ANNUAL REPORT OF THE WYOMING GAME AND FISH LABORATORY

September 30, 2000

WYOMING GAME AND FISH DEPATMENT
CHEYENNE, WYOMING

Game and Fish Laboratory Branch
Summary of Activities For Fiscal Year 2000

The responsibility of this branch is to provide analytical services for various department personnel, and occasionally, other agencies and the general public. For FY00 a total of 31, 878 tests were conducted on 22,575 samples in response to 2,860 requests (cases). These services are conducted at hatcheries, natural streams, lakes, and at the Game and Fish Laboratory and include: fish health inspections and disease diagnostics for hatcheries and natural waters in Wyoming; forensic analyses for law enforcement personnel; analytical services for management personnel; and whirling disease procedure validation for research purposes, all done in a timely and efficient manner.

During FY 00, 146 fish disease cases were studied and 23,473 diagnostic tests were conducted on various specimens of fish. Twenty-six of these cases involved various species of brook, brown, cutthroat, and rainbow trout at State fish culture stations. Four cases involved fat head minnows at the University of Wyoming, Zoology Department and Red Buttes environmental biology research facility and a private hatchery in Casper. In one case, a bluegill was tested from a private hatchery in Nebraska. Bacterial diseases, gas bubble disease, environmental toxicity, physical trauma, malnutrition, predators, skeletal deformity, post spawning stress disorder, kidney mineral deposits, and multiple species of parasites were noted in these cases. The conditions of most lots of fish located at the various facilities returned to normal after corrective measures (disinfectants, antibiotic therapy, diet change, etc.) were taken. There were 19 cases that involved rainbow, brown, brook, and cutthroat trout, largemouth bass and grayling fish from natural habitats. Physical trauma from hooking, malnutrition, bacterial disease, parasites, environmental toxicity, and fungus parasites were identified in various species of fish for these different natural habitats. Forty-six special annual/semiannual fish health inspections, which include 11 state fish-culture stations, three state quarantine units, nine private hatcheries, and nine (wild) broodstock populations, were made during the year. Low levels of furunculosis were detected in one of these cases, and whirling disease was present in another case. Otherwise no pathogens were detected. A total of 2,367 fish in 50 cases were examined for whirling disease organisms. Low numbers of the spores were detected in kokanee, cutthroat, rainbow, brown, and brook trout in Fontenelle Reservoir; kokanee salmon from East Fork River; and in rainbow and brown trout from New Fork River, all from Sublette County.

Pectoral fins from 797 cutthroat trout were collected from Christensen and Laker creeks, tributaries of the Salt River, Lincoln Co. These were tested using DNA-PCR procedures to detect the presence of whirling disease. A total of 163 tested positive for the presence of the parasite. A private lab tested a total of 350 of these same samples histologically, and 211 were positive. Use of the fin clips as a non-lethal sampling tool is not recommended for detection of the whirling disease parasite.

Nine hundred and fifty tests were conducted on 341 different samples in response to 51 wildlife enforcement forensic cases. *In 12 cases defendants pled guilty when lab results were used as evidence.* Tests included identification of species and gender, matching, and minimum numbers of animals present using serological and DNA

techniques. Samples tested included: partial animal carcasses, meat, blood, hair, blanket, leatherman tool, hat, turkey feathers, hide, bones, stains, antlers, saws, gloves, clothes, leaves, knives, and stomach contents.

Teeth from 5,752 game animals, predators, and furbearers were processed and aged using the cementum annuli techniques. Ages ranged from young of the year to 29.6 years for the various species. A total of 82 tests were conducted on 82 different biological samples in response to nine requests from management personnel. These included identification of hair, stomach contents, scat, tissue, and bone.

A total of 756 whole or partial fish heads suspected of containing coded wire tags were received from fisheries biologists in the Casper Region. All samples were recovered from the North Platte River System. Extraction and examination of these resulted in 606 tags recovered. The tags were decoded from a binary number to a data set number and returned to fisheries biologists for analysis of population dynamics.

JOB PERFORMANCE REPORT

PROJECT SEGMENT

STATE **Wyoming**

JOB NO: FXSWCAD551 JOB TITLE: Diagnosis of Diseases in Fish

PERIOD COVERED **April 1, 1999 to March 31, 2000**

Abstract: A total of one hundred forty-six fish disease cases were studied during this period. Disease investigations involved both free-ranging and hatchery reared fish. Annual health inspections for serious pathogens were conducted at 11 state hatcheries, nine private facilities, three quarantine units, and for nine feral broodstock populations. From these culture sources, a total of 10,095 tissue and 2,393 ovarian fluid samples were collected and tested from trout and salmon populations. A total of 23,473 diagnostic tests were conducted and evaluated for the presence of known disease causing organisms. During these routine inspections one nationally important bacteria, *Aeromonas salmonicida*, which causes the infectious disease furunculosis was detected in adult Soda Lake brook trout and East Fork and Green River kokanee salmon. Clinical furunculosis was also identified during these bacterial investigations in mature Bear River cutthroat trout located at Daniel State Fish Hatchery and brook and brown trout tested from 3-M Trout Ranch. Diagnostic disease examinations requested by hatchery and research personnel during this study year also revealed several common bacterial and fungal diseases including; bacterial gill and coldwater disease, fin rot, motile aeromonas septicemia and saprolegniasis. Numerous parasitic fish diseases were also observed during this investigation including cases involving gill and skin infections by the protozoan parasite *Ichthyobodo necator* (Costia), and the trematode flukes *Gyrodactylus sp.* and *Apophallus imperator.* Of special interest was the unusual observance of severe abdominal disease caused by the microsporidian *Glugea pimephales.* Cysts of the common nematode *Contraceum spiculigerum* were also found at one culture facility during routine disease inspections.

Sudden water quality changes also contributed to stressful conditions at many culture facilities and often resulted in significant fish losses during this period. Facilities experiencing these seasonal envirnomental problems included Wheatland High School, Como Bluff Hatchery, Red Buttes Environmental Laboratory, and Dubois Hatchery. Predation also contributed to minor fish losses at Ten Sleep and Clark's Fork hatcheries. The health condition of the fish located at these fish culture facilities returned to normal once the appropriate corrective measures (antibiotics, disinfection, diet changes, and water quality improvements) were initiated.

Detailed investigations into the occurrence of diseases or mortalities in free-ranging populations of fishes revealed the presence of one common bacterial infection, several cases involving fungal infections of the skin, and one case of parasitic infestation. Cases of a non-infectious nature which resulted in minor fish loss or general public concern were also studied

Table 3. Summary of Hatchery Classification and Species Fish Disease Inspections Conducted in Wyoming from April 1, 1999 to March 31, 2000

Case Number	Location	Species Examined	Submission Date	Diagnosis/ Comments
99-028	East Newton Lake	Rainbow Trout	4/3/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-030	Como Bluff Hatchery	Rainbow, Brook, Cutthroat Trout	4/12/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-034	Daniel Hatchery	Rainbow, Cutthroat, Brook, Brown Trout	4/29/99	Positive for furunculosis bacteria. Class B.
99-035	Lindsay Burns Fisheries	Brown, Rainbow, Brook Trout	5/12/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-036	Fish on Ranch	Rainbow and Cutthroat Trout	5/13/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-037	Daniel Hatchery	Cutthroat and Rainbow Trout	5/14/99	No pathogens. *Myxobolus cerebralis* screen only
99-038	J&J Partnership	Rainbow Trout	5/17/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-040	Tillett Quarantine Unit	Rainbow Trout	5/21/99	No pathogens detected. Class A-1. Complete Chapter X Inspection
99-041	Ten Sleep Hatchery	Rainbow, Cutthroat, Brook Trout	5/21/99	No pathogens detected. Class A-2. Complete Chapter X Inspection

Table 3. cont.

Case Number	Location	Species Examined	Submission Date	Diagnosis/ Comments
99-043	Dan Speas Rearing Station	Brown Trout	5/21/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-049	Meadow Lake	Grayling	5/28/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-050	Boulder Isolation Unit	Lake Trout	5/28/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-052	Wigwam Rearing Station	Cutthroat Trout	6/9/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-054	Daniel Hatchery	Cutthroat Trout	6/15/99	*Myxobolus Cerebralis* Screening only, not detected
99-063	Auburn Hatchery	Cutthroat Trout and Kokanee Salmon	7/8/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-063a	Auburn Brood Source	Cutthroat Trout	7/8/99	*Mxyobolus Cerebralis* Positive, Low Level Prevalence. Class C. Complete Chapter X Inspection
99-064	Wyoming Trout Ranch	Rainbow, Brown, Cutthroat, Brook Trout	7/13/99	No pathogens detected. Class A-1. Complete Canadian Schedule II Inspection
99-069	Dubois Hatchery	Rainbow, Cutthroat Trout	7/21/99	No pathogens detected. Class A-2. Complete Chapter X Inspection

Table 3. cont.

Case Number	Location	Species Examined	Submission Date	Diagnosis/ Comments
99-070	Daniel Hatchery	Cutthroat Trout	7/22/99	No pathogens detected, bacterial inspection only
99-071	Daniel Hatchery	Rainbow Trout	7/22/99	*Myxobolus cerebralis* screening only
99-073	Littlefield Creek	Cutthroat and Brook Trout	7/22/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-079	Sjhoberg Creek	Cutthroat Trout	7/29/99	No pathogens detected. (Ovarian fluid only) Class A-2
99-081	Hoemstead Trout Ranch	Brown and Rainbow Trout	8/9/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-093	3-M Trout Ranch	Rainbow, Brown, Brook Trout	8/31/99	No pathogens detected. Class A-1. Complete Chapter X Inspection
99-098	Dan Speas Rearing Station	Rainbow Trout	9/11/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-100	New Fork Lake	Kokanee Salmon	9/17/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-101	Flume Creek	Kokanne Salmon	9/17/99	No pathogens detected, *Myxobolus cerebralis* screening only
99-109	Auburn Hatchery	Cutthroat Trout	9/29/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-110	Fish-on-Ranch	Rainbow and Cutthroat Trout	10/4/99	No pathogens detected. Class A-2. Complete Chapter X Inspection

Table 3. cont.

Case Number	Location	Species Examined	Submission Date	Diagnosis/ Comments
99-111	Story Hatchery	Lake and Rainbow Trout	10/7/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-112	Kessler Spring Suppliers	Rainbow Trout	10/7/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-115	Jenny Lake	Lake Trout	10/8/99	No pathogens detected, viral & R. sal. screening only
99-117	Soda Lake	Brook and Brown Trout	10/14/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-123	Boulder Rearing Station	Rainbow Trout	10/20/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-136	Boulder Lake	Kokanee Salmon	11/10/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-138	Auburn Hatchery	Cutthroat and Rainbow Trout	11/16/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-146	Ten Sleep Hatchery	Rainbow Trout	12/2/99	No pathogens detected. Class A-2. Complete Chapter X Inspection
99-147	Tillett Spring Rearing Station	Rainbow and Cutthroat Trout	12/1/99	No pathogens detected. Class A-1. Complete Chapter X Inspection
99-148	Tillett Spring Rearing Station	Rainbow and Cutthroat Trout	12/1/99	No pathogens detected. Class A-1. Complete Chapter X Inspections

Table 3. cont.

Case Number	Location	Species Examined	Submission Date	Diagnosis/ Comments
99-151	Wigwam Rearing Station	Rainbow and Cutthroat Trout	12/15/99	No pathogens detected. Class A-2. Complete Chapter X Inspections
00-02	Clark's Fork Hatchery	Cutthroat, Rainbow, Brown Trout	1/27/00	No pathogens detected. Class A-1. Complete Chapter X Inspection
00-06	Zn Trout Ranch	Brown, Brook, Cutthroat, Rainbow Trout	2/14/00	Class C. Complete Chapter X Inspection
00-11	Boulder Rearing Station	Rainbow, Cutthroat, Brown Trout	3/16/00	No pathogens detected. Class A-2. Complete Chapter X Inspection
00-12	Dan Speas Rearing Station	Rainbow Trout	3/12/00	No pathogens detected. Class A-2. Complete Chapter X Inspection
00-13	Cedar Ridge Hatchery	Rainbow, Brown, Brook, Cutthroat Trout	3/22/00	No pathogens detected. Class A-2. Complete Chapter X Inspection
00-16	Como Bluff Hatchery	Rainbow, Splake, Brook Trout	3/27/00	No pathogens detected. Class A-2. Complete Chapter X Inspection

Table 4. Summary of Fish Populations Surveyed for the Salmonid Whirling Disease Pathogen *Myxobolus cerebralis* conducted in Wyoming in April 1, 1999 to March 31,2000

Case Number	Location	Submission Date	Species	Total	Results
99-029	Jakey's Fork-above Dubois Hatchery	4/12/99	Rainbow and Brown Trout	35	Negative
99-032	Jakey's Fork-below Dubois Hatchery	4/12/99	Mountain Whitefish and Brown, Rainbow Trout	102	Negative
99-044	Spring Creek at Big Creek Raven	5/11/99	Rainbow Trout	2	Negative
99-045	Green River-below Fontenelle Dam	5/27/99	Mountain Whitefish, Brown, Rainbow Trout	145	Negative
99-047	Green River	5/27/99	Mountain Whitefish, Rainbow Trout	2	Negative
99-051	Green River-below City water treatment plant	5/27/99	Mountain Whitefish	1	Negative
99-053	Ten Sleep Creek	6/9/99	Brown Trout	1	Negative
99-060	N. Platte Rochelle Easement	7/6/99	Water Sample	3	Negative
99-061	N. Platte- 6 mile Gap	7/6/99	Water Sample	3	Negative
99-062	N. Platte-Treasure Island	7/6/99	Water Sample	3	Negative
99-065	Windy Gap Reservoir, Colorado	7/15/99	*TAM Sample	3	Positive
99-066	Fontenelle Reservoir	7/16/99	Kokanee Salmon, Cutthroat, Rainbow, Brown Trout	190	Positive
99-072	Laramie River	7/23/99	Brown Trout	14	Negative

*TAM= Triactinomyxon or infectious stage of the whirling disease parasite.

Table 4. cont.

Case Number	Location	Submission Date	Species	Total	Results
99-074	Ten Sleep Creek	7/29/99	Rainbow and Brown Trout	38	Negative
99-075	Leigh Creek	8/24/99	Rainbow, Brown, Brook Trout	43	Negative
99-083	Bear River at Utah state line	7/16/99	Cutthroat Trout	1	Negative
99-086	Clark's Fork Hatchery	8/24/99	Cutthroat and Rainbow Trout	64	Negative
99-088	Middle Fork Powder River	8/27/99	Rainbow and Brown Trout	75	Negative
99-089	Big Goose Creek	8/27/99	Rainbow and Brown Trout	30	Negative
99-090	Clear Creek	8/27/99	Rainbow and Brown Trout	75	Negative
99-094	North Platte River – 6 mile Gap	9/1/99	*TAM sample	1	Negative
99-102	South Piney	9/17/99	Rainbow Trout and Mountain Whitefish	36	Negative
99-103	LaBarge Creek	9/17/99	Rainbow, Brook, Brown, Cutthroat Trout, Mountain Whitefish	72	Negative
99-104	Green River	9/17/99	Rainbow and Brown Trout	82	Negative
99-105	East Fork River at Boulder R.S. access	9/17/99	Rainbow and Brown Trout	41	Negative
99-106	Pole Creek	9/17/99	Rainbow, Brown, Brook Trout	16	Negative
99-114	Soldier Creek	10/8/99	Rainbow and Brook Trout	28	Negative
99-118	Gilbert Creek	10/19/99	Brook and Cutthroat Trout	47	Negative
99-119	Little Snake River, West Branch	10/19/99	Brook Trout	12	Negative
99-120	Sulfur Creek	10/19/99	Brown and Rainbow Trout	15	Negative

*TAM= Triactinomyxon or infectious stage of the whirling disease parasite.

Table 4. cont.

Case Number	Location	Submission Date	Species	Total	Results
99-121	North Fork Little Snake River	10/18/99	Cutthroat, Rainbow Trout, Mountain Whitefish	45	Negative
99-125	Sand Creek	10/20/99	Brown Trout	65	Negative
99-126	Little Bighorn River	10/20/99	Brook Trout	95	Negative
99-127	Tongue River	10/20/99	Brown, Brook, Rainbow Trout	167	Negative
99-130	Troublesome Creek	10/21/99	Brook Trout	24	Negative
99-131	Laramie River	10/25/99	Brown Trout	24	Negative
99-133	Spring Creek	10/28/99	Rainbow Trout	59	Negative
99-134	South Piney Creek	10/21/99	Brook, Rainbow, Brown, Trout	112	Negative
99-135	Laramie River	10/25/99	Brown Trout	1	Negative
99-137	East Fork River near Boulder Rearing Station	11/10/99	Kokanee Salmon	150	Positive
99-139	Green River	11/16/99	Rainbow, Brown, Cutthroat Trout, Mountain Whitefish	28	Negative
99-143	Snake River	11/18/99	Cutthroat Trout	68	Negative
99-144	Flat Creek	11/18/99	Brook, Cutthroat, Rainbow Trout, Mountain Whitefish	71	Negative for *Myxobolus cerebralis*, *Henneguya sp.* observed in whitefish
99-145	Story Spring Pond	12/2/99	Rainbow and Brown Trout	51	Negative
99-150	Cedar Creek, tributary to the North Platte R.	12/13/99	Brown Trout	2	Negative

Table 4. cont.

Case Number	Location	Submission Date	Species	Total	Results
99-152	New Fork River	11/16/99	Rainbow and Brown Trout	72	Positive
99-153	Green River	11/16/99	Brown, Brook, Rainbow Trout	52	Negative
99-154	Fall Creek	11/16/99	Rainbow and Brook Trout	39	Negative
99-155	Green River at 7 mile bridge	11/16/99	Brown and Rainbow Trout	2	Negative
00-10	Flaming Gorge	3/3/00	Rainbow Trout	60	Negative

JOB PERFORMANCE REPORT
PROJECT SEGMENT

STATE **WYOMING**

JOB NO: FXJNSC9551

JOB TITLE: Detection of Whirling Disease in Salt River Fish by DNA - Polymerase Chain Reaction

PERIOD COVERED: **September 16, 1999 to May 15, 2000**

Abstract: Pectoral fins from 797 cutthroat trout were analyzed using DNA-PCR (polymerase chain reaction) procedures to detect the presence of the whirling disease parasite *Myxobolus cerebralis*. A total of 169 fish were positive for the parasite. In addition, 33 water samples, 16 tissue samples from old cases, and scales from Salt River fish collected in 1981 were also tested. All were negative for the presence of the parasite. A total of 350 of the fish tested by DNA-PCR were also histologically graded by Washington Animal Disease Diagnostic Laboratory (WADDL) and 211 were positive. The correlation between the histological results and the PCR results suggest that fin clips are not a viable sample for non-lethal sampling in order to detect the presence of *M. Cerebralis*.

INTRODUCTION

Native cutthroat trout in the Snake River drainage of Wyoming are known to move into streams created by large springs in order to spawn. The parasite that causes whirling disease has been found in the Salt River, a tributary to the Snake River, and tributaries to the Salt River created by large springs. It is hypothesized that the location of spawning sites of cutthroat trout in spring streams could affect their exposure to the parasite that causes whirling disease and the subsequent occurrence of the disease. Two spring streams where the parasite was known to occur were studied in 1999. High densities of the life stage that infects trout were detected in one of the streams. In that stream, the densities of infective spores and occurrence of whirling disease among small fish held in cages declined with upstream progression. This suggests that the young-of-the-year fish that migrate to the headwater areas of spring streams to spawn may be less likely to be infected by the parasite and more likely to survive. In a second study, stream densities of the parasite were very low and there was no evidence of whirling disease among young cutthroat trout (Hubert et al. 2000).

Fish collected from these streams were subjected to a relative new technique utilizing DNA-PCR as developed by Andree et al. 1997 and 1998. The objective of this was to determine if fin clip DNA-PCR analysis would be a reliable non-lethal technique to detect the presence of *Myxobolus cerebralis* and to determine the prevalence of infection in the streams. This parasite is responsible for causing whirling disease in trout. Current testing for the disease requires lethal sampling of the fish. DNA-PCR results were compared to both histological grading as conducted by WADDL and the presence of clinical signs as observed in the field.

MATERIALS AND METHODS

Five hundred eighty young-of-the-year trout were harvested from Christensen and Laker creeks, Lincoln Co. Two hundred heads were sent to WADDL for histological testing and their pectoral fins were returned to the Game and Fish Laboratory, Laramie, Wyoming, for DNA-PCR testing. Sentinel fish were placed in these creeks in two groups for later analysis. At the time of collection some of the cages had been vandalized, and one was missing. Two hundred and nine sentinal fish were recieved. One hundred fifty heads were chosen randomly from the sentinel group and sent to WADDL. The corresponding fins were tested using DNA-PCR procedures.

The free-swimming form of the whirling disease organism is a triactinomyxon (TAM). This form can enter the fish through the gills or skin when they are released from the spore present in the tubifex worm in the bottom silt of the stream. Thirty-three samples of water filtrates representing 500 gal each collected from the Salt River were subjected to DNA-PCR testing. Known TAM samples were obtained from the Colorado Division of Wildlife. These contained a known concentration of TAM organisms. These were diluted and tested to determine the minimum detection limit. The protocol used for TAM digestion and DNA extraction is a modified version of the Qiagen ™ mouse-tail protocol. Tam detection sensitivity was estimated to be about 10 TAM.

RESULTS AND DISCUSSION

The Game and Fish Laboratory received and anaylzed a total of 846 samples, which included 797 fins collected from the cutthroat trout, 33 water samples, 16 tissue samples from old cases, and scales from Salt River trout collected in 1981. One hundred sixty of the fin samples from young-of-the-year and six sentinel trout tested positive for the presence of *M. Cerebralis.* All of the other samples tested were negative. Clinical signs were present in 137 of the 494 young-of-the-year trout, and 211 were histologically positive.

Detailed results and complete description of samples appear in the final report by Hubert et al. 2000. The correlation that was observed between DNA-PCR testing results, histological grading, and observed clinical signs would suggest that PCR testing on fin clips is not a reliable non-lethal testing method. Future studies may suggest a better location for taking samples for non-lethal testing.

ACKNOWLEDGEMENTS

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JOB PERFORMANCE REPORT

PROJECT SEGMENT

State: **Wyoming**

JOB NOS: CAF and CAT

JOB TITLE: Forensic and Analytical Services

PERIOD COVERED: **June 1, 1999 - May 31, 2000**

Abstract: Various physical and biological properties were tested on 423 samples in response to 60 requests. Tests were conducted on partial animal carcasses, tissue, blood, hair, antlers, saws, gloves, hide, teeth, hamburger, rainbow fish, bones, clothing, blankets, raptor's foot, skulls, gut piles and stomach contents.

INTRODUCTION

This report summarizes the analytical and technical services performed by the Game and Fish Laboratory to aid management biologists and law enforcement personnel. Upon completion of each request, a detailed report is sent to the submitter. Results of specific analyses are on file in the Game and Fish Laboratory.

MATERIALS AND METHODS

Gross, microscopic, serologic, enzyme and DNA methods were employed and include the following:

1. Species identification of hair – microscopic examination of fibers and comparison to the hair standards collection following techniques as outlined in Moore et al. 1974.

2. Species identification of blood and tissues – Electrophoretic analysis follows protocols as outlined in Hawk et al. 1998. This includes Counter Immunoelectrophoresis (CIEP) which is an antigen-antibody reaction test to determine family level identification for pronghorn, deer, bovine, bear, canine, feline, and other important animals. Protein and stained enzyme marker systems for species identification include albumin by Western Blot, phospoglucose isomerase (PGI), erythrocyte acid phosphatase (EAP), and superoxide dismutase (SOD). These will identify samples from mule and white-tailed deer, elk, moose, pronghorn, bighorn sheep, rocky mountain goat, black and grizzly bear, mountain lion, bobcat, domestic cow, horse, sheep and pig, as well as other small game animals and most birds.

3. Gender identification of blood and tissues – DNA extraction, quantitation, polymerase chain reaction (PCR) with gender specific primers and submerged electrophoresis produced gels that are stained with ethidium bromide as outlined in Hawk et al. 1998. This will identify the sex of samples from mule and white-tailed deer, elk, moose, pronghorn, bighorn sheep, black and grizzly bear, mountain lion and bobcat.

4. Individual animal identification and tissue matching – Analysis includes DNA extraction, quantitation and DNA-PCR with appropriate family primers, electrophoresis for detection of DNA microsatellite bands and genotype analysis as outlined in Hawk et al. 1998. The resulting genotype can be used to match samples or to determine minimum number of animals present. Match probabilities are calculated (NRC 1996) from the frequency of occurrence of alleles (DNA bands) in a population of tested animals. These are determined from over 5,000 DNA animal standards present in the laboratory collection or obtained from the field. When DNA standards are not available for the area where the evidence sample originated, then *theta* must be used (NRC 1996) for calculating match probabilities.

5. Results of testing are reported in a timely manner and depends on the number of samples per case and the kinds of tests to be conducted.

6. Appearance in court to testify on the results of testing is arranged through local prosecuting legal council with a subpoena served at least five days ahead of scheduled court date.

Field personnel submit a standard evidence transmittal form, which lists items, description, collection information and requested analysis on evidence as well as database samples for the DNA standards collection. A signature chain documents the transfer of evidence with dates listing evidence transfers. DNA procedures are constantly being updated, developed, and optimized.

RESULTS

Analyses are itemized under two categories: Evidence examination for forensic cases (Table No. 1) and miscellaneous biological analyses (Table No. 2).

Evidence Examination for Forensic Cases

A total of 950 analytical tests were performed on 341 forensic samples for 51 law enforcement cases. In twelve cases, the defendants plead guilty or were found guilty in a court of law. Ten cases are pending legal action. No legal action or the legal action is unknown for 20 cases. Lab results for seven cases showed there was insufficient evidence for further legal action. One case was referred to the Game and Fish Veterinary Services Disease Laboratory for further testing. One case was dismissed for other reasons not involving the laboratory testing.

Miscellaneous Analyses

A total of 82 tests were conducted on 82 biological samples in response to nine requests from management personnel. Tests were done on hair, stomach contents, bone and tissue.

DISCUSSION

DNA Microsatellite optimization and casework is ongoing. Procedures for testing samples of elk, mule deer, white-tailed deer, mountain lion, turkeys and pronghorn are currently validated and cases involving all six species have been analyzed. Currently, laboratory personnel are optimizing moose DNA primers and will begin work on black bear DNA primers. Elk have a suite of nine DNA primers, while eight different DNA primers are optimized for deer. Pronghorn require five different DNA primers and mountain lion use 18 different DNA primers.

Mountain lion DNA microsatellites have been utilized at the Wyoming Game and Fish Laboratory for population genetic studies, as well as forensic applications. This project is being funded in part by the Wyoming Cooperative Fish and Wildlife Research Unit (CO-OP) and in part by the Wyoming Game and Fish Laboratory picking up the rest of the cost. A part time technician is doing the analysis using Wyoming Game and Fish equipment. The results have already been used by the department for forensic applications and will also be available for use in the event of a human-carnivore interaction. In this instance, the database could be used to attempt to match the mountain lion back to bite marks on a human victim. There are approximately 333 mountain lion standards to be tested throughout the state. Paternity testing will also be attempted on mountain lion samples collected from the Snowy Range mountains in Southeastern Wyoming.

Forensic personnel were involved in numerous training sessions listed as follows: Wildlife Forensics lecture to the University of Wyoming Student Chapter of theWildlife Society; DNA Botany class, Mammalogy class, and a Wildlife Management class; training on sample collection and DNA testing to a group of Colorado Conservation Officers; training of department enforcement and management personnel, and other state department officials on the use of the Carnivore Human Interaction Collection kit (CHICK); a three day training course to a Idaho Department of Fish and Game forensic employee on species and gender identification and microsatellite matching; a four-hour workshop to national scientists at the Northwest Association of Forensic Scientists meeting in Cheyenne; and two one-hour presentations to the public at the Wyoming Hunting and Fishing Heritage Expo as well as manning the laboratory booth at the EXPO. Personnel from LI-COR, the manufacturer of the DNA automated sequencer, came to the laboratory to give a three days hands-on training session, and laboratory personnel attended a symposium on whirling disease held by the National Whirling Disease Foundation in Cor d'Alene, Idaho.

DNA standards collection for the state is continuing. The goal is to collect and store at least 20 different standards per species for each region. In many cases, this has been expanded to have 20 standards per hunt area. All standards have been assigned a unique laboratory number and logged into the computer database program. Adequate numbers of standards have been received in all regions to analyze cases involving elk, mule deer, and antelope. Most regions are covered for mountain lions, white-tailed deer and moose. The standards collection numbers over 5,000 samples. It is an ongoing process for meat standards to be cut up, extracted, and tested.

Table No. 3 lists the total number of standards currently in the database. Very few DNA meat standards were submitted this fiscal year because the laboratory had sufficient standards to analyze cases in most regions for most species. The lack of storage room hinders the lab from accepting any more large number of standards from the regions. This will be the case until many DNA meat standards are processed.

ACKNOWLEDGMENTS

Appreciation is extend to the following personnel and agencies who performed or assisted in various aspects of analytical and technical work:

Dr. Danny Walker, Assistant State Archaeologist for discriminate function analysis on bones; Dr. E. S. Williams, DVM, Wyoming State Veterinary Laboratory, Dr. Walt Cook, DVM and Hank Edwards, Wyoming Game and Fish Veterinary Services Disease Laboratory for animal necropsies and numerous other analyses; Dr. John Coffin, University of Alberta for DNA expertise; and Rebecca Russell for editorial corrections of this report.

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Table 1. Evidence examined and results for forensic cases

Case No. and No. of Samples	Submitting Warden & Agency	Date Received	Sample Type and Test Requested	Gender ID	Analysis Results	Completed/No. of Tests Conducted; Court Results if known
99-18/2	DeBoer Montana FWP	4/6/99	Hair, blood and tissue for species, gender and microsatellite matching	Male	All items originated from the same male white-tailed deer.	Yes/17 Unknown
98-27/14	Demaree WGFD	5/5/99	Bones and hairs to match to previously submitted case 98-34	Male	Several items matched previously submitted items.	Yes/50 Pending court summer of 2000
99-29/4	Round WGFD	5/16/99	Three rainbow fish to determine if caught legally or snagged	N/A	It was determined by wound pathology that all fish had been snagged.	Yes/7 Plead guilty
99-30/6	Gilbert WGFD	6/15/99	Three tissue samples, carcass and antlers for species, gender and microsatellite matching	Male	All items originated from same male mule deer.	Yes/26 $5,000, three year loss of privileges, 6 months in jail (suspended)
99-32/8	Haley WGFD	6/2/99	Deer hair and bones to match to previously submitted case 97-38	Male and Female	Items originated from male and female mule deer. Original case was a white-tailed deer.	Yes/14 Was not a case after testing was completed
99-33/3	Antolini West Virginia DNR	7/6/99 &12/1/99	Hairs and skin tissue for species, gender and microsatellite matching	Male	Two items originated from same male WTD, third item was inconclusive.	Yes/34 Unknown
99-36/17	Nelson WGFD	7/16, 7/19, and 7/29	Blood from kill site, hair, clothing, carcass, cotton swabs for species, gender and microsatellite matching	Male	Eleven items originated from the same male pronghorn, other items inconclusive.	Yes/72 Two defendants, both were fined $5,000 for out of season, $1,000 for wanton destruction and $750 court costs and privileges revoked for 6 years
99-37/1	Demaree WGFD	7/26/99	Match to previously submitted case 98-34	Female	This item identified as elk, other items to match to were male.	Yes/3 Not a case as it was identified as originating from a female

Table 1. cont..

Case No. and No. of Samples	Submitting Warden & Agency	Date Received	Sample Type and Test Requested	Gender ID	Analysis Results	Completed; No. of Tests Conducted; Court Results if known
99-38/4	DeSomber WGFD	8/11/99	Blanket, hair, knife and raptor foot for species identification	N/A	No biological material could be located on the knife or blanket. Antelope hair found on blanket. Foot belongs to great horned owl.	Yes/22 Unknown
99-39/1	Lebert WGFD	8/10/99	Hair from truck for species identification	N/A	Hairs identified as mule deer.	Yes/1 Unknown
99-40/4	Edberg WGFD	8/24/00	Three meat samples and a rib cage to determine if they came from same animal	female	All items originate from the same female white-tailed deer. Match probability of >1 in one million.	Yes/18 Case thrown out of court on double jeopardy charge
99-41/4	Pallister WGFD	9/8/99	Deer carcass, trousers, knife and leatherman tool to determine if biologicals exists and match them	Female	All items originate from the same female mule deer. Match probability of one in 185,000.	Yes/30 Found guilty
99-42/12	Conner Montana FWP	9/9/99 & 10/1/99	Extracted DNA and blood to match	Male and female	A minimum of four elk present. Species and gender testing done at the Ashland lab.	Yes/20 Unknown
99-44/1	Matson Wyo. Dept. Ag.	9/14/99	Elk burger to determine if any domestic animal present	N/A	No sheep, beef or pork could be detected.	Yes/2 After testing, was determined no violation had occurred
99-47/2	Schaefer Colorado DW	10/7/99	Skull and elk hide to determine if shot with bullet	N/A	Examination done and reported by WSVL.	Yes/0 Unknown
99-48/2	Lebert WGFD	10/7/99	Gut pile and suspect hair to do species, gender and microsatellite matching	Female	Tissue sample originated from female pronghorn, suspect hairs were actually cotton fibers.	Yes/16 Is not a case without a match

Table 1. cont.

Case No. and No. of Samples	Submitting Warden & Agency	Date Received	Sample Type and Test Requested	Gender ID	Analysis Results	Completed/No. of Tests Conducted; Court Results if known
99-49/11	Lund WGFD	10/10/99	Blood swabs, sweater, towel, hair samples, antlers and gut pile to do species, gender and microsatellite matching	Male	Seven different male mule deer were present.	Yes/53 Pending
99-50/1	Herbel WGFD	10/12/99	Vial of unknown substance to determine if it is a poison	N/A	Sample was sent to WSVL for analysis and they did the reporting.	Yes/0 Unknown
99-51/2	Basagoitia WGFD	10/20/99	Meat and blood for species, gender and microsatellite matching	Male	Both items originated from the same male elk with a match probability of one in 1,135.	Yes/14 Pending, new items submitted in 6/00 to determine if they match these previously submitted items
99-52/5	Cram WGFD	10/23/99	Tissue from carcass and gut pile to do species, gender and microsatellite matching	Male	All items originated from the same male elk with a match probability of one in 3, 884,000.	Yes/24 Found guilty
99-53/1	Marks Montana FWP	10/28/99	Vest with blood in pocket to determine if it came from pheasant, grouse or partridge.	N/A	Testing was started, defendant plead guilty before testing was completed.	Yes/1 Plead guilty
99-54/1	Bashford WGFD	11/1/99	Spike elk head with attached skin to determine how many times the animal was shot	N/A	All testing done at WSVL, who reported the results.	Yes/0
99-55/15	Sax WGFD	11/1/99	Blood, tissue, hide knife, hatchet, rag, head cape and antlers for species, gender and microsatellite matching	Male	Two different male elk were identified.	Yes/31 Fined $1,000 for wanton destruction, $750 for helicopter hazing, $4,000 restitution and loss of privileges for 10 years

Table 1. cont.

Case No. and No. of Samples	Submitting Warden & Agency	Date Received	Sample Type and Test Requested	Gender ID	Analysis Results	Completed/No. of Tests Conducted; Court Results if known
99-56/8	Sarason Colorado DW	11/1/99	Hat, gloves, hair, bone saw, jacket, towel and meat sample for species, gender and microsatellite matching	Male	Biological material on clothing and bone saw were degraded to match to meat sample.	Yes/37 Plead guilty, $12,000 in fines and loss of privileges for 5 years
99-57/1	Harper Colorado DW	11/9/99	Elk meat sample for gender identification	Male	Species was known, only need to know gender.	Yes/2 Was fined for failure to leave evidence of sex attached but had a license for a bull elk so there was violation
99-59/2	Lane Utah DWR	11/10/99	Two tissue samples from elk for gender and microsatellite matching	Male	Both items originated from the same male elk with a match probability of one in 35,700.	Yes/7 Unknown
99-60/1	Haeger Colorado DW	11/17/99	Goat hair with suspect mountain lion saliva	N/A	No mountain lion saliva could be detected.	Yes/5 Unknown
99-61/2	Kroening Colorado DW	11/17/99	Two meat samples for species and gender determination	Male	Both items originated from male elk.	Yes/4 Fined for wrong gender animal
99-62/5	Gumber Colorado DW	11/17/99	Five tissue samples for species, gender and microsatellite matching	Female	Two different female elk were represented in the samples with a match probability of one in 30,000 and one in 43,000.	Yes/28 Unknown
99-64/15	Jauch Alvin Texas PD	11/11/99	Two slides with hair for identification and 13 photographs	N/A	Hair identified as human or Bovidae.	Yes/15 Unknown
99-65/2	Brown USFWS	12/6/99	Hair and extracted DNA for microsatellite match	Male	Both items sent to the US Fish and Wildlife Forensic Laboratory in Ashland, OR.	Unknown

Table 1. cont.

Case No. and No. of Samples	Submitting Warden & Agency	Date Received	Sample Type and Test Requested	Gender ID	Analysis Results	Completed/No. of Tests Conducted; Court Results if known
99-66/2	Smith WGFD	12/6/99	Two tissue samples for microsatellite matching	N/A	The two items originated from separate male elk.	Yes/10 Unknown
99-67/38	Robertson WGFD	12/8/99 & 3/28/00	Hair samples, brain, liver, blood swabs, meat and bone saw for species, gender and microsatellite matching	Male	Elk and mule deer were detected. Submitting warden not interested in elk but three different male mule deer present	Yes/108
99-68/2	Wyllie U. W. COOP	12/13/99	Deer collar to hold for possible match later	N/A	N/A	Hold/0
99-69/1	Schmidlin WGFD	12/99	Tissue sample from white-tailed deer to hold for possible match later	N/A	N/A	Hold/0
00-01/3	Lonneman Iowa DNR	1/5/00	Three tissue samples for species, gender and microsatellite matching	Male	Two different male white-tailed deer present with a match probability of greater than one in one million	Yes/13 Unknown
00-02/3	Sprecher Washing-ton SDFW	1/5/00	Three tissue samples for species, gender and microsatellite matching	Male	All items originated from the same male mule deer with a match probability of one in 235,000	Yes/13 Pending
00-03/10	Queen WGFD	1/12/00	Blood hair and tissue sample for species, gender and microsatellite matching	Male	Two different male mountain lions were present. The blood samples from drag trail only represented one animal.	Yes/25 Defendant plead guilty to taking of lion from wrong area; court pending for fines and restitution.
00-04/6	Penske Idaho FG	1/20/00	Hair, blood and meat samples for species, gender and microsatellite matching	Male	All items originated from the same male pronghorn with a match probability of one in 50,000.	Yes/19 Fined $400 + court costs; informal probation for one year and 5 days in jail (suspended)

Table 1. cont.

Case No. and No. of Samples	Submitting Warden & Agency	Date Received	Sample Type and Test Requested	Gender ID	Analysis Results	Completed/No. of Tests Conducted; Court Results if known
00-05/5	Edwards South Dakota FW	1/28/00	Blood, hair, hunting license and tissue sample for species, gender and microsatellite matching	Male and female	Two male white-tailed deer and one female white-tailed deer present with a match probability of greater than one in one million.	Yes/25 Unknown
00-06/4	Hutton Missouri DC	1/28/00	Cotton swab from Holstein calf and bone fragment from calf to determine if can find canine or feline saliva present	N/A	Canine family biological material found on two of the swabs.	Yes/9 Unknown
00-07/25	Scarafiotti New Mexico GF	2/23/00	Packages of meat for species, gender and microsatellite matching	Male	Two packages were determined to originate from beef, three packages originated from two separate pronghorn, and six male mule deer were represented.	Yes/50 Unknown
00-08/13	Zebro Wisconson DNR	3/22/00	Blood samples for species, gender and microsatellite matching	Male	Three male white-tailed deer were represented with a match probability of one in 500,000.	Yes/41 Unknown
00-09/2	Schamber Wyo.Dept. Ag.	3/9/00	Hair samples and a premolar tooth for species identification	N/A	Hair originated from chipmunk or ground squirrel. Tooth was from a carnivore.	Yes/2 Unknown
00-10/4	Claassen Colorado DW	3/17/00	Antler and tissue samples for species, gender and microsatellite matching	Male	Two male mule deer represented with a match probability of one in ten million	Yes/15 Unknown
00-11/3	Schulze Utah DW	3/22/00	Tissue and skull plates for species, gender and microsatellite matching	Male	All items originated from same male mule deer, a match probability of one in ten million.	Yes/17 Unknown

Table 1. cont.

Case No. and No. of Samples	Submitting Warden & Agency	Date Received	Sample Type and Test Requested	Gender ID	Analysis Results	Completed/No. of Tests Conducted; Court Results if known
00-12/11	Ehrlich Colorado DW	4/4/00	Blood stains, jacket, pants, jeans, knives, meat and lung tissue for species, gender and microsatellite matching	Male	All items originated from the same male elk with a match probability of one in one million.	Yes/29 Unknown
00-13/3	Seigliano WGFD	4/25/00	Blood and hair for species and gender determination	Male	All items originate from male pronghorn.	Yes/6 Unknown
00-15/1	Trebelcock WGFD	5/1/00	Hatchet to determine origin of biological material	N/A	No biological material detected.	Yes/2 Not a case as nothing detected
00-16/4	Choma WGFD	5/11/00	Feathers and tissue sample from turkey for microsatellite matching	N/A	Not started.	No/0
00-17/5	Heicher Colorado DW	5/15/00	Tissue for species, gender and microsatellite matching	Female	All items identified as elk, DNA microsatellite testing not started.	No/7
00-18/6 46 sub-samples	Miller Colorado DW	5/15/00	Freezer search recovered six packages of meat pieces which were subsampled	Male	Species identification complete with all items identified as elk or mule deer, DNA testing not yet complete.	No/62
00-19/9	Conner Montana FWP	5/25/00	Seven samples from bear bait station and two stomach contents of bear for species, gender and microsatellite matching	Male	All items identified as male beef. DNA microsatellite matching has not been started.	No/11

Table 2. Miscellaneous analyses on management samples conducted at the Game and Fish Laboratory

Case No. and No. of Samples	Submitting Person & Agency	Date Received	Sample Type and Test Requested	Gender ID	Analysis Results	Completed/No. of Tests Conducted
99-31/11	Smith USFW	6/21/99	Hair for species ID	N/A	Hairs identified as Canine family, most probably wolf, black bear and fox.	Yes/11
99-34/14	Sawyer COOP	7/15/99	Hair from pronghorn kill site for species ID	N/A	Red fox or coyote.	Yes/14
99-35/2	Bruscino WGFD	7/15/99	Stomach contents and scat from grizzly bear to determine if killing cattle	NA	Stomach contents revealed vegetative material, hair, bone, cartilage, and ungulate calf tooth as well as tissue that tested positive for Bovidae.	Yes/4
99-43/34	Whitman Hornacker	9/8/99	Tissue samples from calf elk and fawn mule deer mortality sites for gender determination	Male and female	18 male elk, nine female elk, five male mule deer, one female mule deer and one male of known species.	Yes/34
99-45/14	Smith USFW	9/16/99	Hair samples from elk calf mortality sites for species ID	N/A	Coyote, black bear, grizzly bear, mountain lion and bobcat	Yes/14
99-46/1	Schaefer CDW	10/5/99	Bear hair from sighting for species identification	N/A	Black bear	Yes/1
99-58/1	Rudd WGFD	11/11/99	Bear tissue for gender determination	Female	Determined to be from a female black bear.	Yes/1
99-63/1	Nixon WGFD	12/6/99	Bone for species identification	N/A	Dr. Danny Walker did the analysis and reported results.	Yes/0
00-14/1	Shanks Orgeon, Grad Student	4/18/00	Hair on stone tool for species identification	N/A	Pronghorn	No/3

Table 3. Total Number of DNA Meat Standards Currently in DNA Standards Database by Region

Species	Region							TOTALS **
	Jackson	Cody	Sheridan	Green River	Laramie	Lander	Casper	
Elk	151	216	211	77	81	92	49	**1063**
Antelope	58	80	152	221	229	175	195	**1184**
Mule Deer	96	302	256	86	187	151	141	**1377**
Moose	99	8	30	26	5	18	0	**191**
White-tailed Deer	1	55	83	0	46	1	73	**321**
Bighorn Sheep	66	190	0	0	0	124	3	**275**
Black Bear	103	46	59	30	24	50	3	**357**
Mountain Lion	22	45	37	26	114	2	27	**333**
Total number of standards in the laboratory								**5,101 ****

** Totals for each species will not equal those in this column because several hunt areas are shared by several regions and in these cases, credit is given to each region for those samples. Also, numerous samples were received without enough information to place them into a region but are included in the total number in the lab.

JOB PERFORMANCE REPORT

PROJECT SEGMENT

STATE: **Wyoming**

JOB NOS: BA and HASWCAY510

JOB TITLE: Animal Tooth Aging

PERIOD COVERED: **June 1, 1999 to May 31, 2000**

Abstract: Cementum annuli tooth aging procedures were conducted on 5,752 different teeth samples from twelve species received in 249 separate shipments from 54 submitters. The submitters included biologists, hunters, wardens and regional offices. Over 282 requests for tooth age reports, for several species of game animals, were completed and sent to biologists and regional coordinators. A total of 2,370 age information cards were sent in response to hunter requests.

INTRODUCTION

This report summarizes the animal tooth aging services performed by the Game and Fish Laboratory to aid biologists in determining age structure and population dynamics for different species of Wyoming game animals. Upon completion of analyses, detailed reports are sent to regional wildlife coordinators and biologists. Results of specific analyses are on file in the laboratory.

MATERIALS AND METHODS

Teeth are sent to the lab in individual "tooth return" envelopes. Hunters are requested to provide information on: animal's gender, date of harvest, hunt area, region, location of kill, name, address, and license number. Upon arrival, all teeth are given a unique laboratory identification number and the above information is entered into a computer database. Teeth from elk, moose, and bison are examined and the root tips removed for further processing. Sharp occlusal surfaces, small size and open apexes help identify calves and yearlings. Once identified and noted as such, these teeth are removed from processing. The remaining teeth are placed in individual "bags" and soaked in a buffered, hydrochloric acid based decalcification solution until they are softened (24 - 72 hours depending on species). After rinsing for 24 hours in tap water, the teeth are placed into a cassette and prepared for paraffin embedding. This step requires a series of alcohol, xylene, and paraffin baths in an Autotechnicon®, followed by paraffin treatment in a vacuum infiltrator. Finally, the teeth are mounted into blocks at a Tissue Tek II® paraffin-embedding center. After hardening, the block is ready to be sectioned. Using a Reichert-Jung Histocut®, the embedded teeth are "faced" and several sections are taken from a tooth block. The two "best" sections are placed onto a labeled slide and allowed to dry on a warming plate overnight. Slides are then deparaffinized by a second series of alcohol and xylene baths. After drying, slides are stained in a Giemsa staining solution.

Currently, mule deer, white-tailed deer, and bobcat teeth samples are frozen sectioned after decalcification. This procedure requires a Reichert-Jung® Cryocut kept at -18^0 C. Sections are mounted on labeled slides, allowed to dry overnight, and stained using Giemsa staining solution. All stained slides are allowed to dry overnight before coverslipping. Each slide is then examined under a microscope at 40X or greater magnification. The number of cementum annuli of each tooth section is counted. Cementum annuli age determination for various species follows the models developed and published by Matson (1981).

The number of annuli, the age of calf and yearling teeth determined by inspection, and the animals' calculated ages are entered into the computer database. Cementum annuli aging reports are sent to all wildlife coordinators and regional biologists. Finished reports include information provided by the hunter as well as age data. Reports are made available by hard copy, computer disc, and by Group Wise e-mail. The laboratory maintains results of past and present tooth age analyses.

RESULTS AND DISCUSSION

Analyses are itemized under two categories: Aging of animal teeth (Table 1) and hunter's requests for age of harvested animals (Table 2). The 5,752 individual tooth ages are kept on file at the laboratory and are not included in this report.

Tests were conducted on 5,752 different teeth samples from twelve species of game animals. Together, elk and moose samples accounted for almost fifty percent of all the teeth analyzed.

Laboratory personnel and a private lab (Matson's in Milltown, Montana) completed the aging of 172 mountain lion teeth from the 1997 and 1998 season. These teeth were originally sent to Matson's Laboratory in Montana for processing and aging. Matson returns the slides and teeth, and inter-lab ages are completed and compared. Validations of mountain lion tooth ages using the cementum annuli aging process are ongoing. This year 197 mountain lion teeth will again be submitted for processing and aging to Matson's Laboratory and tooth age results compared.

A total of 2,370 postcards were sent in response to hunter requests for age data on their harvested animal. During the 1999 - 2000 hunting season every hunter submitting teeth from moose, elk, mule deer, mountain lion, and bear was provided with age determination information when a full mailing address was available.

At their requests criteria and procedures information were sent to two scientists this year. Elephant seal aging information was sent to Amaury Cordero Tapia of Mexico University, and the Wyoming Game and Fish Tooth Aging Manual was sent to Abdallah Mardinin of Quebec, Canada.

ACKNOWLEDGMENTS

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Table 1. Summary of animal teeth aging at the Game and Fish Laboratory for 1999-2000.

Species	Total Number	Age Range (Years)
Antelope	79	3.1 - 9.3
Bighorn Sheep	3	9.3 - 10.3
Bison	11	4.1 - 16.5
Black Bear, Fall	79	1.3 - 31.3
Black Bear, Spring	124	1.3 - 14.2
Bobcat	1390	0.5 - 16
Elk	1961	0.5 - 29.6
Grizzly Bear	32	0.5 - 17.4
Moose	751	0.5 - 15.3
Mountain Lion	203	0.5 - 12
Mule Deer	955	1 - 12.3
Coyote	32	1-9
White-tailed Deer	132	1.3 - 9.3
TOTAL	**5752**	

Table 2. Number of hunter requests for animal age information provided by the Game and Fish Laboratory for the 1999-2000 season.

Species	Total Number of Hunter Age Information Postcards Mailed
Elk	881
Moose	730
Mule Deer	372
Antelope	11
Fall Black Bear	69
Bobcat	0
Bighorn Sheep	3
Spring Black Bear	119
Bison	11
Mountain Lion 1998	153
White-tailed Deer	21
TOTAL	**2370**

JOB PERFORMANCE REPORT

PROJECT SEGMENT

STATE **Wyoming**

JOB NO: FXCREP1510

JOB TITLE: Extraction and Decoding of Binary Coded Wire Tags in Fish

PERIOD COVERED: **April 1, 1999 to March 31, 2000**

Abstract: Seven hundred and fifty six whole or partial fish heads, in numbered bags, were received from fisheries biologists. All samples were recovered from the North Platte River system. Of the 756 sample bags listed on recovery forms, 749 actual samples were received. Of those physically received, 142 (18.9%) contained no tag, 11(1.5%) were lost, and seven (0.9%) were cut too short to read. The data from the remaining 606 was successfully decoded from the tags, and was sent to fisheries biologists in Casper for analysis.

INTRODUCTION

The objective of this study is to supplement information available to fisheries biologists by tagging fish that are stocked in various areas of the North Platte River system including Seminoe, Pathfinder, Alcova reservoirs, Grey Reef and Miracle Mile river sections. When these fish are recovered, they can then be identified and their growth and movement through the system analyzed. Fisheries personnel in the Casper, Wyoming area implant minute binary coded wire tags, supplied by Northwest Marine Technology, in the snouts of various strains of trout, and finclip each tagged fish prior to stocking. The finclip is used to identify tagged fish during the recovery process. Heads from collected fish are then frozen and sent in numbered bags to the Services Division, Game and Fish Laboratory, Laramie, Wyoming for extraction and decoding. The decoded data consists of an agency code and two data words that indicate stocking group and location. This data is then used to obtain biological information regarding the recovered fish which is analyzed by fisheries biologists in Casper.

MATERIALS AND METHODS

Frozen heads in individually numbered Ziploc® bags were passed through a field sampling detector to determine if each sample contained a tag. Heads that did not elicit a response ("deep-sound") were discarded. Heads that did test positive were dissected until the coded wire tag was located. The tag was removed from tissue with fine forceps, rolled on a paper towel to clean, and attached with tape on a form next to the recorded bag number and recovery data for that tag.

The tag was then removed from the mounting tape, placed on the magnetic holder and examined with a dissecting microscope at 40 X magnification. Instructions for decoding of these notches are listed in Binary Coded Tag Format, Northwest Marine Technologies Inc., revised 10/1988. Notches on the surface of each tag represent a binary number. These are arranged on the tag to represent a "data word". After the master word is located it is used as a template to decipher the three integers from the first and second data words and the agency code. These are recorded as a series of numbers on the form next to the actual tag. The decoded numbers are then compared against a list of possible number codes from stocked fish. Any tag numbers with codes not on the list are reread.

Copies of the forms with the decoded numbers were then sent to the fisheries biologist in Casper, Wyoming for data entry analysis. The original forms with the tags were retained in the Game and Fish Laboratory.

RESULTS AND DISCUSSION

The Game and Fish Laboratory has received 756 whole or partial samples from the North Platte River system, mainly Alcova, Seminoe, Pathfinder reservoirs, Grey Reef and Miracle Mile river sections. One hundred and forty-two tags (18.9%) failed to register on the field sampling detector, indicating tags were not present. These heads were not dissected. Eleven (1.5%) coded wire tags were lost at some point during the extraction and decoding process. Of the extracted tags, seven (0.9%) were cut too short to read. A total of 606 coded wire tags were successfully decoded and their numbers verified with the stocking sheet. Of the 296 distinct tag number lots used in fish stocked in the various waters, 276 (93%) have been recovered.

The loss of tags during extraction and decoding was minimal and this has decreased over the course of the study. Various ongoing techniques have been implemented to reduce tag loss. The reduction in the number of samples (2,408 in 97-98, 797 in 98-99, and 750 in 99-2000) indicates the eventual phasing out of this project.

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