallatin River, Montana, where 441 out of 1,847 marked fish

(24%) were recaptured, from late August to early March (McCleave 1964). Again, most of the recaptures were made within <50 m of the original marking site and the maximum distance moved was 181 m. A recent mark-recapture study on 223 Passive Integrated Transponder (PIT) tagged Rocky Mountain Sculpins in Lee Creek, Alberta found that the majority of recaptures had moved no more than 10 m from the tagging location over five months, where the largest distance was 240 m downstream from the tagging/release location (Ruppert *et al.* 2017). Collectively, these findings suggest that Rocky Mountain Sculpin do not migrate great distances and form small home ranges, >5 m^2 for sculpin occupying tributaries in British Columbia (Peden 2000). To date, no quantitative study has examined the movements of young-of-the-year, which is the age group that is most likely to disperse, likely downstream, prior to settling down for a more sedentary adult life. However, Peden and Hughes (1984) did not observe extensive migrations for either juvenile or adult Rocky Mountain Sculpin. Genetic differences among small tributaries based on allozyme electrophoresis did not indicate any movement (or gene flow) among populations in tributaries 10 km or more apart in British Columbia (Peden 2000). Population genetics from the St. Mary River and Lee Creek, however, provided evidence of gene flow suggesting the possibility of the downstream drift of larval and juvenile Rocky Mountain Sculpin in connected systems (Ruppert *et al.* 2017).

The life history and behaviour of the Rocky Mountain Sculpin suggest a relatively sedentary species with limited dispersal (Ruppert *et al.* 2017). Given this feature and the fact that Rocky Mountain Sculpin appear to prefer cooler waters and clean substrates, this species represents an excellent biomonitor of environmental conditions for the rivers in which it resides.

2.3 Known Distribution in Canada

Rocky Mountain Sculpin has a very limited distribution in Alberta and British Columbia, present in only three river systems (St. Mary and Milk river systems in Alberta and the Flathead River system in British Columbia; Figures 2, 3, and 4). West of the Continental Divide it is confined to the North and Middle forks of the Flathead River system in British Columbia and Montana.

Known distribution by Designatable Units (DU)

Based on the COSEWIC National Freshwater Biogeographic Zone (NFBZ) classification, populations of Rocky Mountain Sculpin are found in the Flathead River system in the Pacific Biogeographic Zone, British Columbia (DU1), and in the upper St. Mary River and Lee Creeks pertaining to the Saskatchewan – Nelson River Biogeographic Zone (DU2) and Missouri Biogeographic Zone (DU3) in Alberta. All discussion about the St. Mary River populations refer to the upstream or the portion of the St. Mary River above the dam.

In summary, the former Westslope (BC) population of the Rocky Mountain Sculpin is now DU1, justified by genetic discreteness, the complete separation of the Eastslope and Westslope populations, and the presence of the Westslope population in a different (Pacific) NFBZ. Based on genetic differences and distribution in two NFBZ the former Eastslope DU was separated into DU2 (Saskatchewan - Nelson River NFBZ) and DU3 (Missouri NFBZ).

Designatable Units (DU):

1- Westslope population – Flathead River population (BC)
2- Eastslope population – Saskatchewan - Nelson River populations (AB)
3- Eastslope population – Missouri River populations (AB)

<u>Westslope DU1– Special Concern (COSEWIC 2010)</u>

DU1 occupies the Flathead River and some of its tributaries: Kishinena, Sage, Couldrey, Burnham, Howell, Cabin, and Commerce creeks, where Couldrey Creek is a tributary of Burnham Creek and Cabin Creek is a tributary of Howell Creek. DU1 was designated as Special Concern by COSEWIC in April 2010 and does not carry legal protection under *SARA*. The British Columbia provincial status listing is S2S3 (Imperiled or Vulnerable).

In the Flathead River, Rocky Mountain Sculpin are the only sculpin found in the first 20 km of the main stem upstream of the US border. From ~20-35 km north of the border, Rocky Mountain Sculpin are still the most abundant sculpin species in the main stem. However, the frequency of Slimy Sculpin (*C. cognatus*) gradually increases in the upstream direction. At ~35 km, there is a relatively abrupt increase in the frequency of Slimy Sculpin. It was previously thought that the Slimy Sculpin was the only sculpin species found in the main stem, starting from 28 km upstream of the US border. Recent surveys of the same stretch of river indicate the presence of both Slimy Sculpin and Rocky Mountain Sculpin, extending into the upper reaches. Recent findings by Rudolfsen *et al.* (2019) suggest that Rocky Mountain Sculpin has expanded its range several kilometers (~20 km) upstream since the last study by Hughes and Peden (1984). Similar upstream range extension for Rocky Mountain Sculpin have been seen for Kishinena Creek (Rudolfsen *et al*. 2019).

Three locations where Slimy Sculpin and Rocky Mountain Sculpin hybrids are present within the Flathead River watershed were identified. Rocky Mountain Sculpin showed a distributional range (1200-1902 m) that far exceeded the range limit reported 35 years ago (1200-1372 m), suggesting a contemporary shift to higher elevations and range expansion for DU1 (Rudolfsen *et al*. 2019) (Figure 2).

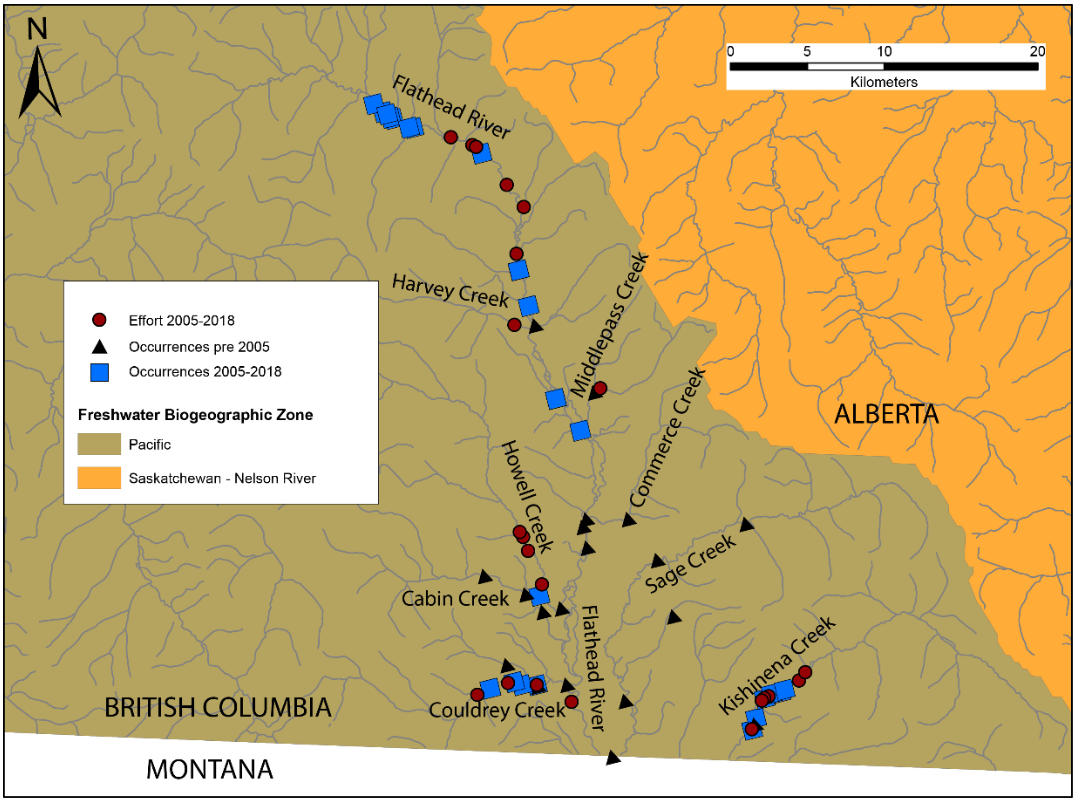

Figure 2. Sampling effort between 2005-2018 and Rocky Mountain Sculpin (*Cottus* sp.) occurrence in DU1 (Pacific Biogeographic Zone) before and after 2005. Figure taken from COSEWIC report draft, 2018.

Eastslope DU 2 and 3 – Saskatchewan – Nelson River and Missouri populations - Threatened (COSEWIC 2010)

COSEWIC recommended of Threatened status for Eastslope DUs in 2005. In 2012, Fisheries and Oceans Canada developed a recovery strategy for both DUs (Fisheries and Oceans Canada 2012), and in 2013 Alberta completed a provincial recovery strategy (The Alberta Rocky Mountain Sculpin Recovery Team 2013). The Alberta provincial status listing is S1 (Critically Imperiled).

No changes in distribution are apparent in the St. Mary River, but COSEWIC (2018) speculated that Rock Mountain Sculpin were present downstream of the reservoir before dam construction (Figure 3). Some changes in distribution have occurred in the Milk River (DU3) since the 1960s, with significant downstream expansion in the river and extirpation in the portion of the Milk River upstream of the confluence with the North Milk (aka, the southfork or upper Milk River), resulting from very low natural and altered water flows (Figure 4).

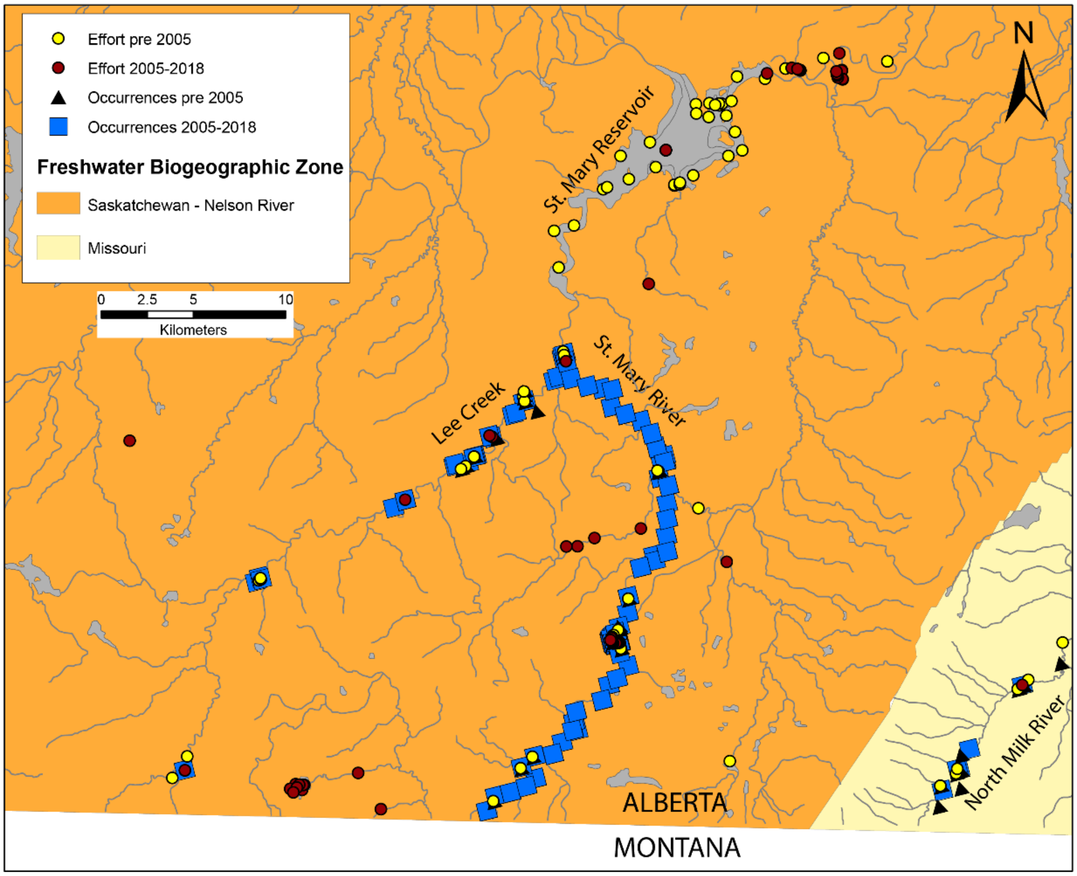

Figure 3. Sampling effort and Rocky Mountain Sculpin (*Cottus* sp.) occurrence in DU2 (Saskatchewan – Nelson Biogeographic Zone) before and after 2005. Figure taken from COSEWIC report draft, 2018.

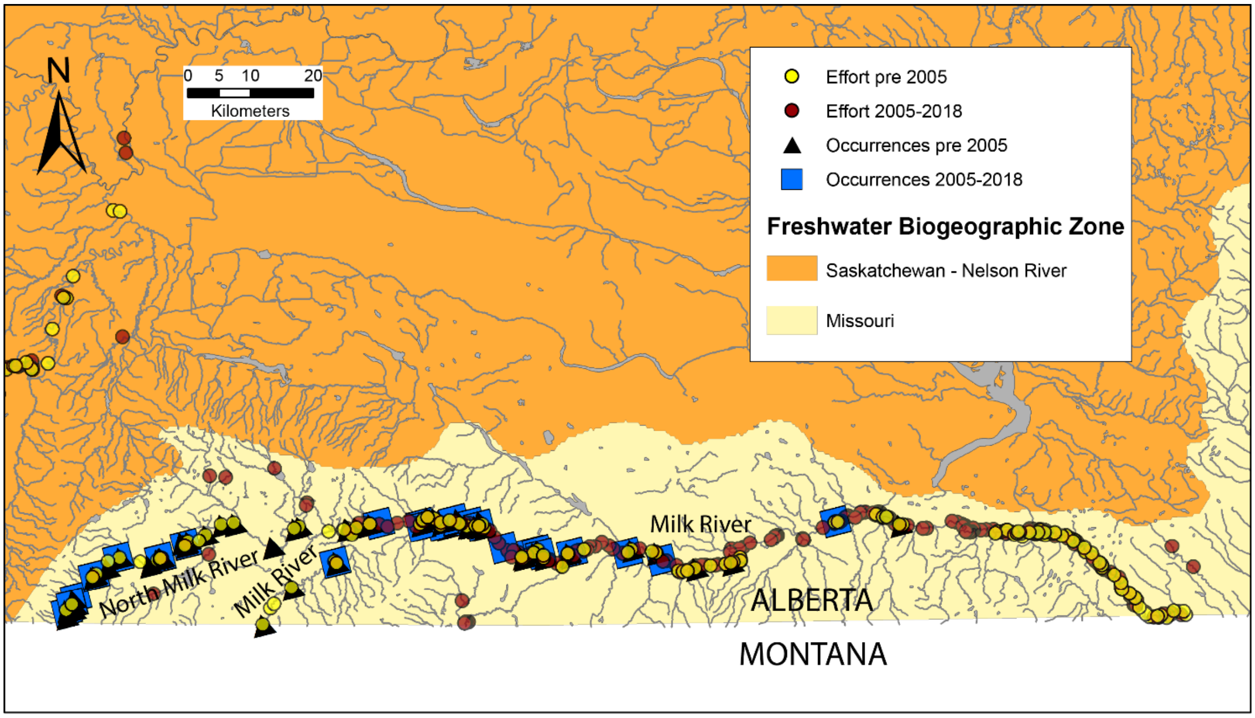

Figure 4. Sampling effort and Rocky Mountain Sculpin (*Cottus* sp.) occurrence DU3 (Missouri Biogeographic Zone) before and after 2005. Figure taken from COSEWIC report draft, 2018.

2.4 HABITAT

Habitat Features

Rocky Mountain Sculpin are a cryptic species (colouration and behaviour), occupying cool, shallow, riffle habitats, with rocky or gravel substrate and moderate current velocity (0.1-1.8 ms^{-1}; Watkinson unpublished data)(Bailey 1952; Paetz 1993). Specific to the North Milk River and St. Mary River, sculpins were most commonly found in stream margin habitats, with rocky substrate and slower water velocity (Paetz 1993). In Lee Creek, sculpins preferred slightly silty stream margins, where water velocities were slower compared to mid-creek section habitats that were silt-free and had higher water velocities (Paetz 1993). Rocky Mountain Sculpin usually use the cover of rocks and boulders during the day as they are nocturnal (McPhail 2001). They are generally absent from pool habitats with sandy and silty substrate, however, a disproportionately large number of juvenile sculpins were observed in muddy areas with little gradient in the Milk River (Willock 1969), as well as in back eddy habitats in the Milk River (Watkinson pers. comm. 2019). Clayton and Ash (1980) also observed greater numbers of Rocky Mountain Sculpin in habitats with clean substrates, but lower numbers were also found in quiet pools with silty substrate. Temperature preference for the species is unknown, but field surveying in British Columbia indicate that they inhabit streams of summer temperatures averaging between 5-15 ºC and winter temperatures between 0-2 ºC (Rudolfsen unpublished data). Willock (1969) stated that the colder temperatures and increased water clarity associated with a higher rainfall and elevation, greater river gradient, less erosion, and more vegetation in the upper Milk River determined the presence of the Rocky Mountain Sculpin. In the Flathead River system, where the Rocky Mountain Sculpin and Slimy Sculpin co-occur, water turbidity and conductivity is thought to drive the distribution of each species (i.e., Rocky Mountain Sculpin prefer more turbid habitats over Slimy Sculpin).

Variability in habitat selection appears to be watershed-specific and dependent on the type of habitat available. Habitat availability within the Milk River can vary significantly with water flow and level, with significant reductions in habitat availability during periods of extreme drought where habitats are dewatered or too warm.

Habitat Trends and Threats

DU1 has undergone relatively few habitat alterations since the 1950s. The first Rocky Mountain Sculpin in the Canadian portion of the Flathead River were collected in 1955 and 1957 (UBC Fish Museum records UBC 55-0277 and UBC 57-0327) and subsequently, at the same sites in the 1980s, 1990s, and in the early 2000s. Although the Flathead River system is often cited as the last remaining pristine large river in southeastern British Columbia (Angelo 2008), there has been commercial logging and mining/oil and gas development in the Flathead Valley since the late 1890s. In 2011, the Government of British Columbia passed the Flathead Watershed Area Conservation Act, which bans any mining and/or oil and gas related activity within the Flathead basin. While logging operations are still allowed to continue, the impacts of these operations in the area appear to be minor. The cumulative effect of these projects and their associated infrastructure, however, may potentially change the ecology of the river.

Water removal for irrigation causes the greatest alterations to sculpin habitat in the St. Mary (DU2), North Milk and Milk rivers (DU3). Water in the Milk and St. Mary rivers are intensively managed for irrigation use both in Canada and the United States. As such, they are subject to provisions in the Boundary Waters Treaty of 1909 (the Treaty) between Canada and the United States, which is administered by a binational organization called the International Joint Commission (IJC) (https://ijc.org/en/aosmmr). The IJC has appointed members by both Canadian and American governments and the Treaty itself provides the principles and mechanisms to resolve disputes concerning shared water.

The context of the apportionment is best considered temporally regarding the irrigation season (April 1 to October 31 annually) and the non-irrigation season (November 1 to March 31). The management approach in the Milk River watershed and St. Mary River has essentially been to divert water from the St. Mary River (~ 18.4 $m^3 \cdot s^{-1}$) into the North Milk River, starting April 1 (or earlier). The natural winter flow in the Milk River is generally very low at this time of year (<1 $m^3 \cdot s^{-1}$), thus, the increase in water flow is significant, rising up to ≥ 15 $m^3 \cdot s^{-1}$ in a relatively short period of time. This higher water flow continues in the Milk River until September or October, when water flow is reduced to natural or close to natural conditions, as the end of the irrigation season approaches. Both rivers have low winter flows, however, water flow in the Milk River watershed in the winter is natural, whereas it is managed in the St. Mary River via storage facilities in Montana (Sherburne Reservoir and St. Mary Lake).

Frequent droughts due to climate change experienced in southern Alberta further affect the availability of sculpin habitat in the river. The construction of the St. Mary Reservoir (completed in 1951) significantly altered the type of habitat available to fish species in the St. Mary River, while the St. Mary's Canal, constructed in Montana to divert water from the St. Mary River to the North Milk River, has caused significant habitat alteration in the North Milk and Milk rivers since 1917. The diversion canal generally diverts water from April to September, increasing the water volume (level and flow) in the North Milk and Milk rivers. Water flows downstream from the canal have become highly regulated, with temporary shutdowns during open water months, causing a substantial reduction in available sculpin habitat in the North Milk and Milk rivers. Ongoing water removal in Montana from the upper Milk River, upstream of the confluence with the North Milk River, may also be responsible for the near disappearance of sculpins in this upstream section of the main stem, as the Milk River can be reduced to isolated pools with little surface flows (Paetz 1993). However, natural flows in the Milk River would likely be the most limiting factor as natural flows are almost non-existent during some periods of the winter. Similarly, the tributaries of the North Milk and Milk rivers in Canada are ephemeral most years. As such, the availability of overwintering habitat in the Milk River system is highly variable from year to year and dependent on sufficient natural water flows.

2.5 POPULATION SIZE AND CPUE TRENDS IN CANADA

<u>Population Trends by Designatable Units (DU)</u>

To better understand population trends over time and establish baseline estimates by river, we first described the effort in terms catch per unit of effort (CPUE - fish m^{-2} min^{-1}) across the different surveys for each of the DUs. We compared species occupancy and abundance estimates as a function of the number of sites and quadrats surveyed using existing Rocky Mountain Sculpin survey data from the Flathead River (DU1) and two sampling locations in the St. Mary River (DU2). Results from these simulations provided information on the sampling effort required to accurately monitor the distribution and abundance of Rocky Mountain Sculpin throughout its Canadian range. Following methods described by Schwartz (2017) and Paul (2018), we conducted a power analysis assessment to 1) inform on the number of sites sampled per survey year and 2) estimate the number of consecutive survey years required to detect changes to the population. Two consecutive years of data (2008-2009) from two sampling locations in the St. Mary River, which included sampling at Kimball Park, were used for the power analysis, as multiple years of sampling is required to determine the random variance among years. For brevity, examples discussed here will consist of the St. Mary main stem and St. Mary River at Kimball Park surveys.

DU1 – Pacific Zone

Although the species has been collected sporadically in the Flathead drainage system since 1955, fish collections were not comparable because surveying methods and effort differed among years and sites. Earliest collections from 1957 were made using rotenone, while current collections consisted of data from different types of electrofishing surveys.

Focusing on the most current collections for the Flathead River and tributaries (2014 and 2015), Rudolfsen *et al.* (2019) sampled 95 sites throughout the Flathead river system and found Rocky Mountain Sculpin in 77 of the sites, with an average CPUE of 0.57 Rocky Mountain Sculpin per $m^{-2}{\cdot}min^{-1}$ of electrofishing. The highest Rocky Mountain Sculpin densities were reported for the Flathead River and Kishinena Creek (Rudolfsen *et al.* 2019), with on average 0.25 and 0.47 fish per m^2, respectively (CPUE 1.5 and 2.82 $m^{-2}{\cdot}min^{-1}$). Howell Creek and Couldrey Creek had the lowest average densities, at 0.12 and 0.19 Rocky Mountain Sculpin per m^2, respectively (CPUE 0.72 and 1.14 $m^{-2}{\cdot}min^{-1}$). The species distribution had a marked expansion upstream since it was last evaluated in the 1980's, on account of gradual migration over the past 35 years (Hughes and Peden 1984; Rudolfsen *et al.* 2019).

We conducted a simulations of the field sampling using the survey data collected in 2014-2015 to determine the minimum effort necessary in terms of the number of sites and quadrats needed to reliably determine species' occupancy and abundance of Rocky Mountain Sculpin in the Flathead system. Since the amount of suitable Rocky Mountain Sculpin habitat varied among the Flathead River and each of the tributaries, Middlepass Creek, Couldrey Creek, Harvey Creek, Howell Creek, and Kishinena Creek and the surveying effort differed among years and systems, the results were considered separately for each of the systems. Most of the sampling effort was allocated to the Flathead River main stem, with a total of 39 sites sampled. Middlepass Creek, Couldrey Creek, Harvey Creek, Howell Creek, and Kishinena Creek were each surveyed over 8, 19, 5, 17 and 22 sites, respectively. Sites consisted of a 300 m long river reach, where 10 or 30 quadrats of 1 m^2 were randomly distributed at depths <0.6 m, throughout each site. Each quadrat was fished for 10 $s{\cdot}m^{-2}$. Rocky Mountain Sculpin were not observed in Middlepass and Harvey creeks, therefore, sampling effort for these systems was not considered (Supplementary information: S1).

DU2 and 3– Saskatchewan – Nelson River and Missouri Zones

A population estimate was not available for the Rocky Mountain Sculpin in Alberta, but the species was abundant in the St. Mary River upstream from the St. Mary Reservoir (~750 000 prorated from surveys 2006-2009); in the lower 13 km of Lee Creek; in the North Milk River; and in the Milk River from Deer Creek upstream to the North Milk River confluence (Paetz 1993; R.L.&L. Environmental Services Ltd. 1987, 2002; P.&E. Environmental Consultants Ltd. 2002; Recovery Strategy for the Rocky Mountain Sculpin 2012; Watkinson, unpublished data). The highest abundances were observed in the upper North Milk River and decreased downstream, where sculpin were absent from the lowest section of the Milk River due to unsuitable habitats in lower reaches (R.L. & L 2002). Sampling effort between 2003 and 2007 for these lower reaches and the confluence with the North Milk showed low capture rates, with 30 Rocky Mountain Sculpin sampled in 116 h of electroshocking (Watkinson unpublished data).

Like for DU1, fish collections from DU2 and DU3 were not comparable because surveying methods and effort differed among years and sites. Rocky Mountain Sculpin had among the highest relative abundance of all fish species sampled in the North Milk and St. Mary rivers in 2001 and 2002, although abundance was dependent on the season sampled and the sampling feasibility (CPUE of 0.7 to 1.8 fish per minute; R.L. & L. 2002). In 2000 and 2001, average CPUE ranged from 2.4-4.2 fish $m^{-2}{\cdot}min^{-1}$ in the St. Mary River and 3 fish $m^{-2}{\cdot}min^{-1}$ in Lee Creek. The Rocky Mountain Sculpin population in the St. Mary River appears stable, but significant increases in abundance in Lee Creek have been recorded (R.L. & L 2002). From 2006 to 2009, the average CPUE was 1.9 fish $m^{-2}{\cdot}min^{-1}$ and 0.62 fish $m^{-2}{\cdot}min^{-1}$ in habitats shallower than 1 m deep, in the St. Mary River and Lee Creek, respectively (Watkinson unpublished data). The North Milk River had an average CPUE of 4.7 sculpin per minute of electroshocking.

St. Mary River at Kimball Park

Rocky Mountain Sculpin surveys conducted in August 2008 and 2009 provided occupancy and relative abundance estimates for a total of 34 and 99 cross sections, respectively. Each cross section consisted of five quadrats that were electroshocked for 20 s and each cross section was distanced at 10 m apart along the river length. The greater the number of cross sections surveyed, the more reliable the estimates as they account for the variability along the river. Increasing the number of quadrats within a cross section also contributes to improving the reliability of estimates within each site (i.e., increase confidence in mean estimates of the site). Increasing the number of cross sections and quadrats sampled in a river come at the cost of increased effort and resources, and thus, the next simulations were used to inform on the amount of effort, corresponding to the number of sites and quadrats recommended to obtain reliable occupancy and abundance estimates.

For each randomly generated combination of the number of cross sections per year (2008: 1-34; 2009: 1-99) and quadrats (1-5) sampled in the St. Mary River at Kimball Park (100 iterations with replacement), the occupancy (presence = 1; absence = 0) and mean abundance of Rocky Mountain Sculpin were calculated. In order to confidently determine the presence or absence of Rocky Mountain Sculpin in Kimball Park, the results from 2008 and 2009 surveys collectively suggested a minimum effort of five quadrats at two to three cross sections (survey area of 10-15 m^2) (Figure 5). Confidence in average Rocky Mountain Sculpin abundance estimates improved with the number of sites surveyed and stabilized beyond 20 sites, irrespective of the number of quadrats surveyed or the year surveyed. Specifically, the variance around the sampling represented by the first quartile (25%), third quartile (75%), as well as the minimum and the maximum confidence lines of the data also decreased with the number of sites surveyed (Figure 6).

Confidence in abundance estimates across the number of sites and quadrats was measured as the proportion of abundance estimates that were within the estimated ranges when surveying was conducted at all cross sections with five quadrats at each cross section (mean abundance estimate in 2008 = 0.60 fish·m^{-2}, minimum = 0.48 fish·m^{-2}, maximum = 0.68 fish·m^{-2}; mean abundance estimate in 2009 = 0.74 fish·m^{-2}, minimum = 0.66 fish·m^{-2}, maximum = 0.82 fish·m^{-2}). Since there was no significant difference in abundance estimates between 2008 and 2009 surveys (Welch's t= -0.71, df= 45.4, p = 0.48), the following simulations were run using 2008 data, for which a more representative sampling effort was conducted (34 sites in 2008 vs. 99 sites in 2009). If abundance estimates for every combination representing the number of sites (1-34) and quadrats (1-5) surveyed were within the minimum and maximum abundance of fish·m^{-2}, they were counted towards the total number of good abundance estimates out of 100 iterations, conveying a measure of confidence (1) or non-confidence (0). The proportion of abundance estimates that were between 0.48 and 0.68 were plotted as a function of sampling effort and confidence thresholds of 0.8 and 0.95 represented the proportion of abundance estimates that were within 20% and 5%, respectively, of this range (Figure 7**)**. These confidence thresholds informed the number of sites and quadrats required to obtain accurate Rocky Mountain Sculpin abundance estimates for this sampling procedure. To achieve at least 80% confidence in species abundance estimates, 4 quadrats at a minimum of 20 cross sections need to be sampled, while 95% confidence in a population estimate requires five quadrats to be sampled at a minimum of 34 cross sections.

In order to estimate power and sample size required to detect trends in CPUE for Rocky Mountain Sculpin in the St. Mary River at Kimball Park, we determined the catch per unit effort per year sampled (natural log of the abundance per m^2 in 2008 and 2009) as the linear model, with years as the explanatory continuous variable. Catch per unit effort data were transformed on the natural log scale and all data were adjusted by adding 1 to account for zeros. Average logged CPUE increased from 0.35 ± 0.46 in 2008 to 0.42 ± 0.49 in 2009, but were not significantly different between years. Therefore, differences in CPUE were assumed to be non-existent between years. According to Schwartz (2017), sampling variance arises through imperfect sampling of the fish population (CPUE) and corresponds to the residual variance from the linear model or the unexplained variation among sites within a year. Another source of unexplained variance in the model refers to the process variance or the variation that occurs from year to year (Schwarz 2017; Paul 2018). A third source of variance may be accounted for to include site-to-site variance from repeat sampling, however our data did not lend itself to estimating site-to-site variance. Given that we have two years of data, it is assumed that there was no trend over the two years and the variance components were extracted after the model where the log (CPUE) corresponded to the natural log of the abundance per m^2 (log+1) and year is a factor of two levels, 2008 and 2009. This model allowed us to estimate process and sampling standard deviations which were used in the power/sample size analysis. For Kimball park surveys in St. Mary, the estimated sampling standard deviation was 0.48 and the estimated process standard deviation was 0.033. Because the analysis was done on the natural log scale, the coefficient of variation for the individual measurements and the yearly means were 0.48 and 0.033, respectively (Schwarz 2017). Using a web-enabled version of the Schwarz (2017) power analysis developed by Alberta Environment and Parks in the statistical computation language R (R Core team 2018) and made interactive and web-enabled through the package shiny (Chang *et al.* 2018), we were able to approximate the sample site requirements and statistical power for surveying Rocky Mountain Sculpin in a river, St. Mary River over five and 10 years (Figure 8). Power analysis revealed that approximately 7 sites per year would be required to have a power of 80% to detect a 100% increase over five years vs. <five sites per year to have over 90% power to detect 100% change over 10 years, when the process standard deviation was 0.03 and the sampling standard deviation was 0.48 at alpha = 0.05. This means that for the St. Mary River, the survey effort to detect 100% change in CPUE over 10 years time is very low at <five sites per year. Moreover, the power remained relatively insensitive to the number of sites once it became large enough (<20 sites per year) over 10 year projections.

COSEWIC quantitative criteria and guidelines for the status assessment of wildlife species indicate 50% and 30% reductions in total number of mature individuals over the last 10 years as benchmarks for listing a species as Endangered or Threatened, respectively (Appendix 3 COSEWIC Assessment Process, Categories & Guidelines 2015). As such, ~10 sites or 26 sites per year would be recommended to confidently (i.e., at 80% power) detect a 50% and 30% increase over 10 years, respectively (Figure 8). Greater survey effort would be required to be able to detect the same percent change over a shorter amount of time (i.e., five years).

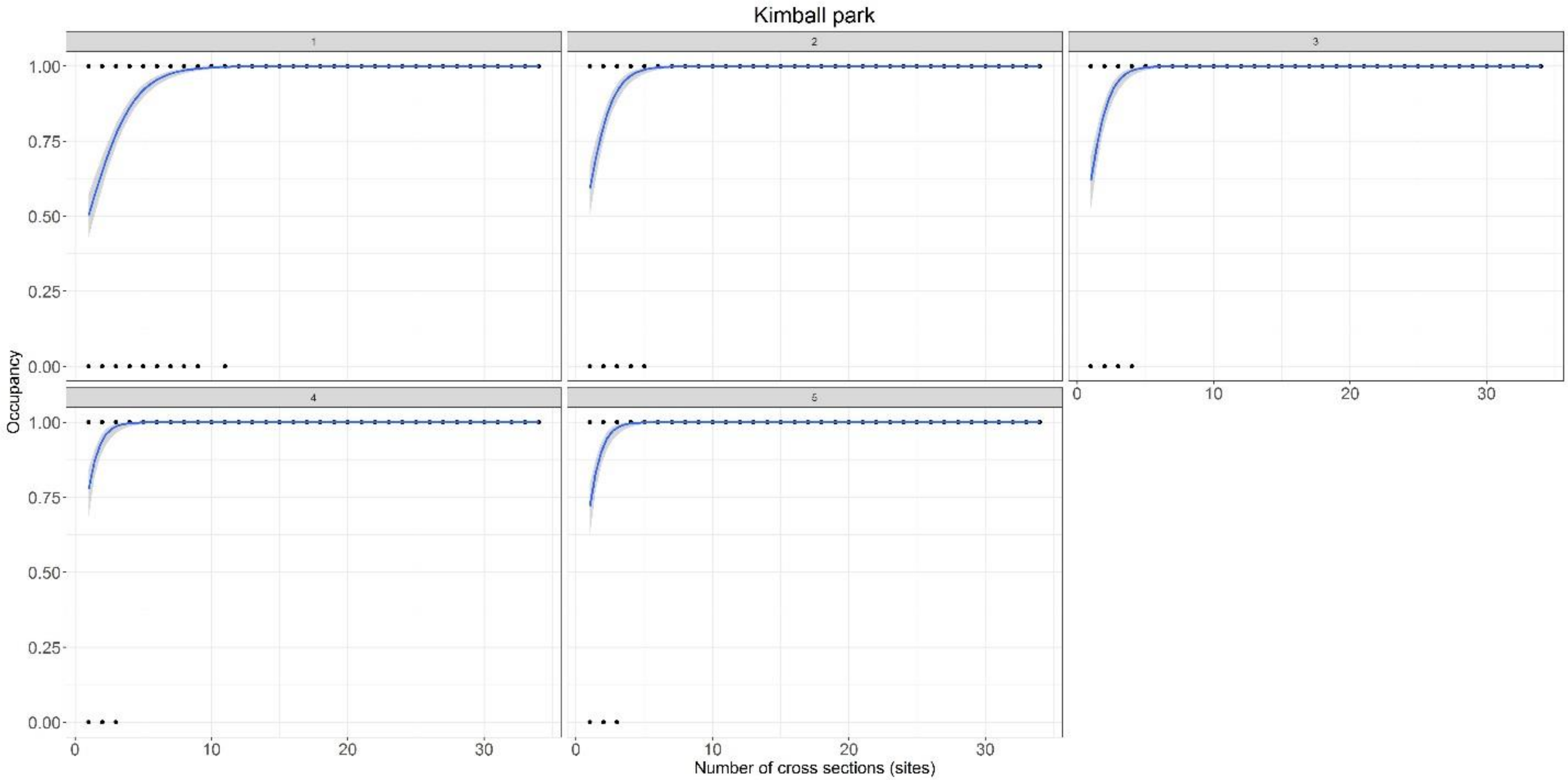

Figure 5. Occupancy (presence = 1; absence = 0) of Rocky Mountain Sculpin (*Cottus* sp.) as a function of the number of cross sections or sites (0-34 sites on x-axis) and quadrats (panels 1-5) surveyed in the St. Mary River, Kimball Park in 2008. Binomial smoother and standard error (blue line and grey shaded area) are represented.

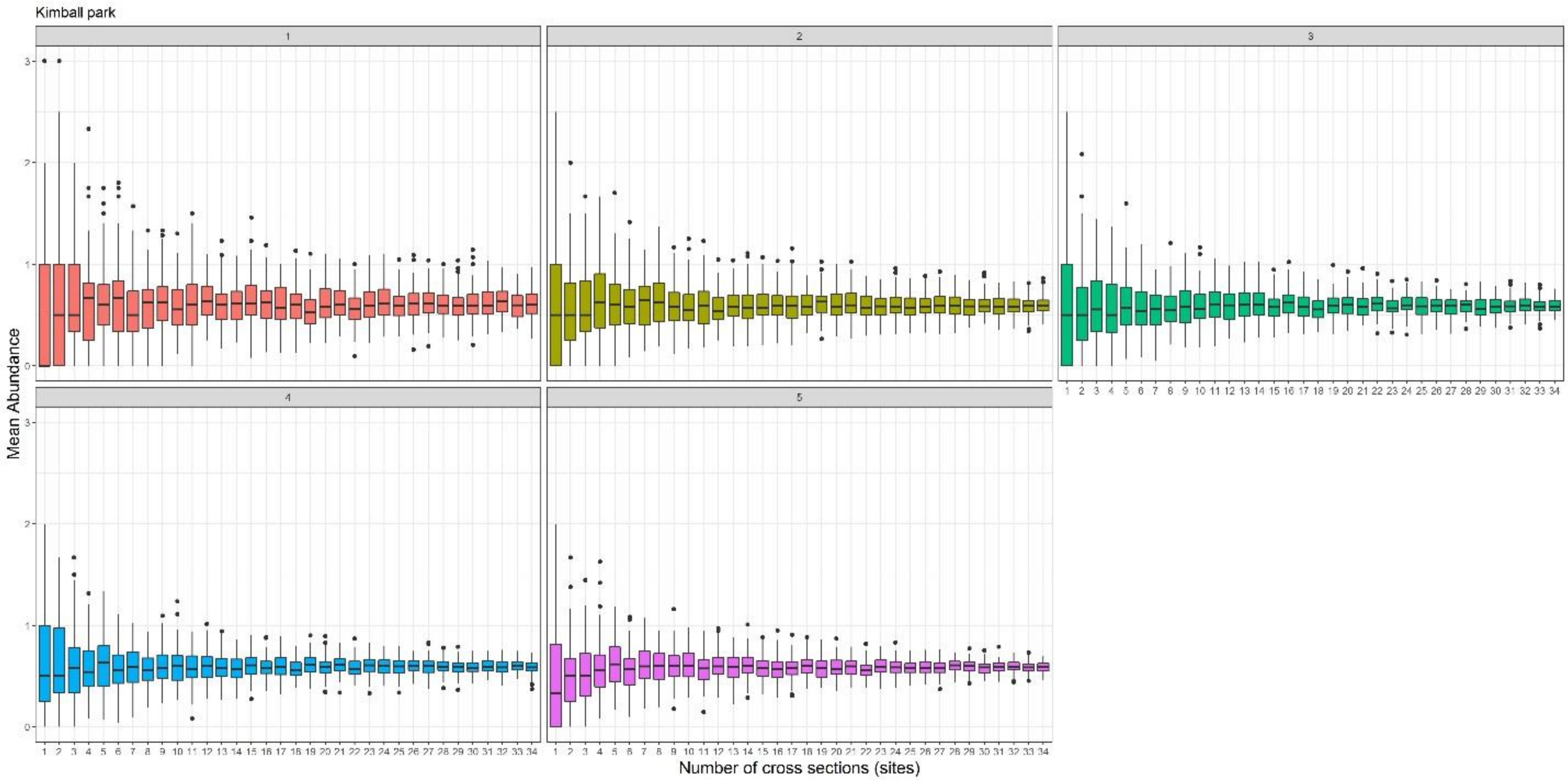

Figure 6. Average abundance of Rocky Mountain Sculpin (*Cottus* sp.) per m² as a function of the number of cross sections or sites (0-34 sites on x-axis) and quadrats (panels 1-5) surveyed in the St. Mary River, Kimball Park in 2008. Boxplots represent the first quartile (25%), median (line), third quartile (75%) of the data, as well as the minimum, maximum (confidence lines), and outliers (points).

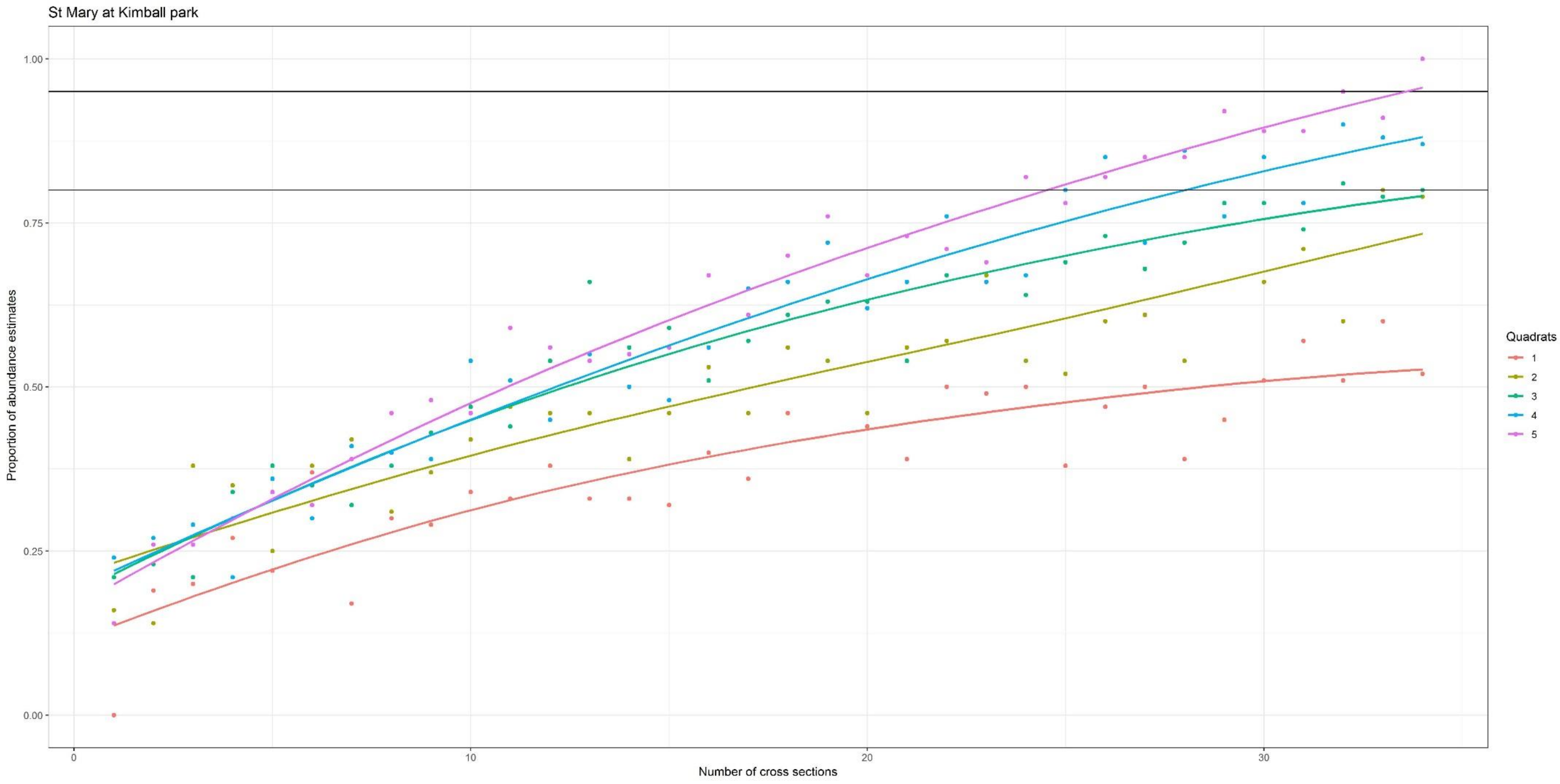

Figure 7. Proportion of abundance of Rocky Mountain Sculpin (*Cottus* sp.) within range of abundance estimates determined for 5 quadrats at each of the 34 sites (minimum= 0.48 fish·m^{-2} and maximum= 0.68 fish·m^{-2}) as a function of the number of sites (0-34 sites) and quadrats surveyed in the St. Mary River, Kimball Park in 2008. "Loess" smoother lines were added per the number of quadrats surveyed. 95% and 80% confidence thresholds drawn.

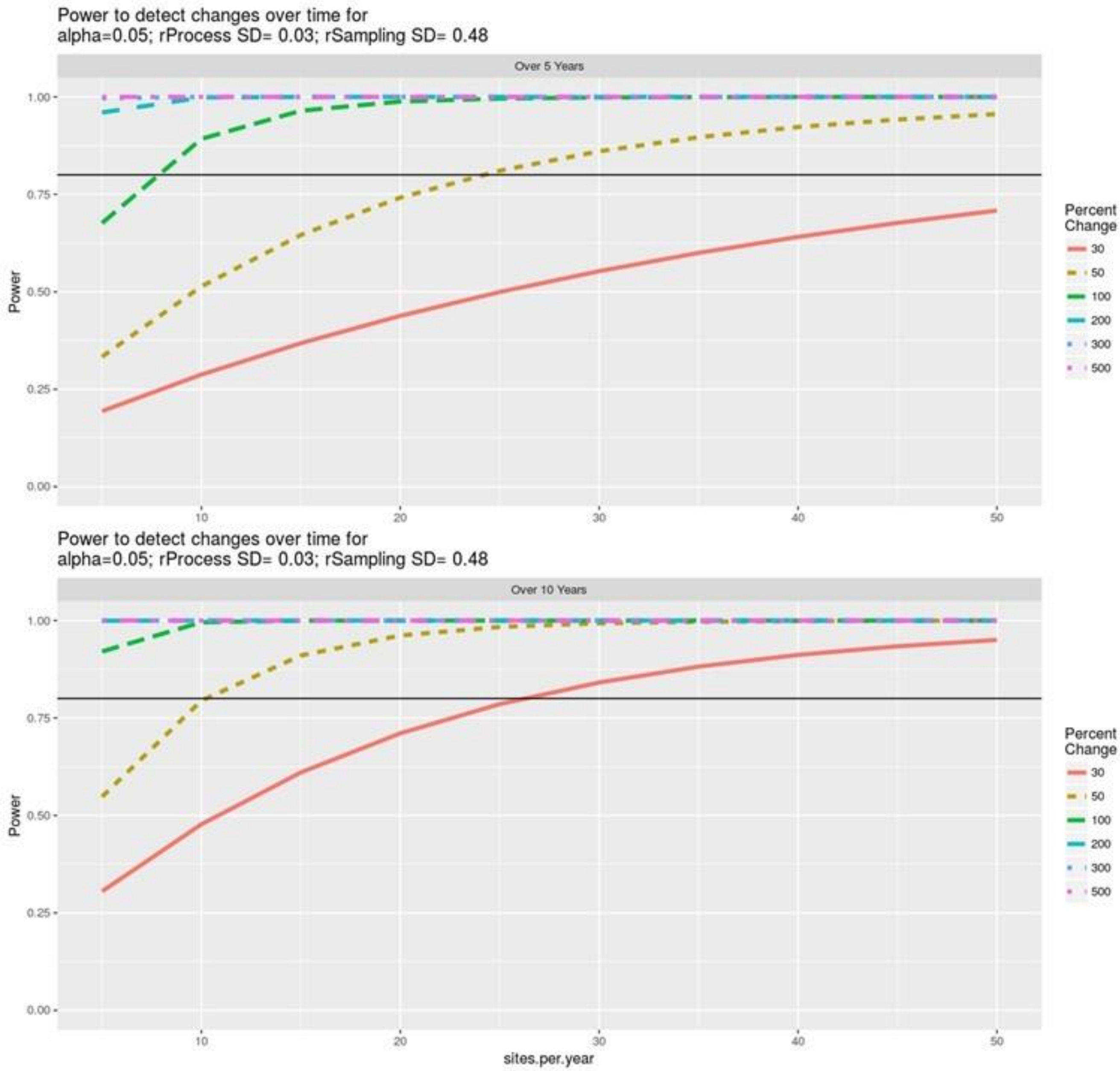

Figure 8. Power to detect changes (% change) across the number of sites per year required to detect these changes, over five and 10 years (Chang *et al.* 2018; Paul 2018).

St. Mary River main stem

Rocky Mountain Sculpin surveys conducted in the St. Mary River main stem in May 2008 and August 2009 provided occupancy and abundance estimates for a total of 42 and 99 cross sections, respectively, with five quadrats per cross section, each cross section distanced at 1 km apart along the river length.

Like for Kimball Park, the occupancy (presence = 1; absence = 0) and mean abundance of Rocky Mountain Sculpin were randomly generated for every combination of the number of cross sections (1-42 or 1-99) and quadrats (1-5) sampled in the St. Mary river main stem, in 2008 and 2009, respectively. Confidence in average Rocky Mountain Sculpin abundance estimates per m^2 increased with the amount of effort represented by the number of quadrats and cross sections surveyed, however, results differed between surveys conducted in 2008 and 2009. We focused on 2008 survey data, where greater abundance estimates were found (mean abundance estimate at all 42 cross sections with five quadrats at each cross section = 0.88 fish·m^{-2}, minimum = 0.71 fish·m^{-2}, maximum = 1.04 fish·m^{-2}). For a total of 42 cross sections surveyed in 2008, a minimum effort of four to five quadrats at two cross sections (survey area of 8-10 m^2) was recommended to confidently determine the occupancy of Rocky Mountain Sculpin in St. Mary River (Figure 9). Confidence in average Rocky Mountain Sculpin abundance estimates (Figure 10) improved with the number of sites surveyed but stabilized at fewer sites (from ~5 cross sections), irrespective of the number of quadrats and year surveyed. The proportion of abundance estimates that were between 0.71 and 1.04 fish·m^{-2} were plotted as a function of sampling effort and confidence thresholds of 0.8 and 0.95 represented the proportion of abundance estimates that were within 20% and 5%, respectively, of this range (Figure 11). To achieve at least 80% confidence in species abundance estimates, four quadrats at a minimum of 20 cross sections need to be sampled, while 95% confidence in a population estimate requires five quadrats to be sampled at a minimum of 23 cross sections.

In order to estimate power and sample size required to detect trends in CPUE for Rocky Mountain Sculpin in the St. Mary River main stem, we determined the catch per unit effort per year sampled (natural log of the abundance per m^2 in 2008 and 2009) as the linear model, with years as the explanatory continuous variable (Figure 12*)*. For St. Mary River surveys, the estimated sampling standard deviation was 0.55 and the estimated process standard deviation was 0.18. Power analysis revealed that approximately eight sites per year would be required to have a power of 80% to detect a 100% increase over 10 years when the process standard deviation was 0.18 and the sampling standard deviation was 0.55 at alpha = 0.05 (Figure 13). Moreover, the power remained relatively insensitive to the number of sites once it became large enough (<20 sites per year) over 10 year projections. Unlike for Kimball Park surveys, results indicated that no amount of sampling effort would allow us to confidently detect 30% and 50% change in CPUE, as per the COSEWIC assessment quantitative criteria and guidelines for the status assessment of wildlife species.

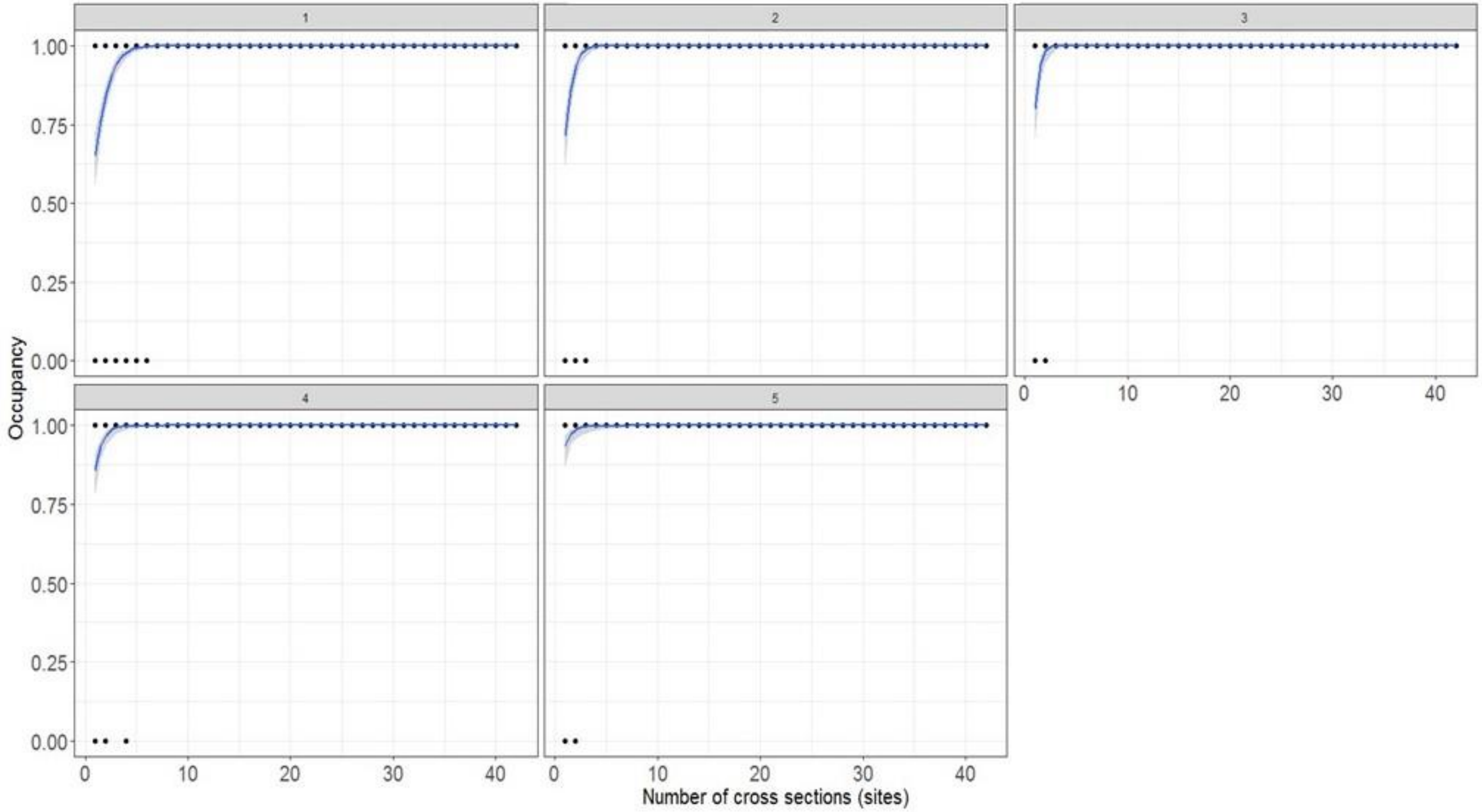

Figure 9. Occupancy (presence = 1; absence = 0) of Rocky Mountain Sculpin (*Cottus* sp.) as a function of the number of cross sections or sites (0-42 sites on x-axis) and quadrats (panels 1-5) surveyed in the St. Mary River in 2008. Binomial smoother and standard error (blue line and grey shaded area) are represented.

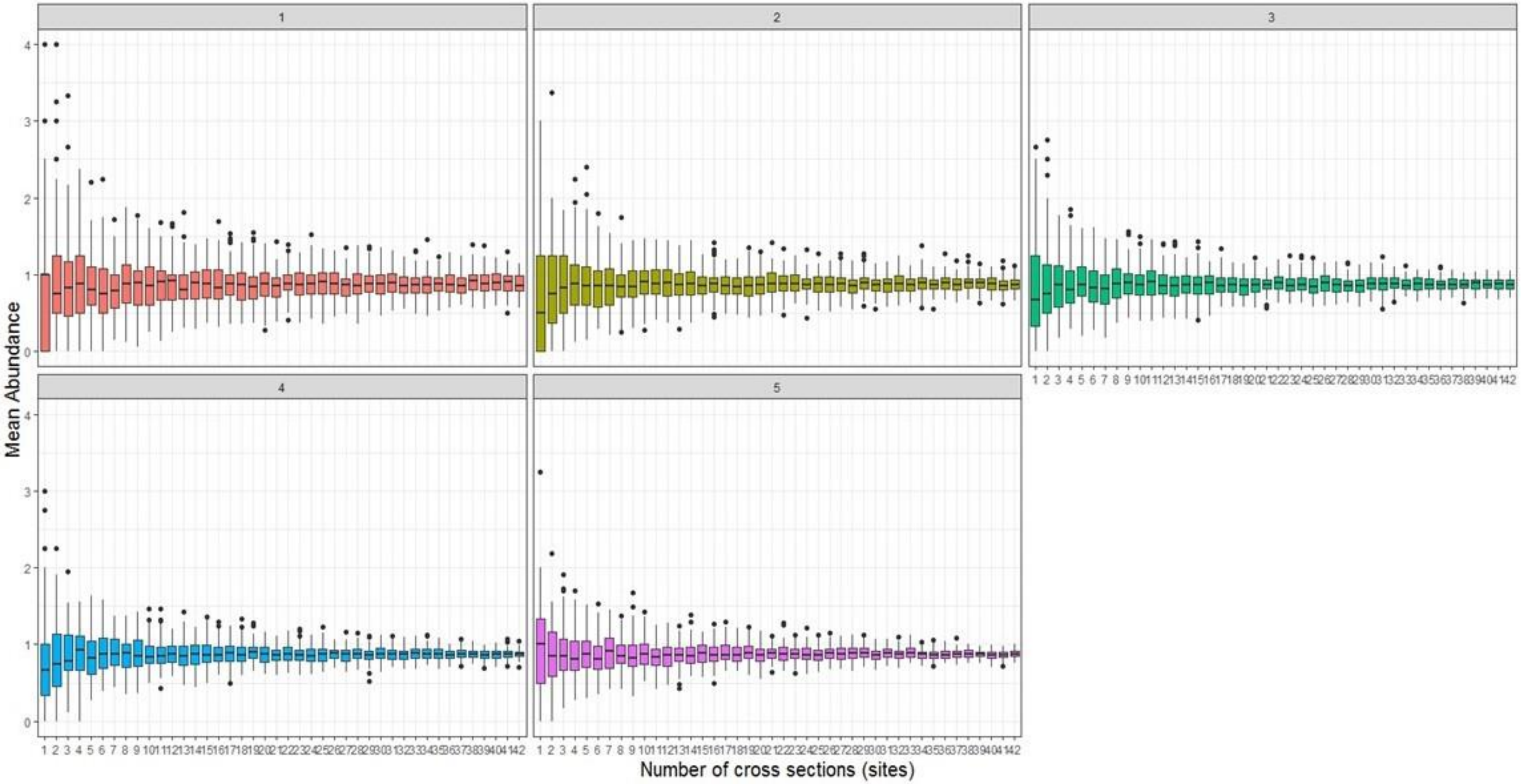

Figure 10. Average abundance of Rocky Mountain Sculpin (*Cottus* sp.) per m² as a function of the number of cross sections or sites (0-42 sites on x-axis) and quadrats (panels 1-5) surveyed in the St. Mary River in 2008. Boxplots represent the first quartile (25%), median (line), third quartile (75%) of the data, as well as the minimum, maximum (confidence lines), and outliers (points).

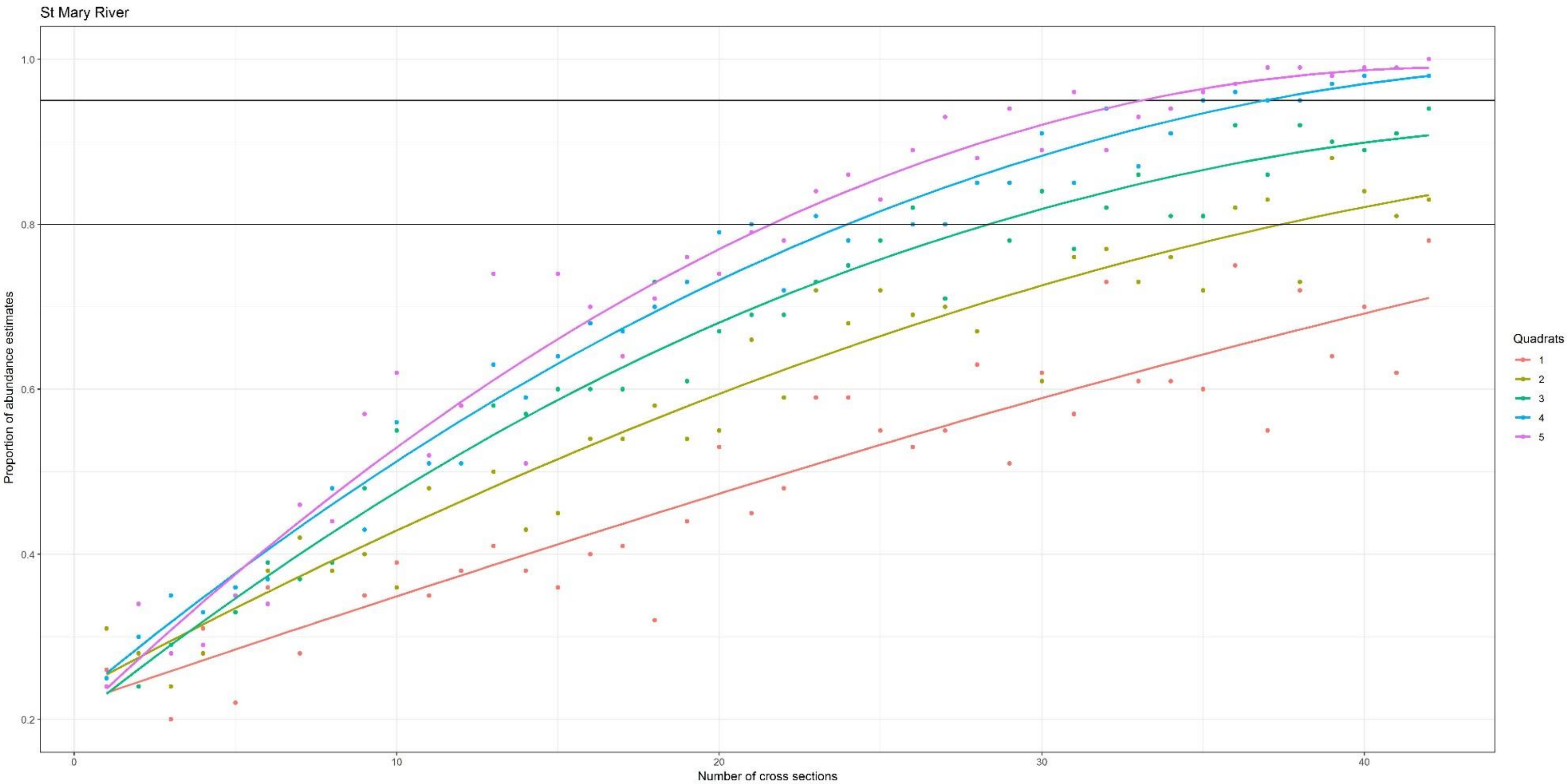

Figure 11. Proportion of abundance of Rocky Mountain Sculpin (*Cottus* sp.) within range of abundance estimates determined for 5 quadrats at each of the 42 sites (minimum = 0.71 fish·m^{-2} and maximum = 1.04 fish·m^{-2}) as a function of the number of sites (0-34 sites) and quadrats surveyed in the St. Mary River in 2008. “Loess” smoother lines were added per the number of quadrats surveyed. 95% and 80% confidence thresholds drawn.

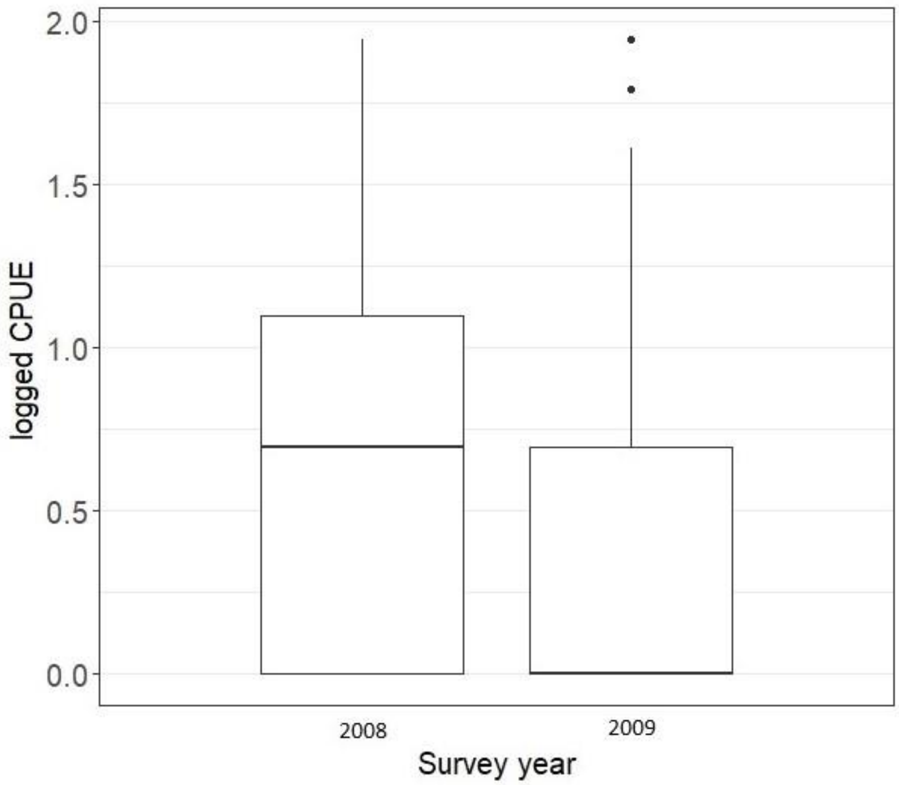

Figure 12. Logged CPUE for 2008 and 2009 Rocky Mountain Sculpin surveys in the St. Mary River main stem. Average logged CPUE decreased from 0.68 ± 0.61 in 2008 to 0.41 ± 0.52 in 2009.

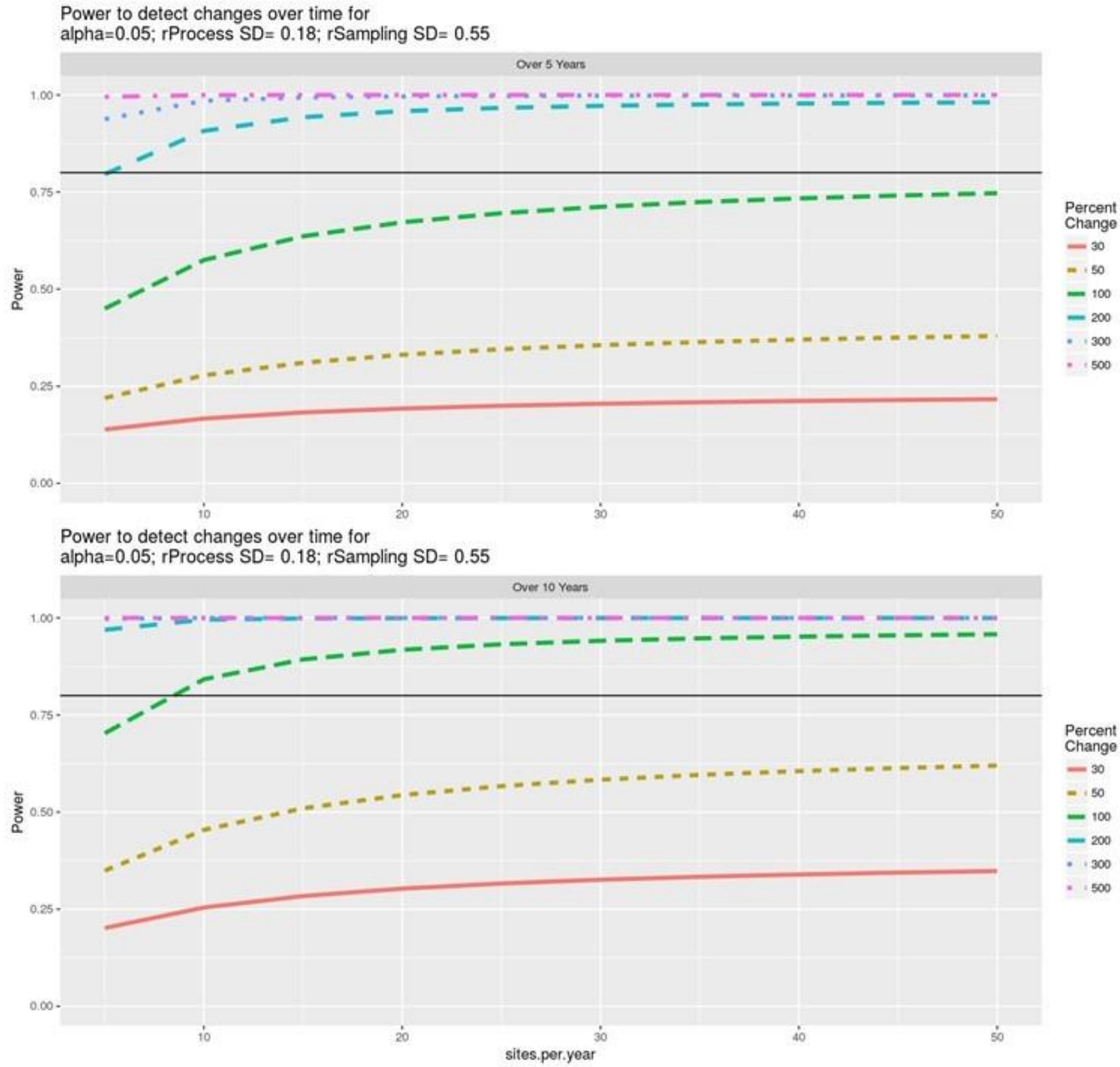

Figure 13. Power to detect changes (% change) across the number of sites per year required to detect these changes in Rocky Mountain Sculpin CPUE for the St. Mary River, over five and 10 years (Chang *et al.* 2018; Paul 2018).

3.0 SAMPLING PROTOCOL

3.1 Sampling Design

We developed a standard sampling protocol using backpack electrofishing has been developed to monitor occurrence and abundance of Rocky Mountain Sculpin in DU1-3 and obtain consistent fish survey data.

Access points

A list of access points has been assembled for river systems in which Rocky Mountain Sculpin occur (see Appendix 1 for the full list of access points and associated coordinates). Recommended access points per river are indicated by triangles, additional access points are illustrated by circles, and locations for possible range extension sampling are depicted by squares (Figures 14 15, and 16). A number of these stratified access points per system are recommended for monitoring population trends and should be resampled over time. Proposed range extension locations will provide information on whether the species' distribution is expanding or contracting. Range extension locations were chosen in the lower sections of tributaries to either the St. Mary River or Lee Creek, where Rocky Mountain Sculpin are known to occur as well as the St. Mary River, downstream of the St. Mary reservoir, where Rocky Mountain Sculpin have never been collected but may be present. Based on sampling experiences, it is recommended that a minimum of 2000 s of directed backpack electrofishing is conducted following a non-random approach at possible range extension sites, with an emphasis on targeting habitat that is likely occupied by Rocky Mountain Sculpin (i.e., cobble, gravel, or woody debris, in water depth of 0.2-0.8 m).

In the Flathead River system, 20 access points have been listed with information about the feasibility of fish surveying described for each access point; of these 10 access points are recommended for ease of sampling and repeatability; five in the Flathead River and one in each of five unique tributaries (Couldrey, Harvey, Middlepass, Howell, and Kishinena creeks; Figure 14). Rocky Mountain Sculpin were not sampled in Middlepass and Howell creeks, however, Slimy Sculpin were, indicating that suitable sculpin habitat may be available.

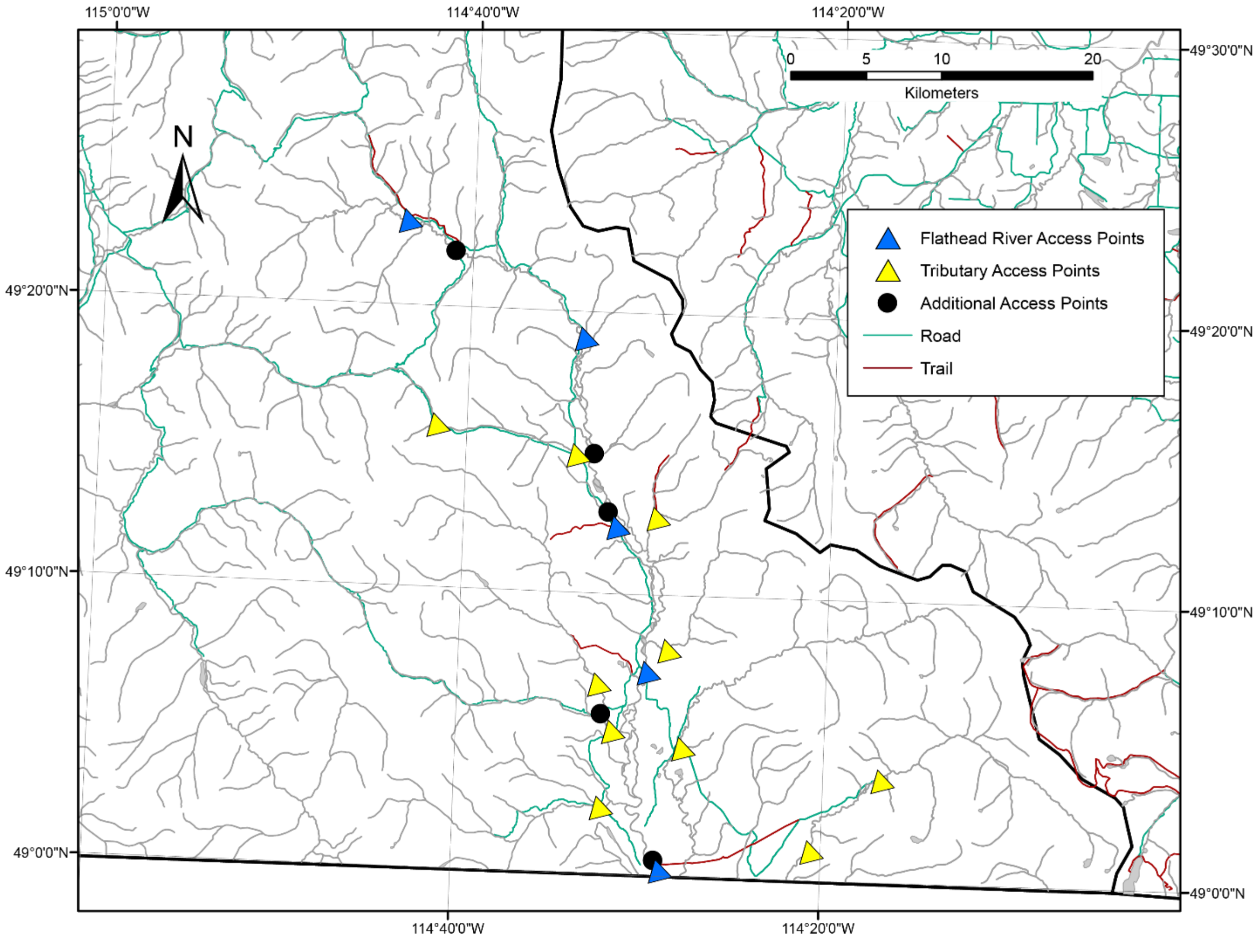

Figure 14. Map of the 20 recommended and additional access points for resampling along the Flathead River main stem and tributaries, Couldrey Creek, Harvey Creek, Middlepass Creek, Howell Creek, and Kishinena Creek, in British Columbia (DU1).

In the St. Mary and Milk rivers systems, 10 access points have been listed for sampling the distribution and relative abundance (Figure 15 and Figure 16). An additional four sites have been identified to investigate range expansion of the species. Rocky Mountain Sculpins have not been sampled in the St. Mary's reservoir or downstream of the reservoir (Roberts, W. pers. comm. 2003; Clayton, T., pers. comm. 2004), although it is unknown whether they once inhabited these sections. Five possible locations for range extension include the downstream river sections from the dam where suitable habitats for sculpin are found and Spoonhead Sculpin (*Cottus ricei*) are known occur. The geographic co-ordinates, site description, a brief description of the access type, and feasibility of sampling is included in Appendix 1. At the possible range extension sites, 2000 s of directed backpack electrofishing is suggested, with an emphasis on targeting habitat that is likely to occupied by Rocky Mountain Sculpin (i.e., cobble, gravel, or woody debris).

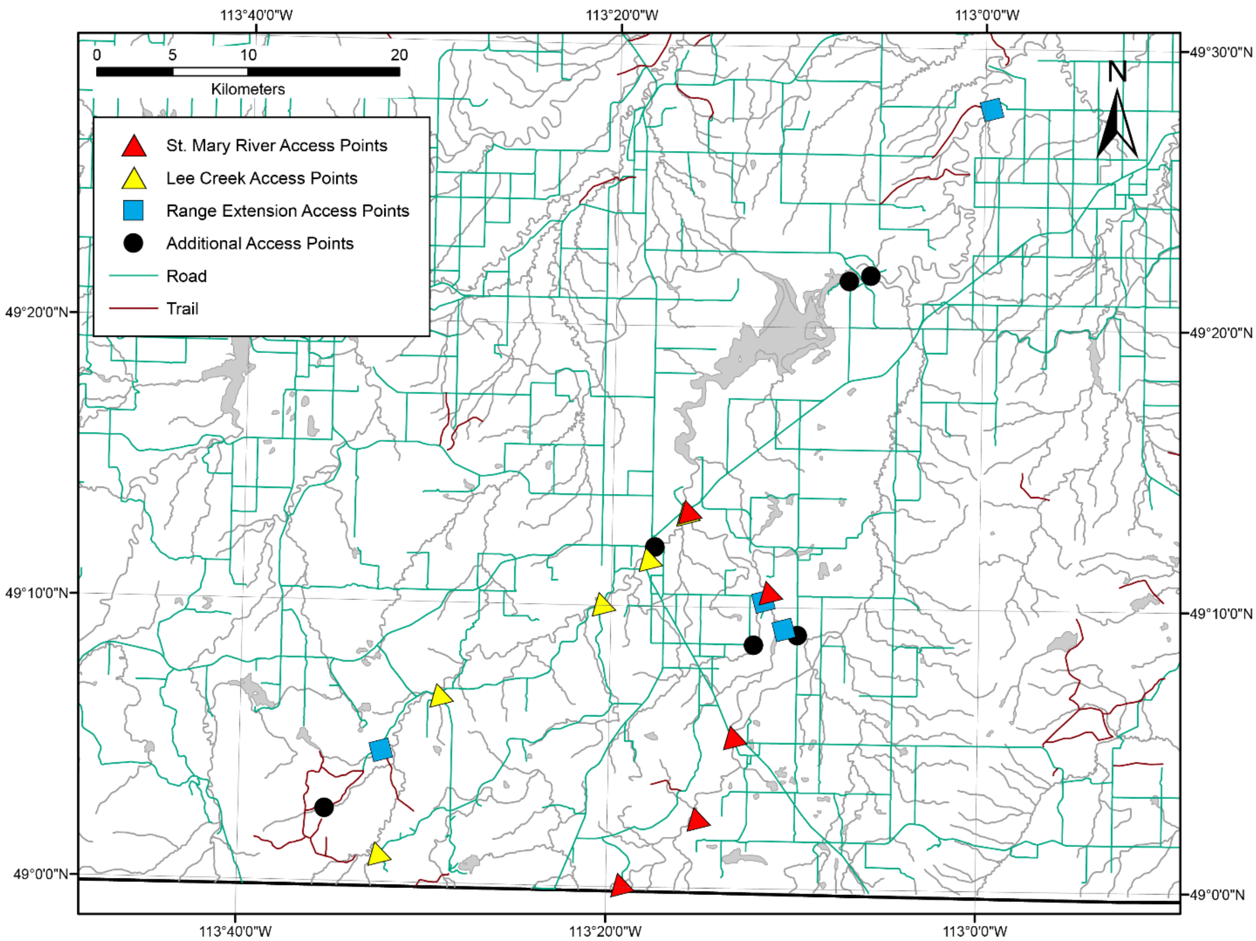

Figure 15. Map of the 19 recommended and additional access points along the St. Mary River watershed in Alberta (DU2) for resampling (triangles) and range extension (square) surveying.

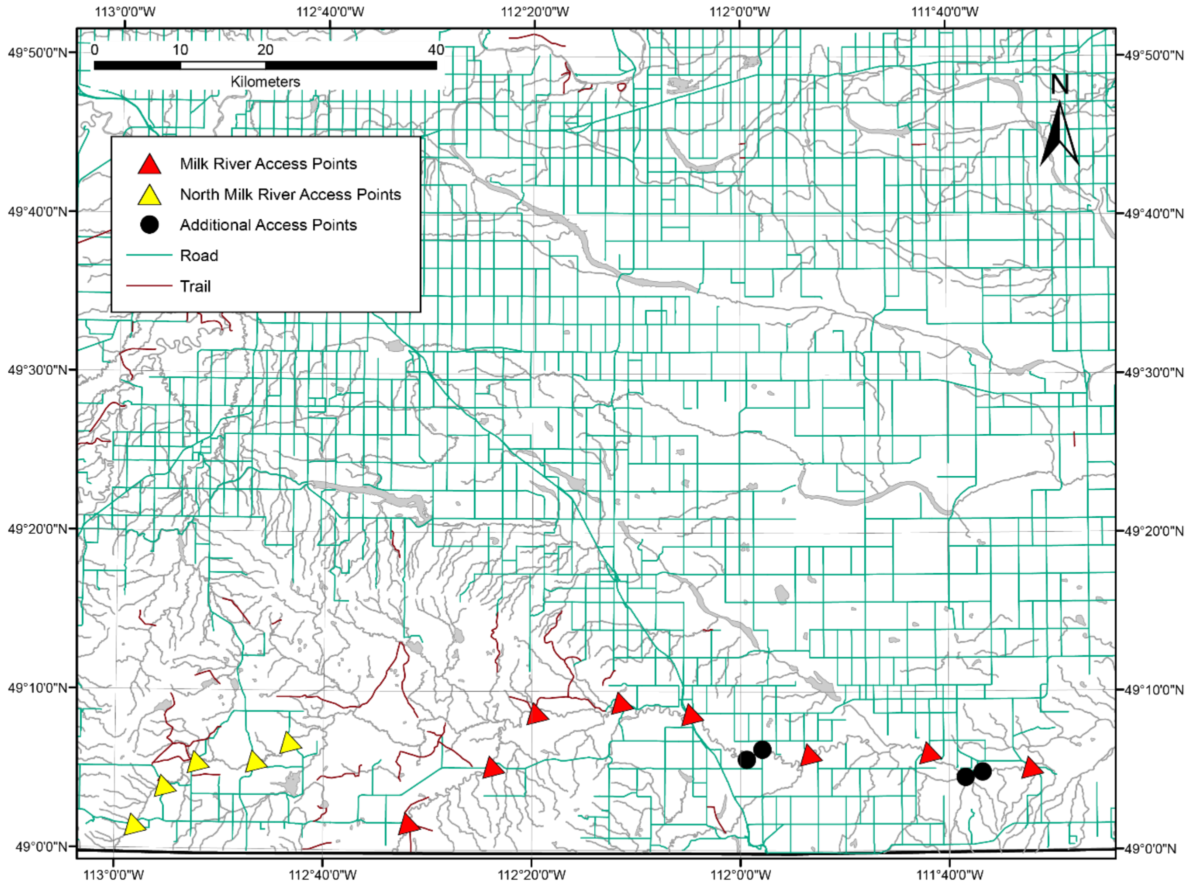

Figure 16. Map of the 17 access points along the Lower Milk River in Alberta (DU3) for resampling (triangles).

<u>Cross sections/Sites</u>

A cross section or site represents the river sampling unit, perpendicular to the river flow, where the quadrats are randomly positioned (i.e., 1-5 quadrats per cross section or site). Cross sections should be evenly distributed among recommended access points along each river, maximizing the spatial extent of the surveying effort (Figure 17). In order to balance the spatial distribution of sampling sites with the effort of moving between these cross sections, we recommend that cross sections are spaced out approximately 20 m apart. To avoid disturbing fish habitats during surveys, sampling should commence at the most downstream cross section or site at any given access point, moving upstream with each new site.

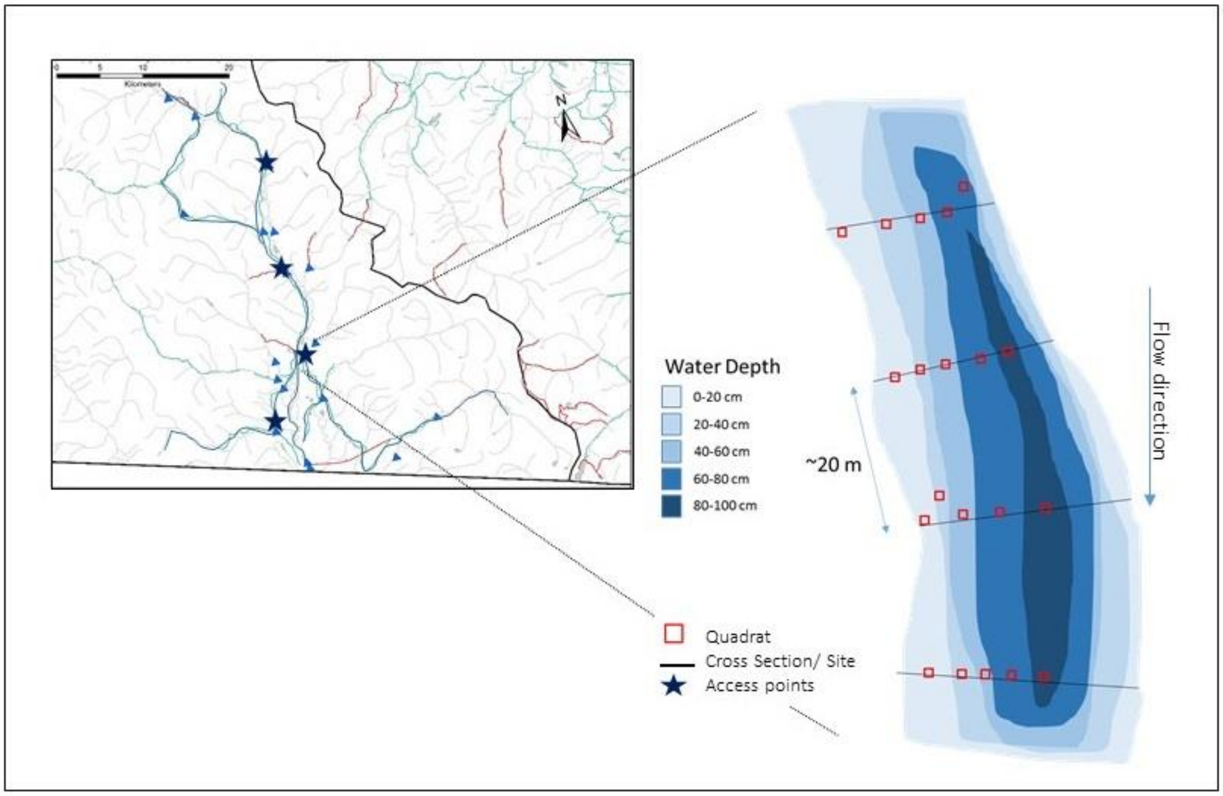

Figure 17. Schematic of the a river and tributaries, with 4 recommended access points (stars) positioned to maximize the spatial extent of cross sections/sites (black lines) and quadrats (red squares).

Quadrats/sampling unit

Quadrats represent the smallest sampling unit (1 m^2). Five quadrats should be distributed across river cross sections, each quadrat randomly occupying one of the water depth groups: 0-20 cm, 21-40 cm, 41-60 cm, 61-80 cm, and 81-100 cm (Figure 17). Starting from the shore, the first quadrat is placed at a depth randomly selected between 0-20 cm. The remaining quadrats are placed at a randomly selected depths obtained between 21-40 cm, 41-60 cm, 61-80 cm, and 81-100 cm, respectively. Realistically, all five quadrats should be sampled to maximize surveying effort at each of the cross sections. If water depth is less than one or more of these groups, the maximum depth should be sampled and any other quadrats randomly allocated to another group. Additionally, if the channel is less than 10 m wide, quadrats should be placed adjacent to one another, staggering them <3 m in an upstream direction to minimize disturbance between quadrats.

3.2 Timing of Sampling

Seasonality

Survey feasibility is contingent on seasonal water levels and water temperatures that allow fishing to consistently and predictably occur. Real-time hydrometric data for the systems are available from the Government of Alberta River Basins network and the Water Survey of Canada to inform on seasonal flow and water level variability (Figure 18).

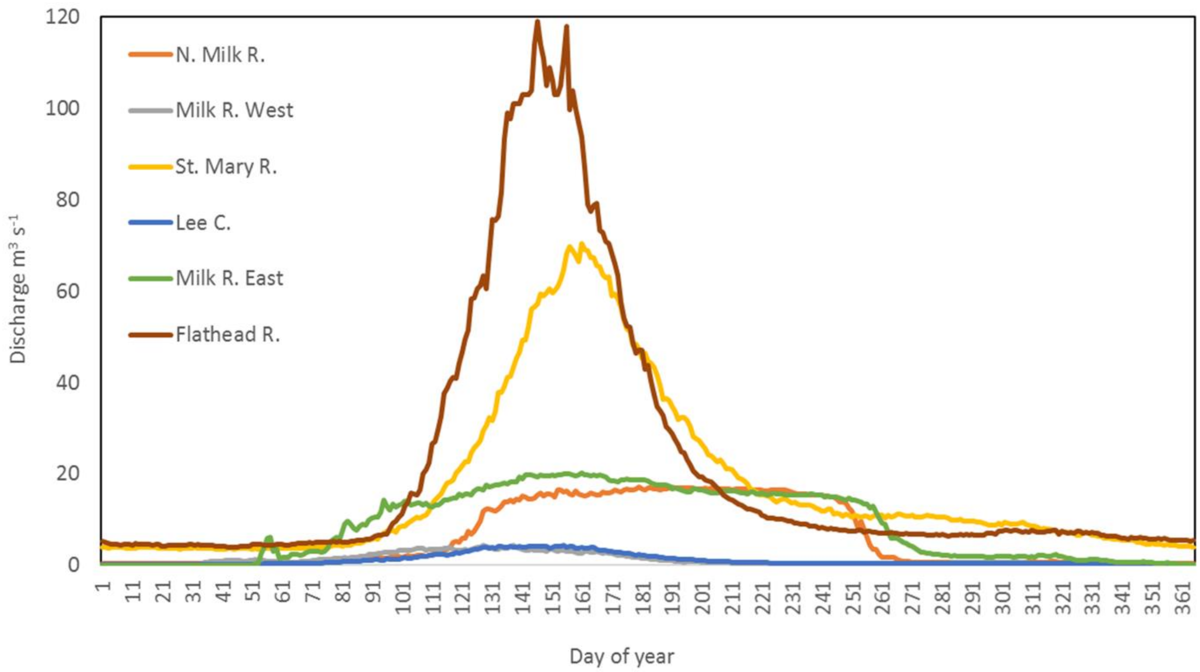

Figure 18. Hydrographs illustrating annual (Day 1= January 1) median discharge ($m^3 \cdot s^{-1}$) for hydrometric data collected by Water Survey of Canada for, in decreasing order of flow magnitude, the Flathead River (burgundy), St. Mary River (yellow), Milk River Eastern crossing (green), North Milk River (orange), Frenchman River (navy blue), Milk River Western crossing (grey), Battle Creek (light blue), and Lee Creek (dark blue).

To accurately monitor Rocky Mountain Sculpin population trends for all of the river systems where the species is known to occur, we recommend that sampling is generally conducted from August 1 to October 31 (~Day 213-304; Figure 19). However, there may be specific timing constraints due to air temperature. For example, low flows persist throughout the late-fall and winter months in most of the Flathead River, Battle Creek, and the Frenchman River, but water temperatures near 0 ºC, may make sampling challenging beyond mid-October. The North Milk River and Milk River downstream of the North Milk River confluence has been severely impacted by changes in its seasonal flow regimes. Water diverted from the St. Mary River in Montana augments flows in the Alberta portion of the Milk River from late March or early April through late September or mid-October. As such, high flows from mid-September (~Day 271; Figure 19) for the Milk River further reduces the window of time that Rocky Mountain Sculpin surveying can be conducted.

Table 1. List of real-time hydrometric stations and recommended sampling time in rivers where Rocky Mountain Sculpin occur.

Waterbody	Hydometric station	Site description	Suggested Sampling time	Source
St. Mary River	05AE027	St. Mary River near International Boundary	August 1-October 15	Alberta River Basins
Lee Creek	05AE002	Lee Creek at Cardston	August 1-October 15	Water Survey of Canada
North Milk River	11AA001	North Milk River near International Boundary	October 1-November 1	Water Survey of Canada
Milk River	11AA025	Milk River at Western Crossing of International Boundary	August 1-October 15	Water Survey of Canada
Flathead River	08NP001	Flathead River Near International Boundary	August 1-October 15	Water Survey of Canada

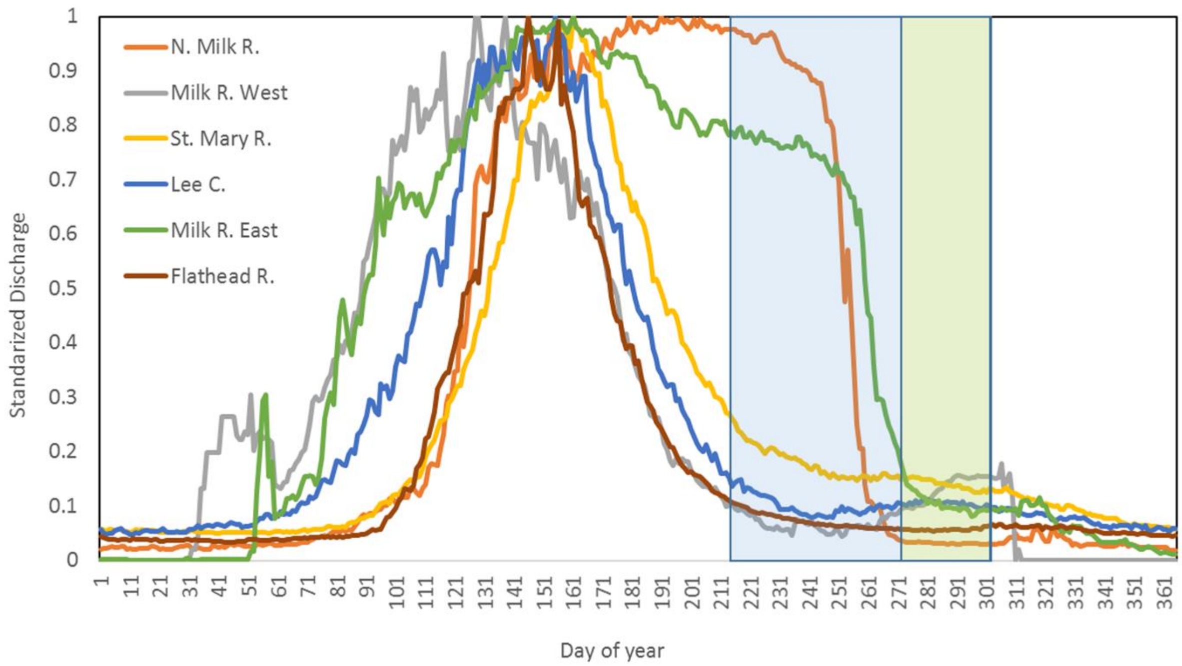

Figure 19. Hydrographs illustrating standardized median discharge ($m^3 \cdot s^{-1}$) over a year (Day 1= January 1) for hydrometric data collected by Water Survey of Canada for the Flathead River (burgundy), St. Mary River (yellow), Milk River Eastern crossing (green), North Milk River (orange), Frenchman River (navy blue), Milk River Western crossing (grey), Battle Creek (light blue), and Lee Creek (dark blue). Windows of time for surveying Rocky Mountain Sculpin for all systems and the Milk River specifically depicted by the blue and green boxes at ~ Days 221 and 271, respectively

Surveying Frequency

COSEWIC assessments determine the status of a species on a 10 year cycle, setting the timeline for when the information is required to update a species' status. To maximize the temporal extent of surveys and to provide a minimum of two estimates of the distribution and relative abundance of the species, sampling should be conducted twice in the 10 year cycle. Ideally, sites should be sampled once every five years, preferably not in consecutive years.

3.3 Sampling Gear and Method

Quadrats (1 m²) should be placed along the river cross sections, according to the schematic previously described (Figure 17). A minimum crew size of two people is required, one to operate an LR-24 backpack electrofisher and one to net (Smith-Root; Washington USA). The person with the backpack electrofisher is positioned upstream of the quadrat, with the netter standing downstream of the quadrat. The netter positions two 60 cm wide, 20 cm high nets downstream of the quadrat. Each quadrat is electrofished for a duration of 20 s, shuffling about the quadrat to dislodge sculpin from under rocks. Sculpin lack a swim bladder and are cryptic by nature, thereby requiring a more active search for individuals during electrofishing surveys, as they will stun and sink further under rocks. Once the electrofishing is finished, fish are placed in a bucket and immediately processed and released at the sampling quadrat.

Many factors affect electrofishing success. The most important environmental factor is the conductivity of the water, that is, its ability to conduct an electrical current due to the concentration of ions in the water. Other variables, including water temperature, depth, and velocity will affect electrofishing efficiency either on their own or via each variable's influence on conductivity. To consistently survey Rocky Mountain Sculpin using an LR24 backpack electrofishing unit across the species' distribution, the recommended range of settings for surveys are listed in Table 2.

Table 2. Recommended backpack electrofishing settings using the LR 24

Conductivity	Frequency	Pulse width	Voltage
150-400 µS cm^{-1}	30 Hz	15% in DC	100-400 V

The Rocky Mountain Sculpin protocol described here uses elements of the existing fish surveying protocol for first-time surveys of small streams in Alberta (Fish and Wildlife Alberta 2008) as a template. This protocol applies to wadeable streams (<1 m in water depth) in Alberta and British Columbia, where the distribution of Rocky Mountain Sculpin is currently being monitored. Refer to Appendix 2 for the database template.

<u>Environmental/Habitat Descriptors</u>

1. Waterbody name – List the name of the river surveyed (e.g., Milk River).
2. Access point – List the location of the access point where sampling is to take place (e.g., Nature Conservation Lot on the Flathead River).
3. Waterbody ID – List a unique number assigned to water bodies in Alberta (Fish and Wildlife Management Information System (FWMIS)).
4. Date of surveying – Use the format (dd/mm/yyyy). Do not abbreviate.
5. Crew – List the names of crew members so that appropriate persons may be contacted to verify data.
6. Latitude and longitude coordinates – Units should be in decimal degrees (WGS84). Provide geographic reference locations of each sample site.
7. Site location notes – Give concise description of the geographic location of the reach or site surveyed using map and site observations (e.g., 10 m upstream from confluence with tributary X).
8. Cross section/site number – Give a unique number to the cross section/site surveyed.
9. Water temperature – Measure the water temperature (ºC) where the water column is thoroughly mixed using an appropriately calibrated thermometer. Temperature influences the distribution of biota and the catchability of certain species. Avoid taking measurements in stream margins, outflows from tributaries or stagnant pools (unless the site is located in these habitats). Record the time of day (24 h).

10. Conductivity – Measure the conductivity, the capacity of transmitting electricity, within the site using a portable conductivity meter ($\mu S \cdot cm^{-1}$, standardized to 25 ºC). Conductivity influences electrofishing efficiency, thus, affects catchability and may provide the means to stratify data.
11. Turbidity – Measure the turbidity within the site using a portable turbidity meter (NTU) and a Secchi disk (cm). Turbidity influences catchability and may provide the means to stratify data.
12. Wetted and rooted width of the cross section – Measure the channel wetted and rooted widths (m) using a tape measure at the downstream (DS) and upstream (US) locations of the river reach surveyed. Wetted width corresponds to the width of the channel at the surface of the water at the time of survey. Wetted width influences electrofishing effort and efficiency, affecting catchability and CPUE. Rooted or bank-full width corresponds to the channel width at the base of permanently rooted vegetation. For braided channels, the measurement should include any islands not covered by permanent vegetation.
13. Maximum depth – Measure the depth of the water at the deepest point between the wetted banks using a meter stick.
14. Water depth – Measure the depth of the water (m) at each of the quadrats within a cross sections using a meter stick, making sure to obtain measurements from the center of the randomly selected quadrat.
15. Water velocity – Measure the water velocity of the water ($m \cdot s^{-1}$) at each of the quadrats within a cross sections using a flow meter metre and wading rod (Marsh-McBirney Flo-Mate), making sure to obtain measurements from the center of the randomly selected quadrat.
16. Site discharge– Measure the water velocity and depth of the water ($m \cdot s^{-1}$) at three points along the upstream-most cross-section of the access point, using a flow meter metre and wading rod (Marsh-McBirney Flo-Mate). Divide the creek/river width into thirds and measure water depth and velocity at each point.
17. Substrate complexity – Calculate the proportion of the substrate at each of the quadrats within a cross sections (visual assessment) that are: bedrock, boulder, cobble, large gravel, small gravel, sand, silt, and clay (modified Wentworth scale).
18. Plant cover – Calculate the proportion of plant cover at each of the quadrats within a cross sections (visual assessment).
19. Site characterization – Characterize the site surveyed based on the pool/riffle/run categories observed to provide a broad idea of productivity and a mechanism for stratifying data.
20. Photo number – Take a picture and record the number of the photograph taken during the stream survey.
21. Photo description – Briefly describe the picture taken for later reference. Indicate whether you are facing upstream (US) or downstream (DS).
22. Comments – Briefly describe any details relating to surveying, location, and sources of error (e.g., outflow from tributary) or change (e.g., seepage or barrier).

Electrofishing Descriptors

23. Time electrofished – Record the time (s) the electrofisher is in use and reset to zero at the start of each survey (quadrat). Electrofishing seconds corresponds to the sampling effort of each survey. This should be standardized for each quadrat (20 s).
24. Pulse width – Note the pulse width used to target the species. Should be standardized for each quadrat (15% DC).
25. Frequency – Note the frequency used to target the species. Should be standardized for each quadrat (30 Hz).
26. Voltage/ Power – Note the voltage (V) and power (W) used. Power should be standardized for each quadrant and the voltage will vary based on the water conductivity (100-400 V).

Fishing Descriptors

27. Capture method – Since the recommended capture method for Rocky Mountain Sculpin is electrofishing, write backpack electrofishing (LR24).
28. Sample Number – Sequentially number fish, one entry per fish sampled.
29. Species – Enter the name code for the Rocky Mountain Sculpin sampled (RMSC).
30. Fork length/total length – Record the fork (tip of the snout to the natural fork of the tail) and total (tip of the snout to the end of the tail) lengths (mm) for each fish sampled. Ensure that fish are placed on a flat measuring board.
31. Injuries/ comments – Note body condition and injury observations (e.g., lesions or parasite burden).
32. Sample picture – Place the fish on a flat, non-reflective surface and take a photograph of the fish on its left side, next to a ruler. Identify the picture number- (RMSC-number-date-river).
33. Sample specimen – retain a voucher specimen at each access point, indicating the location, time and date where the specimen was taken.
34. Refer to specimen collections for archives and life history (Appendix 3).
35. Refer to eDNA sampling protocol (Appendix 4).

4.0 SUMMARY AND RECOMMENDATIONS FOR FUTURE SAMPLING INVESTIGATIONS

The recommended Rocky Mountain Sculpin protocol described (section 3.3.) was established for wadeable rivers (<1 m in water depth) in Alberta and British Columbia, where the distribution of Rocky Mountain Sculpin is currently being monitored. The recommended survey effort was determined, in part, on the survey area (m^2) required to obtain reliable Rocky Mountain Sculpin occupancy and abundance estimates for the St. Mary river system, as well as the power analysis based on methods developed by Schwartz (2017) (Section 2.5). According to our results, the effort required to reliably determine the presence or absence and abundance estimates of Rocky Mountain Sculpin will vary among river systems on account of the variation in sculpin densities.

To reliably determine the occupancy of the species when they are common in a river (e.g., average density of 0.75 fish m^{-2}/ 20^{-s} for the St. Mary River), as little as 10 m^2 of survey area was required. The survey effort substantially increased when the species was less common in a system, from ~ 40 m^2 to 80 m^2 survey area recommended for moderate and low species densities (means of 0.52 and 0.1 individuals m^{-2}/ 20^{-s}, respectively). If five quadrats were sampled per cross section, a total of 2, 8, and 16 cross sections were required to reliably determine species occupancy for high, moderate, and low densities, respectively (Figure 20).

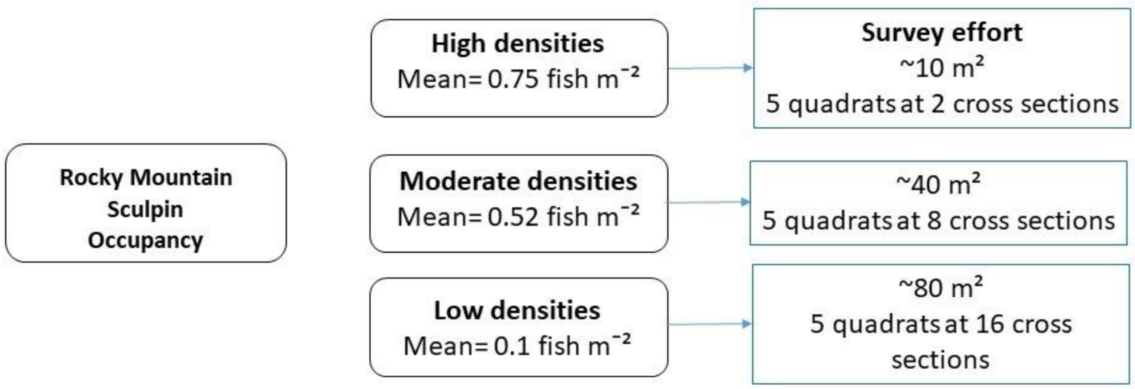

Figure 20. Decision tree summarizing the surveying effort required to reliably determine the occupancy (presence or absence) of Rocky Mountain Sculpin in systems of known high, moderate and low densities.

To accurately obtain Rocky Mountain Sculpin abundance estimates within 5% and 20% confidence of the highest abundance estimate measured for the St. Mary River, a total of 150 m^2 and 80 m^2 survey areas were recommended, respectively. These survey areas corresponded to survey efforts of five quadrats at each of the 30 and 16 cross sections, respectively. Like for the Rocky Mountain Sculpin occupancy results, a greater survey effort was required for systems with lower densities such as the Flathead River and tributaries, ranging from ~285 m^2 to 740 m^2 recommended area surveyed. The confidence in obtaining accurate abundance estimates was also variable among systems on account of sculpin densities, which is why our results focused on achieving abundance estimates with 80% confidence for sites in the Flathead River system (i.e., low and moderate densities). Despite lowering the confidence threshold from 95%, survey effort remained very high for the Flathead River system, from ~50 to 150 cross sections, with five quadrats at each of the cross sections (Figure 21). At this level of effort, sampling may not be feasible to accurately estimate changes in CPUE. Regardless, a minimum sampling effort that addresses occupancy is recommended in any system.

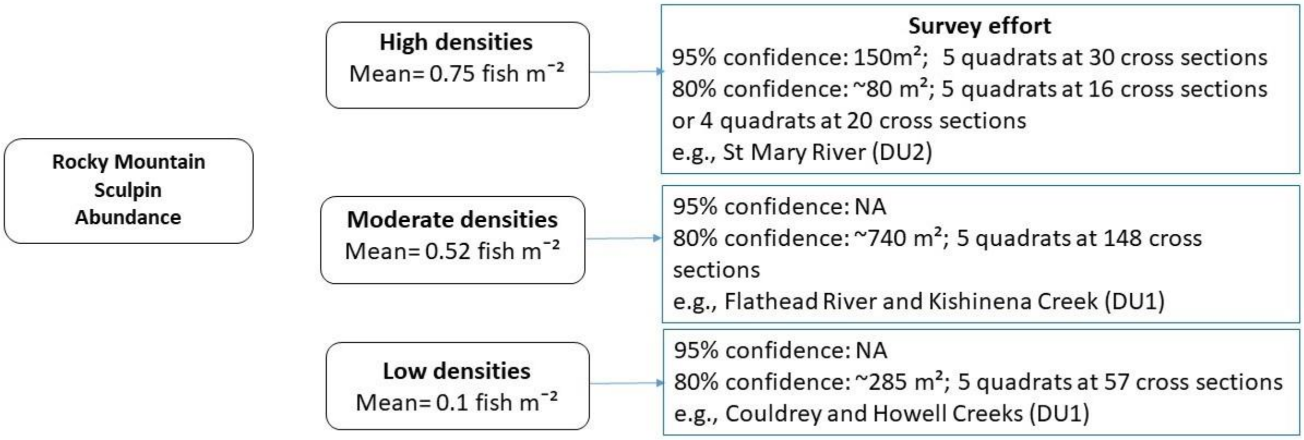

Figure 21. Decision tree summarizing the surveying effort required to accurately determine abundance (with 95% and 80% confidence) of Rocky Mountain Sculpin in systems of known high, moderate and low densities.

Recommendations from the current report directly inform on key objectives of the Species at Risk Recovery Plan by summarizing the CPUE, surveying effort, and proposing a standardized sampling protocol that will assist with monitoring the extent of Rocky Mountain Sculpin distribution and relative abundance across wadeable rivers (Fisheries and Oceans Canada 2012). By recommending that surveys be conducted ~ every five years, data from two survey events fit within the COSEWIC assessment timeline (i.e., 10 years) and allow for a better management of the species over time.

The recommendations established here stem from specific backpack electrofishing surveys conducted on the St. Mary River and Flathead River systems. Sensitivity tests on the length of time (shocking seconds) required to sample Rocky Mountain Sculpin in quadrats are needed to improve standardized protocols. Many other sampling techniques have been undertaken to sample Rocky Mountain Sculpin simultaneously with other *SARA*-listed species in the Milk River system. However, differences in surveying methods and CPUE render these surveys incomparable with the sampling results conducted in the St. Mary and Flathead River systems. As a result, the standard surveying protocol and survey effort described here are speculative for the Milk River system and rely on limited inferred information from the St. Mary and Flathead rivers. To refine the recovery strategy and ensure that the species is adequately monitored in the Milk River system, the sampling protocol and surveying effort described here should be revised following an initial survey to account for discrepancies among rivers.

Beyond the challenges of extrapolating results for different river systems, several advantages/ disadvantages exist for the current sampling protocol. For one, the sampling protocol was developed with the goal of confirming the occupancy and abundance of Rocky Mountain Sculpin in wadeable rivers, precluding its use for different species and/or deeper rivers. While adapting the surveying protocol might be simple for similar benthic species (e.g., Stonecat), additional analyses may be required to refine surveying effort recommendations for pelagic fishes. Another sampling challenge relates to the short window of time when backpack electrofishing surveys are feasible. Augmented flows in the North Milk and Milk rivers further reduce the window of time when surveys may be conducted. Lastly, the effort required to confirm the occupancy and abundance of Rocky Mountain Sculpin varied substantially across rivers, necessitating extensive surveying effort for Flathead River tributaries. Since the species is listed as Threatened by COSEWIC and Alberta, focus on developing a sampling protocol and guidelines as to the effort required to reliably survey the St. Mary River and tributaries was considered most important.

5.0 LITTERATURE CITED

Angelo, M. 2008. BC's Endangered Rivers. News Release, Outdoor recreation Council of BC. www.orcbc.ca/pro endangered.htm.

Bailey, J.E. 1952. Life history and ecology of the Sculpin *Cottus bairdi punctulatus* in southwestern Montana. Copeia 1952: 243-255.

Chang, W., J. Cheng, J.J. Allaire, Y. Xie and J. McPherson (2018). Shiny: Web Application Framework for R. hhts://CRAN.R-project.org/package=shiny.

Clayton, T.D., and G.R. Ash. 1980. A fisheries overview study of the Milk River Basin. Prepared for Alberta Environment, Planning Division by R.L. & L. Environmental Services Ltd. 93 pp.

Clayton, Terry. 2004. Lethbridge and Medicine Hat Fisheries Biologist, Fish and Wildlife Division, Alberta Sustainable Resource Development, South Lethbridge, AB.

COSEWIC 2010. Status report on the Columbia Mottled Sculpin *Cottus bairdi hubbsi* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 57 pp.

COSEWIC 2005. Status report on the "Eastslope" Sculpin, *Cottus* sp. in Canada: St. Mary and Milk rivers populations. Status Report submitted to the Committee on the Status of Endangered Wildlife in Canada.

Fish and Wildlife Management Information System (FWMIS). 2018. Alberta Environment and Parks. Accessed October 2018.

Fisheries and Oceans Canada. 2018. Management Plan for the Rocky Mountain Sculpin (*Cottus* sp.), Westslope populations, in Canada [Proposed]. Species at Risk Act Management Plan Series. Fisheries and Oceans Canada, Ottawa. iv+ 17 pp.

Fisheries and Oceans Canada. 2012. Recovery Strategy for the Rocky Mountain Sculpin (*Cottus* sp.), Eastslope Populations, in Canada. Species at Risk Act Recovery Strategy Series, Fisheries and Oceans Canada, Ottawa. X + 57 pp.

Fisheries Management Standards Committee (FMSC) 2008. Standard for the initial sampling of small streams in Alberta. Small Streams Inventories. Fish and Wildlife Alberta. Final 04/21/2008.

Jane, S.F., Wilcox, T.M., McKelvey, K.S., Young, M.K., Schwartz, M.K., Lowe, W.H., Letcher, B.H., Whiteley, A.R., 2014. Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. Mol. Ecol. Resour. n/a–n/a. http:// dx.doi.org/10.1111/1755-0998.12285.

McCleave, J.D. 1964. Movement and Population of the mottled Sculpin (*Cottus bairdi* Girard) in a small Montana stream. Copeia 1964: 506-513.

McPhail. J.D. 2007. The Freshwater Fishes of British Columbia. University of Alberta Press, Edmonton, Alberta.

NatureServe. 2018. NatureServe Explorer: An online encyclopedia of life. Version 4.0. NatureServe, Arlington, Virginia. Website URL: http://www.natureserve-canada.ca/en/cdcs.htm. [Accessed: November 19, 2018].

Paetz, M.J. 1993. A management plan for the Eastslope Sculpin (*Cottus* sp.) in Alberta. Prepared for World Wildlife Fund Canada and Alberta Fish and Wildlife Division. 21 pp. plus photo plates.

Paul A. 2018. Summary of Statistical Design and Power Analysis for the North Central Native Trout Recovery Program. Fish and Wildlife Habitat Policy. Alberta Environment and Parks.

P&E Environmental Consultants Ltd. 2002. Fish species of concern survey on the Milk River – October 2002. Prepared for Alberta Sustainable Resource Development and the Alberta Conservation Association. P&E Report No. 02018D. 12 pp. plus 3 appendices.

Peden, A.E. 2000 COSEWIC status report on the Columbia mottled Sculpin *Cottus bairdii hubbsi* in Canada. Committee on the Status of Endangered Wildlife in Canada (COSEWIC), CWS, Ottawa. xiv + 57 pp.

Peden, A.E. 2001. Updated COSEWIC status report on Shorthead Sculpin (*Cottus confusus*). Committee on the Status of Endangered Wildlife in Canada (COSEWIC), CWS, Ottawa. v + 55 pp.

R Core Team (2019) R: Language and Environment for Statistical Computation, edn. R. Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

R.L. & L. Environmental Services Ltd. 1987. An inventory of aquatic habitats and fish resources in the Milk River, Alberta. Prepared for Alberta Environment, Planning Division. 80 pp. plus appendices.

R.L.&L. Environmental Services. 2002. Fish species at risk in the Milk and St. Mary drainages. Prepared for Alberta Sustainable Resource Development, Edmonton. Alberta Species at Risk Report No. 45, 92 pp.

Roberts, W. 1988. The Sculpins of Alberta. Alberta Naturalist 18:121-153.

Rudolfsen, T., Ruppert, J. L., Taylor, E. B., Davis, C. S., Watkinson, D. A., & Poesch, M. S. (2019). Habitat use and hybridisation between the Rocky Mountain sculpin (Cottus sp.) and slimy sculpin (Cottus cognatus). *Freshwater Biology*, *64*(3), 391-404.

Rudolfsen, T., Watkinson, D. A., & Poesch, M. (2018). Morphological divergence of the threatened Rocky Mountain sculpin (*Cottus* sp.) is driven by biogeography and flow regime:

Implications for mitigating altered flow regime to freshwater fishes. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *28*(1), 78-86.

Ruppert, J. L., James, P. M., Taylor, E. B., Rudolfsen, T., Veillard, M., Davis, C. S., ... & Poesch, M. S. (2017). Riverscape genetic structure of a threatened and dispersal limited freshwater species, the Rocky Mountain Sculpin (Cottus sp.). *Conservation Genetics*, *18*(4), 925-937.

Savage, T. 1963. Reproductive behavior of the Mottled Sculpin, *Cottus bairdi* Girard. Copeia 1963: 317-325.

Schwartz C. 2017. Estimating power and sample size to detect trends in CPUE.

Scott, W.B., and E.J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada. Bulletin 184. 966 pp.

The Alberta Rocky Mountain Sculpin Recovery Team. 2013. Alberta Rocky Mountain Sculpin Recovery Plan: 2012-2022. Alberta Environment and Sustainable Resource Development, Alberta Species at Risk Recovery Plan No. 27. Edmonton, AB. 61 pp.

Thomsen, P.F., Kielgast, J., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T.P., Orlando, L., Willerslev, E., 2012b. Monitoring endangered freshwater biodiversity using environmental DNA. Mol. Ecol. 21, 2565–2573. http:// dx.doi.org/10.1111/j.1365-294X.2011.05418.x.

Thomsen, P. F. and Willerslev E. 2015. Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. Biological Conservation 183: 4–18.

Troffe, P. 1999. Freshwater fishes of the Columbia Basin in British Columbia – Living landscapes. Royal British Columbia Museum. 102 pp.

Veillard, M. F., Ruppert, J. L., Tierney, K., Watkinson, D. A., & Poesch, M. 2017. Comparative swimming and station-holding ability of the threatened Rocky Mountain Sculpin (*Cottus* sp.) from four hydrologically distinct rivers. *Conservation physiology*, *5*(1), cox026.

Water Survey of Canada 2016. Wateroffice: Historical Hydrometric Data: Environment Canada, Government of Canada.

Whang, A., and J. Jannsen. 1994. Sound production through the substrate during reproduction in the Mottled Sculpin, *Cottus bairdi* (Cottidae). Environ. Biol. Fishes 40: 141-148.

Willock, T.A. 1969. The ecology and zoogeography of fishes in the Missouri (Milk River) drainage of Alberta. M.Sc. Thesis. Carleton University. Ottawa, Ontario.

Wilcox, T. M., M.K. Young, K. S. McKelvey, M. K. Schwartz. 2018. Fine-scale environmental DNA sampling reveals climate-mediated interactions between native and invasive trout species. Ecosphere 9 (11): e02500. Doi. 10.1002/ecs2.2500.

Young, J.A.M. and M.A Koops. 2013. Recovery potential modelling of Rocky Mountain Sculpin (*Cottus* sp.), Eastslope populations, in Alberta. DFO Can. Sci. Advis. Sec. Res. Doc. 2013/061. v + 16 pp.

6.0 APPENDICES

APPENDIX 1. ACCESS POINTS FOR ALL ROCKY MOUNTAIN SCULPIN SURVEYING ACROSS DUS: WATERBODY AND ID, LOCATION, ACCESS TYPE, LATITUDE AND LONGITUDE, AND FIELD NOTES FOR THE FLATHEAD RIVER, ST. MARY RIVER AND MILK RIVER WATERSHEDS.

Waterbody	Waterbody ID	Location of access site	Access Type	DU	Latitude	Longitude	Notes
Commerce Creek	330-95660-77600-84200	FSR Bridge	Tributary	1	49.13475	-114.47794	Easy access on the downstream side of the bridge. Completely wadeable, no known barriers.
Couldrey Creek	330-95660-77600-80100	FSR Bridge	Tributary	1	49.04043	-114.53491	Slightly steep slope to access river bank. Sampling is possible both upstream and downstream of the bridge. River is wadeable across its width.
Flathead River	330-95660-77600-76100	Nature Conservancy Lot	Main stem	1	49.00421	-114.47926	Easy drive and direct access to river. Wadeability is limited due to fast flows.
Flathead River	330-95660-77600-76100	Warden Cabin	Additional	1	49.00957	-114.48369	Road access to river bank. It is a gravel bank, with a slight slope. About half of the wetted width is wadeable (<1 m deep).
Flathead River	330-95660-77600-76100	FSR Bridge 1	Main stem	1	49.12142	-114.4958	Road access to river bank on the northwest side of the bridge. About half of the wetted width is wadeable (<1 m deep).
Flathead River	330-95660-77600-76100	ATV Trail	Main stem	1	49.2068	-114.52821	Direct access off main Forest Service road. Due to the braided nature of the river, some walking on cobble river banks may be required to reach the main braid. Wadeability varies as a function of seasonality.
Flathead River	330-95660-77600-76100	Forest Clearing	Additional	1	49.21511	-114.5357	Very easy access and wadeable during mid-summer (i.e., low flow)
Flathead River	330-95660-77600-76100	Picnic Site	Additional	1	49.24939	-114.55025	Road access to river bank on the west side of the river. About half of the wetted width is wadeable (<1 m deep).
Flathead River	330-95660-77600-76100	FSR Bridge 2	Main stem	1	49.3181	-114.56319	There is a day use/camp area that provides easy access to the river on the southwest side of the bridge. River is completely wadable here, particularly around mid summer.

Flathead River	330-95660-77600-76100	FSR Bridge 3	Additional	1	49.36669	-114.68284	Slightly steep slope to access river bank. Best sampling site would be about 50 m upstream of this bridge. There are fewer deep pools and the river is wadeable.
Flathead River	330-95660-77600-76100	Old Town site	Main stem	1	49.38425	-114.72738	ATV or hike in (~2 to 2.5 h walk) required to access this site. Sandy bank is easy to scale and river is wadeable across its width.
Harvey Creek	330-956600-77600-88400	FSR Bridge	Tributary	1	49.24895	-114.56686	Creek is accessible from both directions. River is wadeable across its width.
Harvey Creek	330-956600-77600-88400	Forest Clearing	Tributary	1	49.26392	-114.69478	Very easy access and wadeable.
Howell Creek	330-95660-77600-82000	FSR Bridge 1	Tributary	1	49.08579	-114.52619	Easily accessible from the bridge. River is wadeable across its width.
Howell Creek	330-95660-77600-82000	FSR Bridge 2	Additional	1	49.09524	-114.53568	River is easily accessible from any direction via the bridge. Water is fast moving and wadeable, but with some difficulty.
Howell Creek	330-95660-77600-82000	Road & Cutline	Tributary	1	49.11343	-114.54045	ATV or 4x4 vehicle required to access. Driver precaution! The road is unstable and soft when wet. Culverts were removed, creating steep drops in the road, but it is driveable. Dead end leads to a cutline that must be walked to reach the creek. ~0.8 km walk of moderate difficulty.
Kishinena Creek	330-95660-77600-76100	Outfitter Camp	Tributary	1	49.01862	-114.34329	Safe, stable road, may experience some fallen trees over the road as it is not actively maintained by forest industry. Access is very easy and the creek is wadeable by mid-summer.
Kishinena Creek	330-95660-77600-76100	FSR Bridge	Tributary	1	49.06187	-114.28128	Slightly steep slope to access river bank. Easiest to park and access from west side of the bridge. Highly recommended to sample upstream, but close to the bridge. There is a waterfall downstream and canyon upstream from the bridge.
Middlepass Creek	330-95660-77600-86100	Road Crossing	Tributary	1	49.21296	-114.49192	ATV or all terrain vehicle required to drive the road, accessing the site. Steep drop and instability of the road requires samplers to stop and walk the road for ~400 m away from the creek. Sampling is possible both upstream or downstream of access point. Bridge at the crossing is unsafe for motor vehicles.

Sage Creek	330-95660-77600-77800	FSR Bridge	Tributary	1	49.07713	-114.46232	Unknown condition of this site.
Burnham Creek	330-956600-77600-80100-1170	FSR Bridge	Tributary	1	49.04098	-114.53608	Unknown condition of this site.
St. Mary River	2232	Private Ranch	Main stem	2	49.0021	-113.32071	Near US border
St. Mary River	2232	HWY 501	Main stem	2	49.09069	-113.22098	Kimball Park
St. Mary River	2232	Woolford Park	Main stem	2	49.17696	-113.19108	Woolford Park
St. Mary River	2232	HWY 5	Main stem	2	49.22408	-113.26537	Bridge
St. Mary River	2232	Township Rd 12	Main stem	2	49.04185	-113.25282	Mile north, gravel road
Lee Creek	934	HWY 5	Tributary	2	49.22281	-113.267	at the confluence
Lee Creek	934	HWY 2	Tributary	2	49.19547	-113.30058	Bridge
Lee Creek	934	HWY 501	Tributary	2	49.16849	-113.34223	Bridge
Lee Creek	934	HWY 501 at Beazer	Tributary	2	49.11258	-113.48742	Bridge
Lee Creek	934	Township Rd 11a	Tributary	2	49.01795	-113.54032	Ford Crossing
St. Mary River	2232	Township Rd 50a	Range Extension	2	49.4647	-112.9945	Downstream of Reservoir half way to Oldman River confluence
Aetna Creek	10	2526 Range Rd 245a	Range Extension	2	49.17079	-113.19545	Woolford Park
Tough Creek	1793	Ford Crossing off Range Rd 272a	Range Extension	2	49.07849	-113.53894	Ford Crossing
Rolph Creek	1476	Farm Yard Access	Range Extension	2	49.15416	-113.17648	Access in Provincial Park
St. Mary Resevoir	3535	Range Rd 240a	Additional	2	49.36168	-113.12184	Campground, SE side of St. Mary Reservoir
Lee Creek	934	HWY 501	Additional	2	49.20216	-113.29422	Bridge in the town of Cardston
Aetna Creek	10	Range Rd 250	Additional	2	49.14476	-113.2034	Woolford Park
Tough Creek	1793	Ford Crossing off Range Rd 272a	Additional	2	49.04361	-113.58876	Ford Crossing
Rolph Creek	1476	Range Rd 244	Additional	2	49.15104	-113.16411	Downstream is Provincial Park
St. Mary River	2232	HWY 509	Additional	2	49.58822	-112.88262	Bridge closer to Oldman River confluence
St. Mary River	2232	Township Rd 50a	Additional	2	49.36501	-113.10237	Immediately downstream of St. Mary Reservoir

Milk River	2136	Township Rd 12	Main stem	3	49.02963	-112.53237	Ford Crossing
Milk River	2136	HWY 501	Main stem	3	49.0895	-112.39801	Bridge
Milk River	2136	Twin River Heritage Rangeland	Main stem	3	49.14592	-112.328	Twin River Heritage Rangeland access road
Milk River	2136	Township Rd 24a	Main stem	3	49.15694	-112.19241	Roadside access
Milk River	2136	HWY 4	Main stem	3	49.14487	-112.08009	Town of Milk River
Milk River	2136	Range Rd 150a	Main stem	3	49.10267	-111.8905	Bridge
Milk River	2136	Township Rd 21a	Main stem	3	49.10424	-111.6998	Bridge
Milk River	2136	Hwy 500	Main stem	3	49.08851	-111.53676	Bridge
North Milk River	2159	HWY 501	Main stem	3	49.02641	-112.96956	Bridge
North Milk River	2159	Range Rd 225a	Main stem	3	49.06737	-112.9224	Bridge
North Milk River	2159	Range Rd 222b	Main stem	3	49.09299	-112.87037	Ford Crossing, Nature Conservatory of Canada
North Milk River	2159	HWY 62	Main stem	3	49.0938	-112.77712	Bridge
North Milk River	2159	Range Rd 212a	Main stem	3	49.11419	-112.72283	Bridge
Milk River	2136	Range Rd 154	Additional	3	49.10646	-111.96447	Near trout ponds
Milk River	2136	Township Rd 20	Additional	3	49.07709	-111.64036	Writing on Stone Picnic
Milk River	2136	Range Rd 130a	Additional	3	49.08265	-111.61297	Writing on Stone Campground
Milk River	2136	Township Rd 20	Additional	3	49.09605	-111.98948	Goldspring Park

APPENDIX 2. DATABASE TEMPLATE DEVELOPED FOR THE STANDARDIZED SAMPLING PROTOCOL OF ROCKY MOUNTAIN SCULPIN IN WADEABLE RIVERS.

Waterbody Name:		Activity Date: (month/day/year)	
Waterbody ID:		Time of Day	
Access Point		Crew:	

Start Latitude (decimal degrees)	Start Longitude (decimal degrees)	Cross Section	Quadrat	Wetted Width (m)	Rooted Width (m)

Water Temperature (°C)	Conductivity (µS/cm)	Depth (m)	Velocity (m/s)	Turbidity (NTU)/ Secchi (cm)	Max. Depth (m)

ELECTROFISHING

Time Fished (s):	Distance Fished (m):	Model Number	Pulse Width (ms/ %)	Frequency (hz)	Volts
	1m2				

Discharge (velocity/ depth) at US cross section	1	2	3

SUBSTRATE %

Bedrock (>1024 mm)		Comments:
Boulder (256-1024 mm)		
Cobble (64-256 mm)		
Large Gravel (34-64 mm)		
Small Gravel (2-34 mm)		
Sand (0.062-2 mm)		
Silt (0.004-0.062 mm)		
Clay (<0.004 mm)		
Plant material		

Photo Number:	Description:

Experience of crew for efishing and identifying species (name/ experience):

Fish #	Species ID	Fork Length (mm)	Photo ID	Specimen Collected (Y/N)	Comments

APPENDIX 3. ROCKY MOUNTAIN SCULPIN SPECIMEN COLLECTION FOR ARCHIVES AND LIFE HISTORY.

Measurements/ samples		Lethal/ Non-lethal sampling	Unit	Method	Purpose of measurement and sample
Morpho-logy and life history traits	Fork Length (FL)/ Total length (TL)/ Standard length (SL)	Non-lethal	mm	• Place fish on its side with the bottom jaw against the bumper of the measuring board/ trough. • Spread the tail and take the length at the shortest point in the tail (Fork), pinch the tail slightly and take length at the tip of the caudal fin (Total), or take the length of a fish measured from the tip of the snout to the posterior end of the last vertebra or to the posterior end of the mid-lateral portion of the hypural plate. This measurement excludes the length of the caudal fin (Standard).	
	Mass (M)	Non-lethal	g	• Weigh fish on manual or electronic scale. Wet mass corresponds to the mass of a live fish.	
	Otoliths	Lethal	Size (mm) and count of annuli	• Otoliths can be collected in different ways but for small fishes, use a sharp fillet knife to cut in horizontally from the snout toward the posterior of the fish, another cut is made straight down behind the head which will meet the horizontal cut. Once the two cuts have met, you may remove this section of the fish, brain matter may be covering the otoliths. Carefully remove the brain with a set of fine forceps, revealing two small pockets in which the otoliths are housed. • Remove the otoliths, clean with a Kim wipe and store in a folded Kim wipe and in a sample envelope or vial.	aging and growth via back-calculation of yearly annuli
	Dorsal Spine/ Fin ray/ Scales	Non-lethal		• Collecting a dorsal spine can be done by slicing the dorsal fin vertically between the second and third spine, then using a set of bone sheers or end cutting pliers to snip off the spines close to the base. • For scales, gently scrape against the grain of the scales with a set of angled forceps. Once loosened, the scales can easily be plucked from the skin. Location of scale extraction is species/ family-specific.	aging and growth via back-calculation of yearly annuli
	Gill raker	Lethal	Length and count	• With a set of bone sheers or end cutting pliers, reach into the gill area, cut the second gill raker as close to top and bottom to collect the entire raker without damaging filaments	respiratory and/or feeding morphology, morphometrics

	Gonads	Lethal	Maturity, size (g), and egg count	● Gonads can be extracted post mortem, once the cavity has been opened and the internal organs are removed. Depending on the time of year and maturity of the fish, the gonads may be very easy to locate (dorsal position).	life history traits such as age at maturity and fecundity
Tissue samples	Fin clip	Non-lethal		● With a small set of surgical scissors, take a thin slice of fin tissue from tip of pectoral fin approximately 0.5 cm by 2 cm on larger fish. take clip of relevant size for smaller fish. ● Fin clips taken for genetics should be stored in ≥70% ethanol to ensure DNA is not degraded.	
	Gill filament	Non-lethal	Minimum sample amount?	● With a set of angled forceps and a small set of surgical scissors, pinch the filament from the 2nd or 3rd gill arch, approximately 0.5 cm from the tip. With surgical scissors, cut the filament as close to the forceps as possible and carefully remove gill filaments. ● Gill filaments should be placed directly into a RNA Later solution and kept cold. Refrigerate samples for approximately 24 h, at ~4°C and transfer to a -80°C freezer until analysis. ● Note: tools must be cleaned thoroughly prior to extracting filaments from different fish. It is very important that these samples are not cross contaminated. Use a combination of bleach bath, 95% Ethanol bath, RNA away spray, Kim wipes and a butane torch lighter to sterilize tools prior to extraction.	environmental stress markers: genomic and transcriptomic analyses
	Blood	Lethal/ Non-lethal sampling	Minimum sample amount?	● For smaller euthanized fish, cut the tail off at the caudal peduncle. Place a capillary tube near the pooling blood and transfer contents to sample vials. ● For larger specimens, non-lethal blood samples may be taken. Turn the fish onto its dorsal side and insert a 20-22 gauge needle with a 5cc, heparinized syringe approximately 5-8 scales posterior to the anal fin. Gently angle the needle at 60°angle until you touch the spine and rotate before initiating suction. ● Blood samples are dropped directly into liquid nitrogen to flash freeze and transferred to a -80°C freezer to prevent degradation.	blood glucose, blood lactose, HSP proteins, plasma cortisol etc.
	Muscle	Non-lethal		● If needed, remove scales with forceps. Press and rotate slightly a sterile biopsy punch to cut into the tissue. Rotate on a wider axis before removing the punch and treat the wound site with a topical anesthetic/ disinfectant. ● Place muscle tissue in a vial.	Isotope analysis, condition factor

	Liver	Lethal	Size (g)	• Make an incision from the anus anteriorly to the gill arches. The liver is the large brown-red organ attached to the rest of the organs in the cavity. • Weigh the liver and place in sample vial.	
	Stomach	Lethal/ Non-lethal sampling	Size (g), food item count	• Stomach contents can be collected via gastric lavage for non-lethal sampling. • Lethal sampling consists of removing the gastric tract from esophagus to anus, taking care to uncoil the stomach and intestine from the fat the surrounds the organs. Store food items in ≥70% ethanol solution to ensure samples are not degraded.	Identify food items and complement isotope analyses

APPENDIX 4. ENVIRONMENTAL DNA (EDNA) SAMPLING PROTOCOL

Environmental DNA is defined as the genetic material obtained directly from environmental samples (e.g., water, soil and sediment) without any evidence of specimens (live or dead fish) on site (Thomsen and Willerslev 2015). It is an emerging tool in conservation for monitoring past and present biodiversity, as well as an efficient tool for investigating potential new range extensions for endangered species. Moreover, crowd-sourced eDNA sampling can also be used to develop species distribution models, which incorporate the effects from other species (Wilcox *et al.* 2018).

Several temporal and spatial constraints exist with the use of eDNA in river systems. For one, the decay of eDNA in freshwater beyond the threshold of detectability has been demonstrated to happen at a scale of days or weeks (Thomsen *et al.* 2012). Despite the short time-frame that eDNA may be relevant, the rapid degradation time in freshwater ecosystems makes eDNA very useful in conservation since a positive detection is likely to be associated with contemporary occurrences of the species, while potentially misleading signals from past species and populations are too low to detect. Second, the distance transport of eDNA in rivers may range from hundreds of meters to several kilometers (Jane *et al.* 2014). Specifically, the spatial scale of eDNA monitoring is essential when considering inferences on the proximity of target species compared to their DNA traces left behind. Transport of eDNA within an ecosystem remains an issue in rivers with higher flows. For eDNA to be relevant in the monitoring and conservation of contemporary biodiversity or range extension of endangered species, it is crucial that sampling and results reflect the current state of an ecosystem (Jane *et al.* 2014).

In the case that future interest in eDNA collection for Rocky Mountain Sculpin arises, we provide here an eDNA sampling protocol (abbreviated protocol provided by M. Docker eDNA lab, University of Manitoba):

1- Put on sterile gloves.
2- Label water bottles and Falcon tubes (or any other sterile tube) with the date, time, river site and collector.
3- Fill one 500-ml water bottle and fiver 50-mL Falcon tubes with water from the river site (total of 6 river water samples per site).
4- Fill one 50-mL Falcon tube with bottled water or distilled water at the site to serve as the negative control (detecting contamination if there is any).
5- Place the samples in a cooler. Samples are not filtered in the field, but need to be refrigerated until transferring samples to the eDNA lab.

Water samples should be collected at every access point to the river, over surveying periods between August and mid-October. Follow-up sampling using traditional fishing methods will be required for verification of Rocky Mountain Sculpin presence and species identification.