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# MORPHOLOGICAL AND GENETIC IDENTIFICATION AND DISTRIBUTION OF SCULPINS IN WESTERN MONTANA

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## ABSTRACT

Sculpins from the Bitterroot, Clark Fork, North Fork Flathead, and Kootenai River drainages were examined for electrophoretic and morphological characters. Using electrophoresis three species of sculpin were detected. Morphological examination showed them to be slimy, torrent, and most likely shorthead sculpins. Slimy sculpins inhabited all of the river drainages that were sampled. Torrent sculpins were collected only in the Kootenai River drainage and the presumed shorthead sculpins were found only in the North Fork Flathead. No evidence of hybridiazation was detected in any of the samples.

# INTRODUCTION

The taxonomy and identification of sculpins within the genus *Cottus* is often confusing. Morphological characters vary within a species and often overlap extensively between presumed species making species determination difficult (Bailey and Dimick 1949, Strauss 1980).

Electrophoresis of proteins often provides a reliable method of identifying species when results from morphological examination may be ambiguous. Electrophoresis refers to the separation of molecules in a medium through which an electrical current is passed. Protein molecules often have a net electrical charge and thus can be separated by electrophoresis. The net charge of a protein is determined by its chemical composition, which in turn is determined by the gene coding for its construction. Differences in the charge of the same protein, therefore, reflect differences in the genes coding for its construction. Because of this, protein electrophoresis can be used to estimate the amount of genetic variation within and divergence between natural populations of organisms.

A common finding of electrophoretic studies is that different species usually are characterized by having distinctive charge states at a number of proteins (Leary and Booke 1990). This implies that the genes (loci) coding for these proteins have distinctive allelic (form of a gene) states. Loci at which such 'fixed' genetic differences exist between taxa are commonly termed diagnostic loci (Ayala and Powell 1972) because an electrophoretic analysis of their proteins can be used to identify individuals and populations.

The purposes of this study were to use electrophoretic analysis of proteins to attempt to find diagnostic loci between sculpins which were

morphologically identified as being slimy, torrent, or shorthead sculpins and to look for hybridization between slimy and torrent sculpins.

## DESCRIPTION OF SPECIES

**Slimy sculpin (Cottus cognatus)** - Montana contains five species of sculpin. The slimy sculpin is primarily located in Montana west of the Continental Divide. It differs from the mottled sculpin (*Cottus bairdi*) and shorthead sculpin (*Cottus confusus*) by lacking palatine teeth and by having 3 instead of 4 pelvic fin rays (if it has 4 one is greatly reduced) (Richardson 1836, Hughes and Peden 1984, Holton 1990).

**Shorthead sculpin (Cottus confusus)** - In Montana, the shorthead sculpin is restricted to waters west of the Divide. It is mostly found in the Flathead River drainage, however, a few isolated populations have been found in the Blackfoot and Clark Fork drainages (Brown 1971). Shorthead sculpins are difficult to distinguish from mottled sculpins. Shorthead sculpins usually have 13 to 14 pectoral fin rays compared to 15 to 16 in the mottled sculpin (Bailey and Bond 1963, Maughan 1978, Holton 1990). However, the number of rays for a particular individual may differ from these ranges. Due to its limited distribution the United States Forest Service (USFS) lists the shorthead sculpin as a sensitive species by in the Kootenai, Flathead, and Helena National Forests.

**Mottled sculpin (Cottus bairdi)** - The mottled sculpin is located in Montana primarily east of the Divide (Holton 1990). However, Peden et al. (1989)

report finding mottled sculpins in the Flathead River drainage. Since both shorthead and mottled sculpins inhabit the Flathead drainage, species identification in this region is particularly difficult. This is especially problematic because of the shorthead sculpin's status as a species of special concern.

**Torrent sculpin (Cottus rhotheus)** - Torrent sculpins are found in the Kootenai River drainage in Montana. The complete lateral line, robust head, and narrow caudal peduncle make it easily distinguishable from the other species of sculpin in Montana (Smith 1882, McAllister and Lindsey 1959, Page and Burr 1991). The USFS also lists the torrent sculpin as a sensitive species in the Kootenai National Forest.

**Spoonhead sculpin (Cottus ricei)** - The spoonhead sculpin has a limited distribution in the State. Although it has been found in other locations, it is only abundant in the St. Mary and Waterton drainages in Glacier National Park. Morphologically it is easily distinguished from the other species of sculpin by the spine on its gill cover (Scott and Crossman 1979, Holton 1990). This species will not be discussed further in this report.

## METHODS

We received 220 individual sculpins from 12 locations in four major river drainages: the Bitterroot, the Clark Fork, the North Fork Flathead, and the Kootenai (Table 1). We analyzed the sculpins at 32 loci coding for proteins present in eye, liver, and muscle tissues by horizontal starch gel

electrophoresis (Table 2). Stains for enzymes followed the recipes of Harris and Hopkinson (1976) and Allendorf et al. (1977). Nomenclature of loci and alleles follows the recommendations of Shaklee et al. (1990).

We analyzed the number of pelvic fin rays on the left side, the completeness of the lateral line, the depth of the caudal peduncle, and presence or absence of palatine teeth of 175 individuals to morphologically identify to species and to look for concordance between morphological identification and gel banding patterns.

## RESULTS

### Identification

We found genetic and morphological similarities between the samples from Pipe Creek, North Fork Bull River, Whale Creek, Nez Perce Creek, Tin Cup Creek; Sleeping Child Creek, Price Creek, and 14 individuals from Quartz Creek We identified these specimens by the number of pelvic fin rays and absence of palatine teeth to be slimy sculpins (Table 3).

We identified a second group of sculpins from Libby Creek, Yaak River, Fortine Creek, and 7 specimens from Quartz Creek to be torrent sculpins by the complete lateral line, narrow caudal peduncle and large robust head (Table 3). When electrophoretically compared to slimy sculpins, the species were electrophoretically distinguisable at eleven loci with fixed differences and two loci with large allele frequency differences.

The remaining sample from Tepee Creek is distinct from either torrent and slimy sculpins in having four pelvic fin rays, palatine teeth and an incomplete lateral line (Table 3). These fish could be either mottled or shorthead sculpins. Based on 14 pectoral fin rays, we presume that they are shorthead sculpins but the possibility of them being mottled sculpin cannot be eliminated. The presumed shorthead sculpins and slimy sculpins differ at seven diagnostic loci. The presumed shorthead sculpins and torrent sculpins have thirteen loci with fixed differences and large allele frequency differences at an additional locus.

### Distributions

The samples from the Bitterroot River drainage and from the North Fork Bull River in the Clark Fork drainage contained only slimy sculpins. Samples from the five sites in the Kootenai River drainage contained either slimy sculpins or torrent sculpins and in one instance both species. In Quartz Creek where both species occured, there was no evidence of hybridization between them. In the North Fork Flathead, the Whale Creek sample contained only slimy sculpins and the Tepee Creek sample had only presumed shorthead sculpins.

### Genetic variation within species

We found low amounts of genetic variation within populations of sculpins based on the 32 protein loci examined. Among the eight samples of slimy sculpins, mean heterozygosity (Nei 1978) ranged from 0% to 2.0% (Table 5). Likewise, mean heterozygosity ranged from only 0 to 0.2% among the four torrent sculpin samples and was only 0.5% in the presumed shorthead sculpin sample (Table 5). The maximum number of polymorphic loci detected in any population of these species was two (Table 5).

## DISCUSSION

Previous studies have found evidence of hybridization within the genus *Cottus*. Strauss (1986) electrophoretically identified hybrids of mottled and slimy sculpins. Lyons' (1990) study based on morphology also reported mottled by slimy hybrids. Zimmerman and Wooten (1981) electrophoretically identified hybrids between the slimy sculpin and shorthead sculpin in the Flathead River drainage. Our results clearly provided no evidence of hybridization, however,

the opportunity for hybridization is rare since Quartz Creek was the only site examined with more than one species.

Field guides (Brown 1971, Holton 1990, Page and Burr 1991) often cite differences in the amount of prickles on the body, the degree of dorsal fin separation, and coloration such as chin mottling, fringe on the dorsal fin, and saddles on the body to identify species. We found these characters to be unreliable since they may be hard to detect, vary within species, or show seasonal variation. The most reliable morphological characters that we found in differentiating torrent, slimy, and shorthead sculpins are complete or incomplete lateral line, presence or absence of palatine teeth, and the number of pelvic fin rays (Table 3).

Torrent sculpins are the only species that we examined with a complete lateral line. The robust head and pinched caudal peduncle also make identifying torrent sculpins relatively easy. Although reports show that slimy sculpins may have four pelvic fin rays (Hughes and Peden 1984), all 112 of the specimens that we examined had only three. All specimens from the other two species have four pelvic fin rays, making the number of pelvic fin rays a reliable character in identifying slimy sculpins. Slimy sculpins are also the only species lacking palatine teeth. This character although reliable is more difficult to examine than pelvic fin rays. The presumed shorthead sculpins have an incomplete lateral line, four pelvic fin rays, and palatine teeth. Based on 14 pectoral fin rays they are most likely shorthead sculpins and not mottled sculpins (Holton 1990). However, Peden et al. (1989) report that the number of pectoral fin rays is not a reliable character in differentiating shorthead from mottled sculpins so the possibility that the specimens are mottled sculpins remains. An electrophoretic comparison of fish

from Tepee Creek with fish known to be mottled sculpins can potentially resolve this issue. Baker et al 1994

The species distributions agree with previous reports (Brown 1971. Holton 1990). Slimy sculpins were located in all four drainages sampled. Torrent sculpins were found only in the tributaries of the Kootenai River and the presumed shorthead sculpins were collected in the North Fork of the Flathead River.

Although the amount of genetic variation detected within sculpin populations was low this does not appear unusual for sculpins. Strauss (1989) examined 20 protein loci and found that mean heterozygosity in populations of 4 species of *Cottus* varied from 0% to 7%. We also found that within a species variant alleles appeared to be confined to single populations. The low amount of genetic diversity within populations and restricted distribution of variant alleles suggests that migrations between populations are rare. This can be attributed to the species limited movements and isolated headwater distribution (Hill and Grossman 1987).

Further investigation is needed to develop a more reliable field key for sculpins and to determine the distribution of each species. Special attention should be given to the shorthead and torrent sculpins due to their status in Montana. Also, the differences between shorthead and mottled sculpins need to be better resolved, especially in the Flathead River drainage, where both are reported to exist.

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Table 1. Number of each species collected at twelve localities.

| Sample | | species | N | Drainage |
|---|---|---|---|---|
| 1 | Tepee Creek | shorthead | 25 | N.Fork Flathead River |
| 2 | Whale Creek | slimy | 25 | N.Fork Flathead River |
| 3 | Fortine Creek | torrent | 25 | Kootenai River |
| 4 | Yaak River | torrent | 25 | Kootenai River |
| 5 | Pipe Creek | slimy | 27 | Kootenai River |
| 6 | Quartz Creek | slimy | 14 | Kootenai River |
| | Quartz Creek | torrent | 7 | Kootenai River |
| 7 | Libby Creek | torrent | 26 | Kootenai River |
| 8 | N.Fork Bull River | slimy | 25 | Clark Fork |
| 9 | Price Creek | slimy | 1 | Bitterroot River |
| 10 | Sleeping Child Creek | slimy | 3 | Bitterroot River |
| 11 | Tin Cup Creek | slimy | 3 | Bitterroot River |
| 12 | Nez Perce Fork | slimy | 14 | Bitterroot River |

Table 2. Enzyme systems examined and number of loci detected in this study. Tissue abbreviations: E = eye, L = liver, and M = muscle. Buffer abbreviations: AC = citric acid - N-3-aminopropylmorpholine buffer (pH 6.5) of Clayton and Tretiak (1972) and RW = Tris - boric acid buffer of Ridgway et al. (1970).

| Abbreviation | Name | # Loci | Tissue | Buffer |
|---|---|---|---|---|
| AAT | aspartate aminotransferase | 1 | M | AC |
| ACP | acid phosphatase | 1 | L | AC |
| ADA | adenosine deaminase | 1 | L | AC |
| ADH | alcohol dehydrogenase | 1 | L | RW |
| AH | aconitate hydratase | 1 | L | RW |
| ALAT | alanine aminotransferase | 1 | L | RW |
| CK | creatine kinase | 3 | E | AC |
| DIA | diaphorase | 1 | L | AC |
| FBALD | fructose-bisphosphatate aldolase | 1 | E | RW |
| FBP | fructose-bisphosphatase | 1 | E | AC |
| GAPDH | glyceraldehyde-3-phosphate dehydrogenase | 1 | E | AC |
| G3PDH | glucose-3-phosphate dehydrogenase | 1 | L | AC |
| G6PDH | glucose-6-phosphate dehydrogenase | 2 | L | RW |
| GPI | glucose-6-phosphate isomerase | 2 | M | RW |
| IDHP | isocitrate dehydrogenase | 1 | L | RW |
| LDH | lactate dehydrogenase | 2 | E | RW |
| MDH | malate dehydrogenase | 2 | L,M | AC |
| MEP | malic enzyme (NADP+) | 2 | E | AC |
| MPI | mannose-6-phosphate isomerase | 1 | L | RW |
| PEPA | dipeptidase | 1 | M | RW |
| PGDH | phosphoglutonate dehydrogenase | 1 | L | AC |
| PGM | phosphoglucomutase | 1 | L | AC |
| SOD | superoxide dismutase | 1 | L | RW |
| TPI | triose-phosphate isomerase | 1 | M | RW |
| XDH | xanthine dehydrogenase | 1 | L | RW |

Table 3. Allozyme loci and morphological characters used to identify sculpins. See Table 2 for enzyme abbreviations and Table 4 for allelic designations of letter codes. PT = palatine teeth. LL = lateral line (i=incomplete and c=complete). P2 = number of pelvic fin rays. CP = depth of caudal peduncle (b=broad and p=pinched).

| Sample | ACP | ADA | ADH | AH | CK1 | CK3 | DIA | G3PDH | GPI1 | GPI2 | LDH2 | MDH1 | MEP1 | MPI | SOD | TPI | PT | LL | P2 | CP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pipe | A | A/B | A | A | A | A | A | A | A | A | A | A | A | A | A | A | n | i | 3 | b |
| Quartz | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | n | i | 3 | b |
| NF Bull | A | A | A | A | A | A | A | A/C | A | A | A | A | A | A | A | A | n | i | 3 | b |
| Whale | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | n | i | 3 | b |
| Nez Perce | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | n | i | 3 | b |
| Tin Cup | A/B | A | A | A | A | A | A | A | A/B | A | A | A | A | A | A | A | n | i | 3 | b |
| Sleep Child | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | n | i | 3 | b |
| Price | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | A | n | i | 3 | b |
| Quartz | B | B | B | B | B | B | B | B | B | B | B | B | B | B | A/B | B | y | c | 4 | p |
| Libby | B | B | B | B | B | B | B | B/D | B | B | B | B | B | B | A | B | y | c | 4 | p |
| Yaak | B | B | B | B | B | B | B | B | B | B | B | B | B | B | A | B | y | c | 4 | p |
| Fortine | B | B | B | B | B | B | B | B | B | B | B/A | B | B | B | A | B | y | c | 4 | p |
| Tepee | C | A | A | A | A | A | A | A | A | B | B/C | B | C | A/B | A | B | y | i | 4 | b |

Table 4. Mobilities of electromorphs at diagnostic loci among slimy, torrent, and shorthead sculpins. When more than one allele was detected at a locus within a species the most common allele is listed first.

| Locus | slimy | | shorthead | | torrent | |
|---|---|---|---|---|---|---|
| ACP | 100 | A | 105 | C | 30 | B |
| | 30 | B | | | | |
| ADA | 100 | A | 100 | A | 110 | B |
| | 110 | B | | | | |
| ADH | - | A | - | A | 100 | B |
| AH | 100 | A | 100 | A | 40 | B |
| CK-1 | 100 | A | 100 | A | 110 | B |
| CK-3 | 100 | A | 100 | A | - | B |
| DIA | 100 | A | 100 | A | 95 | B |
| G3PDH | 100 | A | 100 | A | 90 | B |
| | 110 | C | | | 85 | D |
| GPI-1 | 100 | A | 110 | B | 100 | A |
| | 110 | B | | | | |
| GPI-2 | 100 | A | - | B | - | B |
| LDH-2 | 100 | A | 120 | B | 100 | A |
| | | | 95 | C | 120 | B |
| MDH-1 | 100 | A | 140 | B | 140 | B |
| MEP-1 | 100 | A | 105 | B | 125 | C |
| MPI | 100 | A | 100 | A | 95 | B |
| | | | 95 | B | | |
| SOD | 100 | A | 100 | A | 100 | A |
| | | | 120 | B | | |
| TPI | 100 | A | 400 | B | 400 | B |

Table 5. Percentage of polymorphic loci and mean heterozygosity.

| Species | Sample | N | % of loci polymorphic | mean heterozygosity |
|---|---|---|---|---|
| slimy | Pipe Creek | 27 | 2.9 | .003 |
| | Quartz Creek | 14 | 0.0 | .000 |
| | N.Fork Bull River | 25 | 2.9 | .011 |
| | Whale Creek | 25 | 0.0 | .000 |
| | Nez Perce Fork | 14 | 0.0 | .000 |
| | Tin Cup Creek | 3 | 5.9 | .020 |
| | Sleeping Child Creek | 3 | 0.0 | .000 |
| | Price Creek | 1 | 0.0 | .000 |
| torrent | Quartz Creek | 7 | 0.0 | .000 |
| | Libby Creek | 26 | 5.9 | .002 |
| | Yaak River | 25 | 0.0 | .000 |
| | Fortine Cr | 25 | 2.9 | .001 |
| shorthead | Tepee Creek | 25 | 5.9 | .005 |

Table 6. Number of individuals for each genotype and allele frequencies for polymorphic loci. Allele designated as 1 is the most common allele for that species.

| Species | Site | Locus | Genotypes 11 | 12 | 22 | Allele freq. 1 | 2 | F(IS) |
|---|---|---|---|---|---|---|---|---|
| slimy | Pipe Creek | ADA | 24 | 3 | 0 | .94 | .06 | -0.059 |
| | NF Bull River | G3PDH | 10 | 9 | 6 | .58 | .42 | 0.261 |
| | Tin Cup Creek | GPI-1 | 2 | 1 | 0 | .83 | .17 | -0.200 |
| | | ACP | 2 | 1 | 0 | .83 | .17 | -0.200 |
| torrent | Libby Creek | G3PDH | 25 | 1 | 0 | .98 | .02 | -0.020 |
| | | SOD | 25 | 1 | 0 | .98 | .02 | -0.020 |
| | Fortine Creek | LDH-2 | 24 | 1 | 0 | .98 | .02 | -0.020 |
| shorth. | Tepee Creek | LDH-2 | 23 | 2 | 0 | .96 | .04 | -0.042 |
| | | MPI | 23 | 2 | 0 | .96 | .04 | -0.042 |