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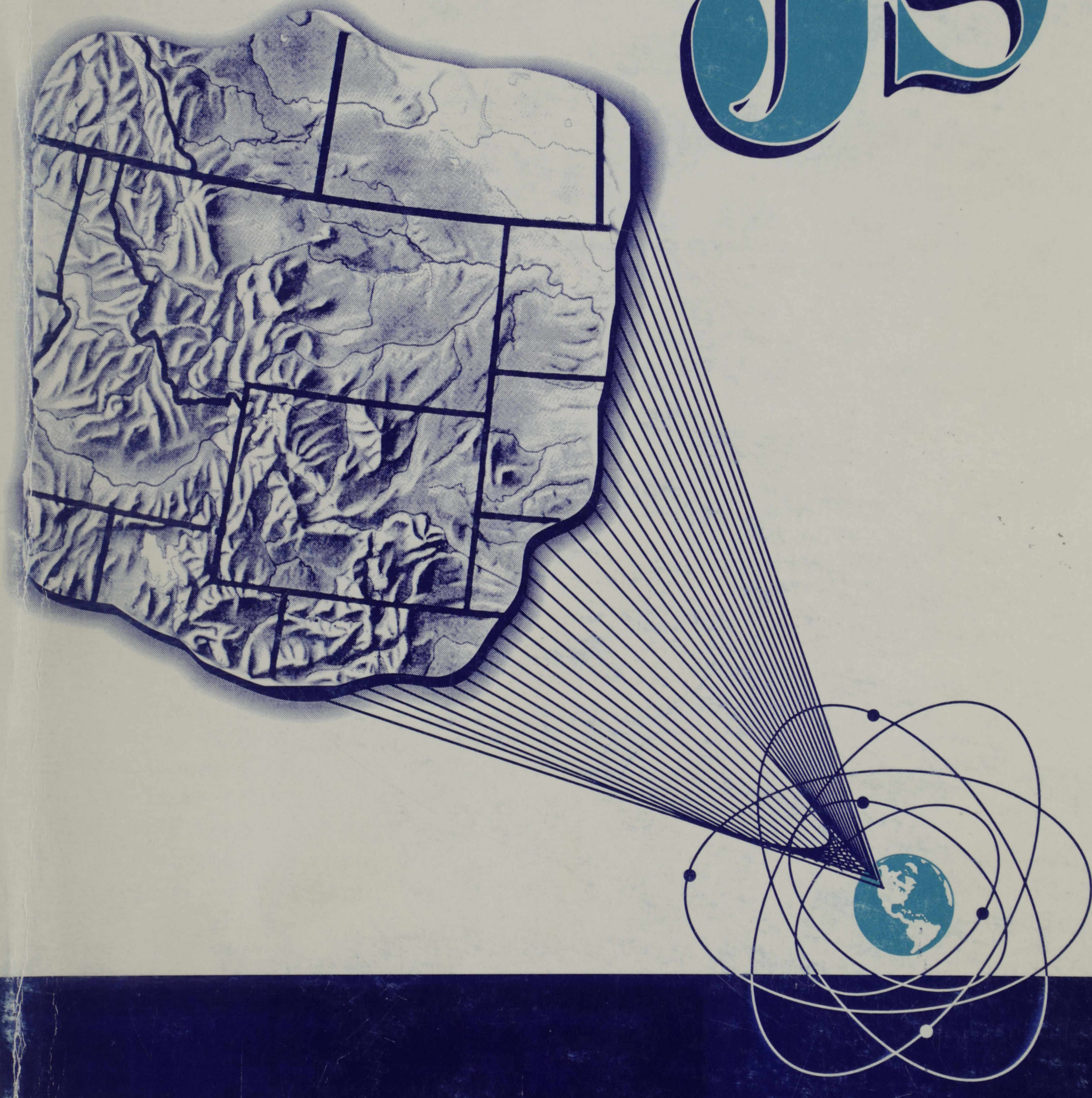

Intermountain Journal of Sciences

The Intermountain Journal of Sciences 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 engineering, mathematical, molecular-cellular, pharmaceutical, physical and social sciences are welcome.

Co-sponsors/publishers include the Montana Academy of Sciences, the Montana Chapter of The Wildlife Society, and the Montana Chapter of The American Fisheries Society. This journal offers peer review and an opportunity to publish papers presented at annual meetings of the co-sponsor organizations. It is the intent of the governing bodies of the co-sponsor organizations that this journal replace printed proceedings of the respective annual meetings. Therefore, it is the policy of the editorial board that presenters at annual meetings of the co-sponsors be given priority in allocation of space and time of publication, although submission of other manuscripts for review and publication without regard to membership is encouraged.

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

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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 this journal, 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). 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 coy 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 required for each manuscript. The two other reviewers are instructed to return the manuscript and their comments to the Associate Editor, who completes and returns to the EIC a blue "Cover Form" and all manuscripts and reviewer comments plus a recommendation for publication, with or without revisions, or rejection of the manuscript. This initial review process is limited to 30 days.

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

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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, four copies if further review is required, or one hard copy plus the computer disk if only minor revision or formatting is necessary. The revised manuscript shall be returned to the EIC within 14 days of the 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.

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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 new manuscript. The new 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.

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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 Chairman of the Editorial Board of IJS, who will serve as the EIC for that manuscript.

Abstracts

Only abstracts 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, key or scan them onto a 3.5" diskette, and submit the diskette and hard copy of each abstract to the EIC 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 assignment to the appropriate discipline section. All abstracts will be published in the December issue only.

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. Guidelines for manuscripts submitted to the *Intermountain Journal of Sciences*. Int. J. Sci. 1(1):61-70.

Send all manuscripts to: Editor-in-Chief, P.O. Box 3014, Bozeman, MT 59772-3014

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The Ichthyofauna of Small Streams on the Charles M. Russell National Wildlife Refuge, Montana

Robert G. Bramblett
Alexander V. Zale

Abstract

*The ichthyofauna of the small streams on the Charles M. Russell National Wildlife Refuge is poorly known because no systematic survey had been conducted previously. We sampled fish and visually evaluated habitat at 18 third and fourth order streams, stratified to ensure good geographic coverage. A total of 13 streams had fish present, two streams had water but no fish, and three streams had no water present. Most streams with water were intermittent; only two streams had flowing water. A total of 19 fish species was captured of which 14 species were native to Montana. From one to 12 fish species, and from one to 899 individual fish were captured per site. Overall, 87 percent of individual fish captured were native species. Introduced species made up over 50 percent of fish captured at only one site and 7 of 13 streams had no introduced species. The most common species were fathead minnow (*Pimephales promelas*), plains minnow (*Hybognathus placitus*), lake chub (*Couesius plumbeus*), white sucker (*Catostomus commersoni*), common carp (*Cyprinus carpio*), flathead chub (*Platygobio gracilis*), and longnose dace (*Rhinichthys cataractae*). Rough positive correlations between a qualitative habitat index and numbers of fish species and individual fish were observed. Because most of the species we captured are rare in the adjacent Missouri River or Fort Peck Reservoir, we suspect that most fish complete their life cycles within the streams we sampled, despite the low quantities of water present. We further speculate that connectivity among streams that enter Ft. Peck Reservoir has been reduced because the reservoir acts as a partial barrier to the movements of most of the fish species we captured.*

Key words: Montana fishes, prairie stream fishes, native fishes, introduced fishes, prairie streams, intermittent streams, Charles M. Russell National Wildlife Refuge, reptiles, amphibians.

Introduction

A systematic survey of the fishery resources of the Charles M. Russell National Wildlife Refuge (CMRNWR) has never been conducted with the exception of the Missouri River and Fort Peck Reservoir. This information is needed to assist in the Federal Reserved Water Rights Negotiation Process because sufficient flows to maintain fish populations are included in Federal Reserved Water Rights. Additionally, assessments of fish assemblages inhabiting the Refuge's small streams are needed to document the Refuge's aquatic biodiversity. The objective of this study was to document the ichthyofaunal assemblages and qualitative habitat conditions of a subset of streams on the Refuge.

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Study Area And Methods

The CMRNWR is a 445,000 hectare National Wildlife Refuge located in northeastern Montana (Fig. 1). The Refuge straddles the Missouri River and the entire 101,000 hectare Fort Peck Reservoir, created by the construction of Fort Peck Dam in 1933. Elevations on the Refuge range from 685 to 988 m; about 80 percent of the landscape is comprised of the "Missouri Breaks"—steep ridges, badlands and coulees (Graetz and Graetz 1999). Vegetation types range from open forests of Rocky Mountain juniper (*Juniperus scoluporum*) and ponderosa pine (*Pinus ponderosa*) with occasional Douglas fir (*Pseudotsuga menzesii*), to riparian gallery forests of plains cottonwood (*Populus deltoides*) along the Missouri River, and sagebrush (*Artemesia tridentata*) and grassland prairies. The dominant geological features south of the river and reservoir are the Hell Creek formation and Fox Hills sandstone, whereas the north side of the Refuge was glaciated in the Pleistocene and is dominated by Bearpaw shale (Graetz and Graetz 1999). Just one large perennial stream, the Musselshell River, enters the Missouri River or Fort Peck Reservoir. The streams we sampled were third and

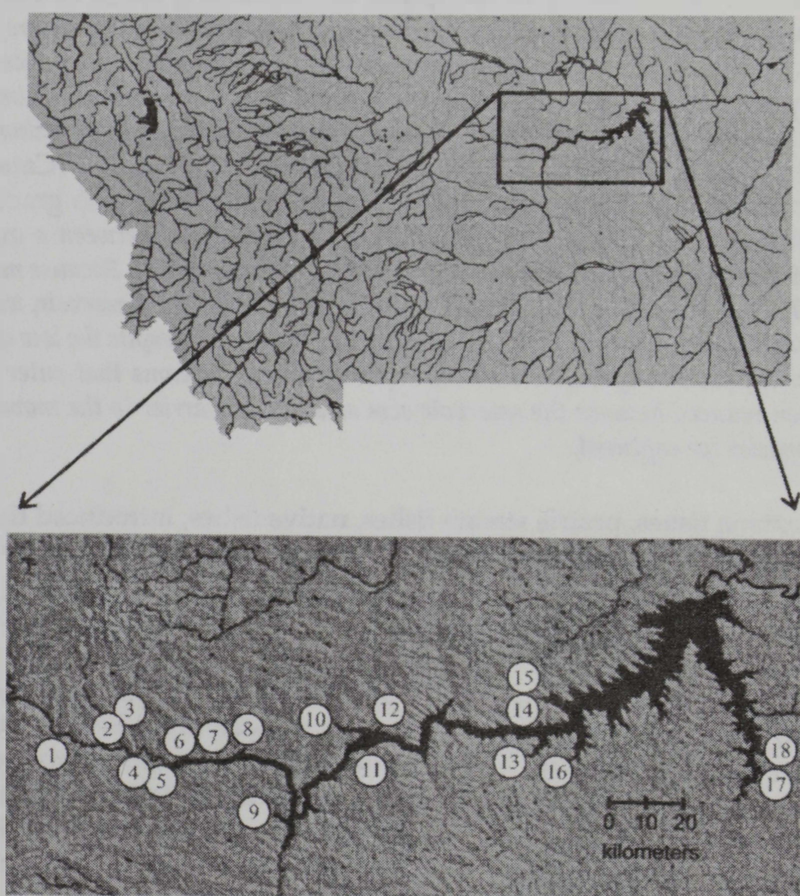

Figure 1. Map of study area. Open circles indicate locations of sites sampled during survey of Charles M. Russell National Wildlife Refuge during July 1999. Site 1 = Armells Creek, Site 2 = Siparyann Creek, Site 3 = Rock Creek, Site 4 = Sand Creek, Site 5 = Carroll Coulee, Site 6 = Sevenmile Creek, Site 7 = CK Creek, Site 8 = Beauchamp Creek, Site 9 = Crooked Creek, Site 10 = Fourchette Creek, Site 11 = Devils Creek, Site 12 = Kill Woman Creek, Site 13 = Snow Creek, Site 14 = Carpenter Creek, Site 15 = Sutherland Creek, Site 16 = Hell Creek, Site 17 = Nelson Creek, Site 18 = McGuire Creek.

fourth order (intermittent tributaries counted when determining stream order) streams. The majority of the streams we sampled have headwaters on the prairie, whereas three streams have headwaters in the Little Rocky or Judith mountains.

Of 29 streams identified by CMRNWR personnel as possibly supporting fish populations, 18 were selected for sampling (Fig. 1). The sites were stratified to ensure good geographic coverage. We sampled the streams in July 1999.

At each site, we walked about 1.6 km along the stream to determine if water was present and to select a representative reach for sampling. We sampled a reach that was 40 times the average wetted width of the stream, a length normally adequate to capture 90 percent of the fish species present in a stream (Lazorchak *et al.* 1998). At sites with mean wetted width of < 4 m, we sampled a minimum reach length of 150 m. All streams were sampled within 3.2 km of the Missouri River or Fort Peck Reservoir, except Rock Creek, which was sampled 6.2 km above the Missouri River and Beauchamp Creek, which was sampled 8.0 km above Fort Peck Reservoir.

Fish were captured by seining with a 3.6, 4.6, or 9.1 m long by 0.9 m tall seine with 6.4 mm mesh. Block nets were placed at the upstream and downstream end of the sampled reach in streams with continuous water. All fish captured were identified to species in the field, except the genus *Hybognathus*. Fishes of this genus are difficult to identify to species in the field, so we preserved 20-36 individuals in 10 percent buffered formalin and determined species identity in the laboratory. The proportion of each *Hybognathus* sp. in the subsample was then multiplied by the total *Hybognathus* spp. in the sample to extrapolate an estimate for the total number of each *Hybognathus* sp. at the site. The single *Phoxinus* sp. we captured was also preserved and identified in the laboratory. While sampling for fish and traveling between sites, we recorded observations of the presence of amphibians and reptiles.

A rapid visual habitat assessment was performed on each of the streams following the U.S. Environmental Protection Agency's (USEPA) wadeable streams protocol (Lazorchak *et al.* 1998). This habitat assessment included 12 parameters; each parameter was evaluated visually and rated in terms of habitat quality on a scale of 0 to 20, yielding a total possible score of 240. Total scores were categorized as poor (0-60), marginal (61-120), sub-optimal (121-180), or optimal (181-240). The relationships between total habitat scores and the number of fish species captured and the number of individual fish captured were examined using linear regression.

RESULTS

Fish Surveys

A total of 4,376 fish comprising 19 species was captured. Fourteen of the species captured were native to Montana; the remaining five were introduced species (Brown 1971, Holton and Johnson 1996). The 19 fish species belonged to six families (Table 1): Cyprinidae (12 species), Catostomidae (three species), Ictaluridae (one species), Cyprinodontidae (one species), Gasterosteidae (one species), and Centrarchidae (one species).

Eighteen streams were sampled; 13 had fish present and five had no fish present (Table 2). Most streams with fish were not flowing during sampling; fish were captured in residual pools separated by dry reaches. Only two streams (Nelson and Rock creeks) had flowing water during sampling. Of the streams without fish, two streams had some water present in isolated pools, and three streams had no water present in the reach that we examined. The

Table 1. *Fish species captured during a survey of the Charles M. Russell National Wildlife Refuge, Montana, 13-27 July , 1999.*

Family/species		Native or introduced
Cyprinidae		
lake chub	*Couesius plumbeus*	native
common carp	*Cyprinus carpio*	introduced
western silvery minnow	*Hybognathus argyritis*	native
brassy minnow	*Hybognathus hankinsoni*	native
plains minnow	*Hybognathus placitus*	native
spottail shiner	*Notropis hudsonius*	introduced
sand shiner	*Notropis stramineus*	native
Northern redbelly x finescale dace	*Phoxinus eos x P. neogaeus*	native
fathead minnow	*Pimephales promelas*	native
flathead chub	*Platygobio gracilis*	native
longnose dace	*Rhinichthys cataractae*	native
creek chub	*Semotilus atromaculatus*	native
Catostomidae		
river carpsucker	*Carpoides carpio*	native
longnose sucker	*Catostomus catostomus*	native
white sucker	*Catostomus commersoni*	native
Ictaluridae		
black bullhead	*Ameiurus melas*	introduced
Cyprinodontidae		
plains killifish	*Fundulus zebrinus*	introduced
Gasterosteidae		
brook stickleback	*Culaea inconstans*	native
Centrarchidae		
green sunfish	*Lepomis cyanellus*	introduced

number of species captured at each site ranged from one to 12, and the number of individuals captured ranged from one to 899. Nelson Creek had 12 species; Crooked Creek had 10 species; Beauchamp and Armells creek had eight species; Fourchette Creek had seven species; Hell Creek had six species; CK and Rock creeks had five species; Sutherland Creek had four species; McGuire Creek had three species; Kill Woman Creek had two species; and Siparyann and Snow creeks had one species. Sand Creek and Carroll Coulee had some water present as isolated pools but no fish, and Carpenter, Devils, and Sevenmile creeks had no water present.

Fathead minnow (*Pimephales promelas*) were the most common fish captured; they were present at 10 of 13 sites with fish present (Table 2). Other common species (captured at five or more sites) were plains minnow (*Hybognathus placitus*), lake chub (*Couesius plumbeus*), white sucker (*Catostomus commersoni*), common carp (*Cyprinus carpio*), flathead chub (*Platygobio gracilis*), and longnose dace (*Rhinichthys cataractae*; Table 2). Rare species (captured at ≤4 sites) included sand shiner (*Notropis stramineus*), longnose sucker (*Catostomus catostomus*), river carpsucker (*Carpoides carpio*), western silvery minnow (*Hybognathus argyritis*), black bullhead (*Ameiurus melas*), brassy minnow (*H. hankinsoni*), spottail shiner (*N. hudsonius*), creek chub (*Semotilus atromaculatus*), brook stickleback (*Culaea inconstans*), green sunfish (*Lepomis cyanellus*), northern redbelly X finescale dace hybrid (*Phoxinus eos* X *P. neogaeus*), and plains killifish (*Fundulus zebrinus*; Table 2).

We observed three species of adult frogs and toads (northern leopard frog, *Rana pipiens*; Woodhouse's toad, *Bufo woodhousei*; and Great Plains toad, *Bufo cognatus*), unidentified tadpoles and toadlets, one salamander species (tiger salamander, *Ambystoma tigrinum*), one lizard species (short-horned lizard, *Phyrnosoma douglasi*), and three snake species (plains garter snake, *Thamnophis radix*; racer, *Coluber constrictor*; western rattlesnake, *Crotalus viridis*).

Habitat Surveys

Habitat assessments were performed on 14 creeks. Composite habitat scores ranged from 79 (33%) to 191 (80%) of 240 possible points (Table 3). One stream had a score in the optimal range, nine streams scored in the suboptimal range, and four streams scored in the marginal range. No streams scored in the poor habitat range.

Relationships Between Habitat and Fish

Linear regression revealed positive relationships between total habitat score and number of fish species and number of individual fish captured. The relationship for total habitat score and number of fish species captured approached statistical significance (P = 0.09, r^2 = 0.22), whereas the relationship for total habitat score and number of individuals captured was weaker (P = 0.27, r^2 = 0.10).

Discussion

Despite low quantities of water, the majority of streams we sampled supported fish, and most streams had multiple year classes of fish. Invertebrates, amphibians, and reptiles also were common in and around these streams. Although at least 14 species of nonnative fish have been introduced into Fort Peck Reservoir (Alvord 1979, Needham and Gilge 1982), the ichthyofauna of the small streams of the CMRNWR was dominated by native species. Fourteen of 19 species, and 87 percent of individual fish captured in this survey were native species. Seven of 13 streams had no introduced species, and no stream had more than two introduced species. Only one site (Armells Creek) had over 50 percent of individuals as introduced species. We expect the extreme environmental conditions typical of intermittent prairie streams, such as low water quantities, high water temperatures, and high flow variability (Paloumpis 1958, Matthews 1988, Zale *et al.* 1989) also are normal in the small streams of the CMRNWR. These conditions probably prevent establishment of all but the most tolerant introduced species, such as common carp, black bullhead, plains killifish, and green sunfish.

Factors that may influence fish species richness and abundance in small streams of the CMRNWR include frequency and magnitude of stream flow, stream size, i.e., stream order, habitat quality, and connectivity to other streams or Fort Peck Reservoir. Despite dry periods when aquatic habitat is limited to residual pools (Paloumpis 1958, Zale *et al.* 1989, Bramblett and Fausch 1991), and disturbances such as floods (Fausch and Bramblett 1991), fishes often persist in the residual isolated pools of intermittent streams. However, intermittent prairie stream pools normally have lower species richness (Paloumpis 1958, Metcalf 1959, Kuehne 1962, Harrel *et al.* 1967, Horwitz 1978) and higher variability in species presence/absence and abundance (Fausch and Bramblett 1991) than larger, more stable, and perennial downstream reaches. Only two streams, Nelson and Rock creeks were flowing at the time of our survey. Nelson Creek had the highest species richness (12 species) and Rock Creek had six species. However, because five non-flowing streams had

Table 2. *Numbers of fish captured in each stream on the Charles M. Russell National Wildlife Refuge, Montana, 13-27 July, 1999.*

Site, Date	BB	BM[2]	BS	CC	CR	FM	FC	GS	JS	LC	LD	LS	PH	PK	PM[2]	RC	SS	SH	SM[2]	WS	Totals
	Species[1]																				
Armells, 7/27/99	0	0	0	158	0	6	40	0	22	0	0	2	0	0	20	3	0	0	31	0	282
Beauchamp, 7/22/99	0	17	5	0	0	92	0	0	0	105	56	0	0	0	235	0	78	0	0	54	642
Carpenter, 7/14/99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carroll, 7/25/99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
CK, 7/22/99	0	0	0	0	0	15	0	0	0	45	0	2	0	0	101	0	0	0	0	2	165
Crooked, 7/24/99	0	0	0	1	0	138	3	3	0	60	9	0	0	0	265	24	153	0	0	68	724
Devil's, 7/26/99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fourchette, 7/23/99	24	0	0	300	0	213	0	0	0	1	0	0	0	0	57	0	0	0	114	8	717
Hell, 7/15/99	0	0	0	0	0	49	144	0	0	191	9	0	0	0	155	0	13	0	0	0	561
Kill Woman, 7/13/99	0	0	0	0	0	3	0	0	0	0	0	0	0	0	40	0	0	0	0	0	43
McGuire, 7/16/99	0	0	0	2	0	2	0	0	0	0	0	0	0	0	16	0	0	0	0	0	20
Nelson, 7/16/99	7	0	0	0	11	149	2	0	0	13	2	2	0	1	572	1	118	0	0	21	899
Rock, 7/21/99	0	1	0	0	0	0	1	0	1	3	85	0	0	0	0	0	0	0	0	8	99
Sand, 7/25/99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sevenmile, 7/21/99	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Siparyann, 7/20/99	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Snow, 7/15/99	0	0	0	0	0	0	0	0	0	24	0	0	0	0	0	0	0	0	0	0	24
Sutherland, 7/14/99	0	0	0	2	0	106	0	0	0	0	0	0	0	0	0	0	0	80	4	0	192
Total captured	31	18	5	463	11	773	190	3	23	442	161	6	1	1	1461	28	362	80	149	161	4376
No. of sites where present	2	2	1	5	1	10	5	1	2	7	5	3	1	1	9	3	4	1	3	6	
Mean total length	83	72	41	64	114	71	71	103	NM[4]	71	61	116	56	55	71	105	52	61	102	115	
SD[3] total length	44	4	3	104	28	7	18	37		20	10	21			9	16	4	11	18	52	
Range of total length	20-198	69-75	39-45	25-500	60-175	32-77	37-132	75-145		35-180	34-90	82-144			30-111	72-132	39-68	52-95	79-136	35-250	

[1] BB = black bullhead (*Ameiurus melas*); BM = brassy minnow (*Hybognathus hankinsoni*); BS = brook stickleback (*Culaea inconstans*); CC = common carp (*Cyprinus carpio*); CR = creek chub (*Semotilus atromaculatus*); FM = fathead minnow (*Pimephales promelas*); FC = flathead chub (*Platygobio gracilis*); GS = green sunfish (*Lepomis cyanellus*); JS = juvenile sucker (*Catostomus spp.*); LC = lake chub (*Couesius plumbeus*); LD = longnose dace (*Rhinichthys cataractae*); LS = longnose sucker (*Catostomus catostomus*); PH = northern redbelly x finescale dace hybrid (*Phoxinus eos x P. neogaeus*); PK = plains killifish (*Fundulus zebrinus*); PM = plains minnow (*Hybognathus placitus*); RC = river carpsucker (*Carpoides carpio*); SS = sand shiner (*Notropis stramineus*); SH = spottail shiner (*Notropis hudsonius*); SM = western silvery minnow (*Hybognathus argyritis*); WS = white sucker (*Catostomus commersoni*).

[2] The number of brassy minnow, plains minnow, and western silvery minnow in each sample was estimated by identifying species of a subsample of up to 36 fish in the laboratory and extrapolating the ratio of each species at the site to the total *Hybognathus* spp. sample for that site.

[3] SD = standard deviation

[4] NM = not measured

Table 3. *Rapid habitat assessment scores for streams sampled on the Charles M. Russell National Wildlife Refuge, Montana, 13-27 July, 1999. Total scores were categorized as poor (0-60), marginal (61-120), suboptimal (121-180), or optimal (181-240).*

	Habitat parameter[1]												
Site, Date		2	3	4	5	6	7	8	9	10	11	12	Total
Armells, 7/27/99	10	11	10	9	20	8	10	5	13	16	16	5	133
Beauchamp, 7/22/99	16	11	12	12	20	13	12	8	12	16	16	5	153
Carroll, 7/25/99			11		20	10	8		8	8	8	2	79
CK, 7/22/99	5	6	12	5	20	7	7	3	6	5	8	2	86
Crooked, 7/24/99	11	11	16	11	20	13	10	5	11	12	19	4	143
Fourchette, 7/23/99	8	7	7	11	20	7	6	4	9	10	16	2	107
Hell, 7/15/99	13	13	11	6	20	7	8	5	12	18	20	5	138
Kill Woman, 7/13/99	15	13	12	16	20	10	14	5	6	11	16	20	158
McGuire, 7/16/99	13	9	8	2	20	3	8	2	16	18	18	5	122
Nelson, 7/16/99	17	17	15	17	20	12	8	13	16	18	18	8	179
Rock, 7/21/99	16	16	16	16	20	15	8	16	18	20	20	10	191
Sand, 7/25/99	9	10	13	8	20	13	15	2	13	15	16	5	139
Snow, 7/15/99		6	8		20	6	7		13	16	16	3	98
Sutherland, 7/14/99	13	3	16	13	20	14	6	4	12	20	20	6	147

[1] Habitat parameters: 1 = Instream cover; 2 = Epifaunal substrate; 3 = Pool substrate characterization; 4 = Pool variability; 5 = Channel alteration; 6 = Sediment deposition; 7 = Channel sinuosity; 8 = Channel flow status; 9 = Condition of banks; 10 = Bank vegetative protection; 11 = Grazing or other disruptive pressure; 12 = Riparian vegetation width. See Lazorchack *et al.* (1998) for detailed description of habitat parameters and scoring methodology.

equal or higher species richness than Rock Creek, flow status alone did not account for species richness.

Fish species richness in a drainage basin generally increases with increasing stream order (Kuehne 1962, Schlosser 1982, Fausch *et al.* 1984, Rahel and Hubert 1991). Increased fish species diversity in higher order streams has been attributed to increased habitat diversity (Gorman and Karr 1978) or moderation of environmental conditions and increased volume of habitat (Rahel and Hubert 1991). However, local geomorphic conditions may reduce species richness in downstream reaches in some prairie streams (Barfoot and White 2000). All of the streams we sampled were third or fourth order, but they varied in the amount of water present. For example, Devil's and Nelson creeks were both third order streams, but Devil's Creek was completely dry in the reach we surveyed whereas Nelson Creek had flowing water and supported 12 species of fish. Because we did not take quantitative depth and wetted width measurements, we could not examine statistical relationships between amount of water present and species richness and abundance. However, our observations suggest a generally positive relationship between the amount of water present and species richness.

The rough correlation between Rapid Habitat Assessment scores and species richness suggests that habitat quality was one factor influencing the number of species that the streams supported. Other studies have demonstrated that fish species richness increases with increased habitat diversity and quality in warmwater streams (Gorman and Karr 1978, Schlosser 1982). The relationship we observed for habitat scores and number of individuals was weaker than the relationship between habitat quality scores and species richness. This is not unexpected, because abundance of individuals generally is more variable than presence or absence of species in streams (Karr and Chu 1999).

Species richness also may be seasonally elevated in adventitious streams, i.e., small feeder tributaries of a much larger stream or reservoir, because of increased connectivity to larger bodies of water that harbor a larger species pool (Gorman 1986). Because all of the sites we sampled (except Beauchamp and Rock creeks) were located roughly the same distance upstream of either Fort Peck Reservoir or the Missouri River, all had similar connectivity to larger bodies of water. Because of their proximity, the fish we captured may complete parts of their life cycles in Fort Peck Reservoir or the Missouri River.

However, most of the species we captured apparently are more common in small streams than in the adjacent Missouri River or Fort Peck Reservoir because only common carp, river carpsucker, white sucker, and flathead chub were abundant (>100 individuals captured) in 142 seine hauls at 19 locations in Fort Peck Reservoir in 1981 (Needham and Gilge 1982), and only three (common carp, river carpsucker, and white sucker) of the 19 species of fish captured in this study are reported as being abundant in Fort Peck Reservoir (Alvord 1979). Also, in the reach of the Missouri River adjacent to the tributaries we sampled, of these 19 species, only western silvery minnow and flathead chub were abundant during a three-year survey of fish using multiple gear types (Lee Bergstedt, Montana Cooperative Fishery Research Unit, personal communication). Thus, we suspect that most fish species we captured are capable of completing their life cycles within the small streams we sampled. Moreover, the presence of multiple year classes in most streams sampled suggests that these streams provide year-round habitat for fish. Possible exceptions to year-round small stream residency on the CMRNWR are spottail shiner, which was introduced to Fort Peck Reservoir in 1985 and prefers large, clear rivers (Holton and Johnson 1996), western silvery minnow, which are thought to prefer larger rivers and creeks (Brown 1971, Cross and Collins 1995), and longnose sucker, which generally prefers cooler water (Brown 1971, Scott and Crossman 1973, Baxter and Stone 1995).

Prior to damming of the Missouri River to form Fort Peck Reservoir, fish populations of the adventitious streams flowing into the reservoir may have had a metapopulation (Hanski and Gilpin 1991) structure. Because of limited quantities of water, fish assemblages in these streams are vulnerable to local extinction from drought, water withdrawals or lowered water tables. Though many of the species we captured are not abundant in the Missouri River, the river probably serves as an occasional corridor between the small streams. Currently, the open lentic waters of Fort Peck Reservoir, with its large populations of introduced piscivorous game fish, including northern pike (*Esox lucius*), walleye (*Stizostedion vitreum*), and smallmouth bass (*Micropterus dolomieui*), may be more of a barrier to movements than a corridor between the streams. Although no obvious relationship between species richness and connection with the Missouri River versus connection with Fort Peck Reservoir is currently evident, future recolonization may be more difficult than before construction of Fort Peck Dam.

We captured three fish taxa that are either of special concern or on watch lists. The northern redbelly dace X finescale dace hybrid is a Class C Montana Fish of Special Concern (Hunter 1997). Class C species have "Limited numbers and/or limited habitats in Montana; widespread and numerous in North America as a whole. Elimination from Montana would be only a minor loss to the gene pool of the species". This taxon is likely very rare on the CMRNWR; we captured only a single individual. The plains minnow and the western silvery minnow are currently listed on the Montana Natural Heritage Program Watch List. The Watch List lists species for one or more of the following reasons: "there are indications that the species may be less common than currently thought; the species is currently declining in Montana or across much of their range; or there is so little information available that they cannot be adequately ranked" (Montana Natural Heritage Program 1999). However, plains minnow populations appear reasonably secure on the CMRNWR because they were the most abundant species captured, and they occurred at 9 of 13 sites with fish present in this survey. The western silvery minnow was fairly rare, as we captured this species at only three sites, although it is probably more common in the Missouri River than in the small streams we sampled (Grisak 1996).

The small streams of the CMRNWR supported fairly diverse assemblages of native fish, amphibians, and reptiles that constitute an important component of the Refuge's biodiversity. Fish abundance and diversity may be related to habitat quality and water quantity. Because of limited water quantities in these streams, biological assemblages are probably vulnerable to extirpation from drought, water withdrawals, or lowered water tables. Moreover, reduced connectivity of streams caused by Fort Peck Reservoir may increase the difficulty of recolonization following local extinctions. Our initial survey represented only a "snapshot" of conditions; a temporally and spatially expanded survey would increase our understanding of the status and variability of fish assemblages in the small streams of the CMRNWR.

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Profile of Recreational Paddlefish Snaggers on the Upper Missouri River, Montana

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Abstract

A written questionnaire was administered to 128 recreational snaggers of paddlefish (Polyodon spathula) *during a creel census on the Missouri River above Fort Peck Reservoir in 1993. We asked snaggers to describe their socioeconomic characteristics, attitudes and motivations regarding fishing for paddlefish, and attitudes on specific fishery regulations. More than 9 of 10 anglers snagged mainly or entirely at this site, and fewer than 1 in 10 had snagged for paddlefish in the past 5 years on the lower Yellowstone River, the other major snag fishery in Montana. Snaggers were most likely to be retirees or people in traditionally blue-collar professions that yielded incomes of US $20,000-29,999. Contrary to stereotypes of snaggers as meat fishers, their motivations for snagging were similar to those of other more traditional anglers. Primary motivations included opportunity to be outdoors, experience and thrill of hooking a paddlefish, experience natural surroundings, and be with friends. Although snaggers thought highly of paddlefish meat, the motivation for acquiring meat for eating ranked low. Paddlefish snagging, as practiced in Montana, is more than a meat harvest for most anglers.*

Key Words: Paddlefish, *Polyodon spathula*, anglers, Montana, Missouri River, survey

Introduction

The paddlefish (*Polyodon spathula*), a large, zooplanktivorous fish native to the Mississippi and Missouri river drainages (Gengerke 1986, Russell 1986), provides popular recreational snag fisheries in several states (Combs 1986). Fisheries in Montana are concentrated in two locations: the lower Yellowstone River at Intake near Glendive that harvests the Yellowstone-Sakakawea stock (Scarnecchia *et al.* 1996a); and, the upper Missouri River from the headwaters of Fort Peck Reservoir upriver to the Fred Robinson bridge (Needham 1979) that harvests the upper Fort Peck stock (Scarnecchia *et al.* 1995). Although the lower Yellowstone River fishery has been studied annually since the early 1960s (Robinson 1966, Rehwinkel 1978, Scarnecchia *et al.* 1996b, Stewart 1997), the Missouri River fishery, which is smaller and more dispersed, has received less consistent effort. The stock was studied by Berg (1981), who investigated life history information, including migration and probable spawning sites. Annual harvest of the stock has generally been between 300 and 900 fish (Needham and Gilge 1986, Gilge 1994, Scarnecchia *et al.* 1995). Annual harvest rates (based on recoveries of tagged fish) have been 1.0-4.5 percent since the early 1970s (Gilge 1994). The bag limit is two fish per person per year, and immediate release of caught fish is permitted.

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Until recently, few studies have focused on understanding the values, attitudes, and motivations of snaggers. Fenske (1983) reported that most salmonid anglers in Michigan supported salmon snagging, at least in restricted areas. Samples and Bishop (1981) reported that 56 percent of Wisconsin's sport anglers snagged for trout and salmon, and 60 percent of the anglers thought snagging sufficiently sporting. Dawson *et al.* (1993) reported that behavioral problems of snaggers at fishing sites in New York resulted in the elimination of snagging. Snaggers also often have been characterized as meat fishers; Catchings (1985), for example, reported that snaggers on the Coosa River, Alabama snagged mainly to obtain food and secondarily for sport. Snaggers also have been stereotyped as having lower socioeconomic status, although evidence has not necessarily supported this claim (Stoffle *et al.* 1983).

Values, attitudes and motivations of paddlefish snaggers on the lower Yellowstone River have been recently investigated in Montana (Scarnecchia *et al.* 1996a, Scarnecchia and Stewart 1997a) as part of the Montana-North Dakota paddlefish management plan (Scarnecchia *et al.* 1995). Increased attention has been directed at understanding the paddlefish snag fisheries because the fisheries are popular with Montana anglers and because snagging often has been disparaged by traditional recreational anglers (Samples and Bishop 1981; Catchings 1985). An improved understanding of the values, attitudes, and motivations of paddlefish snaggers may help alleviate any future user conflicts over snagging. Knowledge of how snaggers might react to specific regulation changes would facilitate efforts to manage the snag fisheries in Montana and perhaps elsewhere. Our objective was to characterize the values, attitudes, and motivations of paddlefish snaggers of the upper Missouri River, and to compare the results with those of a similar survey conducted on the lower Yellowstone River (Scarnecchia *et al.* 1996a).

Methods

The study was conducted from April 1 to June 17, 1993, as part of an on-site creel census. The creel census extended over a 32-km reach of river immediately downstream from the Fred Robinson Bridge (Gilge 1994). Anglers were contacted at ramps and fishing locations. Although the season is open all year, most snagging occurs from late March through June. Snagging is conducted by jerking a large (8/0 to 10/0) treble hook and a 113-170-g lead weight through the water. Fishing occurs either from a boat or from shore. Two fish per person per year could be retained; the other fish were to be immediately released unharmed. Montana regulations require landed paddlefish to be tagged at the front of the dorsal fin with an individually-numbered, locking tag.

We surveyed one randomly-selected, actively fishing person per party, unless the fishing party consisted of both males and females, in which case one male and one female were surveyed. Eighty percent of the snaggers asked to complete the questionnaire did so. Because paddlefish snagging is strenuous, snaggers rest frequently; questionnaires were often completed during rest intervals.

The questionnaire consisted of 38 written questions, including two questions with multiple parts (20 parts for one question and 16 for another question). General questions that were not specific to the paddlefish fishery were modeled after surveys administered in 1986 and 1987 by the Texas Parks and Wildlife Department (Texas Parks and Wildlife Department and Texas A&M University 1986, 1987). Other questions specific to the fishery on the upper Missouri River were added. Questionnaires were reviewed by two specialists in the human dimensions of fisheries (one from a state fisheries agency and another from the University of Idaho) for inconsistencies, wording, and question sequence.

One series of questions addressed the motivations of snaggers (Table 1) and a second series of questions addressed the values, attitudes and preferences on snagging paddlefish and on bag limits (Table 2). Another question asked the respondents to rank the desirability of paddlefish in relation to four other popular game species. Likert scales (five ordered options) were used for responses (Bobko 1995). Although distributions of responses for the Yellowstone River fishery had been analyzed according to age, state of residency, gender, income and education (Scarnecchia *et al.* 1996a), smaller sample sizes in this study prevented such an analysis. A Kruskal-Wallis test (Conover 1980) was used to compare rankings of responses to the questions on motivations and attitudes (Tables 1 and 2), and species desirability preferences. A Chi-square test was used to compare responses to the same, corresponding questions between this sample of snaggers from the Missouri River and a sample of snaggers from the lower Yellowstone River as reported by Scarnecchia *et al.* (1996a). We also used a Chi-square test to investigate the relation between trip catch and satisfaction.

RESULTS

The 128 questionnaires completed (representing an estimated 40 percent of all snagging parties during the creel season) were obtained from 91 percent males and 9 percent females. Ninety-

Table 1. *Motivations of 128 paddlefish snaggers. Responses were rated on a scale of 1 to 5 (1 = not important, 3 = neutral, 5 = very important). Nonresponse to specific questions ranged from 1 percent to 3 percent. Rank refers to level of statistical importance in relation to other motivations (Kruskal-Wallis test, P < 0.05). The lower the numbered rank (i.e., 1) the more important the motivation. Motivations that share a rank or combination of ranks (e.g. 3-4 and 3-4-5) are not statistically different from each other* (P > 0.05).

Motivation		Response distribution (%) for scale values 2	3	4	5	*N*	Mean scale rating	Rank
(a) To be outdoors	0	2	5	12	81	126	4.73	
(b) For family recreation	9	5	8	18	60	124	4.16	3-4-5
(c) To experience new and different things	3	1	11	28	57	125	4.34	3-4
(d) For relaxation	5	2	10	26	57	126	4.29	3-4
(e) To be close to the river	4	6	13	27	50	126	4.14	4-5
(f) To obtain meat for eating	19	15	27	14	25	126	3.11	7
(g) To get away from the demands of other people	9	3	9	24	55	126	4.14	3-4-5
(h) For the experience and thrill of hooking one	1	1	8	12	78	126	4.66	1
(i) To be with friends	3	2	10	21	64	127	4.40	2-3
(j) To eat the eggs	87	5	6	1	1	124	1.25	8
(k) To experience natural surroundings	2	2	10	22	64	127	4.41	2-3
(l) To get away from regular routine	1	0	5	24	69	126	4.61	1-2
(m) To catch a really large fish	7	6	24	20	43	127	3.84	6
(n) For the challenge or sport	3	2	18	19	58	127	4.27	3-4
(o) To catch an unusual fish	7	6	15	25	47	127	4.00	5-6
(p) To meet new people at the fishing site	19	8	27	23	23	127	3.22	7

Table 2. *Attitudes of paddlefish snaggers toward the fish and toward the harvest regulations expressed in percentage of responses to 20 questions (a-t). Responses were recorded on a Likert scale (strongly disagree, SD; disagree, D; neutral, N; agree, A; strongly agree, SA). Percentages do not include nonresponse (0-2%) to specific questions or questions deemed not applicable by respondent (0-4%).*

	Percent respondents that:					
Attitudes	SD	D	N	A	SA	*N*
(a) I enjoy eating paddlefish.		1	16	22	60	124
(b) The bigger the paddlefish I catch, the better the trip.	19	6	33	22	20	128
(c) A successful trip is one in which my limit of two paddlefish is caught.	22	22	17	18	21	125
(d) Paddlefish is as good to eat as trout	7	12	20	14	47	122
(e) I am just as happy if I catch one paddlefish as two fish, as long as I do not get skunked	11	10	22	22	35	125
(f) I would rather catch one big paddlefish than two small paddlefish	19	18	37	11	15	126
(g) I would be just as happy if I didn't keep the two fish I'm entitled to catch, as long as I could be photographed next to them.	30	15	21	14	20	125
(h) Without the opportunity to paddlefish, I wouldn't spend any time in the Slippery Ann/Robinson Bridge Area.	19	11	8	13	49	126
(i) I feel unsuccessful if I catch only one paddlefish.	49	19	19	5	8	124
(j) With less than a two-fish limit, I wouldn't find it worthwhile to come to the SA/RB area for paddlefishing.	32	14	14	10	30	126
(k) I enjoy paddlefish fishing more than other types of fishing.	12	17	44	13	14	126
(l) I would find a one fish annual limit just about as satisfactory as a two fish annual limit.	42	17	17	10	14	128
(m) The paddlefish is an ugly fish compared to a trout.	31	17	25	11	16	123
(n) There is really not that much special about a paddlefish other than that they are large.	55	23	10	6	6	126
(o) The paddlefish is a really special fish and I feel privileged to be able to fish for them.	3		10	19	67	128
(p) I would find a three-fish annual limit just about as satisfactory as the current two-fish limit	25	17	22	13	23	127
(q) Snagging is an acceptably sporting way to catch paddlefish.	2	2	3	17	76	127
(r) I prefer snagging paddlefish at night to snagging during daylight hours.	20	23	49	6	2	120
(s) Paddlefish is as good to eat as walleye.	12	19	22	22	25	116
(t) I enjoy the people and the social atmosphere. It makes paddlefishing more fun.	3	6	13	21	57	127

five of the respondents were Montana residents, 23 were non-residents, and 10 were not identified. Snaggers tended to be men ≥ 35 years of age. The most common age groups (males and females combined) were 30-39 (31%), 40-49 (23%), 50-59 (16%) and 20-29 (11%). Respondents were a mixture of experienced and inexperienced snaggers, but most were experienced; 49 percent had snagged for paddlefish at least 4 of the preceding 5 years (including the current year) whereas only 19 percent had snagged only one year in the past five. More than 9 of 10 respondents characterized their snagging activities as centering mainly or exclusively in the area above Fort Peck Reservoir. Ninety-one percent of them had not snagged on the lower Yellowstone River in the preceding 5 years, and essentially none had fished at the Dredge Cuts below Fort Peck Dam (two snaggers) or in North Dakota (one snagger). The snaggers found out about the fishery mainly from friends and relatives (88%), and occasionally from the newspaper (5%). Most lodged in recreational vehicles and campers (77%) and tents (18%) during their snagging trip. Most snaggers also rated vehicle access good (65%) or fair (32%) and boat access adequate (70%). Snaggers most liked the opportunity to fish for paddlefish and other species (35 responses), the scenic beauty of the area (30 responses), and the privacy of the site because of the lack of people (19 responses). When asked what they would like to see changed about the fishery in the area, 39 respondents said "nothing," 19 respondents said better ramp access to fishing spots, and 10 respondents said more and better maintained campsites.

Socioeconomic Characteristics

Retirees constituted the largest single employment category among snaggers (26 responses). Employed snaggers indicated such professions as self-employed (7), carpenter (6), student (5), coal miner (4), truck driver (4), construction worker (3), plumber (3), and maintenance supervisor (3). Most snaggers tended to have moderate household incomes and educational backgrounds. The most common household incomes before taxes were $20,000-29,999 (27%), $10,000-19,999 (21%) and $30,000-39,999 (17%). Less than 4 per cent of respondents reported incomes of $60,000 or more. Thirteen percent had not graduated from high school, 51 percent had graduated from high school, 20 percent had attended college but not graduated, 8 percent had degrees from 4-year institutions, and 7 percent had advanced degrees.

Motivations for Paddlefish Snagging

Highest ranking motivations (Kruskal-Wallis test, $P<0.05$) for snaggers were to be outdoors, experience the thrill of hooking a paddlefish, get away from the regular routine, experience natural surroundings, and be with friends. Lower ranking motivations were to enjoy the challenge or sport, relaxation, experience new and different things, provide family recreation, and get away from the demands of other people. In contrast, few were motivated by the prospect of meeting new people at the fishing site, obtaining meat for eating, or eating the eggs as caviar ($P<0.05$, Table 1).

Perceptions on Paddlefish and Paddlefish Snagging

When asked to rank the desirability of species in general (i.e., the fish itself, including food value, sport value, and other intangible values; 1 = most desirable, 5 = least desirable) against four other species in multiple comparisons—walleye (*Stizostedion vitreum*), northern pike (*Esox lucius*), cutthroat trout (*Oncorhynchus clarki*), and largemouth bass (*Micropterus salmoides*)—paddlefish (mean, 4.31) and walleye (mean, 4.04) ranked highest, followed by pike (mean, 3.46), trout (mean, 3.44) and bass (mean, 2.94).

Although eating paddlefish did not rank high among all motivations for paddlefishing, 82 percent of the snaggers enjoyed eating paddlefish. Sixty-one percent considered paddlefish as good to eat as trout, whereas only 19 percent thought it inferior. Forty-seven percent thought it equal in palatability to walleye, whereas 31 percent considered it inferior.

Perceptions on Snagging

Ninety-three percent of respondents thought snagging an acceptably sporting way to catch paddlefish; only 4 percent did not think it sporting. Snaggers found snagging for paddlefish about as enjoyable as other types of fishing (29% less enjoyable, 27% more enjoyable, 44% neutral).

Trip Satisfaction and Catch

Among returning snaggers, 80 percent were satisfied with their most recent paddlefish snagging trip. Snaggers that caught one or more fish on their previous trip expressed significantly greater satisfaction with the fishing experience than did those catching no fish (Chi-square test, $P=0.02$).

Attitudes Toward Regulations

Snaggers indicated that they would not find the prospect of a one-fish limit as satisfactory as a two fish limit. Fifty-nine percent of snaggers thought a one-fish limit would be less satisfactory and only 24 percent thought it would be as satisfactory or more satisfactory. If neutral responses are interpreted as satisfactory, the percentage of snaggers that would be satisfied with a one-fish limit increased to 41 percent (Table 2).

The preference for a two-fish bag limit did not, however, indicate that snaggers necessarily felt unsuccessful if they caught only one fish. Thirteen percent felt unsuccessful if they only caught one fish but 68 percent did not feel this way (Table 2). This response was consistent with their response to the statement: "I am just as happy if I catch one paddlefish as two fish, so long as I do not get skunked" (i.e., catch no fish, Table 2). Fifty-seven percent of respondents agreed with this statement and only 21 percent disagreed. They were nearly evenly split on whether a bag limit of less than two fish would necessarily discourage them from fishing for paddlefish at the site (Table 2).

Catch-and-release fishing without any retention was not a favored alternative, although many anglers also supported the idea of releasing all fish. When asked if they would forego harvest in favor of being photographed next to their two fish before releasing them, 34 percent answered affirmatively (Table 2). Snaggers generally preferred the option of catching two small paddlefish to one large paddlefish, but 37 percent of snaggers were neutral on this question (Table 2). Forty-seven percent of snaggers were satisfied with the two-fish bag limit, and only 15 percent were dissatisfied with it.

Compared Responses Between Missouri River and Yellowstone River Fisheries

We found no major differences in responses between snaggers in the Missouri River and Yellowstone River fisheries (Scarnecchia *et al.* 1996a); all significant differences were primarily a matter of degree of preference rather than a completely different preference. For example, 8 of 16 questions on the motivations for paddlefish snagging, which were compared between the two fisheries (Table 1), were more strongly supported as "very important" by Missouri River anglers. These included the motivations: to get outdoors ($P=0.003$), for family recreation ($P=0.001$), to experience new and different things ($P = 0.012$), for relaxation ($P = 0.023$), to be close to the river ($P = 0.005$), to get away from the demands of other people ($P = 0.011$), to experience natural surroundings ($P = 0.001$), and to get away from the regular routine ($P = 0.012$). Overall, responses were more strongly positive by snaggers in the Missouri River fishery.

Other minor degrees of difference were found between snaggers in the two fisheries. Yellowstone River snaggers were significantly more concerned than Missouri river snaggers with catching a large fish ($P = 0.001$), as well as catching a large fish rather than two small fish ($P = 0.003$). Missouri River snaggers were less concerned than Yellowstone River snaggers with catching their two-fish annual bag limit ($P = 0.004$), and more satisfied than Yellowstone River snaggers with the two-fish bag limit ($P=0.001$). Yellowstone River snaggers viewed night snagging more favorably than did Missouri River snaggers although most in both groups were neutral toward it ($P = 0.001$). Although both groups of snaggers overwhelmingly thought snagging a sporting means of catching paddlefish, and enjoyed the social atmosphere of the fishing sites, significant differences in percentage of responses existed between the two sites ($P<0.05$). Snaggers on the Missouri River were significantly ($P<0.05$) more positive about the social atmosphere associated with the fishery.

Discussion

Results of this study corroborate findings of Scarnecchia *et al.* (1996a) that the attitudes and motivations of Montana's paddlefish snaggers are not distinctly different from Montana anglers in general. For example, our study indicated that primary motivations for paddlefish snagging included to be outdoors, the thrill of hooking a paddlefish, and to get away from routine activities. Similar motivations were reported for Yellowstone River paddlefish snaggers by Scarnecchia *et al.* (1996a), for Montana's warmwater anglers (McFarland and Brooks 1993) and for Montana anglers in general (Brooks 1991).

Paddlefish snaggers also displayed many similar socio-economic characteristics as Montana anglers in general. Snaggers that were employed tended to occupy traditionally blue-collar occupations and have educational backgrounds and incomes similar to those reported by McFarland and Brooks (1993).

Missouri River paddlefish snaggers valued the outdoor experience associated with paddlefish snagging that included enjoyment of hooking and landing one, more than the actual consumption of the meat (Table 1). Similar results were reported for Yellowstone River snaggers by Scarnecchia *et al.* (1996a) and for other Montana anglers by McFarland and Brooks (1993). However, our results contrast with studies of snaggers for other species elsewhere (e.g., Catchings 1985) in which snaggers have been condemned as primarily meat fishers. Scarnecchia *et al.* (1996a) concluded that Yellowstone River paddlefish snaggers exhibited what Fedler and Ditton (1986) classified as a low- to mid-consumptive orientation. Our results are consistent with those of numerous studies in which anglers rate nonconsumptive aspects of the experience higher than the number and size of fish caught (e.g., Moeller and Engelken 1972, Duttweiler 1976) although not inconsistent with studies reporting that catching fish was the most enjoyable aspect of the trip (e.g., Cooper 1973).

The low-to-moderate orientation for meat consumption did not imply, however, that the opportunity to acquire meat, nor the meat itself, was irrelevant to the snagging experience. Missouri River paddlefish snaggers rated paddlefish meat highly, at least as highly as walleye and more highly than trout (Table 2). Snaggers also were generally not enthusiastic about a mandatory catch-and-release regulation (Table 2). Similar results were found for snaggers on the Yellowstone River (Scarnecchia *et al.* 1996a), as well as for those pursuing marine species (Matlock *et al.* 1988). We conclude that although acquisition of meat is secondary to other motivations for paddlefish snagging, it is an important component of the total experience. It is the actual *consumption* of the meat that was of lesser importance, even though the snaggers thought highly of the meat (Table 2) and valued the opportunity to harvest fish.

Results of this study also lend support to the merit of existing regulations limiting the harvest to two fish per person per year with optional immediate release of snagged fish. Anglers indicated that a one-fish limit was worse than a two-fish limit (Table 2, Question l), but that a three-fish limit would, overall, not be better than a two fish limit (Table 2, Question p). They did not, however, favor catch-and-release with no harvest opportunity (Table 2, Question g). The existing two-fish annual bag limit plus the opportunity to release fish permits meat harvest without emphasizing it, and accommodates a range of angler preferences and expectations known to exist in recreational fisheries (Fisher 1997). Other factors, however, make the optional release of snagged fish possible in this fishery that prevent its use in the Yellowstone River fishery. In that fishery, a high harvest rate (Scarnecchia *et al.* 1996b) and crowding at the fishing site make mandatory retention the preferable alternative (Scarnecchia and Stewart 1997b). Evidence also exists that less sorting and high-grading (release of a smaller fish in favor of larger ones) occurs in the Yellowstone River fishery, where it is illegal, than in the Fort Peck fishery, where it is not if the fish is released immediately (D. Scarnecchia, Unpublished).

Although the predominance of snaggers in the age group 30-39 was consistent with results from the Yellowstone River (Scarnecchia *et al.* 1996a), snaggers tended to be older on the Missouri River than on the Yellowstone River. On the Missouri River, age groups 40-49 and 50-59 were the next most important, whereas on the Yellowstone River, the age group 20-29 was the second most important. The reasons for this difference were unclear but might involve a greater distance of the Missouri River fishery from population centers and the prevalence of snagging from boats on the Missouri River. Snagging from boats may be less strenuous than the distant casting associated with the Yellowstone River fishery. Older snaggers may also be better able to afford boats.

Although both the Missouri and Yellowstone River fisheries were valued by snaggers for their natural outdoor surroundings, there was some evidence that Missouri River anglers placed more emphasis on the privacy and uncrowded nature of their fishery. Forty-nine of 128 respondents from the Missouri River indicated that what they liked best about the fishery (other than the paddlefish) was either the scenic beauty of the area or the privacy and freedom from crowding that the area afforded. In contrast, 68 of 353 respondents for the Yellowstone River fishery rated the people and social aspects of the fishery as what they liked best (other than the paddlefish). Ironically, the Missouri River snaggers actually rated the social atmosphere at the fishing site significantly ($P < 0.05$) higher than did Yellowstone River anglers. Although these questions are difficult to interpret, the more private nature of the Missouri River fishery may actually create a more desirable social atmosphere for these snaggers and the few friends snagging with them, as opposed to the more public and open atmosphere at the Intake site. Evidently, the two fisheries offer somewhat different intangible rewards for anglers, which might in part explain why regular snaggers in one fishery only rarely snag in the other fishery.

Results of this study and Scarnecchia *et al.* (1996a) support the idea that paddlefish snag fisheries in Montana are not purely meat fisheries but provide other intangible benefits to snaggers similar to those provided by other Montana fisheries (Brooks 1991, McFarland and Brooks 1993) and elsewhere (Hudgins 1984, Falk *et al.* 1989). Because conservation of such a late-maturing, long-lived species can sometimes require restrictive regulations (Combs *et al.* 1986), knowledge that paddlefish snagging is a total outdoor experience and not merely a meat harvest provides managers with more flexibility in managing these fisheries in the future.

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Effect of Suburban Development on Density of Coyotes in Northwest Wyoming

Abstract

*We compared relative densities of coyotes (*Canis latrans*) in a suburban/agricultural area to an adjacent undeveloped area in northwest Wyoming by skiing transects in areas after snowfall and recording number of tracks that crossed each transect. Relative density was the numbers of tracks on each transect divided by the number of hours since the last snowfall. This modification accounted for the increase in track numbers with increased time since last snowfall. The regression equation of study area, surface snow penetration, and night temperature versus relative track density accounted for 74 percent of variation in relative track density. At equal snow penetration and night temperature, the relative density of coyotes was greater in the suburban/agricultural area than the undeveloped area. The presence of remaining open spaces in the suburban/agricultural area combined with high productivity due to both natural and anthropogenic food sources may account for this high relative track density. Coyote densities may increase with development until open space is no longer available to establish and maintain territories.*

Key words: *Canis latrans*, coyote, density, development, suburban, track surveys

Introduction

The densities of coyotes (*Canis latrans*) in a variety of land-use types have been determined throughout North America (Camenzind 1978, Pyrah 1984, Roy and Dorrance 1985, Windberg 1995, McClure *et al.* 1996, Windberg *et al.* 1997). Average densities ranged from 0.23 coyotes/km^2 in northwestern Wyoming (Camenzind 1978) to 3.7 coyotes/km^2 at the interface of a suburban area and a national monument in the southwestern United States (McClure *et al.* 1996).

Ultimately, local prey abundance regulates coyote density (Knowlton and Gese 1995). High levels of prey abundance in undeveloped areas may lead to higher densities of coyotes (Winberg 1995). However, Windberg (1995) and Winberg *et al.* (1997) observed that higher coyote densities and limited prey availability might eventually limit the population size. Thus, within the carrying capacity of an area, coyote density will increase with an increase in prey abundance until behavioral constraints such as territoriality restrict further growth. Other studies have concluded that prey abundance and lack of exploitation (McClure *et al.* 1996), winter ungulate availability (Weaver 1977), exploitation in late winter (Roy and Dorrance 1985), and mortality (Mills and Knowlton 1991) control population density in a given area. Exploitation is defined as intentional human-caused mortality.

Despite extirpation efforts over the last 150 years, coyotes have significantly expanded their range and numbers

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(Gier 1975, Bekoff 1977, Nowak 1979, Crete and Lemieux 1996). This is due to the extirpation of dominant predators such as the wolf (*Canis lupus*) and resulting lack of competition (Nowak 1978), clearing of land for agricultural uses, and adaptability of coyotes to different habitats and food sources (Bounds 1994). Coyotes have adapted well to new environments created by increasing human populations and resulting urban, suburban, and agricultural development (MacCracken 1982, Shargo 1988, Soule *et al.* 1988, Atkinson and Shackleton 1991, Quinn 1992).

Developed areas tend to support higher population densities of coyotes (Shargo 1988, McClure *et al.* 1996). Human-occupied areas provide increased resource availability in the form of human food wastes and domestic animals (Shargo 1988, McClure *et al.* 1996). The reported reduction in home range size with maintenance of social group size may account for these increases. However, coyotes can be exposed to higher levels of exploitation in areas of development, thus potentially decreasing population densities (Knowlton and Gese 1995).

Our study area in northwestern Wyoming provided an opportunity to measure potential differences in coyote density between an undeveloped area and a suburban/agricultural area. We hypothesized that coyote density would be greater in developed areas due to greater food abundance, maintained group size, and reduced home range size (compressed territories).

Study Area

We conducted our research on two adjacent areas in Jackson Hole, Wyoming (43° 40'N, 110° 43'W, Fig. 1). The suburban/agricultural study area (SAA) consisted of primarily private land devoted to agricultural, commercial, and residential uses (0.03 – 0.99 structures/ha). Progressive building development and subsequent reduction of open space have characterized the SAA for the last two decades. Occasional coyote depredation was reported in the SAA. The undeveloped study area (UNDA) was at the southern end of Grand Teton National Park (0 – 0.08 structures/ha). Grazing by domestic livestock and big game hunting were permitted during limited times in this otherwise protected area.

Much of the valley surface is covered with glacial outwash interrupted by four buttes. Elevation ranges from 2000–2333 m. Open portions of both study areas are dominated by big sagebrush (*Artemesia tridentata*). Both study areas contain stands of lodgepole pine (*Pinus contorta*), Douglas-fir (*Pseudotsuga menziesii*), Englemann spruce (*Picea engelmannii*), subalpine fir (*Abies lasiocarpa*), and quaking aspen (*Populus tremuloides*). Narrowleaf cottonwood (*Populus angustofolium*) and Colorado blue spruce (*Picea pungens*) dominate riparian areas throughout the valley. The SAA vegetation is interspersed within an agricultural/suburban matrix. Mean annual temperatures (1961-1990) ranged from -9°C to 16°C in the SAA and 11°C to 15°C in the UNDA. Precipitation was mostly in the form of snow from October to April, with a mean annual precipitation (1961 –1990) of 42 cm in the SAA and 53 cm in the UNDA (High Plains Climate Center, Lincoln, NE).

Methods

We used USGS (United States Geological Survey) 1:24,000 topographic maps to randomly locate 10 transects in the SAA and 12 transects in the UNDA. Randomization was done by selecting random UTM coordinates within the study area boundaries to determine the starting point of each transect. However, true randomization was violated because transects could only be located

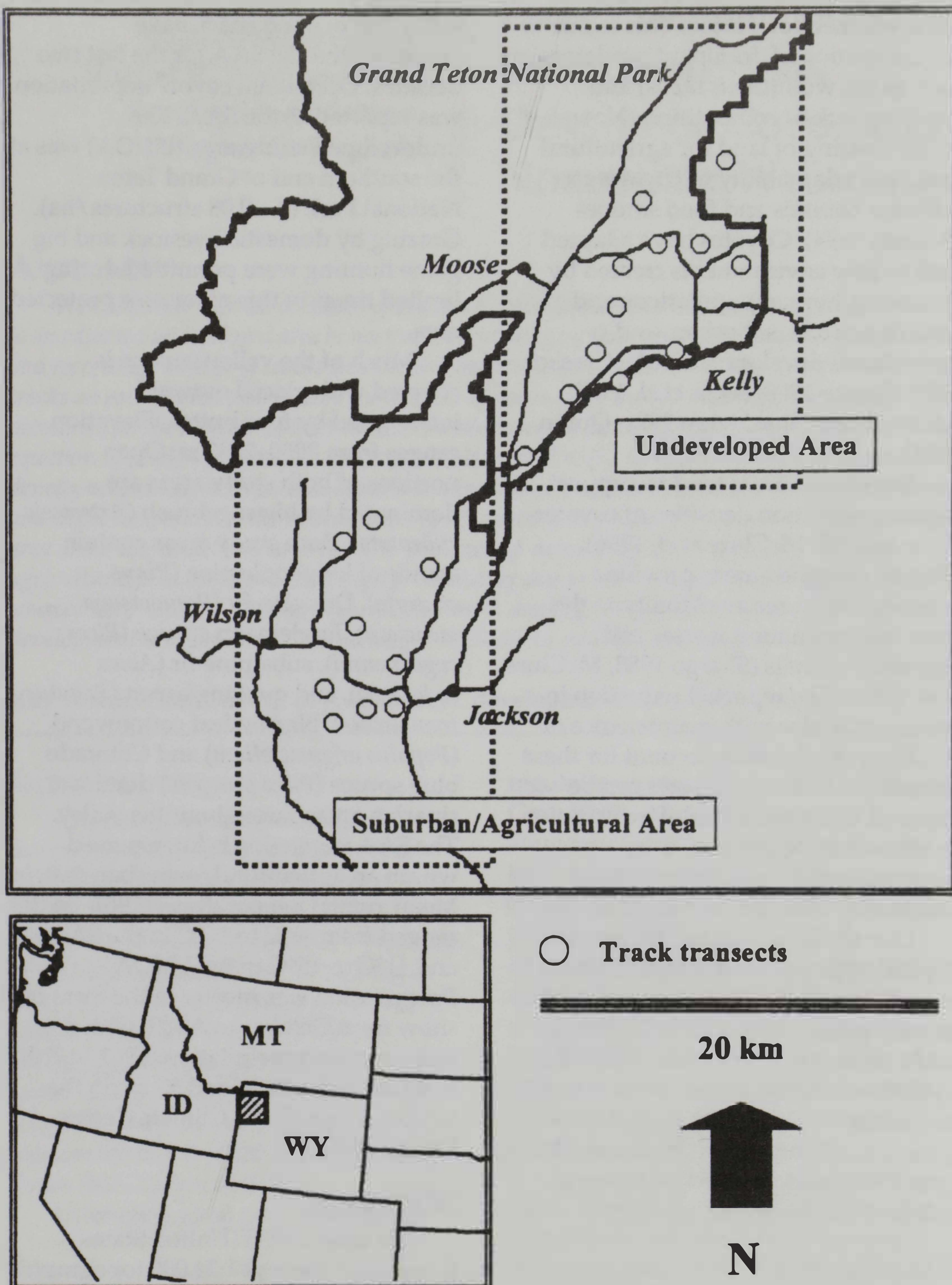

Figure 1. *Location of study areas in Jackson Hole, Wyoming.*

where permission was given for access to private lands in the SAA. Each transect extended 2 km and was aligned on a north-south axis. We skied as many transects as possible after each new snowfall until new tracks were no longer discernable due to snow deterioration. Where obstructions such as trees, rocks, or ponds occurred along the transect, we moved east or west until we could continue north unimpeded. We recorded the number of times that coyote tracks completely crossed a transect, but to avoid double counting tracks we counted only those tracks separated by enough distance on the transect to be discerned from other track crossings. We also recorded time since last snowfall (hours), cloud cover (clear or cloudy), minimum temperature the night before we completed the transect (°C), and percent of each habitat type present for each transect. We classified habitat as riparian, conifer, aspen, or open. Open habitat consisted of sagebrush or grassland covered completely with snow. For each habitat type, we recorded three snow penetration readings as a measure of surface snow density. To measure depth of penetration, a 591-ml plastic bottle (6.5-cm diameter) filled with 300 ml of water was dropped from a 33-cm height. This measurement approximated the snow penetration of a coyote foot in various habitat types (Robison 1999). We then calculated mean penetration for each transect. We determined transect completion order randomly before the start of the study. This ensured that each transect was completed once in each area before a transect was repeated. To avoid independence violations, we used the means of the data from transects that were completed multiple times.

To incorporate the direct relationship between number of tracks and hours since snowfall, we calculated a relative track density value. Relative track density equaled the number of tracks divided by the hours since last snowfall. We compared relative track density between the two areas after accounting for effects of other variables using multiple regression techniques (Minitab Statistical Package, Release 12.21). Mean penetration, night temperature, night cloud cover, area (SAA or UNDA), and percent of habitat categories in each transect were the variables regressed against relative coyote density (coyote tracks/hour).

Results

We completed a total of 27 transects from January to April 1999; 12 transects in the SAA and 15 transects in the UNDA. Because we calculated mean values for repeated transects, total sample size was 22 transects. For the full model (Table 1), only snow penetration (t = - 4.84, P = 0.000) and night temperature (t = 2.59, P = 0.022) were significant predictors of tracks/hr. Although area was not a significant predictor (t = 0.127, P = 0.128), we included it in the reduced regression model because our hypothesis

Table 1. *Comparison of coyote track densities between a suburban/agricultural area and an adjacent undeveloped area in Jackson Hole, Wyoming, 1999. Full regression model with all predictor variables. Response is relative density (tracks/hour).*

	Regression Model				Analysis of Variance		
Predictor	Coef.	*SD*	*t*	*P*	r^2	F	*P*
Intercept	-0.0500	7.3350	-0.01	0.995	0.79	17.21	0.000 [a]
Area	0.1568	0.0960	1.63	0.127			
Penetration	-0.0523	0.0108	-4.84	0.000 [a]			
Night Temp	0.0235	0.0091	2.59	0.022 [a]			
Cloud Cover	-0.2099	0.1629	-1.29	0.220			
% Open	0.0158	0.0725	0.22	0.831			
% Conifer	0.0146	0.0724	0.20	0.844			
% Aspen	0.0132	0.0729	0.18	0.860			
% Riparian	0.0162	0.0726	0.22	0.827			

[a] $P \leq 0.05$

concerned comparison between areas.

The reduced model predicted tracks/hr with area, penetration, and night temperature (Table 2). All regression residuals were normal (Ryan-Joiner correlation test, $P \geq 0.05$, $r = 0.9606$, $n = 22$, H_0: normality). No heterogeneity of variance was observed in the reduced regression model (modified Levene's test, $P = 0.505$, H_0: homogeneity). A plot of residuals against order confirmed the independence of predictor variables. The reduced regression predicted 74 percent of the variation in tracks/hr using area, penetration, and night temperature. All predictor coefficients were significantly different from zero ($P \leq 0.05$) and the F lack-of-fit test confirmed a linear relationship ($F = 17.21$, $P = 0.000$). No interaction terms were significant in either the full or reduced regression model. For fixed values of night temperature and penetration, the model predicted that the SAA would have 0.179 more tracks/hr than the UNDA.

Discussion

After accounting for snow penetration and temperature, the data supported our prediction of increased coyote densities in developed areas. Although snow penetration and night temperature would not affect the actual density of coyotes, these factors affected the number of tracks recorded crossing a transect. The negative coefficient from the penetration variable indicated that as snow penetration increased, detected coyote tracks decreased (if area and temperature were held constant). Similarly, the positive coefficient for the temperature variable suggested that increased temperatures resulted in increased number of coyote tracks (if the other predictors were constant). Thus, relative track comparisons were feasible only after accounting for the decreased travel of coyotes in deep snow or cold temperatures.

In the few studies that investigated coyote density in suburban areas, densities of coyotes appeared to be higher when compared to rural or undeveloped areas. Higher food availability from both natural and anthropogenic sources was cited as the primary cause of this increased density (Shargo 1988, Quinn 1991, McClure *et al.* 1996). Coyote populations in these studies resided in suburban or urban areas, but all had access to adjacent undeveloped areas. In Washington, density of coyotes appeared to be greater in the northern suburbs compared to the central urban area. The northern suburbs, with some high-density housing developments, were adjacent to undeveloped land. However, the data were based on coyote observations by survey participants,

Table 2. *Comparison of coyote track densities between a suburban/agricultural area and an adjacent undeveloped area in Jackson Hole, Wyoming, 1999. Reduced regression model with area, snow penetration (cm), and night temperature (°C). Response is relative density (tracks/hour).*

	Regression Model				Analysis of Variance		
Predictor	Coef.	SD		P	r^2	F	P
Intercept	1.0640	0.1634	6.51	0.000[a]	0.74	17.21	0.000[a]
Area	0.1787	0.0747	2.39	0.028[a]			
Penetration	-0.0437	0.0081	-5.41	0.000[a]			
Night Temp	0.0249	0.0082	3.02	0.007[a]			

[a] $P \leq 0.05$

which may be biased (Quinn 1991). An analysis of coyote scat collected in the SAA revealed a higher percent occurrence of voles (*Microtus* spp.) than the coyote scat collected in the UNDA. Although human-related foods were rarely found in the scat from coyotes in the SAA, scat was collected only in the agricultural areas adjacent to suburban developments (Wigglesworth 2000). Previous studies found higher prey abundance in suburban areas (Shargo 1988, McClure *et al.* 1995). Data collected by Wigglesworth (2000) suggested that there are more voles in the SAA than the UNDA. Plentiful food sources near suburban areas combined with habitat constricted by development may cause increased densities of coyotes in these areas.

Densities of coyotes can be measured with the knowledge of the percent of resident coyotes (belonging to social groups), mean group size, and mean territory size (Knowlton and Gese 1995). Wigglesworth (2000) reported no statistical differences in coyote group size between the SAA and UNDA despite smaller reported home ranges in the SAA (McClennen 2000). Given a consistent percentage of resident coyotes in both study areas, the prey base must be sufficient to support higher densities of coyotes in the suburban/agricultural areas.

Our data supported the hypothesis that densities of coyotes were greatest in areas of development with adjacent refuge areas. These suburban/agricultural areas may provide open areas for pup-rearing as well as additional food sources such as domestic pets, pet food, garbage, and livestock (Shargo 1988, McClure *et al.* 1996, Wigglesworth 2000). Although the food sources may be plentiful in developed agricultural areas, less open area can eventually reduce the habitat where coyotes can defend territory and raise pups.

Due to the lack of permission for access to some private land in the SAA, transects there were not representative of the entire area. We did not include areas of dense subdivisions and small residential plots (approximately 30%) in the surveys. Decreased coyote densities may have been observed in these areas. Thus, increased relative densities of coyotes in the SAA may be only reflective of the remaining habitable land in this matrix of development and not the overall SAA. Densities might not be different between the entire areas.

As agricultural land is converted to development in a suburban/agricultural landscape, we predict that coyote densities will ultimately decrease despite high levels of anthropogenic food sources cited in other studies (Shargo 1988, McClure *et al.* 1996). We believe decreased densities will result from increased potential for mortality from trapping, shooting, or vehicle collisions coupled with lower reproductive success as coyote social structure collapses. Although mortality of some coyotes has been shown to increase pup production in a territorial group (Hodges 1990, Windberg 1995), eventual lack of space for pup-rearing and reduced chances for mated pairs to come together may decrease reproductive success.

Coyotes are highly adaptable animals that survive well in developed areas. Their behavioral plasticity allows them to thrive in areas of suburban development given sufficient refuge to breed and protect young. Although coyotes will exist in urban areas, their densities will be controlled by behavioral and demographic factors that will limit any increases in density caused by increased food sources.

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Landscape Influences on Elk Vulnerability to Hunting

Abstract

*We evaluated landscape elements that we believed influenced elk (*Cervus elaphus*) vulnerability to hunting in western Montana from 1993 to1995. We used six Geographic Information System (GIS) coverages to describe 84 elk-kill locations, 267 live-elk locations, and 166 random locations at three scales (point, 200-m radius, and 700-m radius). We used discriminant function analysis (DFA) to differentiate among these locations using four road variables, three topographic variables, 24 vegetation classes, four vegetation-change classes, hydrography, and a fragmentation index. Road proximity or density discriminated among elk-kill, live-elk, and random locations at each scale. In addition, a vegetation-change variable and two vegetation classes (lodgepole pine [*Pinus contorta*] and open Douglas fir [*Pseudotsuga menziesii*] classes) improved differentiation of the locations ($\bar{x}$ = 50% correct classification). Elk selected locations away from open roads in areas with low road density and large patches of forest with substantial hiding cover. In contrast, elk were killed in areas with higher road density and less hiding cover.*

Key words: *Cervus elaphus*, elk, GIS, habitat, hunting, landscape, mortality, security, vulnerability.

Introduction

A current concern of wildlife managers involves several aspects of elk vulnerability to hunting and specifically a resulting decreased bull:cow ratio. Reduced bull:cow ratios may lead to an increased reliance on immature bulls for breeding and a prolonged calving season. This, in turn may result in increased predation losses and/or decreased survival of elk calves over winter. Although causes of low bull:cow ratios have been studied and discussed by numerous researchers, no individual factor has been consistently isolated. However, three factors have been routinely identified: 1) insufficient hiding cover, 2) increased or unimpeded access of hunters via roads, and 3) hunting seasons that are too long or regulations that are too liberal.

Management of elk hunting in Montana has focused on maintaining a five-week season for bulls without controlling the number of licensed resident hunters. As a result, the number of mature bulls has declined in some populations. In parts of Oregon some elk herds have a distorted population structure (Leckenby *et al.* 1991) that substantially deviates from public expectations and may be biologically unsound (Squibb *et al.* 1991, Prothero *et al.* 1979, Noyes *et al.* 1996).

Our objective was to examine sites where elk were killed by hunters and assess vulnerability and security (Lyon

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and Christensen 1992) of elk in relation to various landscape elements such as vegetation, topography, and proximity to roads and trailheads. Although other factors likely were involved, we presumed that animals killed were associated with inadequate security. Our null hypothesis was that habitat factors at elk-kill sites, live-elk locations, and random points were not statistically different.

Study Area

The 259-km^2 Chamberlain Creek study area lies approximately 56 km east of Missoula, Montana, in the Garnet Mountains. Sport hunting is the primary recreational use of the study area. As part of the Blackfoot Block Management Area, interior roads were closed to motorized traffic from 1 September through 1 December. Bicycles and horses were allowed, but commercial outfitting was prohibited. Hunters wishing to use the walk-in area entered at any of twelve parking and access sites, i.e., trailheads. Two elk herds were identified in the study area, each containing approximately 200-250 elk. During this study (1993-95) a 7-week archery season was followed by one week of no big game hunting, and a five-week general firearm season that ended on the last Sunday in November. During the general firearm season, all hunters possessing a valid license could harvest any antlered bull. The number of antlerless elk permits (n = 250, 250, and 200 in 1993, 1994, and 1995, respectively) issued by Montana Fish, Wildlife and Parks (MFWP) was relatively stable during this study.

Methods

We compared locations of elk kills with random locations and locations used by live radio-collared elk during the same time period using spatial variables at three landscape scales: point, near (200 m radius/17.6 ha), and far (700 m radius/ 125 ha). Both bull and cow elk were radio-collared, and both bull and cow elk were hunted during this study.

Aerial telemetry relocations were made for approximately 30 radio-collared elk (7 mature bulls [>2yrs], 3 immature bulls [≤ 2yrs], and 20 cows) twice/ week throughout the general firearm season. We located most radio-collared elk during each flight; these locations were defined as live-elk locations. Location accuracy was ±100 m (Weber 1996, Burcham *et al.* 1998).

Hunters who killed an elk in the study area were interviewed at a game check station and asked to indicate on a map the exact site where the elk was initially shot and where the viscera were located. We also asked hunters if the elk had run after being shot. Using this information, we searched for viscera and recorded the location of kill using a global positioning system (GPS) receiver. All recorded kill sites represented the point where the animal was initially shot and not necessarily where viscera were found. If the hunter stated the elk had run after being shot we back-tracked using blood trails or other evidence, e.g., tracks, etc., to find the point where the elk was originally shot. Normally, the location of the viscera and the point where the elk was first shot were one-and-the-same. Ninety-five percent of hunters stated the elk did not run after being shot and our investigation found little evidence to suggest otherwise. We did not find all reported elk-kill locations. To determine if a bias existed between elk-kill locations found and sites we did not find, we tested located versus non-located (using the point supplied by the hunter) elk-kill sites for distance to any road, distance to an open road, and vegetation type present at that location.

All locations (elk-kill, live-elk, and random points) on properties closed to public hunting were removed from our analysis to eliminate a potential bias caused by varying hunter accessibility.

Although elk were killed on private land, the landowner frequently limited access to hunters that possessed cow permits. Further, road restrictions often did not exist and hunter numbers were controlled. For these reasons, elk-kill sites located on private land were very different from the elk kill sites found on land open to the general public. Some of the primary factors contributing to the mortality of elk on private land (access, land-owner/hunter relations, etc.) were not landscape related and therefore not of direct interest in this particular study.

We used 84 elk-kill locations, 267 live-elk locations, and 166 random locations in our analysis. The minimum and maximum X- and Y-coordinates describing the geographic extent of our study were used as upper and lower bounds for random coordinate generation using Quattro Pro spreadsheet software. We used six Geographic Information System (GIS) data sets to describe trailheads and roads (created by digitizing USGS 7.5' topographic series maps and aerial orthophotography at a scale of 1:24,000), hydrography (obtained from the MFWP at a scale of 1:24,000), vegetation-change between 1984 and 1992, hunter density, and current vegetation. The vegetation-change coverage used four change classes: no vegetation-change, intermediate vegetation loss, e.g., shelterwood and selection timber harvest treatments, high vegetation loss, e.g., clear-cut and seed-tree timber harvest treatments, and gained vegetation. This coverage was created using methods described by Winne (1996). We created polygon coverages of vegetation from 30-m resolution satellite imagery. The hunter density coverage (Weber 1996) was created using hunter-GPS routes (Lyon and Burcham 1998), a trailhead coverage, and trailhead-use data (684 trailhead-use samples from 11 trailheads during the 1993, 1994, and 1995 hunting seasons). We sampled most trailheads daily throughout three hunting seasons (1993-1995) and recorded the number of vehicles parked at each trailhead and the number of hunters/ vehicle when known. We used the mean (+1 SD) of the maximum distance traveled by a hunter from a trailhead (n = 93 hunter routes) to create a buffer polygon around each trailhead. We then used trailhead use data (n = 71 days) to assign hunter frequency and density values to each trailhead polygon.

We used unsupervised classification of Landsat thematic mapper imagery, remotely sensed in 1992, to distinguish different spectral groups in the study area. We generated polygons from these pixel aggregations (or groups) and ground-truthed them during summer 1994. Ground-truth sites could not be within 70 m of a polygon's edge and had to be representative of the entire polygon. Using data collected from 242 ground-truth samples, the University of Montana, Wildlife Spatial Analysis Laboratory produced a supervised classification with 24 vegetation classes utilizing methods similar to those described by Hart (1994).

To assess the impact of various landscape elements and to better understand the scale at which elk respond to their environment, we chose three landscape scales to analyze each elk-kill, live-elk, and random location. We assembled a point analysis database that contained ten variables describing each location: distance to any road, distance to an open road, distance to a mapped source of water, distance to the nearest trailhead, vegetation class, vegetation-change class, hunter density, elevation, slope, and aspect. We determined the latter three variables using mean elevation, mean slope, and majority aspect for the vegetation polygon where the point was located. The near analysis database contained a description of the landscape within a 200 m radius of each location. We selected this scale because it approximates the distance at which an elk and a hunter might first encounter one another. Further, it represented a reasonably long-range shot for most hunters. Variables in this database were the area of each vegetation class and vegetation-change class, the number of pixels of open and closed roads, the number of non-road pixels, and the number of different vegetation classes within the sampling perimeter (a fragmentation index). The far analysis database contained a description of the landscape within a 700-m radius of each location using the same variables as the near analysis database. We chose this scale to describe the landscape available to the elk within a short spatial-temporal period.

To perform these analyses, we used 30x30-m pixels to rasterize vector coverages of vegetation, vegetation-change, and roads. As a result of the rasterization process, the actual area sampled was different than predicted when computing the area of a circle, e.g., area = πr^2, 3.14 x 200^2 = 12.6 ha compared with 17.6 ha actually sampled, and 3.14 x 700^2 = 154 ha compared with 124 ha actually sampled. MAYA software (Glassy and Lyon 1989) determined the number of pixels of each vegetation class, vegetation-change class, and road type for both near and far analyses.

We used discriminant function analysis (DFA) to differentiate among elk-kill, live-elk, and random locations at each scale. A step-wise procedure that maximized Wilks-lambda was used, i.e., the variable that provided the best discriminating ability was selected first. The three groups (elk-kill, live-elk, and random) were tested simultaneously and in pairs. To compensate for the bias induced by disproportionate sample sizes (Norusis 1990) we corrected classification rates using the Kappa statistic (Titus *et al.* 1984). This technique provides a statistic that indicates how much better (or worse) the classification performed relative to what would have occurred by chance alone.

To examine the importance of the vegetation classes detected by DFA, we made a use-availability comparison using Chi-square analysis (Neu *et al.* 1974, Byers *et al.* 1984). We calculated use as the percent of each vegetation class identified by a live-elk location (site specific) and calculated availability as the percent of each vegetation class contained within the 95 percent isopleth of the home range of each elk herd. We determined herd home ranges with the adaptive kernel method (Worton 1989) using independent cow elk locations (n =112).

Results

During three hunting seasons (1993-95) 257 elk kills were reported, but only 125 of these were located. Of those located, 41 (32.8%) were found on land closed to the general public. Eighty-four elk-kill sites were used in the DFA. The 132 kill sites never located in the field were lost due to weather conditions, and/or errors in map interpretation. We were concerned that elk-kill sites most likely to be found were not randomly distributed, but rather those that were easiest to locate, i.e., close to roads open to vehicular traffic, trailheads, or areas with little or no forested vegetation. We made a concerted effort to locate each elk-kill site, including those in areas difficult to access, but the probability of finding these points, using only verbal instructions from excited hunters, seemingly diminished as the complexity of instructions increased. However, few kills were reported in areas far from open roads and trailheads or in dense forests or in areas that were atypical of the other kill sites. Mean distance to an open road ($\bar{x}$ = 1.54 km vs. 1.28 km) or to any road ($\bar{x}$ = 0.19 km vs. 0.25 km) varied little between found and lost elk-kill sites. Further, maximum distance from an open road is nearly identical for all elk locations (found elk-kill sites =

5.65 km, lost elk-kill sites = 5.50 km, and live-elk locations = 5.77 km). This suggested that in this heavily-roaded study area, elk cannot find areas >6 km from an open road. In addition, we determined the vegetation type found at each lost elk-kill site. Over 20 percent of these sites were located in the Douglas fir vegetation class, which corresponded well with found elk-kill sites where the same Douglas fir vegetation class also was found for 20 percent of the sites. The second most common vegetation class found at lost elk-kill sites was termed foothills and parklands, a non-forested bunchgrass type (19%) while the second most common vegetation class at found elk-kill sites, nearly 19 percent, was open Douglas fir (typified by having <30% canopy closure). Open Douglas fir was the third most common vegetation class found at lost-elk kill sites (16.7%). It is interesting to note that 19 percent of lost elk-kill sites were reported in foothills and parklands. This vegetation class contains no forested vegetation and only the most minimal hiding cover. It also is noteworthy that vegetation classes in which found elk-kill sites and lost elk-kill sites were located approximated their occurrence and order of importance. Thus, we believe that the actual error caused by any bias of lost elk-kill sites was minimal.

Distance to open road and vegetation-change variables provided an overall correct classification of 53 percent using the point analysis database. Elk-kill, live-elk, and random locations were ordinarily associated with areas of no vegetation change. However, 35 percent of kill locations were found in areas of intermediate vegetation loss, e.g., shelterwood and selection timber harvest. Live elk were found 1 km farther, on average, from open roads than elk-kill or random locations. The Douglas fir vegetation class had the highest frequency of kill and random locations (20%) and also was one of the most common vegetation classes (20%, Table 1). Live-elk were most often associated with the lodgepole pine vegetation class (52%). Elk use of lodgepole pine exceeded availability ($\chi^2 = 64.3$, d.f. 11 (critical value [0.05] = 19.7)), whereas elk use of the open Douglas fir vegetation class (≤30% canopy closure) was not different than availability ($\chi^2 = 3.1$, d.f. 11 (critical value [0.05] = 19.7)(Table 1).

The area of lodgepole pine and the

Table 1. *Availability and use of vegetation classes by radio-collared elk during the hunting season, and vegetation class availability.*

Vegetation class	% live-elk use	% Availability	Significance[a]
Cropland/ pasture	0.5	0.5	
Foothills/ parklands	1.6	6.9	
Disturbed grasslands	0.5	0.7	
Other herbaceous	0.5	3.5	
Sagebrush	1.1	5.4	
Mixed grass/ shrub	0.5	0.7	
Lodgepole pine	51.9	17.9	+
Ponderosa pine	7.0	5.3	
Douglas-fir	20.0	20.0	
Mixed coniferous	9.7	20.2	
Open Douglas-fir	4.3	9.9	
Regenerating clearcut	1.6	2.1	

a. + Indicates elk use exceeded availability, - indicates elk use was less than availability.

number of non-road pixels found within the sampling perimeter of each location achieved the best overall classification (50%) in near analyses. Similarly, the area of open Douglas fir and the number of pixels of open road were used to achieve the best overall classification (49%) for far analyses.

The highest correct classifications were achieved using the point analysis database. At this scale, 80 percent of live-elk locations were correctly classified (Table 2).

Discussion

Although elk distributions relative to open roads were generally uniform (Fig. 1), nearly 50 percent of all elk-kills occurred ≤ 1 km of an open road, which suggested elk vulnerability increased close to open roads. The importance of roads as a discriminant factor at each landscape scale illustrated not only the impact of open roads on elk security, but also a discernible benefit of walk-in areas for elk security during the hunting season. Our results concur with findings

Table 2. *Results of discriminant function analysis (DFA) and chance-correction classification (Kappa statistic)*

	% Correctly classified			
Database type	Elk kill locations	Live elk locations	Random locations	Kappa
Point analysis	41	80	39	0.32
Near analysis	31	66	52	0.30
Far analysis	38	63	46	0.26

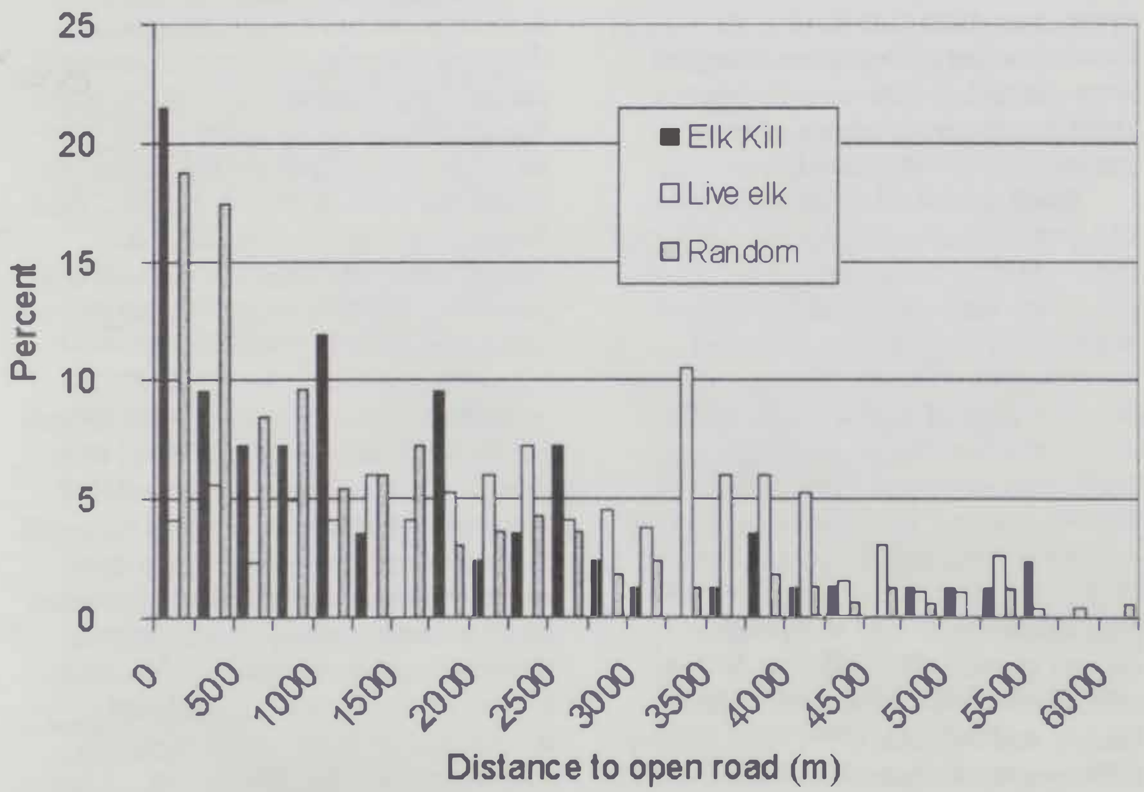

Figure 1. *Frequency distribution of elk-kill, live-elk, and random locations relative to the nearest open road.*

reported by Basile and Lonner (1979), Lyon and Canfield (1991), Unsworth and Kuck (1991), and Unsworth *et al.* (1993).

Although elk-kills were associated most with areas of no vegetation change, 35 percent of kills were found in areas of intermediate vegetation loss, e.g., shelterwood and selection timber harvest, compared to only 4 percent of live-elk locations. This suggested elk vulnerability increased greatly where timber harvests had occurred. However, vegetation change may not be the sole contributing factor to this increase in vulnerability. Although closed to vehicular traffic, roads that lead to these areas provide hunters easier access and greater sight distance.

Only 5 percent of live-elk locations were found in the open Douglas fir vegetation class, compared to nearly 17 percent of elk-kill locations. This agreed with the results of other researchers (Irwin and Peek 1983, Wright 1983, Canfield 1988, Hurley and Sargeant 1991, Vales 1996), who found elk use of open areas decreased during the hunting season. Elk that ventured, or were pushed, into areas with poor security appeared to have a higher probability of being killed.

Based on field data describing the 242 ground-truth samples used to create the vegetation coverage, the lodgepole pine vegetation class had the highest hiding cover estimate and densest canopy cover, which probably explains why elk selected this vegetation class during the hunting season. Marcum (1975) and Edge *et al.* (1987) reported that elk selected sites with high canopy closure and/or dense cover. Irwin and Peek (1983) found that elk preferred pole-timber sites with >75 percent canopy closure with little use of clear-cuts, grass-shrub, or brushfield sites. Hurley and Sargeant (1991) and Hurley (1994) reported that elk in roaded or partially-roaded areas increased their use of dense coniferous cover and subsequently decreased their use of more open sites during the hunting season. Of the 415 individual polygons assigned the lodgepole pine vegetation class, elk in the study area routinely selected ten large polygons with 85 percent of those locations occurring in the largest polygon. These results, when coupled with data we presented regarding use-availability and the results of DFA, indicated selection for large cover patches (Lyon and Canfield 1991, Hillis *et al.* 1991). Elk seem to have selected these sites for the security provided by these forests rather than for lodgepole pine as a species. In other regions sub-alpine fir (*Abies lasiocarpa*) or Douglas fir may provide security. Thus, the vegetation classification becomes less important than the characteristics, i.e., size and structure of the stand, used to describe it.

Management Implications

Implementation of the following suggestions in timber harvest planning, road construction, and property development has the potential to decrease elk vulnerability to hunting: (1) design road closures, i.e., walk-in areas, that provide security cover >1km from an open road, (2) reduce road densities inside the walk-in area by limiting road development and instituting road obliteration projects, and (3) retain large patches of forest with high canopy cover values and hiding cover. These considerations must be applied collectively to be effective because forest patches with dense canopy cover only marginally diminish elk vulnerability when unrestricted use of roads is maintained (Lyon 1979). It does not seem feasible to assign threshold values to act as maximum road density or minimum patch-size guidelines. However, our data suggested that minimum patch size required by elk may be greater than the 100 ha previously recommended by Hillis *et al.* (1991). Because of numerous interacting variables, land managers must assess each landscape individually, considering hunter density and hunter use patterns in conjunction with road and forest variables.

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Identification of an Aromatic Amino Acid Decarboxylase from a Yew-Associated Fungus

Abstract

Several fungi isolated from the inner bark of the Pacific yew tree (Taxus brevifolia) *have been studied as potential paclitaxel producers. Fungal isolate H10BA2, identified as* Penicillium raistrickii, *showed evidence of* de novo *paclitaxel production when grown in liquid culture. This fungus differed in several respects from isolates of* P. raistrickii *obtained from other sources, including the isolates available from ATCC (American Type Culture Collection). Soluble protein extracts of H10BA2 yielded a protein fraction that demonstrated aromatic amino acid decarboxylase activity, converting L-phenylalanine to phenethylamine. Isolation of the decarboxylase enzyme, identified for the first time in a fungus, as well as the characterization of the phenethylamine product is described.*

Key words: decarboxylase, *Penicillium raistrickii*, AADC, enzyme, amino acid decarboxylase.

Introduction

It has been proposed that aromatic amino acids are important precursors of secondary metabolites in higher plants, and that decarboxylation of these amino acids may be involved in regulatory mechanisms for the synthesis of these secondary metabolites (Kawalleck *et al.* 1993, Facchini and DeLuca 1994). Aromatic amino acid decarboxylases (AADC's) have been isolated from plant, insect, bacterial and animal sources, but have not been described from fungal sources (Marques and Brodelius 1988, Choudhury *et al.*1990, Tocher and Tocher 1972, Maneckjee and Baylin 1983). In animals, AADC's are important in the production of norepinephrine from tyrosine (Christenson *et al.* 1970) and the decarboxylation of L-Dopa and 5-hydroxytryptophan to form L-dopamine and serotonin, respectively (Maneckjee 1983, Albert 1987). In higher plants, secondary metabolite production can be correlated with various AADC's. In *Papaver somniferum*, L-Dopa decarboxylase is important in the biosynthesis of alkaloids, including morphine (Roberts and Antoun 1978) and in the production of 3-hydroxytyramine by *Cytisus scoparius* (Tocher and Tocher 1972). Specific L-tyrosine decarboxylases have been isolated from both barley roots and parsley (Kawalleck *et al.* 1993.)

This work identifies aromatic amino acid decarboxylase activity found in soluble protein extracts from cultures of *Penicillium raistrickii* that apparently

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catalyzes the conversion of L-phenylalanine to phenethylamine. An aromatic amino acid decarboxylase may be an important enzyme in the production of other secondary metabolites.

Material and Methods

General Experimental Procedures

Gas Chromatography/ Mass Spectrometer (GCMS) analyses were run on a Hewlett-Packard 5890 GC with a HP-5 capillary column and a HP5971 Mass Spectrometer. Thin layer chromatographies (TLC's) were run on Whatman AL SIL G/UV, 250 mm layer. Anion exchange resin was Whatman, 4057050 DE-52. All solvents were reagent grade. [$^2H_5$]-L-phenylalanine (five aromatic hydrogens were substituted with deuterium, ^2H) was purchased from Cambridge Isotope Laboratories, DLM-1258; and [U-^{14}C]-L-phenylalanine (universal labeled) was purchased from American Radiolabeled Chem. Inc., ARC 675. Fuji RX medical x-ray film was used for autoradiographs.

Fungal Fermentation

Isolates of H10BA2 (identified as *Penicillium raistrickii* by Dr. Zofia Lawrence at the International Mycological Institute) were grown in 5 L M1S medium (5 g Bacto-soytone, 60 g sucrose, 1 g yeast extract, per liter of broth). Both still cultures (20 day) and shaker cultures (6 day) were used for the preparation of soluble protein extracts. Cultures were filtered through Miracloth" (Calbiochem, 475855) and washed twice with 1 L volumes of 50 mM Tris-HCl (pH 7.6), 10 mM EDTA, 1 mM 2-mercaptoethanol, and 0.15 M NaCl (4° C). The mycelium was dried by squeezing in Miracloth" to remove excess moisture, placed in a pre-cooled mortar (-20° C), frozen with liquid nitrogen and ground to a fine powder with a pestle. This powder was resuspended in a 200 mL solution that was 50 mM Tris-HCl and 1 mM 2-mercaptoethanol (pH 7.6) and kept on ice. Cellular debris was removed by centrifugation at 22 800 *g* for 10 minutes. The supernatant, which contained the soluble protein fraction, was saved. This protein preparation was frozen in 1.5 mL aliquots (-20° C). A separate aliquot was saved for Bradford protein assay (Bradford 1976). Typical concentrations of protein as determined by the Bradford assay were in the range of 0.8 to 1.5 mg/mL. These protein preparations were used for enzyme assay procedures.

Enzyme Purification

Powdered $(NH_4)_2SO_4$ was added slowly to the crude supernatant at 4° C while stirring, to a final concentration of 1.0 M $(NH_4)_2SO_4$. The resulting slurry was centrifuged for 15 min at 22 800 *g*. The supernatant was applied to a column of Pharmacia phenyl-sepharose 6 fast flow (2.5 x 11.5 cm), previously equilibrated with HIC buffer 1, at a flow rate of 4 mL/min. The column was rinsed with 200 mL of HIC buffer 1. The enzyme was eluted by a stepwise gradient of HIC buffers [1.0 M, 0.8 M, 0.6 M, and 0.0 $(NH_4)_2SO_4$] using 200 mL of each solution, with 50 mL fractions collected. Five mL of each fraction was dialyzed against buffer C and assayed for enzyme activity by TLC analysis. Active fractions were combined and dialyzed against buffer C. A Pharmacia CM Sepharose Fast Flow cation exchange column (1.5 x 16.5 cm) was equilibrated with buffer C. Dialyzed fractions were loaded onto the column at 4 mL/min. The column was rinsed with 100 mL of buffer C. The enzyme was eluted by a stepwise gradient of CAT buffers (120 mM, 140 mM and 200 mM KCl) using 100 mL of buffer for each step, with collection of 10-mL fractions. Active fractions were dialyzed against buffer C and concentrated by column chromatography on a Pharmacia CM Sepharose Fast Flow cation exchange column (1 x 6 cm). The enzyme was eluted by a stepwise gradient of CAT buffers (100 mM and 200 mM KCl), using 32 mL of buffer for each step, with collection of 4 mL fractions. Fractions were stored at -20°C.

SDS-PAGE analysis was performed according to the method of Laemmli 1970. Gels were made with a 4 percent acrylamide stacking gel and a 6 percent acrylamide separating gel, 1.5 mm thick. Reducing SDS-PAGE analysis was run in the presence of 262 mM 2-mercaptoethanol. Gels were stained by a silver stain method developed by Schoenle and Sammons 1984.

Buffers

We prepared buffers with reagent grade chemicals and adjusted pH with either HCl or NaOH. All chromatography buffers were based on a solution of 1 mM dithiothreitol, 1 mM pyridoxal-5-phosphate (Pxy-P), 50 mM sodium acetate, pH 5.0 (buffer C). *Hydrophobic interaction chromatography* (HIC) buffers 1, 0.8, 0.6, and 0 varied in the concentration of $(NH_4)_2SO_4$ (1.0 M, 0.8 M, 0.6 M and 0.0 M) added to buffer C respectively. Cation exchange chromatography (CAT) buffers varied in the concentration of KCl added to buffer C. CAT buffers 100, 120, 140 and 200 contained 100 mM, 120 mM, 140 mM and 200 mM KCl, respectively.

Enzyme Assay Protocol

We adapted the enzyme assay protocol from a method by Kurylo-Borowska and Abramsky 1972. Enzyme assay mixtures had a total volume of 1 mL. Each assay contained Tris-HCl buffer (pH 8.0, 50 mM), $MgCl_2$ (40 mM) and ATP (10 mM). L-phenylalanine was added to a final concentration of @ 4 mM. If $[^2H_5]$-L-phenylalanine were used, then it was added to a final concentration of 4 mM; if [U-^{14}C]-L-phenylalanine were used, then it was added to a specific activity of 3 µCi/mg (1 µCi/mL) with unlabeled L-phenylalanine. All ingredients except protein were mixed in 1.5 mL Eppendorf tubes on ice. The protein was added to give final concentrations of 0.25 to 0.5 mg/mL, and tubes were placed in a 30°C water bath. Incubation times up to 24 hours were used, but 30 minutes was sufficient to produce detectable product. At the end of the desired incubation time, the assay tubes were placed in boiling water for 1 minute to deactivate enzymes. The assay mixtures were then transferred to glass vials and dried *in vacuo*. Chloroform-methanol [0.5 mL of 1:1 (v/v)] was added to each vial after drying. This was mixed and allowed to incubate for 30 minutes at room temperature. The organic extract was analyzed by TLC for the presence of **1**.

Thin Layer Chromatography

Approximately 3 mL of the 1:1 chloroform-methanol solution from the enzyme assay was spotted on TLC plates. TLC plates were eluted with 1:1 chloroform-methanol. Plates were then air dried, sprayed with ninhydrin reagent (0.3 g ninhydrin in 100 mL *n*-butanol and 3 mL glacial acetic acid, Merck 1976) and warmed with a heat gun to identify the amine containing fractions. Phenethylamine gave a purple spot at R_f 0.86 under these conditions. L-Phenylalanine did not migrate from the origin.

Autoradiography

Enzyme assays using radiolabeled L-phenylalanine as substrate were applied to TLC plates, then analyzed by autoradiography. The plates were overlaid with the film in the dark and exposed for 2 to 6 weeks. Developed film was examined for spots that resulted from exposure to radioisotope.

Gas Chromatography-Mass Spectrometry

Analysis of enzyme assays by GC-MS for the presence of phenethylamine used the following GC parameters: injection port 280° C, column flow rate 0.5 mL/min.; oven temperature program 130° C to 280° C (@ 10° C/min.); MS interface 280° C. TIC's were recorded for natural and synthetic phenethylamine. SIM was used for [$^2H_5$]-phenethylamine due to low concentration of product.

Column Chromatography

DE-52 gel (25 g) was equilibrated with a buffer that was 0.05 M Tris-HCl (pH 7.6), 5 mM dithiothreitol, and 0.1 mM EDTA. The equilibrated gel was packed into a 2.4 x 10 cm glass column at 4° C using a persitaltic pump to maintain a constant flow rate of 1 mL/min. Crude protein preparations were then loaded in a 100 mL volume of the above buffer system. The following stepwise elution system was used: buffer plus: 0.16 M KCl (2 x 10 mL); 0.21 M KCl (2 x 10 mL); 0.35 M KCl (2 x 10 mL); 0.45 M KCl (4 x 10 mL). Fractions were collected (10 mL) and assayed for protein concentration and phenethylamine production.

Isolation of Phenethylamine

Enzyme assay mixtures were dried *in vacuo* and extracted with 1 mL of chloroform-methanol (1:1, v/v). The volume was reduced *in vacuo* to 500 µL and the extract was applied to an HPLC silica gel column run in gradient mode from 10:1 chloroform-methanol to 1:1 chloroform-methanol.

Phenethylamine, 1

Physical characteristics : liquid, bp 196° C; MS m/z 121(14), 105(3), 91(100), 77(31), 65(88); ^{1}H NMR spectral data: (300 MHZ, CD_3OD) d 7.42-7.11 (5H, *m*), 3.18 (2H, *t*, *J*=7), 2.95 (2H, *t*, *J*=7).

Results and Discussion.

We examined the soluble protein extract of *Penicillium raistrickii* isolate H10BA2 for evidence of enzymes involved in paclitaxel biosynthesis. In particular, we were looking for a phenylalanine aminomutase, analogous to the enzyme isolated from the yew tree by Floss and his coworkers (Fleming *et al.* 1993, Walker and Floss 1998). In an attempt to define a parallel enzyme function in fungal production of paclitaxel, we performed enzyme assays on crude soluble protein extracts from H10BA2 using L-phenylalanine as a substrate. Several products were routinely detected in this assay. Product mixtures were applied to thin layer silica gel plates and eluted with chloroform-methanol (1:1, v/v). The mixtures resolved into discreet spots and were visualized with ninhydrin reagent. This assay consistently yielded compound **1** (R_f = 0.86) which generated a purple spot when sprayed with ninhydrin reagent. Enzyme preparations from six separate fungal fermentations, grown both still and as shaken cultures, all yielded protein extracts that produced compound **1** from phenylalanine.

When [U-^{14}C]-L-phenylalanine was used as a substrate, **1** was shown by autoradiography to have incorporated some radiolabel. The spot on the autoradiograms correlated perfectly to the ninhydrin-positive spot on the TLC plate (R_f = 0.86).

To isolate sufficient amounts of compound **1** to facilitate characterization, enzyme assay products were dried *in vacuo* and thoroughly extracted with chloroform-methanol (1:1). The organic extract was purified by silica gel HPLC. TLC analysis of each column fraction demonstrated the presence of **1**; this fraction was analyzed by GC-MS. Total Ion Chromatograph (TIC) exhibited a major peak at 8.1 min; mass spectral analysis of this peak showed a molecular ion at m/z 121, with major fragments at m/z 91 and 77 amu (Fig. 1).

When [$^2H_5$] -L-phenylalanine (five aromatic ring hydrogens substituted with deuterium) was used as a substrate for the enzyme assay, the TLC analyses were identical. [$^2H_5$]-compound **1** was again purified by HPLC and subsequently analyzed by GC-MS, this time using the more sensitive Selective

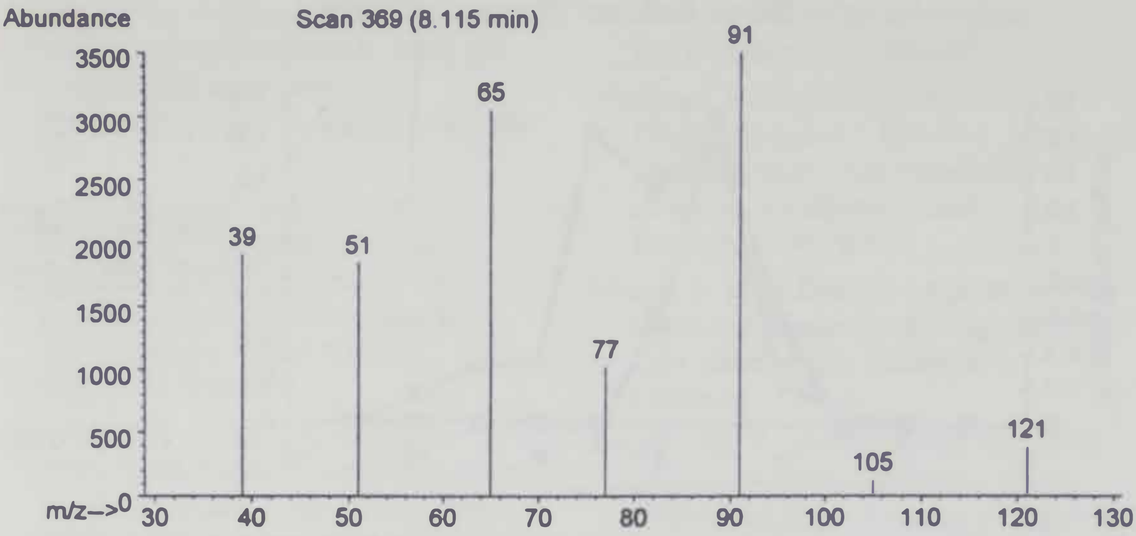

Figure 1. *Mass spectral analysis of the compound that correlated to the total ion chromatograph (TIC) peak at 8.1 min.*

Ion Monitoring (SIM) method instead of TIC. [$^2H_5$]-compound **1** eluted at 8.1 min; mass spectral analysis exhibited prominent peaks at m/z 126, 96 and 82 amu. These masses suggested the same fragmentation pattern as compound **1**, plus 5 amu from [$^2H_5$] incorporation.

Characterization of 1 was accomplished with combined mass spectral and NMR analyses. Reaction with ninhydrin indicated that it was a primary amine, and radiolabeling experiments indicated that it was an L-phenylalanine derivative. Proton NMR and mass spectral data of **1** were identical to that of authentic phenethylamine purchased from Aldrich to provide a TLC standard and to allow direct comparison of spectral data.

The enzyme-catalyzed conversion of L-phenylalanine to phenethylamine (Fig. 2) suggested the presence of an aromatic amino acid decarboxylase, previously unidentified in a fungus. Attempts to purify the AADC began with anion-exchange column chromatography. Crude fungal protein extract (80 mg) was applied to a DE-52 column and eluted with increasing concentrations of KCl. Column fractions (10 mL) were collected and assayed for protein concentration by the Bradford procedure (Bradford 1976) and for AADC activity using the enzyme assay and TLC analysis as described above. Protein fractions 4, 5 and 6 catalyzed the transformation of L-phenylalanine to phenethylamine, **1**, in our enzyme assays. These fractions eluted after the application of 40 mL of solvent, which corresponded to 0.21 M KCl buffer (Fig.3).

A more rigorous isolation scheme confirmed the presence of an AADC in the fungal protein extract. Six-day shaker cultures were harvested as described above. Following ammonium sulfate precipitation and subsequent centrifugation, the supernatant was

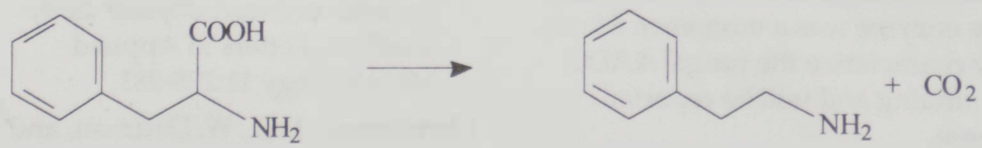

Figure 2. *Decarboxylation of L-phenylalanine to yield phenethylamine,* **1**.

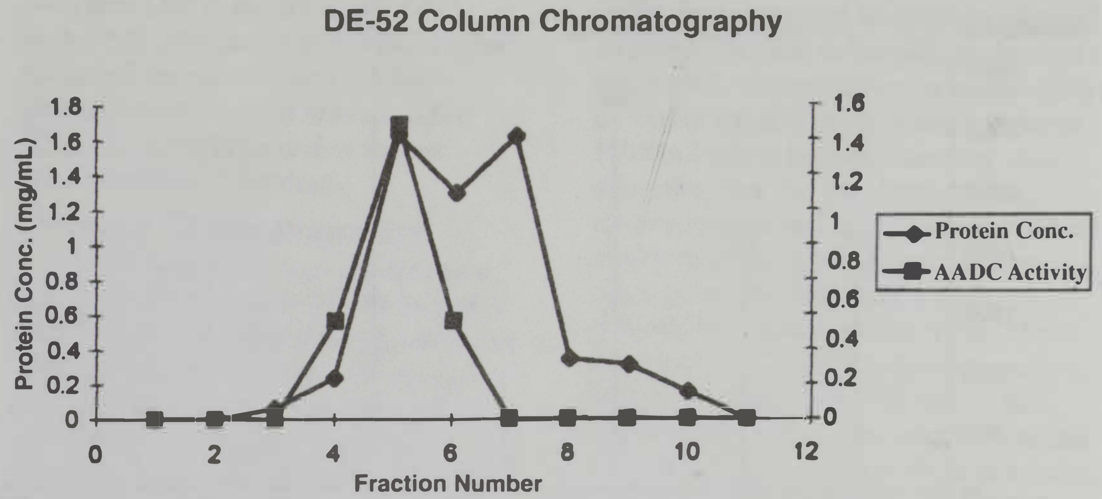

Figure 3. *Comparison of specific amino acid decarboxylase activity and protein concentration in anion exchange (DE-52) chromatography fractions of protein extract.*

applied to a phenyl-sepharose column equilibrated with hydrophobic interaction chromatography buffer 1 (HIC buffer). AADC activity was concentrated in the fractions that eluted with 0.6 M HIC buffer. The fractions were dialyzed and subjected to CM-Sepharose Fast Flow cation exchange chromatography. Active fractions yielded a single enzyme assay product with an R_f of 0.86. AADC activity was concentrated in the fractions that eluted with 140 mM KCl buffer. These active fractions were dialyzed and again applied to a CM-Sepharose Fast Flow cation exchange column, and eluted by a stepwise gradient of CAT buffers. Undialyzed fractions were tested for activity as described. A single enzyme product, **1**, was evident in the TLC assays of the AADC fractions, which eluted with the 200 mM KCl buffer.

The purity of the enzyme was demonstrated by SDS-PAGE analysis. Fungal AADC yielded a single band of 125 000 ± 3000 Da under both reducing and nonreducing conditions, indicating that the enzyme was a monomer. Efforts to fully characterize the fungal AADC are continuing and will be reported elsewhere.

Although AADC's are not involved in the biosynthesis of fungal taxol they may play a role in the formation of other fungal metabolites. The biosynthesis of the *Claviceps purpurea* metabolite ergotamine involves the decarboxylation of isoprenylated tryptophan (Floss 1976). The biosynthesis of b-carbolines by the fungus *Fusarium* sp. probably involves the decarboxylation of tryptophan.

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