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October 10, 1996

Dr. Robert Behnke
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Dr. Behnke,

Enclosed in this packet are five full copies of my thesis. Two copies are for the graduate school, one copy for each committee member and one copy for the department. Judy told me that if you give her the copies for each committee member and the department, then she would see to it that they are all bound in spiral notebooks. She said this in reference to the department copy, but I think she also said that she would do this for the committee member's copies as well. I made the changes you suggested and I found a sentence at the end of the first paragraph, second page that was redundant so I eliminated it from the thesis. In the last paragraph, first sentence on page 15, I claimed RS is native to North America. I believe this is proved by the findings of RS in places where no stocking has ever occurred (and where no stocked fish could have ever come into close proximity to the populations in question). I have stated that RS is probably native to Colorado. I cannot prove that statement, but the evidence supports that conclusion. Since RS is native to North America, it is probable that RS and trout coevolved.

Each copy of the thesis is in its own labeled envelope. Will you see to it that they make the rounds and that all committee members and Dr. Covich sign off on them? Also could you see to it, once all signatures have been obtained, that they are delivered to the proper persons? I would appreciate that very much.

For the thesis copy that I originally sent you, replace pages ix, 1 through 15, and 30 through 32, with the copies that I am enclosing. I suppose that the extra signature page needs to be signed by all committee members and Dr. Covich as well, but this is not stated in the thesis manual. The extra signature page and the two extra title pages then need to accompany the two copies of the thesis that go to the graduate school.

Thank-you for all the time that you have given me in this endeavor. You have helped me broaden my thinking. I have learned not only from doing the research but also from the mistakes that I have made, especially in writing the thesis. Every time I look at the thesis, I see things that I would like to change. These are mostly window dressing at this point, however and I don't think it productive to make all the little changes that I would desire.

Sincerely,
Bob

Bob Kingswood

[Kingswood Env.]

7 bottom
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NW Terr. & Can. and British Col.

last sentence p. 15 all trout waters
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send 5 copies,
3 for comm.
1 " Dept.
1 for grad school
sign all -

bound?

Bob Kingswood
1300 Bluemont Scenic Dr.
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October 3, 1996

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Dr. Behnke,

Enclosed is one copy of my thesis. I have made all the changes that Drs. Flickinger and Ellis requested, at least I think I have met their wishes. I have also reviewed the thesis manual and as much as possible, put the thesis into the proper layout and format. The only instance in which I have veered from the manual is in the page numbering of Tables 1, 2, 3, and Appendix B. The manual states that when tables are turned sideways, the page numbers are to remain in the same position as in the rest of the thesis. Unfortunately, my computer will not do that, so I have all page numbers in the bottom center, <u>when reading that page</u>. I do not think this one deviation will matter that much and hopefully you will agree and can sign off on the thesis in its present form.

The addition Dr. Ellis requested is on page 7, the last sentence of the first paragraph. Dr. Flickinger's changes have been made throughout the document. He was concerned about the formatting for the tables. One item he mentioned was to underline the column headings. I have done this for the tables. For Appendix B, however, I could not underline the headings (the spreadsheets were in quattro pro and I could not convert them to word perfect), so I put the column headings in bold.

If you or the other committee members want me to change anything, let me know and I will do so. If you think the thesis is good enough in its present form, then don't write on it. I will send you five more copies, to make six in all (one each for you, Dr. Flickinger, Dr. Ellis and the department and two for the graduate school). Thank-you for all of you time. I hope that your health stays good.

Sincerely,

Bob Kingswood

Bob Kingswood

THESIS

THE RANGE AND EFFECT OF *RENIBACTERIUM SALMONINARUM* ON TROUT, IN COLORADO

Submitted by

Robert W. Kingswood

Department of Fishery and Wildlife Biology

In partial fulfillment of the requirements

for the Degree of Master of Science

Colorado State University

Ft. Collins, Colorado

Fall 1996

COLORADO STATE UNIVERSITY

October 15, 1996

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY ROBERT W. KINGSWOOD ENTITLED THE RANGE AND EFFECT OF *RENIBACTERIUM SALMONINARUM* ON TROUT, IN COLORADO BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work

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Advisor

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Department Head

ABSTRACT OF THESIS

THE RANGE AND EFFECT OF *RENIBACTERIUM SALMONINARUM* ON TROUT, IN COLORADO

Renibacterium salmoninarum, the causal agent of bacterial kidney disease of salmonids, historically has been considered a serious threat to salmonid fisheries. Due to the large economic impact, most research has focused on the effects of the bacterium on Pacific salmon, while very little research has studied the effects of *Renibacterium salmoninarum* on individuals or populations of inland trout. State wildlife agencies have mandates to manage fishery resources, and thus, have enacted regulations that limit the movement of fish or gametes from hatcheries or populations that have been found positive for *Renibacterium salmoninarum*. This limitation of movement negatively affects private aquaculture, state and federal stocking programs, and programs that are designed to enhance endangered or threatened species or species of special concern. The object of this study is to determine where *Renibacterium salmoninarum* exists in Colorado and what effects it has on wild trout.

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ACKNOWLEDGMENTS

I would like to take this opportunity to thank those people that made this study possible. I would like to thank Larry Harris of the Colorado Division of Wildlife for providing funding and support. Thanks go to Drs. Robert Behnke, Stephen Flickinger, Robert Ellis and David Getzy for their help and patience in guiding me through this process. A tremendous amount of work was conducted by Crystal Hudson of the U. S. Fish and Wildlife Service's, Fish Health Center, in Ft. Morgan, Colorado. Bruce Rosenlund, also with the U. S. Fish and Wildlife Service, was most helpful in assisting in the collection of fish from Rocky Mountain National Park. Thank-you Crystal and Bruce. Finally I would like to thank my wife, Linda Drees, for taking on extra work around the house so I could have time to work on this project and for her critical comments of the thesis.

Dedicated to my wife and my daughter:

Linda R. Drees and Marianna Irene Drees Kingswood

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Key to abbreviations

BKD	bacterial kidney disease
BKT	brook trout (*Salvelinus fontinalis*)
BNT	brown trout (*Salmo trutta*)
BUL	bull trout
CDOW	Colorado Division of Wildlife
CUT	cutthroat trout (*Oncorhynchus clarki*)
DFAT	direct fluorescent antibody test
ELISA	enzyme-linked immunosorbent assay
G	gravid
GOT	golden trout (*Oncorhynchus aguabonita*)
IFAT	indirect fluorescent antibody test
IMM	immature
KOE	kokanee salmon (*Oncorhynchus nerka*)
LAT	lake trout (*Salvelinus namaycush*)
MF-FAT	membrane filtration fluorescent antibody test
NA	not applicable/not available
NFH	national fish hatchery
RBT	rainbow trout (*Oncorhynchus mykiss*)
RS	*Renibacterium salmoninarum*
SFH	state fish hatchery
SPL	splake (lake x brook trout)
STT	steelhead trout (*Oncorhynchus mykiss*)

INTRODUCTION

Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum* (RS), is regarded as a serious disease of wild and cultured salmonids (Fryer and Sanders 1981; Bullock and Herman 1988; Evenden et al. 1993; Fryer and Lannen 1993). Exhaustive summaries of the etiological agent, geographic distribution, susceptible species and epizootiology can be found in papers by Fryer and Sanders 1981; Austin and Rayment 1985; Bullock and Herman 1988; Evenden et al. 1993; and Fryer and Lannen 1993. I will only reiterate those aspects of the pathology important to my study.

Renibacterium salmoninarum is a gram positive bacterium and an obligate intracellular parasite. It is fastidious in its requirements, being very difficult to culture, even on specialized media (Fryer and Lannen 1993). Despite decades of work on developing a suitable growth medium, it can still take three weeks or longer to achieve growth (Evelyn et al. 1989; Evelyn and Prosperi-Porta 1989; Evelyn et al. 1990; Benediktsdottir et al 1991; Olsen et al. 1992).

There is little variation in biochemical properties among RS isolates. Its virulence has been correlated to the presence of a 57 kDa extracellular protein. However, the factor contributing most to the pathogenicity of RS may be the ability to survive and perhaps replicate within host macrophages (Fryer and Lannen 1993).

RS has been detected wherever salmonids are found with the possible exception of Australia and New Zealand (Bullock and Herman 1988). In North America, RS has been found wherever salmonids are cultured (Fryer and Lannen 1993) and it has also been found where no stocking has ever occurred: Alaska (T. Meyers, Alaska Dept. of Fish and

Game, personal communication), the Northwest Territories of Canada (Souter et al. 1987), and British Columbia, Canada (Evelyn et al. 1973). Natural outbreaks of BKD are limited to members of the family Salmonidae (Fryer and Lannen 1993). Fryer and Lannen (1993) report that it has been detected in the genera *Oncorhynchus, Salvelinus and Salmo* as well as other genera of the family Salmonidae. Although BKD primarily affects fish in culture facilities, wild fish can also be affected.

Horizontal (Mitchum and Sherman 1981; Fryer and Lannen 1993) and vertical transmission (Evelyn et al. 1984; Bruno and Munro 1986; Evelyn et al. 1986; Lee and Evelyn 1989) of RS have been demonstrated. There is evidence to show that once RS has been introduced into an environment, it will persist indefinitely, as long as suitable hosts are present (Evelyn et al. 1973; Mitchum et al. 1979; Mitchum and Sherman 1981; Souter et al. 1987).

External pathology associated with BKD is variable and includes exophthalmia, abdominal distension, superficial blebs or blisters, hemorrhagic areas and deep abscesses on various parts of the body. Internally, BKD is usually a systemic infection with a marked affinity for kidney tissue. During early stages of the disease, grayish-white lesions may be found on the ventral side of the kidney or on other organs such as the liver or spleen. As the disease advances the kidney can be swollen, grayish-white, and necrotic (Smith 1964; Fryer and Sanders 1981; Austin and Rayment 1985; Bullock and Herman 1988; Evelyn 1988; Sanders and Fryer 1988; Evenden et al. 1993; Fryer and Lannen 1993).

Detection of RS has been a field of major research in the past. The conventional method for definitive diagnosis of a bacterial pathogen is to culture the bacterium and identify it by its ability (or inability) to metabolize selected compounds. This method, although satisfactory for laboratory study, is inadequate for the rapid detection of RS in the hatchery when stocking or spawning schedules have to be met. Initial isolation of RS may take up to six weeks (Fryer and Sanders 1981; Evelyn et al. 1989; Evelyn and Prosperi-Porta 1989; Evelyn et al. 1990; Benediktsdottir et al. 1991; Olsen et al 1992). A suitable growth medium (KDM2) was created in the 1970's (Evelyn 1977) and research has improved its consistency and reduced the amount of time needed for initial isolation (Evelyn et al. 1989; Evelyn et al. 1990). Despite this, it can still take three weeks or longer to achieve growth (Evelyn et al. 1989).

Because the isolation of RS is time consuming, it is not suitable for the rapid detection needed under field conditions (Bullock et al. 1980). Simple gram staining was initially used in the presumptive diagnosis of RS. Serological techniques have been used since the 1970's: IFAT (indirect fluorescent antibody test) was first developed for RS in 1975 (Bullock and Stuckey); in 1980 Bullock et al. developed a direct fluorescent antibody test (DFAT) that was more sensitive and faster than the IFAT technique; Cipriano et al. adapted a counterimmunoelectrophoresis assay (CIE) and compared it with other methods in 1985; a membrane-filtration DFAT technique (MF-FAT) was developed by Elliott and Barilla in 1987; and an enzyme-linked immunosorbent assay (ELISA) was adapted for use in detecting RS in 1986 (Dixon 1987; Pascho and Mulcahy 1987; Hsu et al. 1991; Gudmundsdottir et al.1993). Of these methods, the ELISA technique is the most sensitive with the MF-FAT technique only slightly less sensitive, for detecting RS (Dixon

1987; Pascho et al. 1987).

DFAT has the ability to detect RS when the level of infection is at least 10,000 bacterial cells per gram of fish tissue (Bullock et al. 1980). With homogenization and centrifugation, levels as low as 1,000 cells per gram of fish tissue have been detected by IFAT (Sakai et al. 1989). MF-FAT can detect levels as low as 100 cells per gram of tissue (Elliott and Barila 1987; Lee 1989), while ELISA can detect levels at 2-20 ng/ml (Pascho and Mulcahy 1987). ELISA sensitivity levels are not convertible to number of cells per gram of tissue, however, several researchers have found ELISA to be more sensitive than MF-FAT, DFAT, IFAT, culture, agarose gel immunodiffusion, counterimmunoelectrophoresis, staphylococcal co-agglutination, and latex agglutination (Dixon 1987a; Pascho et al. 1987; Hsu et al. 1991; Gudmundsdottir et al. 1993).

Most states in the Rocky Mountain region use DFAT for the detection of RS (L. Drees, U. S. Fish and Wildlife Service, personal communication), even though its ability to detect RS at low levels is questionable. The sole use of DFAT in detecting RS leaves open the probability that low level positive carrier fish have been classified as negative for RS. Furthermore, it is highly probable that such carrier fish have been stocked widely in terms of space and time.

Gowan et al. (1994) demonstrated that stream trout move with greater frequency and greater distances than commonly thought. When an organism moves it also carries its pathogens. Despite using inadequate detection techniques, many states attempt to regulate the movement of RS positive fish. These regulations negatively affect private aquaculture, state and federal stocking programs and programs designed to enhance populations of threatened or endangered species or species of special concern (hereafter referred to simply as species of special concern). What are the justifications for these regulations? Commonly it is: "Until we know more, we should be conservative in our approach." Some fish health workers have questioned whether RS is a serious health problem of wild trout (Table 1). Some workers have postulated that RS is a native bacterium in North America which coevolved with inland trout and hence rarely causes disease signs (T. Evelyn, Canada Dept. of Fisheries and Oceans, personal communication). The finding of RS in salmonids, that have never come in contact with hatchery fish is probably the strongest piece of evidence to support this hypothesis. Also, the lack of clinical signs of disease, in the majority of cases when RS has been detected in inland trout (both wild and hatchery stocks)(this study) suggests that the pathogen and host coevolved. If RS coevolved with inland trout, then it could be assumed that different strains of RS could be found that are native to different geographic areas, with perhaps nonnative strains being more virulent to the native fish of a certain area (i.e. strains that the native trout of a particular area did not coevolve with). The literature does not contain any reference to work in this area however, and presently it is only a matter of conjecture.

The objectives of this study are to demonstrate where RS has been found in Colorado, where it has been spread through stocking, where it has probably been spread through fish movement, and what effects RS has on individuals and populations of inland trout (rainbow trout, *Oncorhyncus mykiss*, brown trout, *Salmo trutta,* and brook trout, *Salvelinus fontinalis*).

METHODS AND MATERIALS

To determine where RS has been found in Colorado, records from the Colorado Division of Wildlife's (CDOW) fish health laboratory and the U. S. Fish and Wildlife Service's (USFWS) fish health center were reviewed. Wild populations that were found positive were marked on a map of the state. When state or federal hatcheries were found to be positive, the stocking records for that year were requested and all stocking locations were marked on the map as well as the location of the hatchery. RS persistence in the environment is assumed based on the finding of RS in fish in Rocky Mountain National Park, despite the cessation of stocking in 1968. Fish dispersal is assumed to occur unless barriers block upstream movement (Gowan et al. 1994). On a large scale, RS is assumed to be spread throughout a drainage in which it has been detected or in which infected fish have been stocked.

To determine what effects RS has on individual fish, a fish health assessment (Goede and Barton 1990) was conducted on rainbow trout (*O. mykiss*), brook trout (*S. fontinalis*) and brown trout (*S. trutta*) captured from wild populations in several drainages in Rocky Mountain National Park. Corresponding ELISA was run using the kidney tissue of the individual fish. Linear regressions were used to determine if there were any statistically significant correlations between the level of RS (as determined by optical density readings using the ELISA technique) and selected physiological indicators. For a detailed description of the fish health assessment and the selected physiological indicators see Appendix A. The ELISA tests were conducted by the USFWS Fish Health Center, Ft. Morgan, CO. For a check on false positive or negative results, selected samples were also tested by ELISA at the USFWS Fish Health Center, Olympia, WA. and by DNA probe at the USFWS National Fisheries Research Center, Seattle, WA. and the USFWS Fish Health Center, Ft. Morgan, CO. (the quality control checks verified the accuracy of the original ELISA results). The Ft. Morgan, CO. laboratory uses the ELISA protocol established by Ron Pascho (Pascho and Mulcahy 1987); the antibody used was affinity purified antibody, isolated from a pool of serum from goats immunized with whole cells of *Renibacterium salmoninarum* and labeled with peroxidase by the periodate method of Nakane and Kawaoi (from Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD.).

To determine the effects of RS on populations of wild trout, a survey was mailed to fish health workers who have sampled populations of inland trout to determine if RS exists in self-sustaining populations and if it was having any detrimental effects. Records from the CDOW and USFWS were used to determine if any negative effects have been recorded in wild populations in which RS has been detected in Colorado. Data from Rocky Mountain National Park were reviewed in respect to RS effects. Fish health workers in various states were asked if, in their professional opinion, RS is a serious pathogen of inland trout.

RESULTS

Geographic distribution in Colorado

RS has been detected on 43 separate occasions since 1971, in fish in the White, Colorado, Uncompaghre, Animas, Gunnison, Arkansas, Rio Grande, Cache la Poudre, Big Thompson, St. Vrain river drainages. It has been detected in rainbow trout (*O. mykiss*), Colorado River cutthroat trout (*O. clarki pleuriticus*), Rio Grande cutthroat trout (*O.*

clarki virginalis), brown trout *(S. trutta)* and brook trout (*S. fontinalis*). RS has been found in both wild populations and in private, state and federal fish hatcheries. In 29 cases no clinical signs of disease were observed in any of the fish sampled. In thirteen cases, the records do not mention whether clinical signs of disease were observed or not. In only one documented case were clinical signs of disease observed (Table 2), and in this case, the clinical signs were observed in brook trout confined in a trap in August--fish that were possibly under stress from being crowded, confined, in warm water and gravid. Brook trout that were free, in the stream, showed no ill effects externally. Only those fish in the trap suffered morbidity and mortality.

Fish from RS positive state fish hatcheries have been stocked, into the following drainages: South Platte (301 stocking trips), North Platte (65 stocking trips), Rio Grande (260 stocking trips), Arkansas (325 stocking trips), Colorado (178 stocking trips), San Juan (797 stocking trips), Dolores (122 stocking trips), Gunnison (154 stocking trips), Republican (15 stocking trips), Yampa (40 stocking trips), White (14 stocking trips) (Table 3). These stocking trips represent the stocking of 14,159,445 trout, ranging in size from fingerlings to large broodstock (Table 3). Leadville National Fish Hatchery (NFH) has also stocked trout into the Arkansas River drainage during years when RS was detected in the hatchery. Every Colorado county, that has salmonid habitat, has received fish from RS positive fish hatcheries.

Rocky Mountain National Park Fish Survey

Brown trout, brook trout, rainbow trout and cutthroat trout from nine different locations in Rocky Mountain National Park were tested using ELISA. Overall 86.3% of brown trout (88/102), 65.4% of brook trout (34/52), 100% of rainbow trout (27/27) and 100% of cutthroat trout (1/1) were found to be positive (optical density means of 0.1 or higher). Site 1 (Fall River--Cascade Cottages, So. Platte River drainage) had 77.8% of brown trout (14/18) and 68.8% of brook trout (11/16) positive. Site 2 (Fall River--Endovalley bridge) had 83.3% of brown trout (5/6) and 50.0% of brook trout (9/18) positive. Site 3 (Big Thompson River--So. Platte River drainage) had 100% of brown trout (20/20), brook trout (5/5) and rainbow trout (1/1) positive. Site 4 (Mills Lake--So. Platte river drainage) had 100% of rainbow trout (26/26) positive. Site 5 (No. St. Vrain River--So. Platte River drainage) had 100% of brown trout (20/20) positive. Site 6 (Colorado River) had 55% of brown trout (11/20) positive. Site 7 (Tonahutu Creek--Colorado River drainage) had 100% of cutthroat (1/1) positive, 100% of brown trout (4/4), and 66.7% of brook trout (8/12) positive. Site 8 (North Inlet--Colorado River drainage) had 100% of brown trout (12/12) positive. Site 9 (West Portal--Colorado River drainage) had 100% of brook trout (1/1) and brown trout (2/2) positive (Table 4).

Fish Health Analysis

All of the populations in Rocky Mountain National Park are wild, self-sustaining populations. No clinical signs of disease were observed in any of the fish sampled. No correlations were found between the mean optical density level (RS level) and condition factor, body fat, eye, gill, pseudobranch, thymus, spleen, hind gut, liver, bile, fin, or opercle condition, or hematocrit, leucocrit, and plasma protein levels, for rainbow (due to remote location, blood parameters were not tested for rainbow trout at site 4), brown, or brook trout. No correlations were found between mean optical density level and kidney condition in brook or rainbow trout. Weak correlations were found between mean optical density level and percent of fish gravid in brown trout (r squared = 0.58) and brook trout (r squared = 0.65) and between mean optical density level and percent urolithic kidneys in brown trout (r squared = 0.60) (Appendices C and D).

Survey of Fish Health workers

RS has been detected, usually by DFAT, in every state that has habitat or hatcheries that have salmonids (Table 1). RS has been detected in wild populations of rainbow, brook, brown, and cutthroat trout, in Arizona, Colorado, Nevada, Wyoming, South Dakota, Wisconsin, and Idaho. Most of the surveys indicated that one or more stressors were affecting the fish when RS was detected, in both wild and hatchery populations. Other states that may contain wild populations of inland trout that are RS positive, did not respond to the survey or have not conducted surveys of wild populations.

In survey responses of RS positive, inland trout (both wild and in hatcheries), 21 cases (5 wild and 16 hatchery populations) of clinical signs of disease, morbidity or mortality were reported. In 52 cases (24 wild and 28 hatchery populations), no clinical signs, morbidity or mortality were reported. In western states (AZ, CA, CO, NV, WY, SD), in only eight (4 wild and 4 hatchery populations) out of 52 cases, clinical signs, morbidity, or mortality were reported.

Of fifteen responses, eleven do not regard RS as a serious pathogen of wild or hatchery stocks of inland trout; four fish health workers do consider RS to be a serious fish pathogen. Of these four, one response was “somewhat of a problem”; one response was “a problem only when under stress”; one response was “because inland trout could be carriers, which would spread the pathogen to anadromous salmonids"; only one response was an unqualified yes. The poll indicates that most fish health workers across the country, do not consider RS to be a very serious pathogen of wild or hatchery, inland trout.

Effects in free-ranging populations in Colorado

RS has been detected on 43 separate occasions in Colorado. It has been detected in 27 free-ranging populations, and 16 private, state or federal hatcheries. In one case, clinical signs of disease were observed, in 29 cases no clinical signs of disease were observed, and in 13 cases it is not recorded whether clinical signs were observed or not. Most of the free-ranging populations, in which RS has been detected, are totally self-sustaining (24/27). The Air Force Academy and Farrish Memorial are sustained totally by stocking. Stanley Canyon contains a reproducing population of cutthroat and brook trout but is supplemented with stocking. In all other stream and lake environments, listed in table 1, the populations are completely self-sustaining.

Effects on trout populations in Rocky Mountain National Park

The stocking of fish, for recreational purposes, ceased in Rocky Mountain National Park in 1968 (Rosenlund 1995). Since then only greenback and Colorado River cutthroat trout have been stocked within the boundaries of the park, as part of a reintroduction program. Since 1984, all trout species within the park, have increased in abundance. This has been attributed to a change in fishing regulations that went in effect at that time. RS does not appear to have any detectable effect on trout populations within park boundaries.

DISCUSSION

Geographic distribution within Colorado

RS has been detected in the following river drainage basins: South Platte, Colorado (including the Colorado, White, Gunnison, Uncompaghre and Animas Rivers), Rio Grande, and Arkansas. Fish from RS positive hatcheries have been stocked in the following river drainage basins: South Platte, North Platte, Colorado, White, Yampa, Gunnison, San Juan, Rio Grande, Arkansas and Dolores.

It is now known that trout move much greater distances than previously believed. It is also known that RS is both horizontally and vertically transmitted. Evidence from RMNP (this study), Utah, Wyoming, British Columbia, and the Northwest Territories of Canada demonstrates that once RS is present in the environment, it will persist as long as suitable host fish are present. Furthermore, we can postulate that RS is native to North America (i.e. wherever salmonids were native) and ubiquitous in North America (i.e. wherever salmonids are found).

Therefore, since RS is native or has been spread to every major river system, with salmonid habitat, in Colorado, there is no reason to believe that it is not ubiquitous in Colorado. It is doubtful that there are any RS negative stocks of salmonids within Colorado, unless they are completely isolated populations which were derived from RS-free ancestors, and no RS positive fish were ever stocked at that location.

Effects of RS on inland trout

Although RS/BKD can be a serious problem in hatcheries, especially in salmon hatcheries of the Pacific northwest, a review of all the evidence indicates it is not a serious problem in wild trout populations. The presence of BKD may be related to poor environmental conditions (Mitchum et al. 1979; Banks 1994) or poor nutrition (Bell et al. 1984; Lall et al. 1985; Bowser et al. 1988; Ravndal et al. 1994; Thorarinsson et al. 1994). RS has been detected in wild populations of inland trout. However it is very important to note that these populations have persisted over time; that no impact on population abundance has ever been noted or even suggested. This indicates that RS does not impair a trout population's ability to maintain itself.

RS was present in most of the fish sampled in Rocky Mountain National Park, but no clinical signs of disease, morbidity or mortality were observed. RS has been detected, in the last four years, in five Colorado Division of Wildlife state fish hatcheries and no clinical signs of disease, increased morbidity or mortalities were observed despite the presence of stressors such as chronically poor water quality, crowding, handling, and transporting.

RS levels may be positively correlated to spawning condition. This may be due to stress caused by physiological changes or spawning activity (about 25% of a trout's total energy goes into gonadal development--which is then instantaneously lost), or it may actually show a correlation between the level of RS and the age of the fish.

RS has not had any documented effect on fish populations within Colorado. This has not been thoroughly studied, however no effects have been observed in any of the state's fisheries. No BKD epizootics in Colorado fisheries have been recorded. No effects were observed in Rocky Mountain National Park and other waters, where special regulations increased the size of populations infected with RS.

RS apparently causes few, if any, fish health problems, within Colorado. When compared to *Ichthyophthirius multifilis* (Ich) or gill amoebas (nodular gill disease), RS is a relatively benign pathogen in wild or hatchery trout in the state. RS is probably like the vast majority of fish pathogens (including those listed in the preceding sentence) in that there is usually no problem unless the fish are crowded or otherwise stressed.

CONCLUSIONS

When RS is detected, most western states' regulations may, by restricting the movement of fish or gametes: 1. Cause loss of income to private aquaculturists; 2. Increase the cost and decrease the effectiveness of state and federal stocking programs; or 3. Interfere with the conservation of biological diversity, through the prevention of the use of RS positive broodstocks for enhancement programs for threatened or endangered species or species of special concern. Fish health regulations that restrict movement of RS positive fish or gametes, cannot be supported by the findings of this study. RS is ubiquitous throughout the state and does not appear to cause any measurable harm to Colorado fisheries.

With sole reliance on DFAT, one can predict that RS will be found intermittently. Any private, state or federal hatchery or wild population may, in some years, be found RS positive, but in most years will probably be found negative. Under the present system of fish health regulations and using diagnostic tests that are not the most sensitive available (e.g. DFAT) this means periodic disruption of peoples' income, species of special concern enhancement programs, or normal hatchery operations and stocking programs. This is not a desirable state of affairs for anyone. If BKD is considered a serious threat to the fishery resources (a conclusion this study does not support), then the most sensitive assays available ought to be used to monitor for it. However, if the most sensitive assays available are not employed, it cannot logically be argued that the fishery resources are being protected from BKD by regulations.

RS is native to North America and probably coevolved with inland trout. It is certainly ubiquitous in Colorado, where trout are found. If the most sensitive assays were used to test for RS, then RS would be detected in probably every wild population and hatchery in Colorado. Under these conditions, regulations restricting the movement of RS positive fish or gametes afford little or no protection of the fishery resource.

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Tables

Table 1. Summary of survey responses.

Location	Species	Site type	Habitat quality	Stressors	Signs present	Detection methods	considers serious pathogen
Buford SFH	RBT	Hatchery	fair	unkown	grossly involved kidneys, moderate losses	G-stain, DFAT	
Mt. Lassen TF	RBT	Hatchery	good	low flow, DO, poor nutrition	none	IFAT	no
Idaho	BKT RBT CUT BNT BUL	all	all	various	Yes in presence of anadromous fish	DFAT IFAT ELISA	yes
Clearwater R.	KOE	River	good	variable flows	none	ELISA	somewhat
Pennsylvania	BKT BNT RBT	hatcheries	fair to good overfeeding	Crowding exophthalmia	yes Ascites fluid low level mortality	DFAT	
Missouri	RBT BNT	hatcheries	good	crowding flooding low DO	kidney granulomas no mortalities	DFAT	no
Pisgah SFH	BNT	hatchery	good	low flows low hardness	no	DFAT	no
Nevada	RBT BNT BKT CUT	hatchery lakes	good fair to good	none known	no	ELISA, DFAT	no
Lakes Erie & Ontario	BKT RBT	lake	good	none known	yes	Culture, DFAT	
Lake Oahe	Chinook	lake	fair	fluctuating water levels, fluctuating forage base,	no	ELISA	no

Location	Species	Site type	Habitat quality	Stressors	Signs present	Detection methods	considers serious pathogen
Michigan	BKT	hatcheries			no	DFAT	no
Minnesota	RBT	hatcheries	good		yes, in RBT	DFAT, ELISA	yes, under
	LAT				broodstock only		stress
	BKT						
Wisconsin	LAT	hatcheries	good		yes, furuncles	DFAT, ELISA	yes
	SPL	river			necrotic kidneys		
	BNT	lake					
	BKT						
	STT						
Wyoming	BKT	hatcheries	poor, fair	various	no and yes	GSTAIN,	no
	RBT	rivers	and good			ELISA, DFAT	
	BNT	lakes				CULTURE	
	GOT						
	CUT						
	LAT						
New Hamphire	BKT	hatcheries	good	various	yes and no	G-STAIN	no
	RBT					FAT	
	LAT						
Great Lakes	LAT	hatchery	good	stress	no	DFAT	no
AZ,NM,OK,TX	RBT	hatchery	good	morbidity		G-STAIN	no
	BNT	river			no	ELISA	
Lamar	BKT	hatcheries	fair to good		no and yes	DFAT	no
	BNT	river					
	RBT						

Table 2. Detection of *R. salmoninarum* in Colorado since 1971, from U. S. Fish and Wildlife Service and Colorado Division of Wildlife records.

Year	Location	Drainage	Environment	Self-sus-taining?	Detection method	Species	Clinical signs of disease
1971	Bell-Aire SFH	White River	Hatchery	NA	gram stain	RBT	unrecorded
1975	Glenwood Springs SFH	Colorado River	Hatchery	NA	gram stain	BKT	unrecorded
1975	Pitkin SFH	Gunnison River	Hatchery	NA	gram stain	unknown	unrecorded
1978	Leadville NFH	Arkansas River	Hatchery	NA	DFAT	unknown	unrecorded
1979	Durango SFH	Animas (San Juan)	Hatchery	NA	DFAT	RBT	unrecorded
1980	Durango SFH	Animas River	Hatchery	NA	DFAT	RBT	unrecorded
1980	Trapper's Lake	White River	Lake	yes	DFAT	CUT	unrecorded
1981	Durango SFH	Animas River	Hatchery	NA	DFAT	RBT	unrecorded
1982	Silver Springs TF	Uncompaghre River	Hatchery	NA	DFAT	unknown	unrecorded
1983	Durango SFH	Animas River	Hatchery	NA	DFAT	RBT	unrecorded
1983	Leadville NFH	Arkansas River	Hatchery	NA	DFAT	unknown	unrecorded
1984	Electra Lake	Animas River	Lake	yes	DFAT	BKT	unrecorded
1989	Rock Creek	Arkansas River	Stream	yes	DFAT	CUT, BKT	unrecorded
1992	Mt. Shavano SFH	Arkansas River	Hatchery	NA	DFAT	CUT, RBT	no
1992	Arkansas River (6 sites)	Arkansas River	Stream	NA	ELISA	BNT, RBT	no
1993	Rainbow Springs TF	Animas River	Hatchery	NA	DFAT	RBT	no
1993	Fish Research SFH	Poudre River	Hatchery	NA	DFAT	RBT, CUT	no
1993	Bellvue SFH	Poudre River	Hatchery	NA	DFAT	RBT	no
1993	Watson Lake SFH	Poudre River	Hatchery	NA	DFAT	RBT	no
1993	Rock Creek	Arkansas River	Stream	yes	ELISA	BKT, CUT	no
1993	Leadville NFH	Arkansas River	Hatchery	NA	ELISA	unknown	no
1993	Air Force Academy	Fountain Creek	Lake	no	ELISA	BKT, CUT	no
1993	Stanley Canyon	Arkansas River	Lake	yes	ELISA	BKT, CUT	no
1993	Big Thompson River	South Platte River	Stream	yes	ELISA	BNT	no
1993	Fall River	South Platte River	Stream	yes	ELISA	BKT	no
1993	Ouzel Creek	South Platte River	Stream	yes	ELISA	BKT	no
1993	Farish Memorial	Arkansas River	Lake	no	ELISA	BKT, CUT	no
1993	Jack's Fork	Poudre River	Stream	yes	Histology	BKT	yes
1995	Haypress Lake	Rio Grande River	Lake	yes	DFAT	CUT	no
1995	Fall River (2 sites)	South Platte River	Stream	yes	ELISA	BKT, BNT	no
1995	Big Thompson	South Platte River	Stream	yes	ELISA	BKT, BNT RBT	no

Year	Location	Drainage	Environment	Self-sustaining?	Detection method	Species	Clinical signs of disease observed
1995	Mill's Lake	South Platte River	Lake	yes	ELISA	RBT	no
1995	No. St. Vrain River	South Platte River	Stream	yes	ELISA	BNT	no
1995	Colorado River	Colorado River	Stream	yes	ELISA	BNT	no
1995	Tonahutu Creek	Colorado River	Stream	yes	ELISA	BNT. BKT	no
1995	North Inlet	Colorado River	Stream	yes	ELISA	BNT, BKT	no
1995	West Portal	Colorado River	Stream	yes	ELISA	BNT, BKT	no

Table 3. Stocking of fish from *R. salmoninarum* positive hatcheries, from Colorado Division of Wildlife records.

Year	Hatchery	Drainage stocked	Stocking trips	Counties stocked	Species	Total fish stocked	Total pounds stocked
1971	Bell-Aire SFH	final destination of fish not recorded					
1975	Glnwd Spgs SFH	White River Yampa River North Platte Colorado River South Platte River Arkansas	14 40 1 154 3 1	Garfield, Pitkin, Rio Blanco, Routt, Eagle, Mesa, Grand, Jackson, Summit, Moffit, Gunnison	RBT, BNT, KOE, BKT, LAT, CUT	4,888,844	3,226.6
1975	Pitkin SFH	Gunnison River Rio Grande River	138 12	Rio Grande, Ouray Gunnison, Montrose, Hinsdale, Saguache, Delta, Mineral, Costilla	RBT	309,460	95,303.0
1979	Durango SFH	Rio Grande River Gunnison River San Juan River Dolores South Platte River	70 4 201 38 1	Rio Grande, Conejos Mineral, San Juan, La Plata, Archuleta, Adams, Hinsdale, Ouray, Montezuma, Dolores, San Miguel, Saguache	CUT, RBT, BKT,BNT	1,467,262	65,523.0
1980	Durango SFH	Rio Grande River Gunnison River San Juan River Dolores	67 5 194 26	Rio Grande, Conejos Mineral, San Juan, La Plata, Archuleta, Saguache, Hinsdale, Ouray, Montezuma, Dolores, San Miguel,	CUT, RBT, BKT, KOE, BNT	1,672,127	65,734.85
1981	Durango SFH	Rio Grande River Gunnison River San Juan River Dolores	72 5 209 33	Rio Grande, Conejos Mineral, San Juan, La Plata, Archuleta, Saguache, Hinsdale, Ouray, Montezuma, Dolores, San Miguel,	CUT, RBT, BKT, KOE, BNT	1,657,799	83,614

Year	Hatchery	Drainage stocked	Stocking trips	Counties stocked	Species	Total fish stocked	Total pounds stocked
1983	Durango SFH	Rio Grande River Gunnison River San Juan River Dolores	37 2 193 25	Rio Grande, Conejos Mineral, San Juan, La Plata, Archuleta, Saguache, Hinsdale, Ouray, Montezuma, Dolores, San Miguel,	CUT, RBT, BKT	1,371,230	116,304.0
1992	Mt. Shavano SFH	Arkansas River South Platte River Colorado River	323 45 23	Pueblo, Fremont, Chaffee, Arapahoe, Custer, Huerfano, Jefferson, Eagle, Teller, Las Animas, Clear Creek, Park, El Paso, Bent, Lincoln, Otero	RBT, CUT, KOE, BNT	1,438,340	308,659.14
1993	Bellvue/Watson SFH	South Platte River North Platte River Republican River Arkansas River Colorado River	285 56 15 1 1	Clear Creek, Gilpin, Washington, Weld, Jackson, Boulder, Larimer, Jefferson, Yuma, Chaffee, Sedgwick, Morgan, Grand, Park, Logan	CUT, RBT, BKT, SPL, KOE	1,295,208	177,435.68
1993	Fish Research SFH	South Platte River North Platte River Rio Grande River	12 8 2	Weld, Clear Creek, Adams, Alamosa, Larimer, Boulder, Jackson, Denver	RBT, CUT, GOT	59,175	3616.07
					Totals	14,159,445 fish	919,416.34 pounds

Key:

RBT	rainbow trout (Oncorhynchus mykiss)	LAT	lake trout (Salvelinus namaycush)
CUT	cutthroat trout (O. clarki)	GOT	golden trout (O. aguabonita)
BKT	brook trout (Salvelinus fontinalis)	SPL	splake (brook trout x lake trout)
BNT	brown trout (Salmo trutta)	SFH	state fish hatchery
KOE	kokanee salmon (O. nerka)		

Table 4. ELISA results from Rocky Mountain National Park.

Site no.	Site discription	species	number positive	number caught	percent positive
1	Fall River;Cascade Cottages	BNT	14	18	77.8
		BKT	11	16	68.8
2	Fall River;Endovalley bridge	BNT	5	6	83.3
		BKT	9	18	50.0
3	Big Thompson River	BNT	20	20	100.0
		BKT	5	5	100.0
		RBT	1	1	100.0
4	Mills Lake	RBT	26	26	100.0
5	No. St. Vrain River	BNT	20	20	100.0
6	Colorado River	BNT	11	20	55.0
7	Tonahutu Creek	CUT	1	1	100.0
		BNT	4	4	100.0
		BKT	8	12	66.7
8	North Inlet	BNT	12	12	100.0
9	West Portal	BNT	2	2	100.0
		BKT	1	1	100.0
Totals:		BNT	88	102	86.3
		BKT	34	52	65.4
		RBT	27	27	100.0
		CUT	1	1	100.0

Appendices

Appendix A. Fish Health/Condition Assessment.

The procedures for handling fish and conducting the assessment were followed in this study according to: Fish Health / Condition Assessment Procedures, 1991 by Ron Goede, Utah Division of Wildlife Resources. The fish health/condition assessment was developed by Ron Goede to provide an ordered observation of tissues and organs of fish and is carried out to assess the relative health and condition of a cultured or free-ranging population of fish. It permits inference relative to the fish and the environment. A brief synopsis of the procedures is given here for a more in-depth study the reader is directed to Goede and Barton (1990) and the above listed publication.

The fish are killed by applying an overdose of MS-222. The fish are then measured and weighed and a blood sampled taken by puncturing the bulbous arteriosis with a heparinized micro hematocrit tube. The tubes are placed by sample number into a bed of clay to close one end of the tube. Later the tubes are placed in a hematocrit centrifuge and spun to separate the hematocrit, leucocrit and plasma fractions. The hematocrit, leucocrit and plasma protein levels are then read and recorded.

While the fish are being measured and weighed, the opercles, fins, eyes, gills, thymus and pseudobranch are examined. The fish are then cut open, careful not to damage any internal organs, and the hindgut, fat, spleen, liver, bile, kidney, and sex are observed and conditions recorded. After this was done, the kidney was removed for testing by ELISA.

Key to record codes

Eyes:	N	normal.
	E1 or E2	exophthalmia in one eye or both.
	H1 or H2	hemorrhagic in one eye or both.
	B1 or B2	blind in one eye or both.
	M1 or M2	missing one eye or both.
	OT	other.
Gills:	N	normal.
	F	frayed.
	C	clubbed.
	M	marginate.
	P	pale.
	OT	other.
Pseudobranchs:	N	normal.
	S	swollen.
	L	lithic.

	S&L	swollen and lithic.
	I	inflamed.
	OT	other.
Thymus:	0	no hemorrhage.
	1	mild hemorrhage.
	2	severe hemorrhage.
Fins:	0	no active erosion.
	1	mild active erosion.
	2	severe active erosion.
Opercles:	0	normal.
	1	slight shortening.
	2	severe shortening.
Mesenteric Fat:	0	no fat around pyloric caeca.
	1	less than 50% of each cecum is covered with fat.
	2`	50% of each cecum is covered with fat.
	3	more than 50% of each cecum is covered with fat.
	4	pyloric caeca are completely covered by fat.
Spleen:	B	black (normal).
	R	red (normal).
	O	granular (normal).
	NO	nodular.
	E	enlarged.
	OT	other.
Hindgut:	0	no inflammation.
	1	slight inflammation.
	2	severe inflammation.
Kidney:	N	normal.
	S	swollen.
	M	mottled.
	G	granular.
	U	urolithic.
	OT	other.
Liver:	A	normal color.
	B	lighter or less vivid red color (still considered normal).

	C	fatty liver; cream colored.
	D	nodules in liver.
	E	focal discoloration.
	F	general discoloration.
	OT	other.
Bile:	0	yellow color; bladder empty or partially full.
	1	yellow color; bladder full.
	2	light green to "grass" green.
	3	dark green, dark blue-green.
Sex:	M	male.
	F	female.
	U	unknown.

Appendix B(1). Fish health assessment/condition data and ELISA results for brown trout, in Rocky Mountain National Park (Sites 1,3,5,6,7,8).

ELISA READINGS	Site #	Species	Sam. no.	Length (mm)	Weight (gm)	Ktl	Eye	Gill	Psbr	Thymus	Fat	Spleen	Hind gut
0.000-0.099	1	BNT	1-9	270	186	9.45E-06	N	N	N	0	1	B	0
			1-10	235	114	8.78E-06	N	N	N	0	2	B	0
			1-120	264	183	9.95E-06	N	N	N	0	0	O	0
			1-124	184	66	1.06E-05	N	N	N	1	1	B	0
	6	BNT	7	310	330	1.11E-05	N	N	N	0	0	R	0
			8	355	410	9.16E-06	N	N	N	0	0	R	0
			10	327	318	9.09E-06	N	N	N	0	0	R	0
			12	271	197	9.90E-06	N	N	N	0	1	B	0
			15	308	265	9.07E-06	N	N	N	0	0	R	0
			16	350	444	1.04E-05	N	N	N	0	0	R	0
			17	310	306	1.03E-05	N	N	N	0	0	R	0
			18	295	216	8.41E-06	N	N	N	0	0	O	0
			19	267	188	9.88E-06	N	N	N	0	0	R	0
0.100-0.199	1	BNT	1-1	295	282	1.10E-05	N	N	N	0	0	B	0
			1-2	308	271	9.28E-06	N	N	N	0	0	B	0
			1-4	298	270	1.02E-05	N	N	N	0	1	B	0
			1-5	260	179	1.02E-05	N	N	N	0	0	B	0
			1-11	210	86	9.29E-06	N	N	N	0	2	B	0
			1-122	200	84	1.05E-05	N	N	N	0	1	B	0
			1-123	183	66	1.08E-05	N	N	N	0	1	B	0
	3	BNT	3-46	269	190	9.76E-06	N	N	N	0	1	O	0
			3-47	225	120	1.05E-05	N	N	N	0	2	O	0
			3-48	247	154	1.02E-05	N	N	N	0	1	O	0
			3-50	260	182	1.04E-05	N	N	N	0	0	O	0
			3-51	270	218	1.11E-05	N	N	N	0	1	O	0
			3-52	230	134	1.10E-05	N	N	N	0	1	O	0
			3-61	267	180	9.46E-06	N	N	N	1	3	B	0
			3-62	219	122	1.16E-05	N	N	N	0	2	O	0
	5	BNT	5-164	243	130	9.06E-06	N	N	N	0	3	O	0
			5-165	255	170	1.03E-05	N	N	N	0	2	B	0
			5-166	288	190	7.95E-06	N	N	N	0	3	B	0

Kidney	Liver	Bile	Sex	Hema.	Leu.	Plasma Protein	Fin	Opercle	ELISA READINGS Rep 1	Rep 2	mean	SD	Remarks
N	B	1	F			4.2	0	0	0.093	0.093	0.093	0.0000	G
N	B	1	F				0	0	0.101	0.091	0.096	0.0071	IMM
U	B	0	F	35	3	4.8	0	0	0.097	0.091	0.094	0.0042	G
N	B	0	F	39	2	3.8	0	0	0.083	0.081	0.082	0.0014	IMM
U	A	0	M	44	1	4.4	0	0	0.092	0.084	0.088	0.0057	G
N	A	0	M	42	1	4.0	0	0	0.072	0.069	0.071	0.0021	G
N	A	0	M	48	1	4.2	0	0	0.097	0.095	0.096	0.0014	G
U	A	0	F	49	2	5.0	0	0	0.095	0.087	0.091	0.0057	G
N	A	0	F	44	1	5.2	0	0	0.087	0.083	0.085	0.0028	G
N	A	0	F	34	1	4.6	0	0	0.089	0.081	0.085	0.0057	G
N	A	0	F	41	1	4.4	0	0	0.088	0.087	0.088	0.0007	G
N	A	0	F	39	2	4.2	0	0	0.067	0.067	0.067	0.0000	G
N	A	0	F	48	2	6.0	0	0	0.082	0.076	0.079	0.0042	G
N	B	1	F				0	0	0.189	0.183	0.186	0.0042	G
N	B	1	F				0	0	0.180	0.139	0.160	0.0290	G
N	A	1	M	43	1	3.6	0	0	0.120	0.108	0.114	0.0085	G
N	A	1	F	37	1	3.6	0	0	0.141	0.113	0.127	0.0198	G
N	A	1	F			2.0	0	0	0.114	0.102	0.108	0.0085	IMM
N	A	0	M				0	0	0.106	0.100	0.103	0.0042	IMM
N	A	0	M	44	2	3.0	0	0	0.150	0.131	0.141	0.0134	IMM
N	A	1	M				0	0	0.135	0.156	0.146	0.0148	G
N	A	0	F				0	0	0.177	0.192	0.185	0.0106	IMM
N	A	1	M	38	2	2.0	0	0	0.156	0.186	0.171	0.0212	G
N	A	0	F	27	1	2.8	0	0	0.131	0.166	0.149	0.0247	G
N	A	1	M	46	2	3.2	0	0	0.186	0.197	0.192	0.0078	G
N	A	0	M	45	1	3.0	0	0	0.188	0.195	0.192	0.0049	G
N	A	1	M	42	1	2.8	0	0	0.221	0.068	0.145	0.1082	IMM
N	A	0	F	39	2	2.4	0	0	0.174	0.168	0.171	0.0042	IMM
N	B	1	F	34	3	3.8	0	0	0.216	0.128	0.172	0.0622	G
N	B	1	F	32	1	4.0	0	0	0.106	0.133	0.120	0.0191	G
N	A	1	F	29	1	2.8	0	0	0.135	0.128	0.132	0.0049	G

ELISA READINGS	Site #	Species	Sam. no.	Length (mm)	Weight (gm)	Ktl	Eye	Gill	Psbr	Thymus	Fat	Spleen	Hind gut
			5-167	223	110	9.92E-06	N	N	N	0	2	O	0
			5-168	239	125	9.16E-06	N	N	N	0	4	O	0
			5-169	239	125	9.16E-06	N	N	N	0	2	O	0
			5-170	307	190	6.57E-06	N	N	N	0	1	O	0
			5-171	299	270	1.01E-05	N	N	N	0	1	O	0
			5-173	263	180	9.89E-06	N	N	N	0	1	O	0
			5-174	222	115	1.05E-05	N	N	N	0	3	O	0
			5-175	285	210	9.07E-06	N	N	N	0	1	B	0
			5-176	274	175	8.51E-06	N	N	N	0	2	O	0
			5-177	268	150	7.79E-06	N	N	N	0	1	B	0
			5-178	289	230	9.53E-06	N	N	N	0	1	B	0
			5-179	269	170	8.73E-06	N	N	N	0	1	B	0
			5-180	240	150	1.09E-05	N	N	N	0	1	B	0
			5-181	256	150	8.94E-06	N	N	N	0	2	B	0
			5-182	247	160	1.06E-05	N	N	N	0	1	B	0
			5-183	253	155	9.57E-06	N	N	N	0	1	B	0
	6	BNT	1	295	278	1.08E-05	N	N	N	0	0	O	0
			2	370	454	8.96E-06	N	N	N	0	0	R	0
			3	340	470	1.20E-05	N	N	N	0	0	O	0
			4	380	500	9.11E-06	N	N	N	0	0	B	0
			6	335	400	1.06E-05	N	N	N	0	0	B	0
			11	317	304	9.54E-06	N	N	N	0	0	O	0
			14	320	305	9.31E-06	N	N	N	0	0	O	0
			20	277	200	9.41E-06	N	N	N	0	0	O	0
	7	BNT	1-76	305	370	1.30E-05	N	N	N	0	2	B	0
			2-77	275	230	1.11E-05	N	N	N	0	2	O	0
			5-80	218	110	1.06E-05	N	N	N	0	1	R	0
	8	BNT	131	395	595	9.65E-06	N	N	N	0	0	O	0
			132	340	440	1.12E-05	N	N	I	0	0	O	0
			135	340	392	9.97E-06	N	N	N	0	0	R	0
			147	404	630	9.55E-06	N	N	N	0	0	B	0

						Plasma			ELISA READINGS				
Kidney	Liver	Bile	Sex	Hema.	Leu.	Protein	Fin	Opercle	Rep 1	Rep 2	mean	SD	Remarks
N	A	1	F	39	2	3.6	0	0	0.139	0.184	0.162	0.0318	G
N	A	1	F	35	2	3.6	0	0	0.105	0.139	0.122	0.0240	G
N	A	1	F	41	2	3.4	0	0	0.138	0.119	0.129	0.0134	G;Jaw injury
N	A	1	F	13	1	0.4	0	0	0.150	0.121	0.136	0.0205	G
U	A	1	F	29	1	4.8	0	0	0.123	0.120	0.122	0.0021	G
N	A	0	M	30	2	2.0	0	0	0.115	0.096	0.106	0.0134	G
N	A	1	M	42	1	4.2	0	0	0.153	0.110	0.132	0.0304	G
N	A	0	M	47	1	3.8	0	0	0.112	0.106	0.109	0.0042	G
N	A	1	F	38	1	3.6	0	0	0.123	0.137	0.130	0.0099	G
U	A	1	F	37	1	2.6	0	0	0.151	0.123	0.137	0.0198	G;Jaw injury
N	A	1	M	28	2	1.2	0	0	0.117	0.098	0.108	0.0134	G
N	A	0	U	25	1	2.0	0	0	0.140	0.081	0.111	0.0417	G
U	A	1	F	34	2	4.6	0	0	0.129	0.108	0.119	0.0148	G
N	A	0	M	40	2	3.0	0	0	0.128	0.123	0.126	0.0035	G
N	A	1	M	55	1	4.6	0	0	0.104	0.096	0.100	0.0057	G
N	A	1	F	31	1	3.4	0	0	0.156	0.140	0.148	0.0113	G
U	A	0	F			4.5	0	0	0.159	0.179	0.169	0.0141	G
N	A	0	M	39	1	2.0	0	0	0.134	0.137	0.136	0.0021	G
N	A	0	F	47	1	5.5	0	0	0.140	0.145	0.143	0.0035	G
N	A	0	M	40	1	4.2	0	0	0.131	0.168	0.150	0.0262	G
U	A	0	F		1	4.2	0	0	0.106	0.095	0.101	0.0078	G
U	A	0	F	38	2	4.8	0	0	0.118	0.116	0.117	0.0014	G
N	A	0	M	38	1	4.6	0	0	0.143	0.132	0.138	0.0078	G
N	B	0	M	43	1	4.2	0	0	0.108	0.097	0.103	0.0078	G
U	C	1	M	45	1	3.6	0	0	0.122	0.130	0.126	0.0057	G
N	C	1	M	54	2	4.4	0	0	0.137	0.145	0.141	0.0057	G
N	C	1	M	36	1	3.2	0	0	0.153		0.153		G
N	C	1					0	0	0.166	0.185	0.176	0.0134	G
	C	0	M				0	0	0.139	0.153	0.146	0.0099	G
N	B	1	F				0	0	0.137	0.140	0.139	0.0021	G
N	B	0	M				0	0	0.181	0.203	0.192	0.0156	G

ELISA READINGS	Site #	Species	Sam. no.	Length (mm)	Weight (gm)	Ktl	Eye	Gill	Psbr	Thymus	Fat	Spleen	Hind gut
0.200-0.299	1	BNT	1-6	197	75	9.81E-06	N	N	N	0	1	B	0
			1-7	193	74	1.03E-05	N	N	N	0	1	B	0
			1-8	198	78	1.00E-05	B-2	N	N	0	3	B	0
			1-3	270	190	9.65E-06	N	N	N	0	1	B	0
	3	BNT	3-44	237	138	1.04E-05	N	N	N	0	1	O	0
			3-45	239	138	1.01E-05	N	N	N	0	1	O	0
			3-49	259	170	9.78E-06	N	N	N	0	0	O	0
			3-54	269	191	9.81E-06	N	N	N	0	2	B	0
			3-55	343	498	1.23E-05	N	N	N	0	2	O	0
			3-57	226	112	9.70E-06	N	N	N	1	2	B	0
			3-59	275	215	1.03E-05	N	N	N	0	3	B	0
			3-60	232	132	1.06E-05	N	N	N	0	2	B	0
			3-63	216	106	1.05E-05	N	N	N	0	3	B	0
	6	BNT	5	340	400	1.02E-05	N	N	N	0	0		0
			9	331	342	9.43E-06	N	N	N	0	0	R	0
	7	BNT	3-78	273	240	1.18E-05	N	N	N	0	1	B	0
	8	BNT	133	350	425	9.91E-06	N	N	N	0	0	O	0
			134	340	432	1.10E-05	N	N	L	0	1	O	0
			136	275	210	1.01E-05	N	N	N	0	0	O	0
			144	352	440	1.01E-05	N	N	S	0	0	O	0
			145	315	300	9.60E-06	N	N	N	0	0	O	0
0.300-0.399	1	BNT	1-117	310	312	1.05E-05	N	N	N	0	0	O	0
			1-118	315	322	1.03E-05	N	N	N	0	1	B	0
	3	BNT	3-56	295	266	1.04E-05	N	N	N	0	1	O	0
			3-58	238	120	8.90E-06	N	N	N	0	3	B	0
	5	BNT	5-172	225	110	9.66E-06	N	N	N	0	0	O	0
	6	BNT	13	328	330	9.35E-06	N	N	N	0	0	R	0
	8	BNT	139	330	416	1.16E-05	N	N	N	0	1		0
			141	286	290	1.24E-05	N	N	N	0	0	B	0
			142	320	285	8.70E-06	N	N	N	0	1	B	0
			148	304	260	9.25E-06	N	N	N	0	2	O	0

Kidney	Liver	Bile	Sex	Hema.	Leu.	Plasma Protein	Fin	Opercle	ELISA READINGS Rep 1	Rep 2	mean	SD	Remarks
N	A	1	F	48	2	3.4	0	0	0.230	0.176	0.203	0.0382	IMM
N	B	1	M			3.6	0	0	"	"	"	"	IMM
N	A	1	F			3.6	0	0	"	"	"	"	IMM
N	B	1	F	35	1	5.4	0	0	0.273	0.246	0.260	0.0191	G
N	B	1	F	28	1	3.6	0	0	0.191	0.209	0.200	0.0127	G
N	A	1	M	42	1	2.0	0	0	0.235	0.255	0.245	0.0141	G
N	A	1	M	50	2	2.0	0	0	0.222	0.240	0.231	0.0127	G
N	A	0	M				0	0	0.247	0.280	0.264	0.0233	IMM
N	A	0	M	36	0	2.6	0	0	0.226	0.199	0.213	0.0191	G
U	A	1	F	37	1	2.2	0	0	0.207	0.239	0.223	0.0226	G
N	A	0	M	36	3	2.6	0	0	0.287	0.282	0.285	0.0035	IMM
U	A	0	M	43	1	2.2	0	0	0.274	0.283	0.279	0.0064	G
U	A	0	F	29	2	1.6	0	0	0.192	0.210	0.201	0.0127	IMM
U	A	0	M	32	2	3.8	0	0	0.227	0.226	0.227	0.0007	G
N	A	0	F	45	1	7.4	0	0	0.193	0.210	0.202	0.0120	G
N	B	1	F	50	2	4.6	0	0	0.188	0.215	0.202	0.0191	G
N	B	0	F				0	0	0.197	0.202	0.200	0.0035	G
N	C	1	F				0	0	0.193	0.230	0.212	0.0262	G
N	B	1	F				0	0	0.201	0.241	0.221	0.0283	G
U	B	0	M				0	0	0.227	0.219	0.223	0.0057	G
N	B	0	F				0	0	0.182	0.225	0.204	0.0304	G
U	A	1	M	44	2	3.6	0	0	0.332	0.330	0.331	0.0014	G
N	A	0	F	34	2	4.0	0	0	0.413	0.281	0.347	0.0933	G
N	A	1	M	36	3	2.0	0	0	0.314	0.358	0.336	0.0311	G
N	A	1	F	35	1	2.4	0	0	0.315	0.306	0.311	0.0064	G
N	A	1	M	49	3	4.2	0	0	0.427	0.265	0.346	0.1146	G
N	A	0	M	48	1	3.8	0	0	0.347	0.353	0.350	0.0042	G
N	B	0	M				0	0	0.299	0.352	0.326	0.0375	G
N	B	1	F				0	0	0.317	0.331	0.324	0.0099	G
N	B	1	F				0	0	0.340	0.360	0.350	0.0141	IMM
N	B	0					0	0	0.407	0.264	0.336	0.1011	IMM

ELISA READINGS	Site #	Species	Sam. no.	Length (mm)	Weight (gm)	Ktl	Eye	Gill	Psbr	Thymus	Fat	Spleen	Hind gut
0.400-0.499	1	BNT	1-116	274	216	1.05E-05	N	N	N	0	1	B	0
			1-119	285	261	1.13E-05	N	N	N	0	1	O	0
			1-121	243	133	9.27E-06	N	N	N	0	1	B	0
	3	BNT	3-53	242	152	1.07E-05	N	N	N	0	1	O	0
	8	BNT	140	295	270	1.05E-05	N	N	N	0	1	O	0
			143	275	200	9.62E-06	N	N		0	1	O	0
0.500-0.599	8	BNT	137	300	280	1.04E-05	N	N	N	0	2	O	0
			138	295	280	1.09E-05	N	N	N	0	3	O	0
			146	315	310	9.92E-06	N	N	N	0	0	B	0
0.600-0.699	8	BNT	149	265	180	9.67E-06	N	N	N	0	2	O	0

Kidney	Liver	Bile	Sex	Hema.	Leu.	Plasma Protein	Fin	Opercle	ELISA READINGS Rep 1	Rep 2	mean	SD	Remarks
N	A	0	F	35	4	4.2	0	0	0.419	0.398	0.409	0.0148	G
U	A	0	F	37	2	3.6	0	0	0.477	0.425	0.451	0.0368	G
U	A	0	F	39	2	3.0	0	0	0.487	0.436	0.462	0.0361	IMM
N	A	0	F	30	1	2.0	0	0	0.421	0.428	0.425	0.0049	G
U	B	1	F				0	0	0.405	0.433	0.419	0.0198	G
U	B	0	F				0	0	0.361	0.522	0.442	0.1138	IMM
N	B	0	F				0	0	0.489	0.517	0.503	0.0198	IMM
N	B	0	F				0	0	0.502	0.508	0.505	0.0042	G
U	B	0	F				0	0	0.543	0.572	0.558	0.0205	G
U	B	0	F				0	0	0.543	0.798	0.665	0.1881	IMM

Appendix B(2). Fish health assessment/condition data and ELISA results for brook trout, in Rocky Mountain National Park (Sites 1,3,7).

ELISA READINGS	Site #	Species	Sam. no.	Length (mm)	Weight (gm)	Ktl	Eye	Gill	Psbr	Thymus	Fat	Spleen	Hind gut
0.000-0.099	1	BKT	1-13	209	87	9.53E-06	N	N	N	0	2	B	0
			1-14	185	69	1.09E-05	N	N	N	0	1	B	0
			1-15	202	82	9.95E-06	N	N	N	0	0	B	0
			1-129	191	66	9.47E-06	N	N	N	0	1	B	0
			1-130	187	70	1.07E-05	N	N	N	0	1	B	0
	7	BKT	5-70	196	75	9.96E-06	N	N	N	0	3	O	0
			9-74	202	75	9.10E-06	N	N		0	2	B	0
			11-82	210	120	1.30E-05	N	N	N	0	0	B	0
			12-83	202	75	9.10E-06	N	N	N	0	1	B	0
0.100-0.199	1	BKT	1-12	224	108	9.61E-06	N	N	N	0	2	B	0
			1-16	194	77	1.05E-05	N	N	N	0	0	B	0
			1-17	185	63	9.95E-06	N	N	N	0	0	B	0
			1-18	188	56	8.43E-06	N	N	N	0	1	B	0
			1-126	232	146	1.17E-05	N	N	N	0	0	B	0
			1-127	213	96	9.93E-06	N	N	N	0	1	B	0
	3	BKT	3-106	193	90	1.25E-05	N	N	N	0	2	B	0
			3-107	190	85	1.24E-05	N	N	N	0	3	B	0
			3-109	179	56	9.76E-06	N	N	N	0	2	B	0
	7	BKT	7-72	200	80	1.00E-05	N	N	N	0	3	B	0
			8-73	194	65	8.90E-06	N	N	N	0	2	R	0
0.200-0.299	1	BKT	1-19	179	50	8.72E-06	N	N	N	0	2	B	0
			1-20	141	24	8.56E-06	N	N	N	0	0	B	0
	3	BKT	3-108	204	97	1.14E-05	N	N	N	0	3	B	0
	7	BKT	2-67	220	110	1.03E-05	N	N	N	0	2	R	0
			4-69	210	105	1.13E-05	N	N	N	0	2	B	0
			10-75	230	120	9.86E-06	N	N	S	0	2	B	0
0.300-0.399	3	BKT	3-105	255	198	1.19E-05	N	N	N	0	3	B	0
			1-66	230	120	9.86E-06	N	N	N	0	2	R	0
			3-68	210	105	1.13E-05	N	N	N	0	2	B	0
			6-71	222	110	1.01E-05	N	N	N	0	2	B	0
0.600-0.699	1	BKT	1-131	218	105	1.01E-05	N	N	N	0	1	B	0
			1-132	191	83	1.19E-05	N	N	N	0	1	B	0
			1-133	184	66	1.06E-05	N	N	N	0	1	B	0

						Plasma			ELISA READINGS				
Kidney	Liver	Bile	Sex	Hema.	Leu.	Protein	Fin	Opercle	Rep 1	Rep 2	mean	SD	Remarks
N	A	1	F			4.6	0	0	0.107	0.088	0.098	0.0134	G
N	A	1	F	38	1	4.8	0	0	"	"	"	"	G
N	A	1	F				0	0	"	"	"	"	G
N	B	0	F	40	4	6.6	0	0	0.073	0.071	0.072	0.0014	G
N	B	0	M	40	3	5.4	0	0	"	"	"	"	IMM
N	B	1	F				0	0	0.077	0.076	0.077	0.0007	IMM
N	B	1	F	38	1	4.0	0	0	0.071	0.067	0.069	0.0028	IMM
N	C	1	M	42	2	5.6	0	0	0.073	0.074	0.074	0.0007	G
N	C	1	M	48	1	4.2	0	0	0.069	0.070	0.070	0.0007	G
N	A	1	M				0	0	0.108	0.099	0.104	0.0064	G
N	A	1	F				0	0	0.148	0.118	0.133	0.0212	G
N	A	1	F				0	0	"	"	"	"	G
N	A	0	M	45	1		0	0	"	"	"	"	IMM
U	A	0	M	39	4	4.0	0	0	0.174	0.151	0.163	0.0163	G
N	A	0	M	46	4	5.4	0	0	0.121	0.138	0.130	0.0120	G
N	A	1	F	30	5	3.0	0	0	0.142	0.140	0.141	0.0014	G
N	A	1	M	46	3	3.6	0	0	0.168	0.171	0.170	0.0021	G
N	A	3	U	30	6	4.2	0	0	0.184	0.210	0.197	0.0184	IMM
N	B	1	F	44	1	6.0	0	0	0.163	0.137	0.150	0.0184	G
N	B	1	F	34	1	4.8	0	0	0.172	0.127	0.150	0.0318	IMM
N	A	1	M	33	1	3.8	0	0	0.237	0.189	0.213	0.0339	IMM
N	A	1	M				0	0	"	"	"	"	IMM
N	A	1	F	26	1	0.8	0	0	0.265	0.261	0.263	0.0028	G
N	C	1	M	39	1	3.4	0	0	0.291	0.171	0.231	0.0849	G
N	B	1	M	38	1	2.2	0	0	0.296	0.290	0.293	0.0042	G
N	C	1	M	35	1	2.6	0	0	0.198	0.242	0.220	0.0311	G
N	A	1	F	31	2	4.0	0	0	0.339	0.366	0.353	0.0191	G
N	C	1	M	52	1	4.6	0	0	0.336	0.368	0.352	0.0226	G
N	B	1	M	54	1	4.8	0	0	0.305	0.367	0.336	0.0438	G
N	B	1	M	40	1	3.0	0	0	0.366	0.428	0.397	0.0438	G
N	A	0	F	37	2	5.4	0	0	0.705	0.552	0.629	0.1082	G
N	A	0	M	40	3	3.0	0	0	"	"	"	"	G
N	A	1	M	42	4	4.8	0	0	"	"	"	"	G

Appendix B(3). Fish health assessment/condition data and ELISA results, for rainbow trout, in Rocky Mountain National Park (Sites 3,4).

ELISA READINGS	Site #	Species	Sam. no.	Length (mm)	Weight (gm)	Ktl	Eye	Gill	Psbr	Thymus	Fat	Spleen	Hind gut
0.100-0.199	3	RBT	3-114	237	136	1.02E-05	N	N	N	0	2	B	0
	4	RBT	4-140	269	185	9.50E-06	N	N	N	0	3	B	0
			4-142	288	220	9.21E-06	N	N	N	0	3	B	0
			4-147	245	140	9.52E-06	N	N	N	0	3	B	0
			4-148	322	280	8.39E-06	N	N	N	0	2	B	0
			4-156	256	150	8.94E-06	N	N	N	0	4	B	0
			4-159	246	140	9.40E-06	N	N	N	0	2	B	0
			4-161	260	170	9.67E-06	N	N	N	0	4	B	0
			4-162	200	75	9.38E-06	N	N	N	0	3	B	0
			4-163	235	125	9.63E-06	N	N	N	0	1	B	0
0.200-0.299	4	RBT	4-146	260	180	1.02E-05	N	N	N	0	4	B	0
			4-150	255	150	9.05E-06	N	N	N	0	3	B	0
			4-151	235	130	1.00E-05	N	N	N	0	4	B	0
			4-152	245	145	9.86E-06	N	N	N	0	3	B	0
			4-154	312	250	8.23E-06	N	N	N	0	2	B	0
			4-155	270	185	9.40E-06	N	N	N	0	1	B	0
			4-157	247	155	1.03E-05	N	N	N	0	4	B	0
			4-158	236	130	9.89E-06	N	N	N	1	4	B	0
			4-160	290	210	8.61E-06	N	N	N	0	2	B	0
0.300-0.399	4	RBT	4-138	322	270	8.09E-06	N	N	N	0	3	B	0
			4-145	290	240	9.84E-06	N	N	N	0	4	B	0
			4-149	280	230	1.05E-05	N	N	N	0	3	B	0
0.400-0.499	4	RBT	4-141	320	280	8.54E-06	N	N	N	0	3	O	0
			4-153	253	170	1.05E-05	N	N	N	0	3	B	0
0.500-0.599	4	RBT	4-139	242	130	9.17E-06	N	N	N	0	2	B	0
0.600-0.699	4	RBT	4-144	232	120	9.61E-06	N	N	N	0	3	B	0
0.700+	4	RBT	4-143	240	160	1.16E-05	N	N	N	0	3	B	0

						Plasma			ELISA READINGS				
Kidney	Liver	Bile	Sex	Hema.	Leu.	Protein	Fin	Opercle	Rep 1	Rep 2	mean	SD	Remarks
N	A	0	F	39	1	3.2	0	0	0.177	0.199	0.188	0.0156	IMM
N	A	0	F				0	0	0.194	0.158	0.176	0.0255	
N	A	0	M				0	0	0.151	0.165	0.158	0.0099	
N	A	1	F				0	0	0.201	0.181	0.191	0.0141	
N	A	1	F				0	0	0.135	0.120	0.128	0.0106	
N	A	0	F				0	0	0.176	0.195	0.186	0.0134	
N	A	3	M				0	0	0.128	0.145	0.137	0.0120	
N	A	0	F				0	0	0.166	0.142	0.154	0.0170	
N	A	1	F				0	0	0.208	0.160	0.184	0.0339	
N	A	0	F				0	0	0.159	0.206	0.183	0.0332	
N	A	1	F				0	0	0.244	0.222	0.233	0.0156	
N	A	1	M				0	0	0.173	0.325	0.249	0.1075	
N	A	0	F				0	0	0.245	0.205	0.225	0.0283	
N	A	2	M				0	0	0.237	0.206	0.222	0.0219	
N	A	0	F				0	0	0.258	0.322	0.290	0.0453	
N	A	2	M				0	0	0.237	0.249	0.243	0.0085	
N	A	0	F				0	0	0.224	0.192	0.208	0.0226	
N	A	0	F				0	0	0.252	0.181	0.217	0.0502	
N	A	1	F				0	0	0.309	0.271	0.290	0.0269	
N	A	1	U				0	0	0.325	0.420	0.373	0.0672	
N	A	1	F				0	0	0.346	0.281	0.314	0.0460	RBTxCUT
N	A	2	M				0	0	0.422	0.361	0.392	0.0431	
N	A	0	F				0	0	0.409	0.412	0.411	0.0021	
N	A	0	M				0	0	0.471	0.450	0.461	0.0148	
N	A	1	M				0	0	0.584	0.516	0.550	0.0481	
N	A	2	M				0	0	0.699	0.686	0.693	0.0092	
N	A	1	F				0	0	2.131	1.911	2.021	0.1556	

Appendix C(1). Linear regression analysis for brown trout, in Rocky Mountain National Park.

OD v Ktl

Regression Output:		
Constant		9.8E-06
Std Err of Y Est		9.8E-07
R Squared		0.022138
No. of Observations		100
Degrees of Freedom		98
X Coefficient(s)	1.2E-06	
Std Err of Coef.	8.1E-07	

OD v hematocrit

Regression Output:		
Constant		39.36739
Std Err of Y Est		7.42739
R Squared		0.002015
No. of Observations		70
Degrees of Freedom		68
X Coefficient(s)	-3.41957	
Std Err of Coef.	9.228107	

OD v plasma protein

Regression Output:		
Constant		3.999675
Std Err of Y Est		1.175762
R Squared		0.042434
No. of Observations		74
Degrees of Freedom		72
X Coefficient(s)	-2.57471	
Std Err of Coef.	1.441413	

OD v % urolithic kidneys

Regression Output:		
Constant		-0.02809
Std Err of Y Est		0.226126
R Squared		0.602934
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	1.210177	
Std Err of Coef.	0.439198	

OD v fat

Regression Output:		
Constant		0.776571
Std Err of Y Est		0.980558
R Squared		0.018475
No. of Observations		100
Degrees of Freedom		98
X Coefficient(s)	1.103681	
Std Err of Coef.	0.812619	

OD v leucocrit

Regression Output:		
Constant		1.115929
Std Err of Y Est		0.684259
R Squared		0.09552
No. of Observations		71
Degrees of Freedom		69
X Coefficient(s)	2.284526	
Std Err of Coef.	0.846297	

OD v % gravid

Regression Output:		
Constant		0.781146
Std Err of Y Est		0.185256
R Squared		0.575553
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-0.93691	
Std Err of Coef.	0.359816	

OD v % creamy livers

Regression Output:		
Constant		0.05561
Std Err of Y Est		0.036532
R Squared		0.281363
No. of Observations		7
Degrees of Freedom		5
X Coefficient(s)	-0.09928	
Std Err of Coef.	0.070956	

Appendix C(2). Linear regression analysis for brook trout in Rocky Mountain National Park.

OD v Ktl

Regression Output:		
Constant		1.02E-05
Std Err of Y Est		1.25E-06
R Squared		0.027792
No. of Observations		20
Degrees of Freedom		18
X Coefficient(s)	1.97E-06	
Std Err of Coef.	2.74E-06	

OD v fat

Regression Output:		
Constant		1.397095
Std Err of Y Est		0.890246
R Squared		0.098628
No. of Observations		19
Degrees of Freedom		17
X Coefficient(s)	2.725545	
Std Err of Coef.	1.99839	

OD v hematocrit

Regression Output:		
Constant		39.15431
Std Err of Y Est		8.061376
R Squared		0.000646
No. of Observations		18
Degrees of Freedom		16
X Coefficient(s)	1.921367	
Std Err of Coef.	18.89675	

OD v leucocrit

Regression Output:		
Constant		2.942388
Std Err of Y Est		1.613663
R Squared		0.073022
No. of Observations		18
Degrees of Freedom		16
X Coefficient(s)	-4.24661	
Std Err of Coef.	3.782604	

OD v plasma protein

Regression Output:		
Constant		4.808712
Std Err of Y Est		1.239308
R Squared		0.122978
No. of Observations		18
Degrees of Freedom		16
X Coefficient(s)	-4.35137	
Std Err of Coef.	2.905074	

OD v % urolithic kidneys

Regression Output:		
Constant		0.038016
Std Err of Y Est		0.043194
R Squared		0.136251
No. of Observations		5
Degrees of Freedom		3
X Coefficient(s)	-0.06865	
Std Err of Coef.	0.099794	

OD v % creamy livers

Regression Output:		
Constant		0.210367
Std Err of Y Est		0.154658
R Squared		0.21833
No. of Observations		5
Degrees of Freedom		3
X Coefficient(s)	-0.32709	
Std Err of Coef.	0.357319	

OD v % gravid

Regression Output:		
Constant		0.475759
Std Err of Y Est		0.16465
R Squared		0.654213
No. of Observations		5
Degrees of Freedom		3
X Coefficient(s)	0.906279	
Std Err of Coef.	0.380404	

Appendix C(3). Linear regression analysis for rainbow trout, in Rocky Mountain National Park.

OD v Ktl

Regression Output:		
Constant		9.17E-06
Std Err of Y Est		6.98E-07
R Squared		0.236195
No. of Observations		27
Degrees of Freedom		25
X Coefficient(s)	1.05E-06	
Std Err of Coef.	3.78E-07	

OD v fat

Regression Output:		
Constant		2.877992
Std Err of Y Est		0.909133
R Squared		0.000173
No. of Observations		27
Degrees of Freedom		25
X Coefficient(s)	0.032413	
Std Err of Coef.	0.492262	