Intermountain Journal of Sciences

Vol. 22, No. 1-3 - September 2016

(ISSN 1081-3519)

IJS

Intermountain Journal of Sciences

The Intermountain Journal of Sciences (IJS) is a regional peer-reviewed journal that encourages scientists, educators and students to submit their research, management applications, or viewpoints concerning the sciences applicable to the intermountain region. Original manuscripts dealing with biological, environmental, health and human development, mathematics, molecular-cellular, pharmaceutical, physical and social sciences are welcome.

Co-sponsors/publishers include the Montana Academy of Sciences, the Montana Chapters of The Wildlife Society and The American Fisheries Society. It is the intent of the governing bodies of the co-sponsoring organizations that this journal replace and standardize printed proceedings from the respective annual meetings. Format and style should follow the *Guidelines for Meeting Abstracts Submitted to the Intermountain Journal of Sciences, 1st revision 2016.** It is the policy of the editorial board that abstracts from presentations at annual meetings be published in the last issue of ***IJS*** for that year of the annual meeting. Submission of manuscripts for review and publication without regard to membership is encouraged.

Baseline funding is provided by the co-sponsoring organizations. Long-term funding will be derived from page charges assessed manuscript authors at $60/page, sponsoring organizations at $40/page for annual meeting abstracts and annual subscriptions: student - $6, regular member - $15, patron member - $25, international member - $25 and library - $25. One time subscriptions are: life member - $150 and sustaining subscriber - $2,500.

The intent of the co-sponsors and editorial board is that ***IJS*** be expanded to a quarterly journal. Achieving that objective depends upon numbers of acceptable manuscripts received and available funding. The editorial board's policy is that contributing authors be assured of publication within 12 months of acceptance of their manuscript. It is also intended that ***IJS*** be converted to an eJournal.

The organizational staff is voluntary and consists of an editorial board, an editor-in-chief, a managing editor, associate editors, a business manager and a panel of referees. The editorial board is responsible for establishing policy and the chair of the editorial board serves as liaison to the editor-in-chief and managing editor. The editor-in-chief is responsible for determining acceptability and level of revision of manuscripts based on referees' comments and recommendation of an associate editor. The managing editor serves as supervisor for layout and printing and liaison to the sponsoring organizations. Associate editors and referees are selected on the basis of their field and specific area of knowledge and expertise.

Associate editors and referees judge submitted manuscripts on originality, technical accuracy, interpretation and contribution to the scientific literature. Format and style should follow the *Guidelines for Manuscripts Submitted to the Intermountain Journal of Sciences, Dusek 1995, 2nd revision 2016.** Organization may vary to accommodate the content of the article, although the text is expected to elucidate application of results.

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ISSN #1081-3519

Editorial Review Policy

The *Intermountain Journal of Sciences* (IJS) is a fully refereed journal.

Manuscripts are submitted to the Editor-in-Chief (EIC) for initial consideration for publication in the IJS. This review shall include, but not be limited to, appropriateness for publication in IJS, correct formatting and inclusion of a letter of submittal by the author with information about the manuscript as stated in the "Guidelines for manuscripts submitted to the *Intermountain Journal of Sciences*" (Dusek 1995, 2007). This cover letter must also include a statement by the author that this paper has not been submitted for publication or published elsewhere. The EIC notes the date of receipt of the manuscript and assigns it a reference number, IJS-xxxx. The EIC forwards a letter of manuscript receipt and the reference number to the corresponding author. The corresponding author is the author who signed the submittal letter.

Three hard copies of the submitted manuscript, with copies of the "Guidelines and checklist for IJS referees" attached are forwarded to the appropriate Associate Editor. The Associate Editor retains one copy of the manuscript and guidelines for his/her review, and submits a similar package to each of two other reviewers. A minimum of two reviewers, including the Associate Editor, is recommeded for each manuscript. The two reviewers are instructed to return the manuscript and their comments to the Associate Editor. The Associate Editor then returns all manuscript copies and reviewer comments plus a recommendation for publication, with or without revisions, or rejection of the manuscript to the EIC. This initial review process is limited to 30 days.

The EIC then reviews the recommendations and all comments and notifies the corresponding author of the results of the review and the publication decision.

Acceptance

For accepted manuscripts, each copy of the manuscript containing comments thereon and other comments are returned to the corresponding author. Revised manuscripts are to be returned to the EIC in hard copy and four copies if further review is required. These copies can be submitted in digital form by email. The revised manuscript shall be returned to the EIC within 14 days of notification. Review of the revised manuscript by the Associate Editor and reviewers shall be completed and returned to the EIC within 14 days. An accepted manuscript will then be forwarded to the Managing Editor (ME) for final processing.

Rejection

Each manuscript that is rejected for publication is returned by the EIC to the corresponding author along with the reasons for rejection. The author is also advised that the manuscript may be resubmitted, provided all major criticisms and comments have been addressed in the resubmitted manuscript. The resubmitted manuscript may be returned to the initial review process if deemed appropriate by the EIC. If the manuscript is rejected a second time by either the EIC or the Associate Editor and reviewers, no further consideration will be given for publication of the manuscript in IJS. The corresponding author will be notified of this decision.

Reviewer Anonymity

The identity of all reviewers shall remain anonymous to the authors, called a blind review process. All criticisms or comments by authors shall be directed to the EIC; they may be referred to the ME or the Editorial Board by the EIC for resolution.

Manuscripts Submitted by Editors

Each manuscript submitted by an Associate Editor shall be reviewed by the EIC and a minimum of two other reviewers with expertise in the subject being addressed. Each manuscript submitted by the EIC shall be forwarded with the necessary review materials to the ME or chairman of the editorial board, who will serve as the EIC for that manuscript.

Abstracts

Only abstracts submitted from the annual meetings of the sponsoring organizations will be published in IJS. Other submissions of abstracts shall be considered on a case-by-case basis by the Editorial Board. Sponsoring organizations shall collect abstracts, review them for subject accuracy, format them in Microsoft Word and email them to Rick Douglass, the EIC (RDouglass@mtech.edu), on or before November 1. Each abstract shall be reviewed by the EIC to assure proper grammar, compliance with IJS "Guidelines for Abstracts Only" and for publication in the December issue of IJS.

Commentary

Submissions concerning management applications or viewpoints concerning current scientific or social issues of interest to the Intermountain region will be considered for publication in the "Commentary" Section. This section will feature concise, well-written manuscripts limited to 1,500 words. Commentaries will be limited to one per issue.

Submissions will be peer reviewed and page charges will be calculated at the same rate as for regular articles.

Literature Cited

Dusek, Gary L. 1995, revised 2007. Guidelines for manuscripts submitted to the *Intermountain Journal of Sciences*.Int. J. Sci. 1(1):61-70. Revised guidelines are available on the Intermountain Journal of Sciences web site: (www.intermountainjournal.org)

Macroinvertebrate Communities in the Big Hole River (Montana): A Comparison of Sites Sampled ~ 50 years apart

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Abstract

We replicated quantitative sampling of macroinvertebrate communities in two reaches of the Big Hole River in Montana from approximately 50 years ago. Our objective of this study was to compare macroinvertebrate densities, composition and biological integrity reported in 1960 to the present and to determine if any changes have occurred. Recent anecdotal reports from guides and fisherman have suggested that salmonfly, *Pteronarcys californica*, hatch numbers in the Big Hole River have been noticeably less than in previous years. We report that the salmonfly populations at these two sites were not different from 50 years ago; although some significant changes have occurred in the macroinvertebrate communities. Taxa richness measured at the family level has significantly increased at both sites since 1960, as has the benthic densities (numbers per m2). Biological integrity, as measured by decreased community tolerance scores, has also increased since 1960. At the upstream Site 1, the percent contribution of filtering caddisflies and the filtering trophic guild has significantly increased since 1960 with concurrent decreases of collector gatherers (e.g. Chironomidae). The macroinvertebrate community sampled at the downstream Site 2 was more similar to the 1960 community than the upstream Site 1, but community similarity at the same site sampled on consecutive years tends to be lower than taxa similarity. Overall, macroinvertebrate communities at these two Big Hole River sites provide significant evidence, both in increases of diversity and sensitive taxa that the health of the benthic community in this river section has improved over the last 50 years.

Key Words: macroinvertebrate community, *Pteronarcys californica*, salmonfly, Big Hole River, Montana

Introduction

The Big Hole River in southwest Montana is a popular recreational trout fishery that receives an average of 77,579 angler days per year; in 2011, the Big Hole ranked fourth for river fishing pressure in the state (MFWP 2011). It is one of the last rivers in the lower 48 states still inhabited by the fluvial arctic graying, *Thymallus arcticus* (Rens and Byorth 2010). The Big Hole watershed also contains the most viable populations of the western pearlshell mussel, *Margaritifera falcata*, in the state (Stagliano 2015). The Big Hole River is regionally famous among fisherman for its hatch of salmonflies, *Pteronarcys californica*, that typically emerge during early to mid-June (Gaufin et al. 1972). Recent drought conditions over the last decade (2000-2009) in Montana have been suggested by anglers as a reason for perceived lower numbers of salmonflies seen during their visits (Stagliano 2011). The most frequent response by guides and anglers to the polled question of "How have the Big Hole's recent salmonfly hatch numbers been?" was "they are not what they used to be" (Stagliano 2011). Though anecdotal, on-stream observations reported by anglers can often lead biologists to address these questions. Despite keen interest from anglers and natural resource managers, few studies on Montana's heavily-fished rivers have evaluated long-term changes of aquatic macroinvertebrate communities, especially

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salmonflies (McGuire 2003, MDEQ 2007, Stagliano 2011 and McGuire 2014). Several studies in the Big Hole River have provided cursory long-term information (1999-2012) on the status of macroinvertebrate communities within the basin, but these studies were qualitative and could not compare or compute population densities (McGuire 2003, MDEQ 2007, Bias 2014).

In 1960, a pre-impoundment study of the insect fauna was conducted on two reaches of the Big Hole River in southwest Montana (Averett 1961). The purpose of the 1960 study was to quantify baseline benthic macroinvertebrate composition and densities prior to a proposed Bureau of Reclamation dam project near Melrose, Montana, which was to be named Reichle Reservoir (Averett 1961). Our study objective was to replicate macroinvertebrate sampling at the two sites from the 1960 study and compare macroinvertebrate presence, density and community composition to current conditions, with particular emphasis on caddisfly, mayfly and stonefly families.

Study Area

The study area is located in southwestern Montana within the Big Hole River watershed (HUC 10020004) (Fig. 1). We sampled the upstream reach at the Hecla Bridge (Salmonfly Fishing Access site 1) at

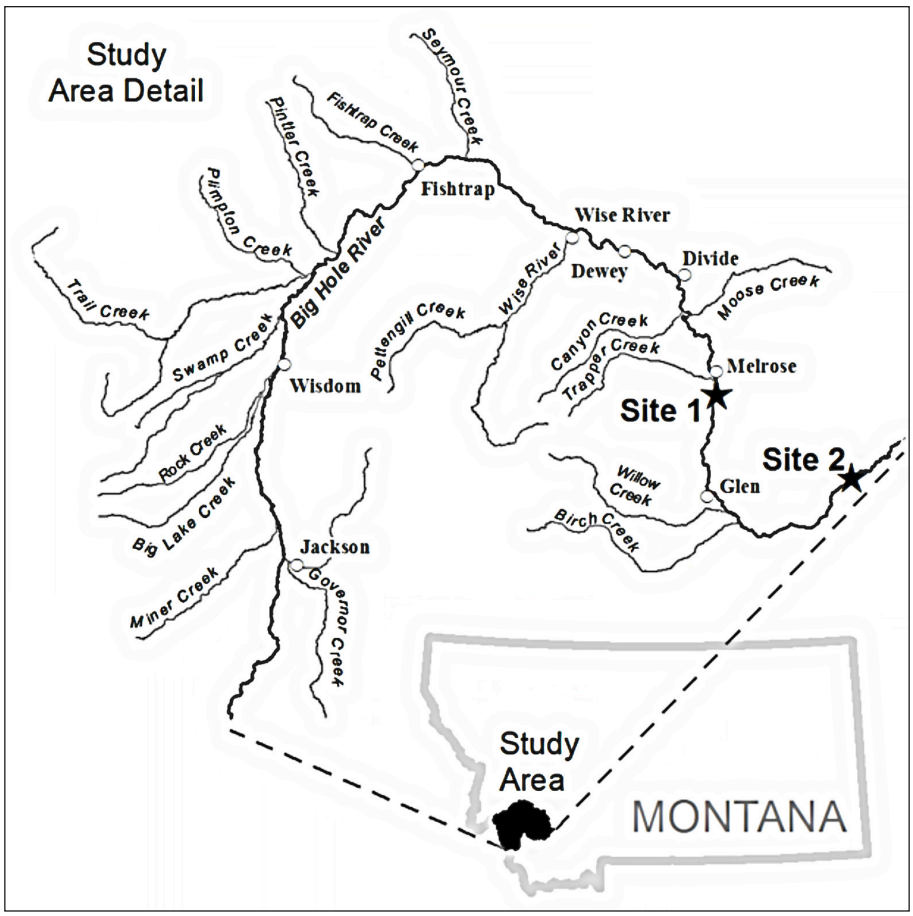

Figure 1. Location of sites sampled on the Big Hole River in Montana (stars).

an elevation of 5180 feet and a lower reach site 2 near Pennington Bridge at an elevation of 4720 ft. separated by 45 river km (Fig. 1). The original study sampled the downstream site (site 2) at Ziegler Hot Springs, which is now private, so the Pennington Bridge reach, 1.5 km downstream, represents a surrogate, publically-accessible site. Riffle habitats were sampled at both sites, with bottom substrate consisting primarily of large to medium cobbles mixed with gravels and silt in C3 classified channels determined using the Rosgen Level II Stream Designation (Rosgen 1994).

We sampled both sites on August 26, 2011, 51 years and 1 day from the previous study (August 25, 1960) during stable, summer hydrologic conditions. River discharge was measured at 405 cubic feet per second (cfs) compared to 381 cfs reported in 1960 (USGS Site 06025500 at 10:00 am). Previous years flows (2010) on this date were reported to be much lower at 310 cfs and similar in 2009 at ~400 cfs (USGS Site 06025500). We did not measure discharge at Pennington Bridge in 2011, although Averett (1961) reported a discharge of 413 cfs at the lower reach (Site 2) in 1960. With conservative calculations based on the prior study, we modeled stream flow at the Pennington Bridge site to be approximately 437 cfs (Table 1). The Big Hole River is a snow-melt dominated system and stream flows in 2011 were above average with a maximum peak flow of 11,700 cfs recorded at the Big Hole River, Melrose gauge on June 10 (USGS 2011).

Methods

Habitat and Physical Water Sampling

We recorded air temperature and basic physical water parameters (temperature, pH and conductivity) using an Oakton 10 water quality monitoring meter, calibrated for the lower conductivity range prior to macroinvertebrate sampling. We staked a 100 m survey tape on both banks across the stream channel to record stream wetted width and this also served as a guide for sampling locations at similar distances from shore as reported by Averett (1961). We recorded stream depths along the survey tape at each Surber sample point (n=8).

Macroinvertebrate Sampling

We used a standard Surber sampler (30.5cm x 30.5cm) with 500 micron (µm) mesh to quantitatively collect macroinvertebrates at eight points along each designated riffle transect at spaced distances from the bank corresponding to the 1960 study (Averett 1961). Eight discrete samples were collected at the downstream transect (X) of Site 1 before moving upstream to sample the next transect (W). We performed the same procedure at Site 2 for two transects (Y) and (Z) following Averett (1961). Each series of eight samples constituted a benthic area of 0.75 m^2. At each sampling point, we pushed the Surber sampler into the stream bottom and all cobbles (> 64 mm) within the sampling frame were scrubbed clean of organisms

Table 1. Comparison of physical stream parameters measured or recorded at Sites 1 and 2, in 1960 and 2011. Q = stream discharge in cubic feet per second.

Site	Year	Survey Time (MT hr.)	Air Temp (^{0}C)	Water Temp (^{0}C)	Wetted Width (m)	Average Depth (m)	Q (cfs)
1	1960	1000	12.1	12.1	44.9	0.42	381
	2011	1000	17.8	14.5	47.5	0.33	405
2	1960	1530	14.3	14.3	43.1	0.41	413
	2011	1300	24.8	18.1	48.7	0.37	447

and removed; then the entire area within the sampler frame was raked (disturbed) for approximately one minute until all organic matter and macroinvertebrates were washed into the collection net of the Surber sampler. Macroinvertebrates, organic and inorganic matter were then composited in a 40 liter bucket until all eight samples were collected. In the bucket, organic material was elutriated from the inorganic portion onto a 500µm sieve, so that only macroinvertebrates and organic matter were transferred into 1 liter labeled sampling jars filled with 95% ethanol (ETOH). The inorganic portion remaining in the bottom of the bucket was thoroughly examined for rock caddisfly cases before being discarded back into the stream.

Taxonomic Analysis

We processed and analyzed the samples at the Stag Benthics Helena laboratory. All macroinvertebrates were picked from the samples, placed into vials and identified to the lowest taxonomic level possible (genus/species) with a dissecting microscope (10-40x) following protocols developed by the Montana Department of Environmental Quality (MDEQ 2012). The macroinvertebrate taxonomic resolution of the 1960 study was primarily to the Family and Ordinal level, however, based on Montana distributions, we were able to classify some 1960 taxa to genus or species (e.g. Lepidostomatidae = *Lepidostoma sp*.; Rhagionidae = *Atherix sp*.). Prior studies have reported that Pteronarcys californica was the only species of salmonfly collected in the lower Big Hole River (McGuire 2003, MDEQ 2007, Stagliano 2011); therefore, we considered individuals of the Pteronarcyidae family to be this species. We assigned functional feeding guilds to the taxa groups based on Merritt and Cummins (2008) and pollution tolerance values (TV) based on MDEQ (2012). Analysis of functional feeding guilds attempts to link available food sources to responses in aquatic macroinvertebrate assemblages. TV are based on a 0-10 scale, where zero-ranked taxa are most sensitive and 10-ranked taxa are most tolerant to pollutants (Hilsenhoff 1987; MDEQ 2012). TV of 0.0-3.5 indicate no apparent organic pollution (excellent quality), 3.5-4.5 possible slight organic pollution (very good), 4.5-5.5 some pollution, 5.5-6.5 fairly significant pollution, 6.5-7.5 significant pollution (fairly poor), 7.5-8.5 very significant organic pollution 8.5-10 severe organic pollution. These taxa TVs have also been found to act as a surrogate for sediment impairment (MDEQ 2012). The combined mayfly, caddisfly and stonefly species (EPT taxa) and the relative percentage of these in the sample (%EPT) are always informative metrics, as EPT taxa contain some of the more intolerant aquatic insects, usually requiring clean substrates (Plafkin et al 1989, Barbour et al. 1999). Thus, EPT metrics typically decrease with increasing sediment in the benthic substrates (Plafkin et al 1989, Barbour et al. 1999); although, Tricos (*Tricorythodes* and *Caenis*) and burrowing mayflies (*Ephemeridae*) are silt tolerant and can increase in numbers with increasing siltation. We calculated % EPT, instead of EPT taxa richness, because there is limited value of this metric at the family level of taxonomic resolution reported in 1960. We analyzed metrics of the macroinvertebrate data using a one-way ANOVA and the Percent Similarity Index as the comparison of macroinvertebrate communities, families, functional guilds and tolerance values between years. Differences were considered significant at p values less than 0.05.

Results

Habitat and Physical Water Characteristics

The Hecla Bridge site (Site 1) had an average stream width (two transects) of 47.5 m. Average stream depth across transects (n=16) was 0.33m (1.1 ft.) and water and air temperatures recorded at 10:00am August 26th, 2011 were 14.5°C (58°F) and 17.8°C (64.4°F) respectively (Table 1). The Pennington Bridge site (Site 2) had an average stream width of 48.7 m (n=2). Average stream depth across transects points

(n=16) at Pennington bridge was 0.37m (1.2 ft.). Water and air temperatures at this site in 2011 (1:00 pm) were 18.1°C (64.6°F) and 25°C (77°F), respectively. The same stream physical parameters were collected for both sites in 1960 (Table 1).

Macroinvertebrate Communities

Hecla Bridge: Upper Site 1

We reported nine macroinvertebrate orders and 22 families at Site 1 in 2011, while in 1960, eight orders and 11 families were present; three of those families present in 1960 (Nemourdae, Tanyderidae, Corixidae) were not represented in 2011; however, we collected 13 new families in 2011 (Table 2). This change in macroinvertebrate family composition represents a significant increase in family-level diversity compared to 1960 (ANOVA, p=0.02). Chironomidae was the dominant family in 1960, while in 2011 the caddisfly family, Hydropsychidae was dominant (Table 2). The percent community similarity index between W transects between years was 47.8%, while taxa similarity was only 33.3%. The percent similarity index between X transects between years was 41.7%, while taxa similarity was 40.0% (Table 2).

Numbers of the caddisfly families, Brachycentridae and Glossosomatidae were significantly higher in 2011 than in 1960 (F-test, $p < 0.05$); these taxa were not reported in the 1960 samples (Fig. 2). The percentage of the community comprised of the filtering caddisfly, Hydropsychidae was 19% higher in 2011 (avg. 23%) compared to 1960 (avg. 4%), but this was not significantly different (ANOVA, $p = 0.07$). Increases in these two caddisfly families contributed to the significantly higher percentage of the filtering-collectors trophic guild in 2011 (Fig. 3). We reported significant (p=0.05) declines in the numbers of the caddisfly family, Leptoceridae in 2011 (avg. 2) compared to 1960 (avg. 54), but not in the percentage of the sample comprised by that family (ANOVA, p=0.06) (Fig. 2). Other taxa with non-significant decreases in community percentage since 1960 are the Simuliidae (6%), Chironomidae (7%) and Baetidae (9%) (Fig. 2). Decreases in the Chironomidae and Baetidae families have led to large, but not significant decreases in the gatherer–collector trophic guild between years (Fig. 3). Large increases of numbers of individuals were seen in three caddisfly families at Site 1, while one caddis family (Leptoceridae) significantly decreased (Table 2).

A significant proportion of individuals that comprised these samples in 2011 were less tolerant (i.e. more sensitive) than in 1960 (Fig. 4). Taxa with tolerance values of 0-3 increased up to 16%, while taxa ranked 4-6 decreased 13% since 1960 (Fig. 4). Community tolerance values decreased significantly (ANOVA p=0.03) from 4.1 to 3.2 for this site (Fig. 5).

Total densities of macroinvertebrates were significantly higher in 2011 (average 451 per m2) than in 1960 (average 347 per m^2) (F-test, p=0.04) (Table 2). Densities of salmonflies in 1960 were reported to be 41.2 per m^2, while in 2011 we reported 39.9 per m^2. No significant differences were detected between numbers of salmonflies between years (ANOVA $p = 0.89$, 1960 avg. n=31; 2011 avg. n=30).

Pennington Bridge: Lower Site 2

Eight macroinvertebrate orders and 20 families were recorded at Site 2 in 2011, while in 1960, six orders and 12 families were present; all families from 1960 were represented in 2011 samples plus eight new families (Table 2). Macroinvertebrate family richness was significant different between years (ANOVA, p=0.04). The filtering caddisfly family, Hydropsychidae was the dominant taxa in both years at this site with a large decrease reported between 1960 (avg. 52%) and 2011 (avg. 33%) (Fig. 2), but his was not significant ($p = 0.39$). The percent community similarity index between Y transects between years was 60.8%, while taxa similarity was 57.9%. The percent similarity index between the Z transects between years was 54.7%, while taxa similarity was 42.1% (Table 2). Increases in taxa densities at the lower reach from 1960 to 2011 were dominated

Table 2. Macroinvertebrate raw sample data and family characteristics. FC= Filterer-Collector, SC= Scraper-Collector, GC = Gatherer–Collector, SH=Shredder, PR=Predator. Habitat: BU=Burrower, CN=Clinger, CL=Climber, SP=Sprawler, SW=Swimmer. TV=Tolerance Value. EPT= Ephemeroptera, Plecoptera and Trichoptera.

					Upper Site 1				Lower Site 2			
					Transect W		Transect X		Transect Y		Transect Z	
Order	Family	Feeding Guild	Habitat Guild	TV	1960	2011	1960	2011	1960	2011	1960	2011
Trichoptera	Brachycentridae	FC	CN	1	0	38	0	29	4	32	9	25
Trichoptera	Hydropsychidae	FC	CN	4	8	39	15	132	161	136	137	107
Trichoptera	Hydroptilidae	SC	CL	4	0	5	0	1	0	1	0	1
Trichoptera	Glossosomatidae	SC	CN	0	0	25	0	33	0	3	0	4
Trichoptera	Leptoceridae	GC	CL	4	72	2	36	2	4	1	3	1
Trichoptera	Lepidostomidae	SH	CN	1	0	7	0	1	0	0	0	0
Plecoptera	Pteronarcyidae	SH	CN	0	26	31	36	29	0	0	2	0
Plecoptera	Perlidae	PR	CN	1	0	20	3	14	7	39	15	45
Plecoptera	Perlodidae	PR	CN	2	0	1	0	1	0	8	0	3
Plecoptera	Nemouridae	SH	SP	2	0	0	5	0	0	0	0	0
Diptera	Simuliidae	FC	CN	6	38	7	0	1	0	1	0	3
Diptera	Chironomidae	GC	BU	6	58	48	57	52	20	26	64	42
Diptera	Rhagionidae	PR	CN	2	0	5	0	6	2	25	0	38
Diptera	Tipulidae	SH	BU	3	5	1	5	3	9	14	2	8
Diptera	Tanyderidae	SH	BU	6	1	0	0	0	0	0	0	0
Ephemeroptera	Baetidae	GC	SW	4	30	55	78	12	60	10	32	16
Ephemeroptera	Ephemerellidae	GC	CN	1	0	7	0	5	0	6	0	12
Ephemeroptera	Heptagenidae	SC	CN	4	0	13	0	2	0	1	0	2
Ephemeroptera	Ephemeridae	GC	BU	4	0	0	1	0	9	1	0	0
Ephemeroptera	Leptophlebiidae	GC	SW	2	0	2	0	1	0	0	0	0
Ephemeroptera	Leptohyphidae	GC	SW	2	0	3	0	6	0	8	0	9
Coleoptera	Elmidae	GC	CN	4	20	31	27	45	12	41	2	56
Hemiptera	Corixidae	PR	SW	5	0	0	26	0	0	1	0	0
Lepidoptera	Pyralidae	SH	CL	5	1	0	5	1	7	4	6	0
Gastropoda	Gastropoda*	SC	CN	7	0	5	2	1	0	0	0	2
Nematoda	Nematoda*	PR	BU	8	0	2	0	0	0	0	0	0
Oligochaeta	Tubificidae	GC	BU	10	0	3	0	1	0	0	0	0
Total Number in Sample					**259**	**345**	**296**	**377**	**295**	**358**	**272**	**374**
Number per m^2					**323.8**	**431.3**	**370.0**	**471.3**	**368.8**	**447.5**	**340.0**	**467.5**
Number of families					**10**	**22**	**13**	**22**	**11**	**19**	**10**	**17**
% EPT					**52.5**	**71.9**	**58.8**	**71.1**	**83.1**	**68.7**	**72.8**	**60.2**
% Community similarity					**47.8%**		**41.7%**		**60.8%**		**54.7%**	

* No family determined

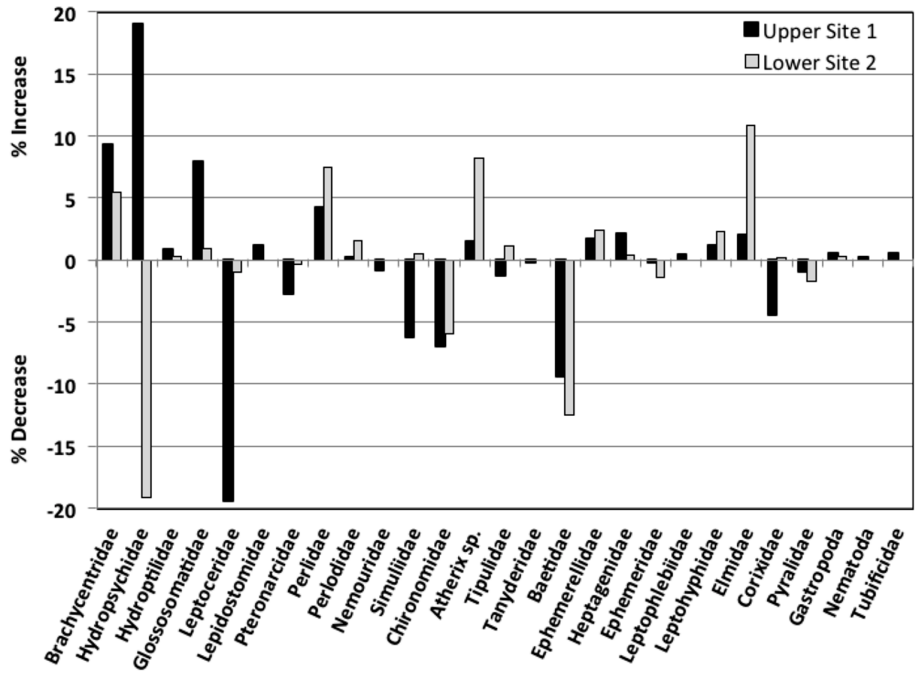

Figure 2. Differences in macroinvertebrate taxa (family level) composition between 1960 and 2011 samples.

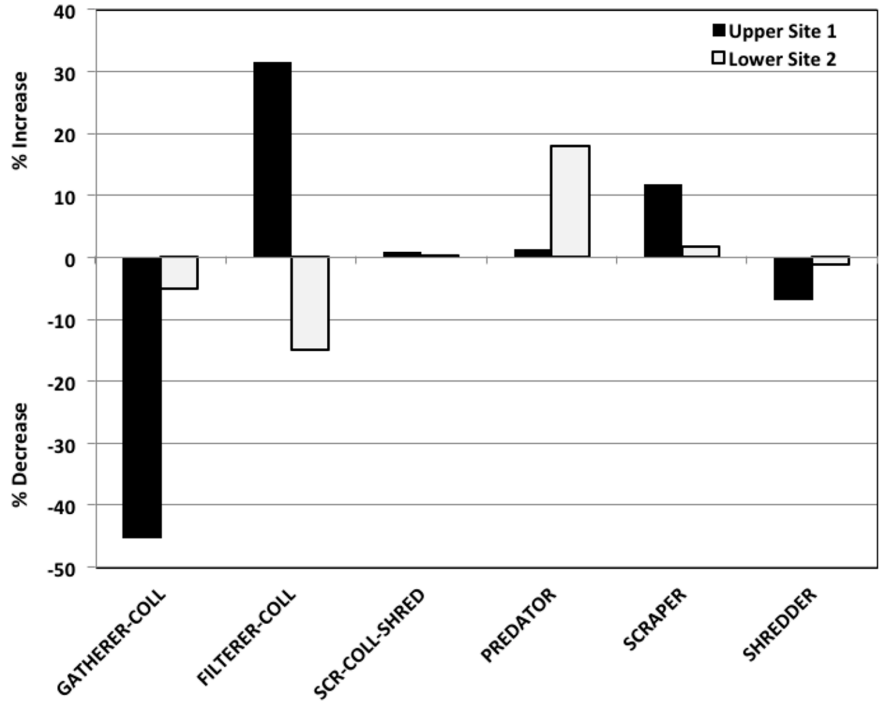

Figure 3. Differences in trophic guild composition change between 1960 and 2011. COLL=Collectors, GATH= Gatherers, FILT= Filterers, SCR= Scrapers.

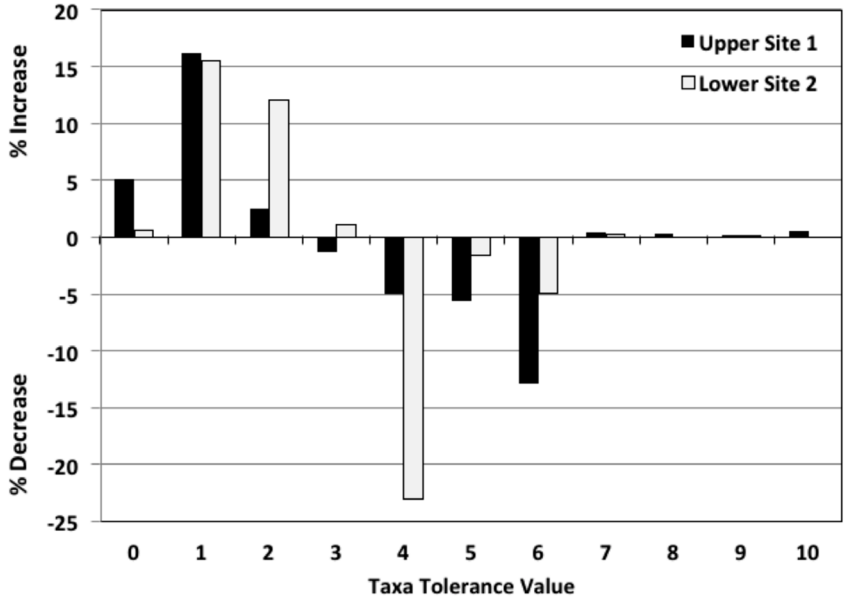

Figure 4. Differences in macroinvertebrate taxa tolerance scores between 1960 and 2011.

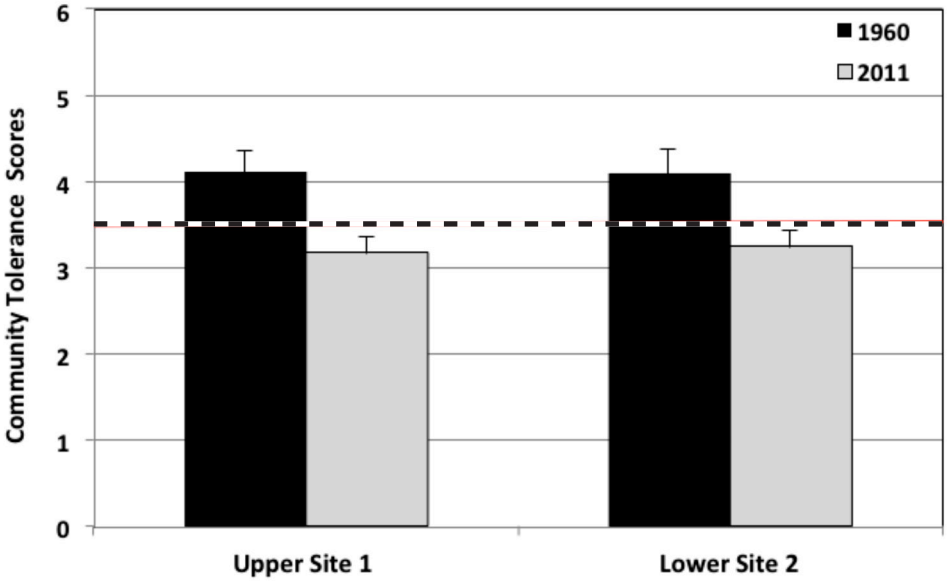

Figure 5. Comparison of average macroinvertebrate tolerance scores for composite samples in 1960 and 2011. Error bars are standard error (SE). Dashed line at 3.5 is the HBI slight organic impairment threshold.

by the riffle beetles, Elmidae (11%); the stonefly family, Perlidae (8%) and the dipteran, *Atherix* (8%), but none of these increases were significant ($p > 0.05$) (Table 2). Taxa with non-significant decreases in community percentage since 1960 are the Chironomidae (6%) and Baetidae (12.5%) (Fig. 1). Decreases in the numbers of Hydropsychidae, Chironomidae and Baetidae families led to large decreases in the number of filterer-collector and gatherer–collector trophic guilds since 1960 (Fig 3). The proportion of predatory macroinvertebrates collected in 2011 averaged 18% higher than 1960 but this was not significant ($p=0.22$) (Fig. 3). A significant proportion of individuals that comprised these samples in 2011 were less tolerant (i.e. more sensitive) than in 1960 (Fig. 4). Up to 16% increases of taxa ranked 0-3 (TV) and 23% decreases in taxa ranked 4-8 have occurred since 1960 (Fig. 4). Community tolerance values (HBI) decreased significantly (ANOVA, $p=0.01$) from 4.1 to 3.3 for this site since 1960 (Fig. 5).

Total densities of macroinvertebrates were significantly higher in 2011 (avg. 458 m^{-2}) than in 1960 (avg. 354 m^{-2}) (ANOVA $p=0.01$) (Table 2). Salmonflies were not collected in the lower reach in 2011, while two nymphs (2.7 m-2) were reported in the Z transect in 1960; this was not significantly different between years (ANOVA $p = 0.5$, 1960 avg. n=1; 2011 avg. n=0).

Conclusions & Discussion

This study compared quantitative macroinvertebrate samples across time at two sites on the Big Hole River and detected significant changes in the densities, family level diversity, pollution tolerance and functional feeding guilds of the benthic macroinvertebrate community. We did not detect any significant difference in salmonfly populations. Despite a lack of taxonomic specificity in the original 1960 study by reporting taxa to the family level, instead of genus and species level, we were able to classify and compare results, allowing for a meaningful comparison of historic and current samples.

Macroinvertebrate Densities, Family Diversity and Community Stability

A variety of factors may influence the relative stability or instability of a benthic macroinvertebrate community through time (Miller et al. 2010). These include changes in environmental conditions (climate, water chemistry), anthropogenic caused changes (habitat degradation, pollution, dewatering, dams) (De Jalon et al. 1994; Fore et al. 1996; Miller et al 2010) or natural stochastic events (floods, fires, drought etc.) (Boulton 2003, Boyero 2003). Benthic densities, measured by the number of macroinvertebrates per meter squared, are some of the most variable measures of an invertebrate community (Dole´Dec et al. 2000 and Boyero 2003). Benthic macroinvertebrate densities in this study were significantly higher at both sites in 2011 when compared to 1960 samples. But even so, these densities reported (< 500 per m^2) are low for large freestone, trout streams in Montana (McGuire 2014, Pierce et al. 2015). Diversity at these two Big Hole sites, measured by the numbers of macroinvertebrate families recorded at a site, was more similar to other Montana rivers in 2011 and much less when compared to the 1960 sample diversity (McGuire 2014, Pierce et al. 2015).

Studies have demonstrated that macroinvertebrate community similarity between years at “non-impacted” sites was higher than at human-impacted (managed) sites (Miller et al. 2010). Vinson et al. (2010) also reported that macroinvertebrate communities have still not stabilized a decade after treatment with the piscicide rotenone. The macroinvertebrate community sampled at the Pennington Bridge Site 2 in 2011 remained more similar to the 1960 community than the upstream Site 1. While community similarity between years in the upstream site is lower than 50%; this was much more similar than reported between Big Hole River sites separated by only a few river miles (McGuire 2003).

Comparison of Functional Feeding Guilds

Although few significant changes were observed in most of the functional feeding guilds, we did document a significant decrease in the gatherer-collector functional feeding guild across both sites over time. This was primarily due to a reduction in the numbers of midges from the family, Chironomidae and Baetidae mayflies. Gatherers typically consume fine detritus that is deposited in substrate and are generalists that have a fairly broad range of food choices, when compared to other feeding guilds (Merritt et al., 2002). As such, their increased presence in the 1960 study is somewhat indicative of poorer water quality or at least increased sediment. Given that all functional feeding guilds were present and roughly in similar proportions, it can be assumed that stable food dynamics exist within the watershed during the course of both sample periods. A similar proportion of predatory macroinvertebrates were collected during both time periods and at both sites; this is also an indication of good water quality since predators have a narrower range of food choices and are well represented in healthy streams (Merritt et al. 2002).

Comparison of Macroinvertebrate Tolerance Measures

In terms of macroinvertebrate biointegrity, the HBI tolerance metric decrease, averaging 1 point at each site indicates a qualitative community shift from slight organic pollution to no apparent organic pollution (Hilsenhoff 1987). This metric has also been used as a surrogate for sediment impairment; thus, a reduction in benthic sediments may have also occurred over this time period (MDEQ 2012). This tolerance value decrease reflects both an increase in the community's sensitive taxa and subsequent decreases in pollution tolerant taxa in 2011. Tolerance values generated from samples taken at an adjacent MDEQ site (Maiden Rock) near Site 1 in 2002 (average 2.95) (MDEQ 2007) were slightly better than those reported in 2011 (avg. 3.2) and much improved since 1960 (avg. 4.11); while 2002 samples taken at Notch Bottom Fishing Access near Site 2 scored more tolerant (average 4.7) than this study (avg. 3.3) or even the values reported from 1960 (avg. 4.1). This implies that improvements in the benthic biological integrity of this lower Big Hole section may have occurred more recently or were more localized in scope.

Taxa decreases common to both sites include the mayfly family, Baetidae and the dipteran, Chironomidae; this has contributed to decreased (healthier) community tolerance scores. However, taxonomic resolution at the family level in the original study is slightly problematic because of the lack of ability to discern trends that may be reflected as taxa shifts that occurred at the genus or species level within the same family (Bailey et al 2001). It has been demonstrated that the assignment of a site to a biological integrity class may change if different taxonomic levels are used (Bailey et al. 2001, Schmidt-Kloiber and Nijboer 2004). Deviations in both directions (higher/lower biological integrity classes) have been observed. For example, in 2011 we identified three taxa within the family Hydropsychidae; *Cheumatopsyche*, the most tolerant of the three reported, may have decreased since 1960 which would have indicated improvements in water quality, but we would not detect this change at the family-level resolution. Nevertheless, the macroinvertebrate taxonomic changes we did observe at the family level provide compelling evidence for potential increases in water quality occurring over time.

One large change in the Big Hole Watershed that may be contributing to improved water quality since 1960 is the increase in riparian fencing, bank stabilization and bank re-vegetation along the mainstem and tributaries in response to the Candidate Conservation Agreement with Assurances program (CCAA) (Rens and Byorth 2010). But, many of these CCAA projects are located as far as 70 km upstream from Site 1; therefore, local riparian conditions in the mainstem or contributing

waters from nearby tributaries (Trapper, Canyon and Moose Creeks) may be more directly influencing the macroinvertebrate communities in this study reach. Although, an ongoing macroinvertebrate study occurring in the CCA region of the Big Hole is reporting some similar improvements in the biological integrity of the benthic macroinvertebrate community (Bias 2014).

Comparison of Stream Flow Measures

Despite slightly higher stream discharges in August 2011 compared to 1960 (24 cfs higher or 6%); the water temperatures recorded at the same time of day averaged 3°C (9%) higher during this study (Table 1). This is an unexpected observation. Typically higher stream discharge equates to lower stream temperatures, because larger volumes of water take longer to warm up through ambient heating (Kaushal et al. 2010). But, the Rocky Mountains have been experiencing a decades long, warming trend (IPCC 2007) and this inverse relationship may no longer be valid, as snowpack is leaving the mountains earlier each year. Average monthly air temperature recorded for August 2011 (63.5°F) at the NOAA monitoring station in Divide, MT (US COOP: #242421) was 4.7°F (2.6°C) higher than in August of 1960 (58.8°F) (NOAA 2015). Air temperature recorded at 1 pm (1300 hr.) at our lower Site 2 was ~10°C warmer than recorded during the 1960 study at 3:30pm (typically the warmest part of the day). By 3:30 pm on August 26th, 2011, air temperatures approached 30°C (90°F). However, snow pack and water quantities have steadily improved from 2007 to 2012 (Fig. 4); average 88% snowpack, Montana Basin-Wide Snowpack Summaries, NRCS 2015). This improving water quantity over time may be causally reflected in steadily increasing EPT relative abundances seen in this study and in a Big Hole River Foundation study of the upper basin (Bias 2014).

Based on this quantitative, snapshot-in-time sampling event, we report some significant changes within the macroinvertebrate communities in the lower Big Hole River. Although these sample results represent a comparison of only two sites in time, the community differences observed do indicate that an increase in water quality or benthic habitat has likely occurred, since macroinvertebrates typically reflect conditions at least a year prior to the sample taken. A similar trend in biological integrity is also being reported in a macroinvertebrate monitoring study in the upper reaches of the Big Hole (Bias 2014). We may need additional years of replication before a definitive conclusion can be reached. There is some additional evidence that the upper Site 1 community improvements have occurred over a decade from this study, while biotic integrity increases in the lower Site 2 may have happened more recently (MDEQ 2007). We found no evidence that salmonfly densities were significantly lower in 2011 than in the 1960's. In fact, macroinvertebrate communities at these two sites in the lower Big Hole provide substantial evidence (increases in both diversity and sensitive taxa) that the health of the river's benthic community has improved in the last 50 years.

Acknowledgements

The original salmonfly data compilation report that precipitated this study was funded by Montana Fish Wildlife and Parks facilitated by Travis Horton and Don Skaar. We thank Steve Fernandes for field sampling help. Brian Lehl and Bill Schenk assisted with macroinvertebrate sample processing. Editorial review and comments from Dave Feldman and an anonymous reviewer greatly improved this manuscript.

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Received 06 January 2016
Accepted 08 May 2016

Effect of Diluent Type, Diluent:Sperm Ratio and Extender Use on Rainbow and Cutthroat Trout Egg Fertilization

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Abstract

Premature sperm activation can reduce fertilization. Sperm extenders are a potential remedy. In Test 1, rainbow trout (*Oncorhynchus mykiss)* sperm motility and motility duration were compared among three diluent types, three milt:diluent dilutions and between extended and un-extended milt. Dilutions ≥ 1:1 were sufficient for complete activation of un-extended rainbow trout sperm with all three diluents. For extended milt, complete activation was observed in 4 of 5 replicates at 1:2 and all replicates of 1:3, but not at 1:1. Sperm motility lasted from 21 to 52 s and was unaffected by extender, diluent type, or dilution. In another test using extended and un-extended sperm to fertilize eggs at high female to male ratios (4:1), no significant difference in percent fertilization was observed between 4:1 and 1:1 ratios or between extended and control sperm treatments. For cutthroat trout (*O. clarkii pleuriticus*) eggs fertilized with extended sperm, there was no significant difference in survival to eye-up. The data indicated extender requires three-fold dilution, but did not negatively affect fertilization or duration of motility when common activating solutions were used.

Key words: fertilization, diluent, extender, spermatozoa, *Oncorhynchus*

Introduction

Several subspecies of cutthroat trout (*Oncorhynchus clarkii*) are endemic to Utah. The Utah Division of Wildlife Resources annually collects eggs from wild cutthroat trout at various locations around the state for the purpose of propagation and stocking for both sport fish enhancement and for re-establishing populations within their historic range. A review of the historical production at these wild traps in Utah was made recently in response to concerns about egg survival (Wagner and Oplinger 2013). A data meta-analysis of egg survival among all the trap sites suggested that although site specific variables such as travel distance (trap to hatchery) and reservoir size were significant, within-site variance in egg survival to the eyed stage (0 to 100%) indicated other variables that were not measured were likely affecting egg survival. These variables include weather, air temperature, gamete quality and operational variables such as personnel and gamete handling (despite uniform spawning protocols), which may vary at a given trap site. Chief among variables biologists can control, are those associated with gamete handling.

Sperm motility is a critical factor for fertilization and is easily compromised. It is known that water dripping into the milt from the fish or a spawning glove can lead to premature activation (Piper et al. 1982). Blood or urine contamination of the milt may also reduce fertilization by prematurely activating sperm (Poupard et al. 1998, Linhart et al. 1999, Ingermann et al. 2010). Ovarian fluid can also stimulate premature activation of sperm (Rucker et al. 1960, Billard 1983, Ingermann et al. 2010), which can be a problem when several males are

added sequentially to a pool of eggs. Poor milt production by males can contribute to poor fertilization via reduced sperm numbers (Stockley et al. 1997). Although not usually measured, the volume of milt produced per male spawned at the wild traps has typically been lower and more variable than in captive broods.

The primary goals of our research were to determine whether sperm extenders can be used to prevent premature activation of sperm and to determine the best dilution ratios and diluents for the activation of extended sperm. The wild cutthroat trout collected by the Utah Division of Wildlife Resources typically produce a low volume of sperm (< 1 mL) and we were concerned that this limited volume exacerbates issues of water, urine, or blood contamination. Sperm extenders are solutions that maintain physiological conditions similar to seminal fluid (Henderson and Dewar 1959, Baynes et al. 1981) and prevent flagella activation until a diluent (activation) solution is added. Sperm extenders developed for salmonids typically have high potassium ion (K+) concentrations (Morisawa et al. 1983a). Short term sperm storage in extender solutions has been described (e.g., Henderson and Dewar 1959, Baynes et al. 1981) but the use of extenders during the spawning process has not been described to our knowledge. Theoretically, stripping sperm directly into an extender solution could offset contamination by water, urine, or blood and could prevent premature flagella activation and thus increase fertilization rates. However, to our knowledge, no research on the optimal solution (diluent) or dilution ratio for the activation of extended sperm has been performed. Water is the natural diluent, activating sperm as K+ concentrations drop, but solutions have been developed that significantly extend the duration of motility of salmonid sperm (Billard 1985). A saline solution called D532, buffered to pH 9 and containing 20 mM Tris and 50 mM glycine, is known to increase motility duration of salmonids and extend the time that the micropyle remains open for receiving sperm (Billard 1985). Steyn et al. (1989) found that a Borax-boric acid buffer diluent resulted in higher fertilization percentages than water, saline, or Tris-glycine buffer. However, sodium chloride alone has proven to be as effective as sodium chloride solutions containing other ingredients (Petit et al. 1973, Scott and Baynes 1980, Krise et al. 1995). Rock salt, although comprised primarily of NaCl (>99.4%), contains other cations and anions in small amounts and is cheaper than purified NaCl. A rock salt solution of 0.5-0.7% has been used routinely at the Utah wild trap sites and Utah brood hatcheries as a diluent.

In addition to preventing premature flagella activation, sperm extenders could be used to improve sperm distribution during fertilization. Increasing the volume of sperm by producing a dilute sperm+extender solution could improve the coverage of eggs and increase fertilization. Poon and Johnson (1970) showed that dilution of sperm at the time of fertilization improved percent fertilization (72-80% versus 39-40% in undiluted controls). Similar results were observed by Plosila et al. (1972) for brook trout (*Salvelinus fontinalis*) eggs. These studies diluted sperm with water, but we are not aware of similar studies being conducted diluting sperm with extender solutions. One potential advantage to diluting sperm with extender solutions rather than activating solutions (diluents) is that extenders do not activate the sperm; i.e., the dilution occurs before activation, providing a few more seconds of motility in a situation where flagella are only active for a limited period (Billard and Cosson 1992).

We hypothesized that improvements could be made in fertilization success (or at least no negative impact), in both trap and hatchery settings, by collecting sperm in an extender solution that would buffer against premature activation. We also hypothesized that when milt quantity is low, as is typical when spawning wild males, dilution in extender solution better distributes the sperm to the eggs, leading to higher percent fertilization. Prior to implementing the use of extender solutions at the wild traps, we

conducted some preliminary tests. Three objectives were targeted in these tests: 1) determine the dilutions needed to activate extender solutions with common diluents, 2) determine the effectiveness of extender solutions for fertilizing under low milt:egg scenarios and 3) evaluate extender use in the field for cutthroat trout in a wild trap setting. We conducted this research with the goal of improving the fertilization and hatch rates of eggs from cutthroat trout, but since availability of wild brood fish is limited and fisheries managers were unwilling to risk using extenders without some preliminary testing, two experiments were performed with hatchery rainbow trout as a surrogate. One field experiment did evaluate extender use with cutthroat trout.

Methods

We conducted two tests to determine the effect of extenders and various fertilization variables on egg survival of rainbow trout (4 year-old females and 3 year-old males) from the Mantua State Fish Hatchery, Mantua, Utah. A third test evaluated extender use for cutthroat trout collected from Lake Canyon Lake, Duchesne County, Utah. The extender solution used in each test was derived from Negus (2008) and was comprised of 6.02 g/L NaCl , 2.98 g/L KCl, 4.77 g/L HEPES; It was mixed with the sperm at a 1:1 (v/v) ratio.

For data analysis, we used NCSS Version 2007 (J. Hintze, Kaysville, Utah) or R (Hornik 2015). We conducterd normality tests with the Martinez-Iglewicz and Kolmogorov-Smirnov tests (Hintze 1995). These tests were followed by appropriate tests (GLM, ANOVA, or t-tests) detailed within each experimental test section below. A two-tailed probability of <0.05 was considered significant. We used Scheffé's test for mean separation (Scheffé 1959).

Test 1:Effects of Dilution, Diluent Type and Extender use on Sperm Motility and Duration

We evaluated the effect on sperm motility and motility duration of three different dilution ratios (1:1, 1:2, 1:3; v/v, sperm:diluent) and three diluent types (0.75% rock salt[Solar Salt, Western Sun, Salt Lake City, Utah; label states sodium chloride content >99.4%], 0.75% NaCl and Fisheries Experiment Station [FES] well water) using both extended and un-extended milt (control). The FES well water had a hardness of 222 mg/L as $CaCO_3$, total alkalinity of 222 mg/L and a pH of 7.6 and the rock salt and NaCl diluents were prepared in de-ionized water. We collected milt on 20 October 2014 by hand stripping the milt from males into a Styrofoam cup using a metal sieve (1.6 mm mesh) to prevent feces from entering the cup. By holding the cup at an angle away from the fish, only expressed milt reached the cup, avoiding dripping water from the fish and gloved hand. A total of three pools of milt were made, with three males in each. The pools were placed into 50 mL centrifuge tubes and kept in a cooler for transport to FES (30 min drive). Extender was not added to the sperm until motility tests were initiated.

We checked motility 3-5 h after sperm collection. There were three replicates of each treatment. Each pool of sperm was divided into two even volume portions and an equal volume of extender was added to one of the portions whereas no extender was added to the other portion. The sperm was activated by adding the appropriate amount of sperm and then 100 µL of diluent in 1.7 mL microcentrifuge tubes. For the 1:1, 1:2, 1:3 dilutions, 100, 50, or 33.3 µL of milt or the sperm + extender mixture was added, respectively. After dilution, we mixed each solution with a pipette by drawing it in and out twice and then 10-20 µL of the mixture was placed onto a microscope slide and viewed at 100x magnification (10x objective). An estimate of the percent motility was made as quickly as possible (usually about 7-10 sec after activation) by visual inspection and the time (sec) until sperm motion ceased was recorded. Motion was defined as >2 sperm body (head + tail) lengths/sec. The sperm + extender mixture was allowed to sit for 15 min prior to estimating motility. We attempted to

keep milt and diluent solutions at the same temperature (14.1°C) during the experiment. We analyzed duration of motility with a general linear model; diluent type, extender use and dilution ratio were considered fixed factors in the model.

Test 2: Effects of Extender With Low Sperm:Egg Ratios

In this experiment, the effect of high egg numbers relative to the amount of sperm was evaluated, with or without the use of extender. We hypothesized extender would help dilute the sperm and improve fertilization. Rainbow trout of the West Virginia strain were used for the experiment.

We divided a pool of eggs from 10 females into 4 lots: two with 1/10 of the eggs each and 2 with 4/10 of the eggs each. One male was used to fertilize one 1/10 lot and another male was used to fertilize a 4/10 lot. The remaining two lots were also fertilized by one male each, but the milt had been diluted in 10 mL of sperm extender (about 1:1 v/v). After egg pooling, this approximates male: female ratios of 1:1 and 1:4, each fertilized by either extended sperm or an un-extended control. A separate Styrofoam cup was used to collect sperm from each male. The four lots were fertilized at the same time and 0.75% rock salt solution was used to initiate fertilization, pouring enough solution to cover the eggs within a small pail. The eggs were rinsed after 2 min and left to water harden in hatchery well water at 9°C. We repeated this process five times to generate 5 replicates per treatment.

A subsample of 50 mL of eggs from each treatment was retained for the extender experiment and the rest were given back to the hatchery for production needs. Each subsample was placed into a 1 L plastic beaker and water hardened for 1-1.5 hr. After hardening, the eggs were disinfected for 10 min in 100 mg/L of iodine. The subsamples were randomly assigned to one of 20 egg incubation trays supplied with about 15 L/min of hatchery well water (pH = 7.8, total hardness = 185 mg/L, total alkalinity = 179 mg/L).

Survival to hatch was determined by hand counting and removing dead eggs on three separate dates and expressed as a percentage of total eggs at the start (also hand counted). The number of deformed fry was also determined and expressed as a percentage of the number of live fry at hatch. Data were analyzed with two-way ANOVA using R (Hornik 2015). Extender use (yes, no) and milt:egg ratio (1:1, 1:4) were considered fixed variables.

Test 3: Effect of Extender on Cutthroat Trout

On 27 May 2015, we captured Colorado River cutthroat trout from Lake Canyon Lake, Duchesne County, Utah, for the annual spawning operation. See Wagner and Oplinger (2013) for more details on the site and its history. For the experiment, eggs from each female were divided into two separate plastic bowls. Eggs from a second female were similarly split into two roughly equal aliquots that were added to the bowls from the first female, creating two groups with the same genetic composition. We stripped milt from two males sequentially onto eggs within one of the bowls, which served as a control. Milt from two other males was collected in a 50 mL centrifuge tube with 10 ml of extender solution (Negus 2008). The milt-extender solution was added to the second bowl of eggs. Efforts were made to keep the bottle of extender solution at the same temperature (11-13°C) as the lake water and spawning fish. A rock salt diluent (0.75% in hatchery well water), also kept at lake temperature, was used to initiate fertilization in both bowls, adding enough to cover the eggs (about 100-200 mL). After 5 min, we rinsed the bowls with fresh hatchery well water to remove excess sperm and dead eggs. Eggs were added to coolers with hatchery well water, one cooler for the "Extender" treatment and second cooler for "Control" treatment. We repeated this process four more times to acquire eggs from a total of 10 females (5 fertilization groups per treatment). This was considered a single replicate. A total of three replicates were obtained per treatment, each

contained in a separate cooler. This resulted in a total of 30 females and 60 males being used.

After transport to the isolation station at Fountain Green State Fish Hatchery, Fountain Green, Utah, we treated eggs in each cooler with 100 mg/L iodine for 10 min. Eggs were put into six separate egg incubation jars, one for each replicate. Eggs were treated daily with 1,667 mg/L formalin until reaching the eyed stage. Upon reaching the eyed stage, the egg survival was determined based on the proportion of the volume of live eggs over total volume of live + dead eggs. Live and dead eggs were separated using a commercial egg sorter. Percent survival of eggs in the treatment and control was evaluated for significance using a t-test.

Results

Test 1:Effects of Dilution, Diluent Type and Extender Use on Sperm Motility and Duration

The dilution ratio needed to obtain complete activation of sperm in extender solution was 1:2 when water was used as an activator and 1:3 when the rock salt diluent was used (Table 1). Un-extended sperm needed less dilution, activating at dilutions of ≥ 1:1 for all three diluent types.

Duration of sperm motility ranged from 21 to 52 sec among individual replicates and mean values ranged from 29.3 to 42.3 s among treatments (Table 1). Use of sperm extender had a significant ($P = 0.04$, F = 5.0, d.f. = 1), positive effect on the duration of motility (mean of 35.9 s versus 30.6 s

Table 1. Comparison of the percent motility and duration of motility (mean ± SD, N = 3) among different dilution ratios and diluents for extended or un-extended rainbow trout sperm.

Extender treatment	Diluent Type	Dilution Ratio	Motility (%)	Duration of motility (sec)
Extended	Water	1:1	0.0 ± 0.0	-
		1:2	100.0 ± 0.0	42.3 ± 9.1
		1:3	100.0 ± 0.0	33.3 ± 0.6
	0.75% NaCl	1:1	100.0 ± 0.0	37.3 ± 1.5
		1:2	100.0 ± 0.0	34.0 ± 1.0
		1:3	100.0 ± 0.0	29.3 ± 6.7
	0.75% rock	1:1	63.3 ± 28.9	32.7 ± 4.0
	salt	1:2	73.3 ± 46.2	27.3 ± 5.5
		1:3	100.0 ± 0.0	39.3 ± 10.6
Un-extended	Water	1:1	100.0 ± 0.0	35.7 ± 2.5
		1:2	100.0 ± 0.0	29.3 ± 3.2
	0.75% NaCl	1:1	00.0 ± 0.0	35.0 ± 3.6
		1:2	100.0 ± 0.0	32.3 ± 2.3
	0.75% rock	1:1	100.0 ± 0.0	28.0 ± 0.0
	salt	1:2	100.0 ± 0.0	25.7 ± 0.6

for unextended, pooling across treatments). Duration of motility was not significantly affected by the type of diluent activator solution ($P = 0.88$, F = 0.12, d.f. = 2) or the milt dilution ratio ($P = 0.22$, F = 1.6, d.f. = 2). There were no significant interaction terms in the general linear model ($P > 0.06$).

Test 2: Effects of Extender With Low Sperm:Egg Ratios

The mean percent survival to hatch ranged from 83.8 to 88.7% among extended milt treatments and from 81.5 to 86.5% among un-extended controls (Fig. 1). There was no significant effect of extender use ($P = 0.56$, F = 0.3, d.f. = 1) or sperm:egg ratio ($P = 0.22$, F = 1.6, d.f. = 1) on the percent hatch. Similarly, the percentage of deformities was not significantly affected by extender use ($P = 0.98$, F < 0.01, d.f. = 1) or sperm:egg ratio ($P = 0.11$, F = 2.9, d.f. = 1).

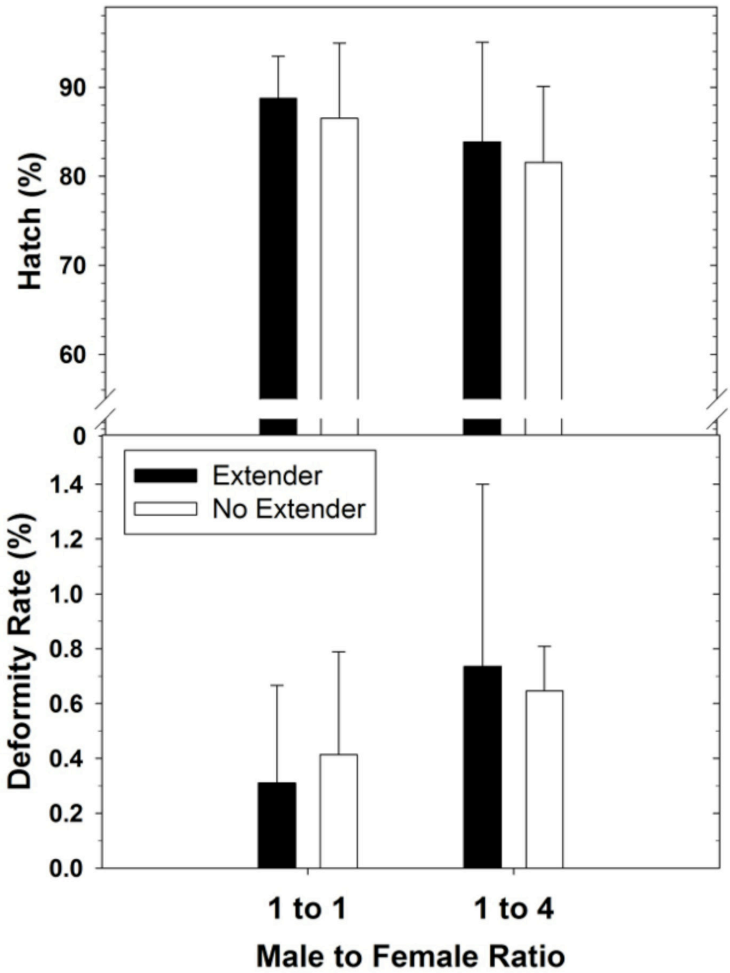

Figure 1. Comparison of the percent hatch (top panel) and percent deformities (bottom panel; mean ± SD, N = 5) among eggs fertilized with extended milt or non-extended milt, using male:female ratios of 1:1 (control) or 1:4.

Test 3: Effect of Extender on Cutthroat Trout

There was no significant difference in survival to the eyed egg stage between eggs fertilized with extended sperm (84.0 ± 9.5%) and controls fertilized by adding milt directly to the bowl of eggs (74.7 ± 30.0%). However, the high variance in the control group was notable, largely due to one replicate which had only 40% survival to the eyed stage.

Discussion

Our results from Test 1 indicated that non-extended sperm can be activated when mixed with an equal volume of water. In that test, we observed that male milt quantity varied from about 0.3 to 20 mL per fish. So, especially at lower milt volumes, it would not take much contamination with water to activate at least pockets of sperm, which could inhibit fertilization. Blood (Ingermann et al. 2010), urine (Poupard et al. 1998) and ovarian fluid (Rucker et al. 1960, Billard 1983, Ingermann et al. 2010) may also prematurely activate sperm prior to thorough mixing.

Fortunately, extender solutions have been developed to store sperm in an un-activated state, extending the storage life of sperm (Billard 1983, McNiven et al. 1993, Henderson and Dewar 1959). Extenders typically try to match the osmolality and pH of seminal fluid (Petit et al. 1973, Ingermann et al. 2002). For example, the best extenders for striped bass (*Morone saxatilis*) (Jenkins-Keeran and Woods 2002) and sea lamprey (*Petromyzon marinus*) sperm (Ciereszko et al. 2002) were those that matched the osmolality of the seminal fluid. Sperm extenders were developed for preserving sperm for extended periods of time, i.e., several weeks. What has received little attention, however, is the use of sperm extenders during routine spawning to prevent premature activation. Proper handling procedures can minimize water dripping into the milt, but blood and urine in the milt are harder to mitigate with handling per se.

In our studies, we evaluated the use of sperm extenders during stripping or for diluting small volumes of sperm to promote better coverage over eggs and fertilization. The extender used in this study required at least a 1:2 dilution if activated with water and a 1:3 dilution to get consistent 100% activation with rock salt diluent. Graybill (1968) also observed a ≥1:2 dilution was needed for activation of extended coho salmon (*Oncorhynchus kisutch*) sperm using fresh water; The extender solution had a K^+ concentration of 2.98 g/L or 83 mM, so activation occurred at about 41.5 mM when using water as an activator (did not measure K^+ concentration in water) and 27.7 mM K^+ (1:3 dilution) using rock salt diluents (K^+ concentration of salt diluents unknown). This concentration is much higher than the threshold for activation reported by Ingermann et al. (2010). Baynes et al. (1981) observed that KCl concentrations as low as 1 mM inhibited activation of rainbow trout sperm in the absence of NaCl, but when present (150 mM), at least 13 mM KCl was required to inhibit activation. So, our findings corroborate previous studies that have demonstrated that variables other than K+ concentration alone are involved with sperm activation, such as changes in osmotic pressure (Stoss 1983, Orfão et al. 2011). For example, Bates et al. (1996) noted for channel catfish (*Ictalurus punctatus*) sperm that a drop in osmolality from physiological levels of 273 mosmol/kg to 132 mosmol/kg (about a two-fold dilution) led to complete activation. Changes in transmembrane potential have been shown to activate rainbow trout sperm (Blaber and Hallett 1988); the electrical potential was three times greater when K+ concentrations dropped, than for the same drop in Na+ ion concentration. Sperm from cyprinid species also has been shown to activate after decreasing osmolality by half (Morisawa et al. 1983b).

In this study, sperm motility duration did not differ between hatchery well water and either of the 0.75% salt diluents. However, sperm of Atlantic salmon (*Salmo salar*) studied by Ellis and Jones (1939)

and of salmonids in studies reviewed by Scott and Baynes (1980) remained motile longer if activated by dilute salt solutions rather than freshwater. Ginsburg (1972) reviewed several studies and found this relationship as well, but there were other studies he reviewed in which this motility difference was not observed. The discrepancy among studies may be related to the salt concentration used, which may have a profound effect on motility duration, which decreases as salinity exceeds a narrow optimum concentration (Ginsburg 1972; Billard 1978).

The goal of our second test was to determine whether the dilution of sperm in extender helped improve egg fertilization in a situation where milt volume and presumably sperm number were limited compared to the number of eggs fertilized. A few studies (e.g., Billard et al. 1974, Scott and Baynes 1980) have described how the dilution of sperm using diluent can influence motility. What has received little attention, however, is the uniform distribution of sperm, which has been identified as a factor that can influence egg fertilization (Snook 2005). Rainbow trout sperm motility has been shown to decrease precipitously after 15 sec (Stoss 1983) and in principle the time required to thoroughly mix sperm with eggs after activation may be sufficient to prevent some eggs from coming in contact with motile sperm. This issue is likely most prevalent when the volume of milt used is low relative to the number of eggs fertilized. In our test, the pre-dilution of sperm in extender did not significantly improve fertilization rates. Although not statistically significant, hatch rates were approximately 2% higher when the extender was used. Oplinger and Wagner (2015) evaluated the use of sperm extenders containing antibiotics and found a 0.75% greater hatch rate among eggs fertilized using extended sperm compared to controls where the sperm was not extended. Thus there is some evidence that extender use leads to slight increases in fertilization, albeit more evaluation is required. Slight increases in viability could translate into significant increases in fish numbers in large production hatcheries or could be beneficial in situations where species conservation is of interest and there is a need to produce as many fish as possible.

The use of extender for cutthroat trout sperm indicated no negative effect of extender use on survival to the eyed stage. The variance observed in the controls indicated that while some batches have high egg survival when milt is stripped directly onto the eggs, others do not. So, although extender use did not significantly improve fertilization, egg survival was more consistent in eggs fertilized with extended sperm. A similar reduction in variance was observed in the small-scale trials performed by Oplinger and Wagner (2015). There are many factors that can affect fertilization percentages such as nutrition, stress, genetics, overripe eggs and age of female (Coward et al. 2002). Sperm quality has also been shown to vary temporally, typically declining later in the spawning season (Büyükhatipoglu and Holtz 1984, Hajirezaee et al. 2010, Johnson et al. 2013). For the cutthroat trout in this study, the gametes were collected during the middle of the spawning season. While these factors may also be influencing fertilization success at the wild traps that were the impetus for this study, factors relating to premature activation of sperm may be mitigated with the use of extender. In addition to standard hatchery practices minimizing water dripping into milt, extender use in routine hatchery spawning scenarios could also lead to less variance and incremental improvements in fertilization. We recommend further testing of extender use on a production scale. Also a controlled quantitative study to assess the effect of contaminants (e.g., blood, water, ovarian fluid) added to extender solutions is recommended.

The literature on extenders is extensive for evaluations of sperm storage methods, storage duration and motility after storage, but our current research is the first application of extender use reported in the literature that we are aware of for mitigating

premature sperm activation during normal spawning operations. Extender use did not compromise sperm motility and is recommended for use for preventing premature sperm activation. Sufficient dilution to achieve sperm activation, e.g. ≥ 1:3, is easily achieved if small amounts of extender solutions are used.

Acknowledgments

We thank Q. Bradwisch and the Mantua Hatchery staff for their assistance with the studies and the use of their egg incubator trays for the study. We also thank G. Birchell, T. Hedrick and M. McCarty for their cooperation and help with the experiment at Lake Canyon Lake. We also thank technicians T. Hanson and A. Winegar for their help conducting the tests. The study was funded by the Federal Aid in Sport Fish Restoration program, Project F-74-R and the Utah Division of Wildlife Resources.

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Received 12 December 2015
Accepted 18 February 2016

Ecological Model for Seral Stage Classification and Monitoring within a Greasewood/Western Wheatgrass-Blue Grama Ecological Type

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Abstract

A multivariate statistical model was developed to classify seral stages and to monitor vegetation within a greasewood-western wheatgrass-blue grama (*Sarcobatus vermiculatus*/*Pascopyrum smithii*-*Bouteloua gracilis*) ecological type. Two key plant species, greasewood and western wheatgrass, provide the required information for the model to classify seral stages and monitor trends based on index values of both plants (canopy cover (%) and frequency of occurrence (%)). Three seral stages were quantitatively identified. Classification had an overall accuracy of 94% and all seral stages were significantly different ($P < 0.05$). Three seral stages (late, intermediate and early) provide resource managers quantitative options to evaluate alternatives and objectives associated with steady states and transitions between and among seral stages. Application of this model within the greasewood ecological type is simple to apply, repeatable, accurate and cost effective for field applications.

Key words: succession, seral stages, diversity, monitoring, greasewood, western wheatgrass, blue grama, management, state and transition.

Introduction

Monitoring natural resources and predicting impacts has received much attention in recent years for developing management plans and environmental impact analyses. Quantitative ecological models with input from field data can accurately predict impacts on resources and document current conditions (Uresk 1990, Uresk and Mergen 2014). However, subjective monitoring of the resources provides no quantitative information on the natural resource being impacted and is only available after a visual impact is observed (Kershaw 1973, Block et al. 1987). Quantitative monitoring is required to determine current condition and better predict future impacts to the natural resource. Increased public awareness of our natural resources and management has influenced public and private land managers to be provided with methods and models that are economical, accurate and simple to apply in the field yet powerful enough to monitor trends and predict resource effects prior to observing visual impacts.

Ecological statistical models offer a quantitative approach with input from field data to evaluate and monitor trends of resources based on patterns of plant succession (Uresk 1990, Mclendon and Dahl 1983, Huschle and Hironaka 1980, Friedl 1991, Uresk and Mergen 2014). Plant succession concepts have been used for many years on rangelands for resource management and monitoring (Sampson 1919, Dyksterhuis 1949, Stoddart and Smith 1955, Dyksterhuis 1985). Recently, USDA-Forest Service, Natural Resource Conservation Service (NRCS) and Bureau of land management have implemented state and transition models in the Ecological Site Descriptions (Briske et al. 2005, Bestelmyer et al. 2010 and USDI-USDA 2013). However, these models are qualitative and are based primarily on expert opinion (Twidwell et al. 2013).

The greasewood (*Sarcobatus vermiculatus*)/western wheatgrass (*Pascopyrum smithii*)-blue grama (*Bouteloua gracilis*) ecological type is generally limited to low lands, associated with high water tables and soils with relatively high levels of sodium (Thilenius et al. 1995, USDA-NRCS 2015). Greasewood primarily exists in the Great Basin and eastward to Wyoming and southward to New Mexico (Kuchler 1964), but occurs in most western states (USDA-NRCS 2013). Greasewood occurrence is limited to arid and non-saline sites. Greasewood and associated vegetation can occur as narrow bands adjacent to open water. Shrub density and associated understory plant species will vary in abundance. The greasewood/ western wheatgrass/blue grama is a unique vegetation type important to wildlife (Wallestad 1971, Ryder and Irwin 1987, Welch 2005) and provides forage to livestock (Costello 1944, USDA-NRCS 2015). Knowledge of the current seral status and successional trends of the greasewood type is necessary for resource managers when they determine desired management options and implement guidelines to meet compliance standards. The objectives of this study were: 1) to develop an ecological classification and monitoring model for the greasewood ecological type, 2) define and describe seral stages and 3) to provide sampling and monitoring protocols.

Study area

This study was conducted on the Thunder Basin National Grasslands (TBNG), Wyoming, in a greasewood ecological type on gently sloping saline lowland sites in the Cheyenne River valley (Thilenius et al. 1995). The Thunder Basin National Grasslands encompasses about 153,780 ha of National Forest Service lands. Small drainages include the Little Powder River, Antelope Creek, Little Thunder Creek and School Creek. Elevations in Thunder Basin range from approximately 1100 m to a maximum of 1800 m (Thilenius et al. 1995).

The climate of Thunder Basin is interior continental with hot summers and cold winters. The mean annual precipitation at the Dull Center is 32.8 cm in central TBNG for an 87-year period (HPRCC 2015). Short duration intense thunderstorms, sometimes accompanied by damaging hail, occur from May to September. The average minimum temperature is 0.1°C with a mean annual maximum temperature of 16.4°C. The frost-free period averages 120 days (Martner 1986).

Greasewood is located on low flood plains with soils having relatively high levels of sodium (Thilenius et al. 1995). However some areas with greasewood lack high levels of sodium in the soil. The dominant plants within the greasewood ecological type are western wheatgrass, blue grama, Sandberg bluegrass (*Poa secunda*), alkali sacaton (*Sporobolus airoides*), sand dropseed (*Sporobolus cryptandrus*), saltgrass (*Distichlis stricta*), plains pricklypear (*Opuntia polyacantha*) and big sagbrush (*Artemisia tridentata*).

Methods

I conducted a field reconnaissance of the greasewood study area to assess the full range of variability from early to late plant succession within the ecological type based on Thilenius et al. (1995). The experimental design, data collection and analyses follow procedures developed by Uresk (1990). Site selection encompassed the entire greasewood ecological type to include the full range of natural variability. Sites were stratified into three pre-defined visual seral stages, early, mid and late based on key plant species and their previously described changes through plant succession (Cochran 1977, Thompson et al. 1998, Levy and Lemeshow 1999).

I collected data on 104 sites during the summer of 1995. I randomly selected each site within one of three perceived seral stages based on major plant species abundance defined for each seral stage by experienced range professionals. First, an area was located within a perceived seral stage for site selection. Once the area was located, a random direction and a random number of paces were established prior to

actual site location for establishment of transects. This procedure was repeated for all sites. At each site, two, 30 m parallel transects were established 20 m apart. Canopy cover (six cover classes) and frequency of occurrence of plant species were estimated within 0.1 m^2 (20 x 50 cm) quadrats (Daubenmire 1959). These quadrats were located at 1 m intervals along each of the two transect for a total of 60 quadrats. Total plant cover, litter cover and bare ground were estimated within each quadrat. Once all data were collected for the site, it was assigned a perceived seral stage. All data were averaged by transect. The two transect means were then averaged for each site to generate a grand mean for data analyses. An index for plant species was created based on canopy cover means time the frequency means: Index = ((transect 1 cover + transect 2 cover)/2)* ((transect 1 frequency + transect 2 frequency)/2) (Uresk 1990, Uresk et al. 2010). Note that averaging canopy cover and frequency of occurrence over several sites and then multiplying the two variables will not provide the same indices for seral stage classification and monitoring. Additional details for macroplot establishment and transects may be obtained from USDA Forest Service website (Uresk et al. 2010): http://www.fs.fed.us/rangelands/ecology/ecologicalclassification/index.shtml

Preliminary data examinations of the overall index mean for the ecological type resulted in the removal of minor plant species (variables) from analyses with mean index values of <50. The remaining` plant species on 104 sites were used as variables for analyses in the following sequence (Uresk 1990, Uresk and Mergen 2014). The variables remaining after preliminary data reduction of variables were analyzed by principal component analyses for further variable reduction. The extraction method was used and the component matrix, component scores coefficient matrix and the mean index value for each variable were examined. There were no further analyses with principal component procedures. A non-hierarchical cluster analysis (ISODATA) defined groupings based on the four variables for seral stages (Ball and Hall 1967, del Morel 1975). Stepwise discriminant analysis was used to estimate compactness of the cluster, to identify key variables that accounted for the differences between and among clusters and to develop Fisher classification coefficients (SPSS 2003, Uresk 1990). Discriminant analyses identified two key variables for model development and for classifying seral stages and monitoring. Misclassification error rates were estimated with a cross validation using a jackknife or "leave one site out" procedure (SAS 1988, 2012). In the cross validation procedure, each site was classified by the discriminant functions derived from all other sites other than the site left out. This was repeated for each of the sites and gave a true hold out prediction for each of the sites. The developed model was field tested the following years in 1992-93.

Results

A total of 71 plant species and categories for graminoids, forbs, litter and bare ground were sampled on 104 sites. Plant species remaining after initial data reduction, reduced the number of variables to 7 plant species: western wheatgrass, blue grama, prairie junegrass (*Koeleria macrantha*), plains pricklypear, sand dropseed, needle and thread (*Hesperostipa comata*) and greasewood. Principle component analysis further reduced the variables to four species: western wheatgrass, blue grama, prairie junegrass and greasewood. The clustering procedure grouped the 104 sites into 3 distinct clusters (seral stages). Then stepwise discriminant analysis further reduced the number of variables to two plant species, greasewood and western wheatgrass. These two key plant species based on cover x frequency indices were used for predicting seral stage classification and monitoring changes within and among the seral stages. Three seral stages (early, intermediate and late) were significantly different from each other ($P < 0.05$). The distributions of the two key variables throughout the seral stages show

the ecological and biological dynamics from late to early succession (Fig. 1, Table 1). Both greasewood and western wheatgrass were minor components in the early seral stage with mean indices of 700 and 854, respectively. Greasewood dominated the late seral stage with a mean index 6769 and western wheatgrass with 1086. However, the mid seral stage of succession western wheatgrass was greater with an index 2946 compared to greasewood with an index 1964. Each variable individually and collectively describe the dynamics of the model within the greasewood/ western wheatgrass-blue grama ecological type. Blue grama was not a key variable for predicting seral stages within the greasewood ecological type. Indices for blue grama were low with little change through the system, with 85, 183 and 680 for late, mid and early seral stages, respectively. Blue grama decreased from early to late seral stage.

Fisher's discriminant function coefficients (SPSS 2003) for two key variables provided the biotic based potential for predicting and classifying seral stages within the greasewood/western wheatgrass-blue grama ecological type (Table 2). Applying Fisher's discriminant functions to

Table 1. Mean indices of key plant species for three seral stages in a greasewood/ western wheatgrass-blue grama ecological type in Eastern Wyoming.

		Mean Index	
Seral	n	Greasewood	Western wheatgrass
Late	16	6769	1086
Intermediate	31	1964	2946
Early	57	700	854

n=number of sites

Table 2. Fisher's discriminant function coefficients for classification of seral stages with key species within a greasewood/ western wheatgrass-blue grama ecological type in Eastern Wyoming.

Species	Late	Intermediate	Early
Greasewood	0.00945	0.00338	0.00116
Western wheatgrass	0.00293	0.00407	0.00121
Constant	-34.664	-10.408	-2.021

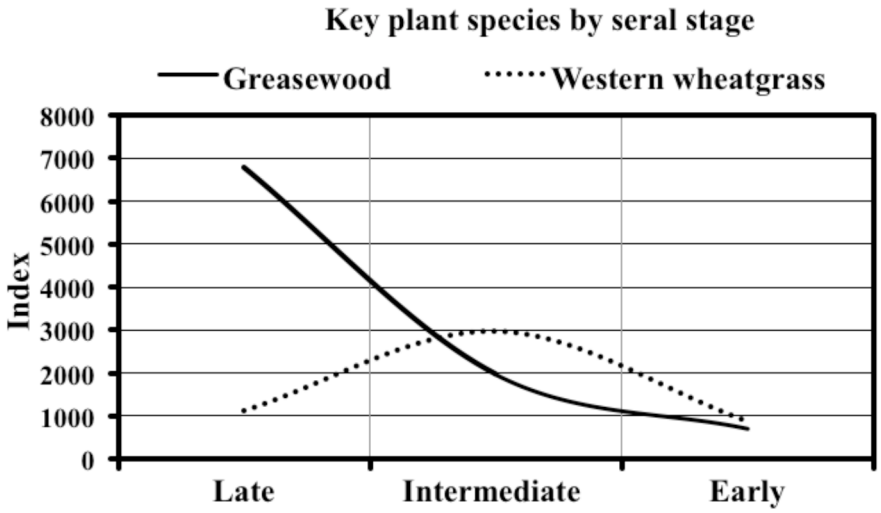

Figure 1. Index means of key variables, greasewood and western wheatgrass displayed throughout three seral stages in the greasewood/western wheatgrass-blue grama ecological type in Eastern Wyoming.

classify and monitor with new data collected for two key variables is presented in Table 3. Site index values for greasewood were 814 and 2418 for western wheatgrass, respectively. To determine seral stage assignment, multiply greasewood and western wheatgrass by the coefficients for each seral stage (row) and the products are summed (+ and -) including the constants for a score. The greatest positive or least negative score assigns the seral stage. In this example, the seral stage assignment was intermediate with a score of **2.18**.

The cross validation result for this model was 94% accurate for seral stage assignment (SAS 2012). Additional information on plot establishment, data collection, direct assignment of seral stage classification and trend monitoring with programs may be downloaded from USDA Forest Service website (Uresk et al. 2010): http://www.fs.fed.us/rangelands/ecology/ecologicalclassification/index.shtml. Programs may be used on most computer systems for data collection and summaries, seral stage classification and monitoring.

Late Seral Stage

The late seral seral stage was dominated by greasewood with a mean of 71% canopy cover and 95% frequency of occurrence for 16 sites (Table 4, Table 5). Western wheatgrass provided 13% canopy cover and 64% frequency of occurrence. Other common grasses were field brome (*Bromus arvensis*) also known as Japanese brome and cheatgrass (*Bromus tectorum*), both annuals. Perennial grasses included blue grama, Kentucky bluegrass (*Poa pratensis*) and crested wheatgrass (*Agropyron cristatum*). Total graminoid cover was 32%. The forb component was dominated by common pepperweed with 22% canopy cover and 63% frequency of occurrence followed with lesser amounts by burningbush (*Bassia scoparia*) also known as kochia . Total forb cover was 19%. Plant species richness in the late seral stage included 4 forbs, 17 graminoids and 4 shrubs (Fig. 2).

Intermediate Seral Stage

The intermediate seral stage was dominated with western wheatgrass with 36% canopy cover and 86% frequency of occurrence (Table 4, Table 5). Greasewood canopy cover was 33% and a frequency 55%. Canopy cover of other common grasses with this seral stage ranged from 0% to 4%. Frequencies of occurrences were low and ranged from 0% to 14%. Forbs were minor components present in the intermediate seral stage. Total canopy cover for graminoids, forbs and shrubs was 53%, 3% and 36%. Plant species richness was 21 forbs followed by 19 graminoids and 6 shrubs (Fig. 2).

Early Seral Stage

Both western wheatgrass and greasewood showed low canopy cover and frequency of occurrence within the early seral stage. Western wheatgrass canopy cover was 15% and frequency 47%. Greasewood cover and frequency was

Table 3. An example of assigning seral stages by using Fisher's discriminant coefficients with data collected from the field and a new index. Index =((transect 1 cover + transect 2 cover)/2)* ((transect 1 frequency + transect 2 frequency)/2).

	Greasewood				Western wheatgrass					
Seral Stage	**(Coeff[1]**	*****	**Index**	**+**	**Coeff**	*****	**Index)**	**Constant**	**=**	**Score**
Late	(0.00945	*	814	+	0.00293	*	2418)	-34.664	=	-19.87
Intermediate	(0.00338	*	814	+	0.00407	*	2418)	-10.408	=	**2.18[2]**
Early	(0.00116	*	814	+	0.00121	*	2418)	-2.021	=	1.85

[1] Coeff = coefficient
[2] Assigned seral stage

Table 4. Average canopy cover (%) and standard errors (in parentheses) for common plant species and other variables by seral stages in Eastern Wyoming.

	Late[1]	Intermediate	Early
Species or variable	**n = 16**	**n = 31**	**n = 57**
Western wheatgrass *Pascopyrum smithii*	12.9(2.5)	35.8(2.4)	15.1(1.2)
Blue grama *Bouteloua gracilis*	1.5(1.3)	3.5(1.1)	11.8(1.5)
Prairie Junegrass *Koeleria macrantha*	0	3.7(1.3)	2.6(0.9)
Sand dropseed *Sporobolus cryptandrus*	0	3.0(1.1)	4.0(1.0)
Needle and thread *Hesperostipa comata*	0	0	2.7(1.0)
Kentucky bluegrass *Poa pratensis*	2.9(1.2)	0	0
Field brome (Japanese) *Bromus arvensis*	7.2(1.9)	2.0(0.8)	1.9(0.6)
Crested wheatgrass *Agropyron cristatum*	3.8(2.5)	0	0
Cheatgrass *Bromus tectorum*	1.3(0.9)	2.5(1.0)	3.7(0.8)
Burning Bush *Bassia scoparia*	1.3(0.5)	0	0
Common pepperweed *Lepidium densiflorum*	20.8(3.5)	0	0
Plains pricklypear *Opuntia polyacantha*	0	1.1(0.3)	3.3(0.6)
Greasewood *Sarcobatus vermiculatus*	70.9(2.0)	33.0(2.3)	16.7(1.4)
Big sagebrush *Artemisia tridentata*	0	1.6(0.6)	0
Other species<0.1	**16**	**37**	**56**
Graminoid cover[1]	31.9(4.0)	53.2(2.7)	44.1(2.3)
Forb cover[1]	19.1(3.9)	2.6(0.4)	6.0(0.9)
Shrub cover[1]	71.7(1.8)	35.6(2.3)	17.5(1.5)
Litter	3.8(2.6)	32.1(2.4)	19.7(1.3)
Total cover[1]	97.3(2.8)	86.7(2.8)	66.8(2.0)

[1] Two dimension cover and not the sum of the individual plant species.

Table 5. Frequency of occurrence averages (%) and standard errors (in parentheses) for common plant species by seral stages in Eastern Wyoming.

	Late[1]	Intermediate	Early
Species or variable	**n = 16**	**n = 31**	**n = 57**
Western wheatgrass *Pascopyrum smithii*	64.1(7.0)	85.7(4.0)	47.2(2.7)
Blue grama *Bouteloua gracilis*	4.1(3.9)	10.7(3.0)	35.9(3.7)
Prairie Junegrass *Koeleria macrantha*	0	14.3(4.5)	10.2(2.6)
Sand dropseed *Sporobolus cryptandrus*	0	10.7(3.3)	13.7(2.9)
Needle and thread *Hesperostipa comata*	0	0	7.3(2.0)
Kentucky bluegrass *Poa pratensis*	22.7(6.3)	0	0
Field brome *Bromus arvensis*	34.1(6.2)	6.7(2.2)	6.4(2.4)
Crested wheatgrass *Agropyron cristatum*	13.4(7.5)	0	0
Cheatgrass *Bromus tectorum*	5.3(2.1)	9.4(2.7)	10.2(1.8)
Burning Bush *Bassia scoparia*	10.8(4.0)	0	0
Common pepperweed *lepidium densiflorum*	62.8(7.1)	0	0
Plains pricklypear *Opuntia polyacantha*	0	7.2(1.9)	14.5(0.6)
Greasewood *Sarcobatus vermiculatus*	94.6(1.2)	54.7(3.4)	32.9(2.2)
Big sagebrush *Artemisia tridentata*	0	6.6(2.9)	0
Other species <0.1	16	37	56

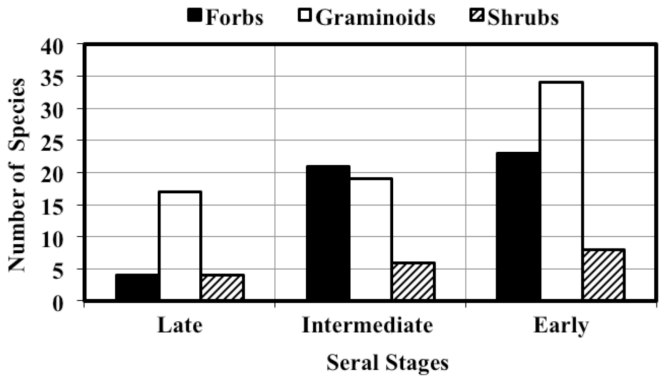

Figure 2. Number of plant species by life form category and seral stages in a greasewood/wheatgrass-blue grama ecological type in Eastern Wyoming.

17% and 33%. Blue grama grass exhibited 12% canopy cover and 36% frequency of occurrence. All other grasses were minor components within the early seral stage. Forbs were also minor components. Total graminoid cover was 44%, forb cover 6% and shrub cover 18%. Species richness was greater in this seral stage compared to other stages (Fig. 2). Forb richness was 23 species, graminoids 34 species and shrubs 8 species.

Discussion

The multivariate model developed for the greasewood ecological type can be used for seral stage classification and monitoring. The model is quantitative, accurate, repeatable and cost effective for describing plant ecological dynamics and plant species changes between and among seral stages using two key variables, greasewood and western wheatgrass, with a 94% accuracy. Current state and transition models used to describe successional dynamics through an ecological type are conceptual (Bestelmyer et al 2003, Briske et al. 2005). These models are qualitative, primarily derived from personal judgements and observations (Twidwell et al. 2013). Model coefficients developed herein can be incorporated into the conceptual state and transition models currently being used by USDA Forest Service, Natural Resource conservation Service and Bureau of Land Management (USDA-NRCS 2013).

Trends within the greasewood ecological type over time based on the two key variables as affected by livestock grazing, fire or climatic changes can be quantitatively documented to monitor if management goals and objectives are achieved. These disturbances may change plant species associations or seral stages from early to late within the greasewood ecological type. Depending upon the management objectives, livestock grazing can be used for modifying seral stages (Severson and Urness 1994, Costello 1944). Grazing intensity may be adjusted to modify a successional seral stage or transition from a non-preferred stage to a desired seral stage to meet the planned management objective. However, the successional process for change to meet desired management objectives can be slow (USDA-NRCS 2015).

The greasewood/western wheatgrass-blue grama ecological type described by Thilenius et al. (1994), based on five stands, reported that western wheatgrass and blue grama were widely distributed throughout the greasewood type. Canopy cover of blue grama reported by Thilenius et al. (1994) was 21 %. In our study, blue grama cover was 8% (104 sites). Although the blue grama cover was less than originally described by Thilenius et al., our data support the greasewood/western wheatgrass-blue grama type. Blue grama was variable throughout all seral stages in our study and not statistically defined as a key plant in the model for predicting seral stages.

Management of all three ecological seral stages within the greasewood ecological type provides the greatest plant and animal diversity. Non-game birds commonly use greasewood communities. Welch (2005) presented the importance of greasewood for small birds finding 4.4 species per mile and 17.9 birds per mile. Greasewood was also an important habitat for sage grouse broods during July-August (Wallestad 2015). Several species of small mammals (deer mouse (*Peromyscus maniculatus*), least chipmunk (*Eutamia minimus*)) were abundant in the greasewood type (Douglass 1989). Pronghorns were observed in the greasewood type near draws or bottomlands during winter months (Ryder and Irwin 1987). Overall, the greasewood ecological type is important for livestock grazing, non-game and game birds, small mammals and big game. However, current literature does not describe the importance of a seral stage for groups of animal species or individual species of birds, mammals and livestock for this ecological type. Plant species richness was greatest in the early seral stage.

Using individual seral stages is not practical for multiple use management because plant and animal species vary

among seral stages. Fritcher et al. (2004), Uresk and Mergen (2014) recommend a mosaic of desired seral stages that will apply within the greasewood type across the landscape as ideal for management of plants, birds, mammals and livestock. To meet plant and animal species diversity, a 10-15% of greasewood type in the early and late seral stages was recommended, with the remainder managed for the intermediate seral stage (Kershaw 1973, Mueller-Dombois and Ellenberg 1974).

Canopy cover and frequency of occurrence for the two key plants (greasewood and western wheatgrass) for calculation of indices are the only field requirements for field data collections on a site to determine seral stage assignment and monitoring. It is recommended for data collection that western wheatgrass is near or at full expression for growth. Indices must be calculated for each individual site (See methods). Collection of data may be yearly or every few years with a minimum of two sites (macroplots) per section (640 acres) within the greasewood ecological type. Additional information may be obtained from USDA-Forest Service website (Uresk et al. 2010) at: http://www.fs.fed.us/rangelands/ecology/ecologicalclassification/index.shtml.

Aknowledgements

Thanks are extended to Lakhdar Benkobi and Jody Javersak for data collections and analyses. George Wiggins provided invaluable advice for locating greasewood, providing historical aspects and support from the Medicine Bow-Routt National Forest. Rudy King, Rocky Mountain Research Station statistician (retired), provided invaluable statistical advice throughout the study and statistical analyses. Partial funding was provided by the Medicine Bow-Routt National Forest.

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Received 19 October 2015
Accepted 27 January 2016

Historic Distribution and Abundance of Bison in the Rocky Mountains of the United States

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Abstract

Scant public awareness of the early distribution and abundance of bison (*Bison bison*) in the Rocky Mountains of the United States inhibits discussion of possible restoration of wild bison. A review of written evidence, largely from 1805-1845, indicates bison were widely distributed in intermountain valleys, with a major regional concentration spanning parts of Idaho, Montana and Wyoming. However, several interacting factors caused large spatial and temporal variation in bison abundance. Native American predation was likely a major influence on bison distribution and abundance during and shortly before 1805-1845. The area where bison were observed by early explorers underestimates the area where restoring productive herds of wild bison is possible.

Key words: Bison, *Bison bison*, Rocky Mountains, Historic Distribution

Introduction

Although bison (*Bison bison*) are abundantly widespread as private livestock, there are proposals to reestablish wild herds that will be influenced by a preponderance of natural selection in diverse habitats (Gates et al. 2010, Bailey 2013). Developing a constituency for restoring wild bison requires broader awareness of former bison distribution, particularly in the Rocky Mountains.

Previous summaries of early bison distribution in the Rocky Mountains are from Allen (1877), Hornaday (1889) and Roe (1951). Meaney and Van Vuren (1993) compiled a list of early bison observations and of collected bison specimens for the Rocky Mountains of Colorado. For the northwest United States, reviews of bison in late-prehistoric to early historic time are in Kingston (1932), Butler (1978), Daubenmire (1985), Van Vuren and Bray (1985), Van Vuren (1987), Van Vuren and Dietz (1993), Lyman (2004), Williams (2005) and Grayson (2006). However, most of these references focus on bison west of the Rocky Mountains in southwest Idaho, eastern Washington and Oregon and the Great Basin.

Early literature based largely on 2nd hand descriptions, suggested that a unique strain of mountain bison once occupied the Rocky Mountains (Allen, 1877:447-448; Hornaday, 1889:407-412; Roe, 1951:33-56; Meagher, 1973:14-17). Described characteristics of "mountain bison" may have been phenotypic with little or no genetics distinct from sympatric bison on the Great Plains. However, unique bison characteristics elicited by distinct environmental conditions in the Rocky Mountains constitute an ecotype, a portion of biodiversity without which the full expression of the *Bison bison* genotype would not occur.

Literature review

For the "Rocky Mountains" I included parts of Idaho, Montana, Wyoming, Colorado and northeast Utah. To emphasize continuous mountain habitat, I excluded island mountains in the plains of eastern Montana and the Bighorn Mountains of Wyoming. I did not include the Colorado Plateau of southwest Colorado and Utah.

I searched reports of fur trappers, trapping brigade leaders, missionaries, military expeditions and other explorers, largely from 1805-1845. Sources are the most accessible literature and are mostly edited versions of original writings. I noted the year, month and general location (usually a major river drainage) of bison observations (Tables 1-5). I omitted sightings with unclear locations. However, a few locations

were inferred from clearly described travel routes. Nothing can be inferred from diary entries lacking references to bison. Clearly, where bison were everyday abundant, writers often failed to record them. Records are organized by state and county to be most useful for today's readers.

Meaney and Van Vuren (1993) plotted locations of about 89 specimens (mostly skulls) and about 36 early recorded observations of bison in the Rocky Mountains of Colorado. Almost all these records are in northwest, north-central and central Colorado. I report additional records for Colorado and repeat observations, not specimens, from Meaney and Van Vuren.

Findings

Bison were found throughout the Rocky Mountains, except in the northern, more forested, mountains of Idaho and northwest Montana (Fig. 1, Tables 1-5). It is widely known, but misleading, that Meriwether Lewis and William Clark saw no bison in the mountains in 1805-06. In contrast, they noted old bison sign on the Jefferson River, in the Big Hole and Gallatin Valleys and on Bozeman pass, all in Montana. In 1806, Sacajawea said to Clark that her nation, the Shoshones, had "gathered cows" in the Big Hole Valley not many years before and that bison had recently been numerous in the Jefferson and Gallatin Valleys (Table 2).

There was a major abundance of bison in the central Rocky Mountains of the United States (Fig. 1). This area - broadly straddling the continental divide - encompasses parts of southwest Montana, east-central and southeast Idaho and southwest Wyoming, including the upper valleys of the Jefferson-Beaverhead, Salmon, Snake, Bear and Green rivers and their numerous tributaries. Fremont (1845:144) reported "mountain man" Tom Fitzpatrick's description of "immense numbers" of bison over this country "in about 1824". Numerous, mostly later, reports confirm Fitzpatrick's description (Tables 1-3).

Bison were observed in the Rocky Mountains during every month. A preponderance of summer observations, especially in Wyoming and Idaho, reflects observations of seasonal cross-country travelers on the Oregon and Overland Trails. Most winter observations were in Idaho and Montana, also reflecting the distribution of observers. Mountain men favored wintering not far north of Fort Hall, Idaho where trapping brigades and Native Americans, often wintered on both sides of the Idaho/ Montana border in part because bison were abundant in this area (Lewis and Phillips, 1923:114-119, 123, 130; Haines, 1965:108-109; Ferris, Ch. XXII, XXIII). These records provide very little information on habitat selection by bison in the mountains, or about elevational migrations.

Rocky Mountain bison were quite mobile and could be absent from large portions of the area at any time. Roe (1951:261-266) discussed locally inconsistent observations of bison abundance. For example, bison were reported as abundant in the Jefferson-Beaverhead Valleys, Montana, in the 1830s, where Lewis and Clark saw no bison in 1805-06, although Sacajawea said bison were once numerous there (Table 2). In 1812, Stuart found no bison in southeast Idaho where bison were plentiful in the 1820s and 1830s (Table 1). Stuart (Spaulding 1953) found few bison in the upper Green River valley of Wyoming where Hunt (Irving 1836) had observed many herds during the same season of the previous year (1811). In the 1830s, even more bison were recorded in this area (Table 3). Stuart had observed large, recently used Native American camps and trails and bones from recent abundant bison kills. At least three nations had recently hunted in the Wyoming area.

Mass movements of bison, sometimes over long distances, were reported by several diarists as responses to attacks by hunters. Many records of Euro-American brigades and accounts of Native American hunting parties describe cautions taken not to disturb bison, lest they leave an area, before an entire hunting party was brought up and prepared to kill a sufficient number of animals.

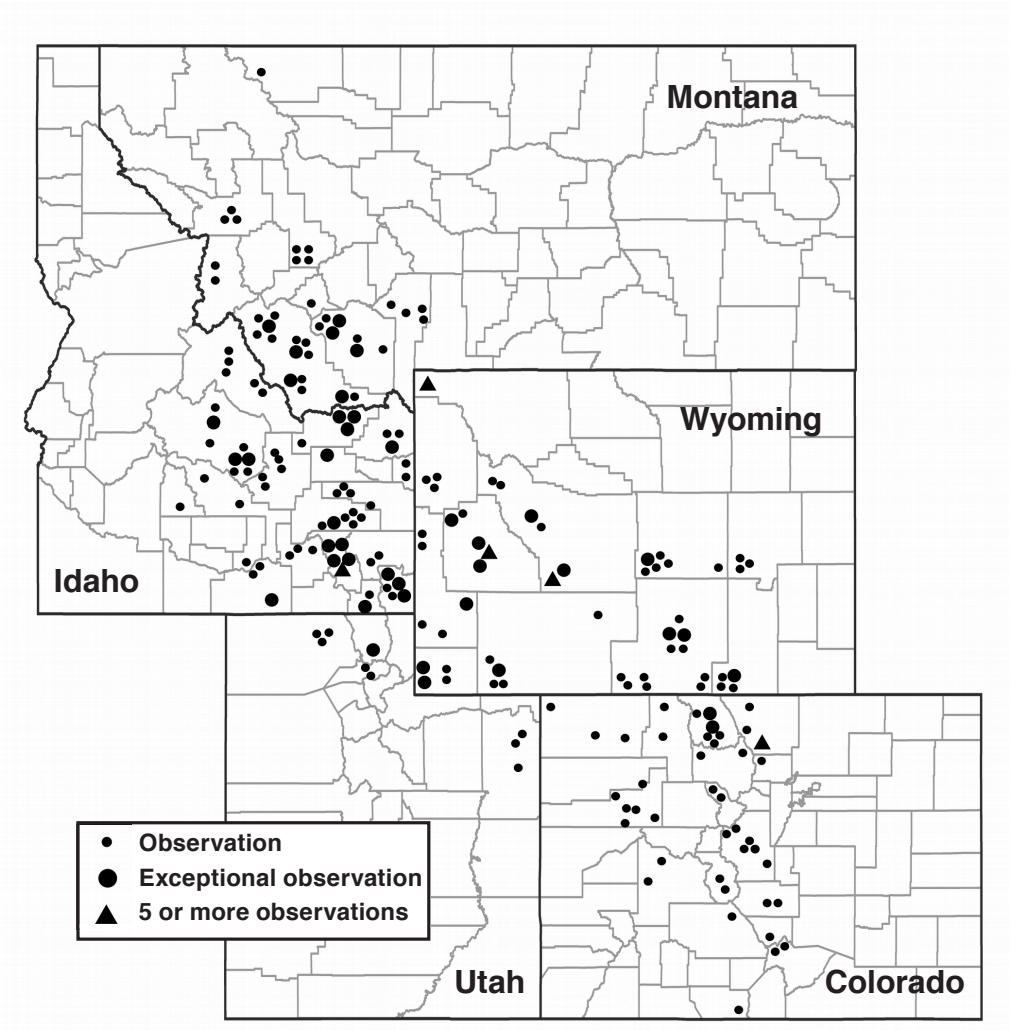

Figure 1. Early historic observations (Tables 1-5) of bison in the Rocky Mountains, USA. Exceptional observations are observer estimates of 1000 or more bison, or descriptions such as "vast herds".

Even before obtaining horses (about 1730) and more so thereafter, Native Americans had effective methods for killing bison (Point nd:121-125; Hornaday 1889:465-484). When large numbers of bison were accessible, both Native- and Euro-Americans often preferred the flesh and hides of cows (Hornaday 1889:465). This selective harvesting would have negatively influenced regeneration of bison numbers.

Native Americans often killed very large numbers of bison. Stuart (Spaulding 1953:116-117) found immense numbers of bison bones in every direction of the upper Green River Valley, Wyoming, in 1812 and Bonneville observed similar conditions in the same place in 1833 (Irving 1837:95). Clyman (1984:25) observed Crows killing "upwards of a thousand" bison in a day of 1824. Russell (Haines 1965:36) describes one village of Shoshones killing, without guns, "upwards of a thousand cows" in one day of 1835. On the Great Plains, 500 or more Sioux killed 1400 bison in less than a day of 1832 (Catlin in Roe 1951:631) and 100 or more Minatarees and Mandans killed several hundred bison in 15 minutes (Catlin in Hornaday 1889:482). Native Americans often attempted to kill whole herds of bison.

Table 1. Early historic observations of bison in the mountains of Idaho.

County	Area	Year	Month	Comments	Citation
Bannock	Portneuf Valley	1825	May	Kittson: "bulls killed"	Miller (1954:142)
				Ogden: "buffalo in abundance"	Miller (1952:186)
		1826	April	Ogden: "large herd of buffalo"	Elliott (1909:358)
		1827	Dec.	Ogden: "Never seen buffalo so numerous, killed 12	Elliott (1910a:367)
		1831	Apr.	"buffalo numerous", killed "upwards of 50"	Ferris (1983:Ch. XIV)
		1833		Bonneville:"the buffalo range"	Irving (1837:77)
		1833	Nov.	Bonneville: "several large herds"	Irving (1837:135)
		1834	June	Bonneville: Killed 2 bulls.	Irving (1837:177)
		1834	July	With Nez Perce, killed "many buffalo"	Townsend (1978:100)
		1834	autumn	Bonneville: "immense herds" of buffalo	Irving (1837:220)
		1836		Russell: "large bands could be seen"	Haines (1965:123)
		1840	Nov.	Russell: "only traces were scattered bones"	Haines (1965:123
Bear Lake	Bear River Valley	1824		Fitzpatrick: "immense numbers"	Fremont (1845:144)
		1830	Sept.	Valley "covered with buffalo"	Ferris (1983:Ch. X)
		1832	April	"killed several from a large herd"	Ferris (1983:Ch. XXIV)
		1834	June	Bonneville:"country covered with buffalo"	Irving(1837:180)
		1834	July	Bonneville: Killed "some buffalo"	Irving (1837:192)
		1834	July	Killed "only a few buffalo"	Townsend (1978:90)
		1834-35	winter	Bonneville: "vast herds"	Irving (1837:222)
Bingham	Blackfoot Valley	1832	May	"buffalo continuously in sight"	Ferris (1983:Ch. XXV)
		1833		Bonneville: "the buffalo range"	Irving (1837:77)
		1834	July	Bonneville killed buffalo.	Irving (1837) p. 206
	Snake River Valley	1811		Hunt: "old bison traces"	Rollins (1995:291)
		1832	Dec.	Saw "buffalo running"	Ferris (1983:Ch. XXXIV)
		1835	May	Russell: "thousands; killed a great number"	Haines (1965:13)
Blaine	Big Wood R. Valley	1827	Nov.	Ogden: Killed 10 cows.	Elliott (1910a:364)
	Little Wood Valley	1832	May	Work: "a good many" buffalo	Lewis, Phillips (1923:153)

Table 1. continued.

County	Area	Year	Month	Comments	Citation
Bonneville	Snake River Valley	1831	May	"killed numbers of buffalo" Saw "hundreds of carcasses" in Snake River	Ferris (1983:Ch. XV)
		1833	Jan.	Bonneville: "buffalo in herds"	Irving (1837:76)
		1839	Jan.	Russell: "a few bulls"	Haines (1965:94)
	Gray's Lake Outlet	1833	Apr.	"a herd; killed several"	Ferris (1983:Ch. XXXVI)
Butte	Little Lost R. Valley	1827	Nov.	Ogden: Killed 5 cows along Day's Defile.	Elliott (1910a:364)
		1830	Oct.-Nov.	Work: "large herds are about" Killed 4	Elliott (1912:369)
		1832	Dec.	Bonneville killed 2 bulls.	Irving (1837:73-74)
	Big Lost R. Valley	1831	Dec.	"several herds; shot 1"	Ferris (1983:Ch. XXXIV)
	near Three Buttes	1832	Dec.	Bonneville killed a bull.	Irving (1837:73-74)
Camas	Camas Creek	1832	May	Work: "started 8 buffalo"	Lewis, Phillips (1923:153)
Caribou	Bear River Valley	1812	Sept.	Stuart: "considerable fresh sign of buffalo"	Spaulding (1953:95)
		1824		Fitzpatrick: "immense numbers"	Fremont (1845:144)
		1825	May	Kittson: "plenty buffalo, many killed"	Miller (1954:142)
	Portneuf Valley	1839	July	"killed 5 buffalo" west of Beer Spring	Wislizenus (2005:104)
Cassia	Raft River Valley	1831	May	Work: "large herds" Killed many	Elliott (1913a:287-288)
	Snake River Valley	1826	Mar.	Ogden: "buffalo are near" Killed 2	Elliott (1909:355)
		1824		Fitzpatrick: "buffalo west to Fishing (Twin) Falls	Fremont (1845:144)
		1830?		Kit Carson: 3 or 4 bulls	Fremont (1845:166)
Clark	Camas Creek	1831	May	"large herds, killed many"	Ferris (1983:Ch. XV)
		1834	May	"immense herds in every direction"	Ferris (1983:Ch. L)
		1835	Sept.	Russell: "traveled amid thousands; immense bands as far as eye could reach" Bannock killed "upwards of 1000 cows"	Haines (1965:34)
	Birch Creek	1832	May	"numerous in all directions; killed numbers"	Ferris (1983:Ch. XXV)

Table 1. continued.

County	Area	Year	Month	Comments	Citation
Custer	Salmon River Valley	1824		Ross: "over 10,000 in 1 herd" Killed 60	Roe (1951: 267)
		1832	Apr.	Work: Killed 21.	Lewis, Phillips (1923:144)
	East Fork, Salmon R.	1835	Sept.	"large band" Killed 50-60	Parker (1842:107)
	Big Lost R. Valley	1827	Nov.	Ogden: Killed 10 buffalo	Elliott (1910a:364)
		1831	July	"valley covered with buffalo; killed many"	Ferris (1983:Ch. XVII)
		1832	Feb.	"large herds, killed several"	Ferris (1983:Ch. XXIII)
		1832	Apr.	Work: "a band; plenty ahead" Killed 17	Lewis, Phillips (1923:146)
		1833	Apr.	Bonneville: "buffalo recently driven from area"	Irving (1837) p. 84
		1834	Aug.	Killed a bull, calf and 3 others	Townsend (1978:125)
Franklin	Bear River Valley	1825	May	Ogden: "plains covered with buffalo, killed many"	Miller (1952:169-172)
				Kittson: "several bulls, calves killed"	Miller (1954:132)
Fremont	Henry's Fork Valley	1811	June	Hunt: "numerous tracks of buffalo in all directions"	Rollins (1995:290)
		1832	June	"killed hundreds daily"	Ferris (1983:Ch. XXVI)
			Sept.	"plains covered with buffalo in all directions"	
Jefferson	Camas Creek	1835	Sept.	See Clark County comments for 1835	Haines (1965:34)
Jerome	Snake River Valley	1824		Fitzpatrick: "buffalo west to Fishing (Twin) Falls	Fremont (1845:144)
Lemhi	Lemhi River Valley	1831-32	Dec.-Jan.	Work: "a few - large herds far off"	Lewis, Phillips (1923:118-119)
		1832	Mar.	Work: "buffalo road, some herds"	Lewis, Phillips (1923:136-137)
	Salmon R. Valley	1831		"a favorite resort of buffalo"	Ferris (1983:Ch. XXIII)
		1827	Dec.	McKay to Ogden: "buffalo numerous"	Elliott (1910a:375)
		1831		"buffalo numerous" Killed "upwards of 100"	Ferris (1983:Ch. XXII)
Minidoka	Snake River Valley	1824	Apr.	"buffalo on both sides of river west of Fort Hall"	Fremont (1845:144)
Power	Bannock R. Valley	1831	Apr.	Work: "few", but "numerous recently" Killed >16.	Elliott (1913a:282-283)
	Snake River Valley	1824		Fitzpatrick: "buffalo west to Fishing (Twin) Falls"	Fremont (1845:144)
		1826	Apr.	Ogden: Killed 12 buffalo.	Elliott (1909:360)
		1831	Apr.	Work: "some buffalo seen, killed 2 or 3"	Elliott (1913a:284)
Teton	Teton River Valley	1833	July	"killed a bull"	Ferris (1983:Ch. XLI)
		1834	May	"killed several bulls"	Ferris (1983:Ch. LI)

Table 2. Early historic observations of bison in the mountains of Montana.

County	Area	Year	Month	Comments	Citation
Beaverhead	Beaverhead Valley	1806		Sacajawea: bison once numerous to sources.	Biddle (1962:510)
		1824	April	Ross: "large herd, took 22"	Elliott (1913b:379)
		1831	Nov.	Work: "large herds" Killed 45.	Lewis, Phillips (1923:105-108)
		1832	Jan.-Feb.	Work: "buffalo numerous" Killed >100.	Lewis, Phillips (1923:123-131)
		1853?		Suckley: "bison still existed, immense numbers"	Allen (1877:516)
	Bighole Valley	1806		Clark: "old bufalo paths" and skulls. Sacajewea recalled "gathering cows" here.	Biddle (1962:506)
		1824	Apr.	Ross: "herds of buffalo"	Elliott (1913b:379)
		1831	Nov.	Work: "herds seen" Killed 8-10.	Lewis, Phillips (1923:101-102)
		1832	Aug.	"herd of buffalo" Killed 1.	Ferris (1983:Ch. XXIX)
		1834	May	Killed a cow and a bull.	Ferris (1983 Ch. XLIX)
		1834	May	"large herds, killed several"	Ferris (1983:Ch. XLIX)
		1853	Dec.	bison "in great numbers"	Allen (1877:539)
	Horse Prairie	1831	Dec.	Work: "large herds all around" Killed >22.	Lewis, Phillips (1923:109-111)
		1832	Feb.-Mar.	Work: "large herds, immense number" Killed 56.	Lewis, Phillips (1923:132-135)
		1832	Oct.-Nov.	Bonneville: "killed buffalo"	Irving (1837) p. 53
	Red Rocks Valley	1835	Sept.	Russell: "valley full of buffalo"	Haines (1965:34)
		ND		In 1895, noted old bison trails, wallows.	Brower (1896:Chart)
Gallatin	Bozeman Pass	1806		Clark: "pursued the buffalo road"	Biddle (1962:511)
		1840		DeSmet followed a buffalo trail.	Carriker (1995:38-39)
	Gallatin Valley	1806		Clark: "buffalo roads in every direction" Sacajawea: bison "once numerous here"	Biddle (1962:510)
	Three Forks	1840		DeSmet: Indians killed >500 bison	Carriker (1995:37)
Glacier	Glacier Nat. Park	ND		records of bison	Fryxell (1928)

Table 2. Continued.

County	Area	Year	Month	Comments	Citation
Madison	Jefferson Valley	1805, 06		Clark: "old signs, bones, excrement of buffalo"	Biddle (1962:206, 509)
				Sacajawea: bison once numerous to river sources.	Biddle (1962:510)
		1831	Sept.	"plains alive with buffalo" Killed "great numbers"	Ferris (1983:Ch. XX)
		1832	Sept.	"plains covered with buffalo at the Beaverhead" "several fine herds"	Ferris (1983:Ch. XXXI)
			Oct.	"buffalo in abundance"	Ferris (1983:Ch. XXXIII)
	Madison Valley	1832	Oct.	"discovered 2 herds" Killed 6.	Ferris (1983:Ch. XXXI)
	Ruby Valley	1831	Sept.	"buffalo numerous in all directions" Killed many.	Ferris (1983:Ch. XXI)
		1832	Oct.	"hills covered with vast herds"	Ferris (1983:Ch. XXXII)
		1835	Sept.	Russell: "large numbers of buffalo"	Haines (1965:33)
Missoula	Hellgate vicinity	1832		Wyeth: Buffalo coming here are "killed at once."	Kingston (1932:168)
		1853	Nov.	A "lost bull" killed.	Pacific R. R. Report (1860:138)
		1860		Bison skulls seen "daily"	Allen (1877:539)
Powell	Little Blackfoot V.	1831	Nov.	Work: "some bulls" Killed 2.	Lewis, Phillips (1923:98)
	Upper Clark Fork V.	1831	Nov.	Work: "bulls only" Killed a few.	Lewis, Phillips (1923:99-100)
		1831		"buffalo now rare"	Ferris (1983:Ch. XIX)
		1832	Sept.	"buffalo seldom seen" Killed 2.	Ferris (1983:Ch. XXX)
Ravalli	Bitterroot Valley	1824	Feb.	Ross mentions game, but no buffalo.	Elliott (1913b:373-374)
		1831	Oct.	"a few buffaloes"	Roe (1951:268)
		1833		"buffalo never found" this far west.	Ferris (1983:Ch. XLI)
		1841		DeSmet: Bison "abundant in Bitterroot Mountains".	Roe (1951:270)
		1853		"great numbers" of skulls observed.	Allen (1877:516)
Silverbow	Lower Big Hole Valley	1831	Nov.	Work: "bulls, some cows" Killed several.	Lewis, Phillips (1923:101-102)

Table 3. Early historic observations of bison in the mountains of Wyoming..

County	Area	Year	Month	Comments	Citation
Albany	Laramie River Plains	ND		Bison bones found	Fryxell (1928)
		1825	Mar.	Ashley: "innumerable herds of buffalo"	Dale (1918:131)
		1831		"buffalo and other game"	Leonard (1978:18)
		1839	Sept.	Smith: "a great many buffalo"	Hafen (1950:13)
		1843	Aug.	"a buffalo bull"	Fremont (1845:123)
		1844-45	winter	Dodge: "snow crusts and winter kill"	Allen (1877:544)
		1868		Observed numerous bison skulls.	Allen (1877:544)
Carbon	Medicine Bow Mtns.	ND		Bison bones found	Fryxell (1928)
		1840	Apr.	Smith: "saw a great many buffalo"	Hafen (1950:22)
		1843	Aug.	"ten or 12 buffalo bulls"	Fremont (1845:124)
	North Platte Valley	1812	Oct.-Dec.	Stuart: "many buffalo in bottoms" Killed at least 48.	Spaulding (1953:127-134)
		1839	Sept.	Smith: "probably about 2000 buffalo"	Hafen (1950:13)
		1840	Feb.	Smith: "valley filled with herds, killed 3 fat bulls"	Hafen (1950:20)
		1843	Aug.	"bands; country well-stocked with buffalo"	Fremont (1845:125)
		1844	June	"buffalo frequent"	Fremont (1845:282)
	Savery River Valley	1839	Aug.	"small herds"	Wislizenus (2005:136)
		1844	June	Saw herds, killed 4 bison.	Fremont (1845:281)
	Muddy Creek	1839	Sept.	Smith: "saw only a few bulls"	Hafen (1950:14)
		1840	Feb.	Smith: "killed some buffalo"	Hafen (1950:19)
Converse	North Platte Valley	1812	Dec.	Stuart: "very few buffalo seen"	Spaulding (1953:135)
		1834	Aug.	Anderson: hunter killed buffalo in this area.	Morgan (1987:179, 187)
		1844	Aug.	"saw quantities of buffalo"	Clyman (1984:101)
Fremont	Popo Agie Valley	1829	summer	Meek: "plenty of buffalo" near rendezvous site.	Vestal (1963:36)
		1833	Sept.	Bonneville: "numerous droves" Killed 2	Irving (1837) p. 119
	Upper Wind River	1823-24	winter	"buffalo plenty" Killed "upwards of 1000 one day"	Clyman (1984:24-25)
		1835		"abundance of buffalo"	Leonard (1978:255)

Table 3. Continued.

County	Area	Year	Month	Comments	Citation
	Sweetwater River[1]	1812	Oct.	Stuart: much "sign on pass, few seen, very wary"	Spaulding (1953:124)
		1824	Feb.	Killed a bull.	Clyman (1984:29)
		1832	July	Bonneville: "immense herds of buffalo"	Irving (1837) p. 26
		1834	Aug.	Anderson: records of many buffalo killed.	Morgan (1987:179)
		1839	June	"several buffalo, quantities of buffalo"	Wislizenus (2005:79, 81)
		1842	Aug.	"several bands of buffalo"	Fremont (1845:57)
		1843	Aug.	"saw buffalo again" Killed at least 1	Fremont (1845:71)
		1844	Aug.	"buffalo in great abundance"	Clyman (1984:106)
		1846	June	"several male buffalo"	Clyman (1984:258)
Lincoln	Salt River Valley	1833	May	"valley covered with buffalo"	Ferris (1983:Ch. XXXVII)
		1835	May	Russell: "thousands of buffalo"	Haines (1965:12)
	Green River Valley	1824		Fitzpatrick: "immense numbers"	Fremont (1845:144)
	Bear River Valley	1830	July	"herds of buffalo, killed a great many"	Ferris (1983:Ch. VIII)
Natrona	Platte River	1839	June	"a herd grazing on the shore, drove herds before us"	Wislizenus (2005:77-78)
	Sweetwater River	1812	Oct.	Stuart: "numerous herds" Killed 5.	Spaulding (1953:125-126)
		1834	June	Anderson: "immense numbers of buffalo"	Morgan (1987:117)
		1842	Aug.	"tolerably abundant" near Independence Rock	Fremont (1845:56)
		1844	Aug.	"buffalo in great abundance"	Clyman (1984:102)
		1846	June	"plenty of buffalo, several herds"	Clyman (1984:259)
Park	Yellowstone Park	ND		Bison bones found	Fryxell (1928)
	Yellowstone Park	1860-79		Eleven records of bison in the Park	Meagher (1973:116-118)
	Yellowstone Park	1877-1928		Annual Rpts. 600 bison in 1880; 25 in 1907	Skinner, Alcorn et al.
Sublette	Ham's Fork Valley	1834	June	Russell: "country abounds with buffalo"	Haines (1965:3)
	Upper Green River	1811	Sept.	Hunt: "numerous herds of bison graze the valleys"	Rollins (1995:287)
		1812	Oct.	Stuart: "a few bulls" Many fresh kills. Killed some.	Spaulding (1953:116-122)
		1832	Aug.	"plain covered with buffalo"	Ferris (1983:Ch. XXVII)
		1833	June	"plains covered with buffalo in all directions"	Ferris (1983:Ch. XL)

Table 3.Continued.

County	Area	Year	Month	Comments	Citation
		1833	July	Bonneville: "valley strewed with buffalo carcasses"	Irving (1837) p. 95
		1833	Sept.	Bonneville: "buffalo as far as he could see"	Irving (1837) p. 128
		1834	June	"large herds in every direction"	Townsend (1978:78)
		1834	May-June	"killed several buffalo along lake" "killed plenty of buffalos in plains"	Ferris (1983:Ch. LI)
		1838	July	Russell: "large band of buffalo"	Haines (1965:90)
	Hoback Valley	1832	Aug.	Valley "covered with herds of buffalo"	Ferris (1983:Ch. XXVII)
		1833	July	"killed a bull"	Ferris (1983:Ch. XLI)
		1834	May	"buffalo found here"	Ferris (1983:Ch. LI)
Sweetwater	Green River Valley	1824		Fitzpatrick: "immense numbers"	Fremont (1845:144)
		1824	Feb.	Killed a buffalo.	Clyman (1984:30)
		1825	Apr. -May	Ashley: "got hides for boat; buffalo abundant"	Dale (1918:138, 141)
		1834	June	Anderson: "killed some buffalo"	Morgan (1987:130, 134)
	Great Divide Basin	1843	Aug.	"a few straggling bulls"	Fremont (1845:127)
Teton	Jackson Hole	ND		Bison bones found	Fryxell (1928)
		1833	June	"large herd, killed several"	Ferris (1983:Ch. XXXIX)
		1835	Aug.	Buffalo ran through party"	Parker (1842:92)
		1877		Hayden: "a few bulls persist"	Allen (1877:516)
Uinta	Bear River Valley	1824		Fitzpatrick: "immense numbers"	Fremont (1845:144)
		1834-35	winter	Bonneville: "vast herds"	Irving (1837) p. 222
	Black's Fork Valley	1846	June	Valley "covered" with bison bones and campsites.	Clyman (1984:256)
		1849		Stansbury: "bison near Bridger's Fork of Muddy Creek"	Allen (1877:513)

[1] including South Pass

Table 4. Early historic observations of bison in the mountains of northeast Utah.

County	Area	Year	Month	Comments	Citation
Wasatch Front		1836		"many buffaloes".	Allen (1877:512)
		1837		"all destroyed with deep snow".	Allen (1877:512)
		1840		Russell: Buffalo "long since" gone.	Haines (1965:121)
Box Elder	Promontory Point	1832		Marsh: "Bison present until 1832."	Roe (1951:279)
	Salt Lake, northwest	1828	Dec.	Ogden: Killed 2 buffalo.	Elliott (1910b:390)
		1833	Sept.	Killed last buffalo, heading west.	Leonard (1978:106)
Cache	Ogden Valley	1825	May	Ogden: "plains covered with buffalo, killed some"	Miller (1952:175)
Uintah	Green River	1776	Sept.	Escalante expedition: Killed 1 bison.	Bolton (1950:168)
		1825	May	Ashley: "a number of buffalo."	Dale (1918:146)
	White River	ND		"few bison descend to mouth of White River"	Fremont (1845:144).

Table 5. Early historic observations of bison in the mountains of Colorado.[1]

County	Area	Year	Month	Comments	Citation
Boulder	Wild Basin	ND			Meaney and Van Vuren (1993)
Chaffee	Arkansas Valley	1806	Dec.	Pike: Killed 8 buffalo.	Hart and Hulbert (2006:156)
Conejos	San Luis Valley	1694		"over 500 buffalo"	Meaney and Van Vuren (1993)
Custer	Grape Cr. Valley	1807	Jan.	Pike: Saw "2 gangs" of buffalo. Killed 2.	Hart and Hulbert (2006:164)
Fremont	Arkansas Valley	1806	Dec.	Pike: killed buffalo near 4-Mile Creek	Hart and Hulbert (2006:148)
		1844	June	Noted buffalo trails.	Fremont (1845:287)
Garfield	Flat Tops	ND		G. Schoonveld collected bison horn in 1980s.	Pers. Comm.[2]
		ND		Prof. J. V. K. Wagar: "last bison in Colorado"	Pers. Comm.[3]
	N of Glenwood Sprgs.	ND			Meaney and Van Vuren (1993)
	Piceance/R	ND			Meaney and Van Vuren (1993)
	Rifle Creek	ND			Meaney and Van Vuren (1993)
Grand	Grand Lake	ND			Meaney and Van Vuren (1993)
Gunnison	near Irwin	ND			Meaney and Van Vuren (1993)
	West Elk Mtns.	ND			Meaney and Van Vuren (1993)
Huerfano	Huerfano Valley	1807	Jan.	Pike: "discovered buffalo" Killed 4 at Muddy Creek	Hart and Hulbert (2006:166)
	East of sand dunes	1807		Pike	Meaney and Van Vuren (1993)
Jackson	North Park	ND		Bison bones.	Fryxell (1928)

Table 5. Continued.

County	Area	Year	Month	Comments	Citation
		1825	Mar.	Ashley: "valleys filled with numerous herds"	Dale (1918:129)
		1839	July	Guides: "Arapahos hunting bison"	Farnham (1843:43, 50)
		1839	Sept.	Smith: "probably about 2000 buffalo"	Hafen (1950:13)
		1844	June	"killed some buffalo"	Fremont (1845:283)
		ND		between Illinois and Grizzly Creeks	Meaney and Van Vuren (1993)
	Higho	ND			Meaney and Van Vuren (1993)
Larimer	Chambers Lake	ND			Meaney and Van Vuren (1993)
	North Fork, Poudre	1839	Sept.	Smith: "saw a great many buffalo" Killed 7.	Hafen (1950:13)
	Rocky Mtn. Nat. Pk.	ND		Bison bones.	Fryxell (1928)
		ND		bison noted at several sites in the Park.	Meaney and Van Vuren (1993)
Moffat	Little Snake Valley	1839	Sept.	Smith: "shot old buffalo", probably on Powder Wash	Hafen (1950:15)
	Yampa River Valley	1849		Stansbury: "bison on northern tributaries of Yampa:	Allen (1877:513)
	Brown's Hole	ND			Meaney and Van Vuren (1993)
Park	South Park	ND			Meaney and Van Vuren (1993)
		ND		Dodge: a bison hunt in mtns. near South Park	Hornaday (1889:410)
		1806	Dec.	Pike: "killed a buffalo"	Hart and Hulbert (2006:151)
		1839	July	"herds, small bands" Killed 2.	Farnham (1843:43-46)
		1844	June	"buffalo charged through camp"	Fremont (1845:286)
	Mt. Lincoln	ND		"far above timberline"	Meaney and Van Vuren (1993)
	Mosquito Gulch	ND			Meaney and Van Vuren (1993)
	Tarryall Creek	ND			Meaney and Van Vuren (1993)
Rio Blanco	Mud Springs	ND			Meaney and Van Vuren (1993)
Routt	California Park	ND			Meaney and Van Vuren (1993)
	Yampa R. Valley	<1839		Guide: "buffalo plenty some years before"	Farnham (1843:51)
Saguache	San Luis Valley north	ND			Meaney and Van Vuren (1993)
Summit	Blue R. Valley	1839	July	"small bands, swells covered with buffalo" Killed 1.	Farnham (1843:47-48)
		1844	June	"country alive with buffalo"	Fremont (1845:284)

[1] Colorado records of subfossil specimens in Meaney and Van Vuren (1993) are not repeated here.
[2] G. Schoonveld: Wildlife biologist, Colorado Division of Wildlife.
[3] J. V. K. Wagar: Retired professor, wildlife biology, Colorado State University.

In the cited Minataree/Mandan slaughter, every animal of the herd was killed. Using the same hunting technique, the "surround" or "running hunt", Flatheads (Salish) "usually carried a hunt to the point of extermination." (Point, nd:141). Literature cited here contains descriptions of pre-hunt ceremonies of Native Americans. Many appear to have believed that providence, more than prudence, determined the continued availability of bison.

These observations of bison slaughter occurred after Native Americans had been greatly and widely diminished by diseases, especially smallpox, which preceded Euro-Americans to the interior of North America. Pandemics eliminating entire local populations and more than half of regional populations have been inferred for smallpox epidemics that included the Rocky Mountain tribes in 1781, 1801 and 1837 (Russell in Haines 1965:86; Thompson in Hopwood 1971:93, 97, 198; Dobyns 1983:15). It is reasonable to assume that much larger populations of Native Americans routinely killed even larger numbers of bison over larger areas prior to arrival of European diseases, affecting where the earliest Euro-American travelers did not find bison.

Unusually deep and persistent snows sometimes greatly depleted local bison herds and may have caused local extirpations, especially in conjunction with continued human harvest. Colonel Dodge reported that thousands of bison starved on the Laramie Plains, Wyoming during an extraordinary winter with deep and crusted snow in 1844-45 (Allen 1877:544). The Plains were never repopulated. Allen (1877:512) noted reports that nearly all the bison in the Salt Lake area, Utah, were destroyed by unusually deep snow about 1837. In 1840, Russell (Haines 1965:121) noted that bison had "long since" been gone from this area.

Williams (2005) used local weather records to conclude that abundant, dense snow, combined with a greater frequency of droughts, had contributed to limiting bison distribution and abundance in the northwest United States during late pre-historic and early historic times. These weather effects were most notable west of the Rocky Mountains in eastern Washington and Oregon.

Native Americans likely caused local, or even regional, extirpations of bison. In 1806, Sacajawea (Biddle 1962: 510) informed Clark that bison were once numerous in the Gallatin, Jefferson and Beaverhead valleys of Montana, but had disappeared in "but a few years" with concentrated Shoshone hunting. In 1833, near the mouth of the Bitterroot River and the "home base" of Salish, Wyeth noted: "Buffalo have come here and even further but they are killed at once and do not get wonted here." (Kingston, 1932:168).

Discussion

During the first half of the 19th century, bison were widely distributed in the intermountain valleys of the Rocky Mountains in the United States. A major regional concentration once occurred in the upper Snake and Salmon River drainages of southeast Idaho, in the upper Green River drainages of southwest Wyoming and over the continental divide along the uppermost tributaries of the Jefferson River in southwest Montana (Fig. 1, Tables 1-3).

Previous authors proposed multiple interacting factors to explain local or regional absence of bison in the northwest United States during early historic time (Van Vuren 1987; Laliberte and Ripple 2003; Lyman 2004; Williams 2005). Three of these references focus on areas west of the Rocky Mountains where bison were absent as Europeans arrived. For this area, authors had no access to recorded observations of Native American/bison interactions. Still, all recognized Native American predation as a factor explaining the early historic absence of bison, as did Meaney and Van Vuren (1993) for southwest Colorado. In contrast, early historic literature from the Rocky Mountains provides abundant descriptions of bison and of Native American predation. These observations provide compelling evidence that human predation was a major, perhaps preponderant, factor limiting bison distribution in the Rocky Mountains. While

other factors varied geographically and temporally, Native American predation was more persistent, mobile and widespread.

Native American ability to extirpate bison or to prevent reestablishment of bison would have been enhanced by:

(1) low bison abundance and productivity in areas with little and inconsistent forage production, due to aridness with frequent droughts and perhaps exacerbated by competitive foraging from wild and Native American horses;

(2) a large Native American population supported by alternative food resources, including salmon, other big game, small game and invertebrates;

(3) periodic major bison declines due to severe winters or prolonged droughts, accentuating the numerical ratio of human predators/prey;

(4a) patchily distributed bison habitat limiting bison mobility as an escape strategy, (4b) isolating source populations for reintroductions, (4c) limiting long term inter-population genetic support and (4d) allowing hunters to predictably locate their prey.

In the reviewed literature, I found no descriptions of epidemic disease in bison before there was contact with Euro-American domestic livestock.

In contrast, the ability of Native Americans to reduce bison numbers would have been limited by distance from permanent villages, especially in areas contested by dangerous enemy tribes (Martin and Szuter 1999; Laliberte and Ripple 2003; Kay 2007). The distance from permanent villages may have contributed to the above noted major abundance of bison in the central Rocky Mountains of the United States.

These interacting factors seem to have characterized much of the land in and especially west of, the Rocky Mountains. No doubt interacting factors varied in time and space. Interacting factors resulted, when Euro-Americans first arrived, in few or no bison west of the Rocky Mountains and, at least periodically, few or none in some areas within the Rockies. But human predation must have been a preponderant factor in many areas (Urness 1989; Martin and Szuter 1999; Laliberte and Ripple 2003; Kay 2007). After 1824, continued harvesting by both Native- and Euro-Americans rapidly eliminated most bison from the Rocky Mountains. By the 1840's Salish from the Bitterroot Valley, Montana, had to march 24 winter days to find any trace of bison (Point nd:120). In summer, on a more direct route, they traveled 15 days before finding bison (p.166). Eventually, bison remained only as a relict herd in Yellowstone National Park (Skinner, Alcorn, et al. 1951; Meagher et. al 2002).

Early historic records indicate bison were once widespread in non-forested intermountain valleys of the Rocky Mountains. It is less clear if bison persistently used upper mountain elevations in large numbers. Some areas where bison were not recorded by Euro-American explorers likely were lands where bison had been extirpated – for short or long periods - largely by Native Americans. Early historic records provide few geographic limits for restoring the mountain ecotype of bison in the Rocky Mountains.

Acknowledgements

Thanks to Kathryn QannaYahu for producing Fig. 1 and to two anonymous reviewers for helpful suggestions. This work was supported in part under 42 U. S. Code, Chapt. 7. Paul Griffen and The Gallatin Wildlife Association defrayed printing costs.

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Received 08 February 2016
Accepted 10 May 2016

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Design and Layout ~ **Media Works Publishing, Bozeman, Montana**
Printing ~ **Bozeman Printing Company, Bozeman, Montana**

Intermountain Journal of Sciences

Vol. 22, No. 1-3 - September 2016

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