

## FINAL REPORT

## SAN JUAN RIVER TROUT FISHERY MONITORING PLAN: FISH HEALTH ASSESSMENT



NEW MEXICO COOPERATIVE FISH AND WILDLIFE RESEARCH UNIT

## COOPERATORS:

New Mexico Department of Game and Fish
New Mexico State University
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# San Juan River Trout Fishery Monitoring Plan: Fish Health Assessment 

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## EXECUTIVE SUMMARY

A Health Condition Profile (HCP) was conducted on rainbow trout (Oncorhynchus mykiss) in the San Juan River below Navajo Dam, northwestern New Mexico. The purpose of the HCP was to provide baseline data from which to assess the effect of the 4 -month low-flow test conducted during the winter of 1996-97. Approximately 30 each of juvenile and adult fish were collected at two sites on each of five sample dates from October 2000 to August 2001. After lengths and weights were recorded, a necropsy-based fish health assessment was conducted. Blood was collected for hematocrit and protein analysis, and dorsal epaxial muscle was collected for lipid analysis. Data from the low-flow test (1996-97) and baseline study (200001) were analyzed to compare the health of fish population between the two sample collections.

Statistical comparisons of the data between the low-flow test and baseline study revealed relatively few significant differences. No relevant differences were observed in condition factor, normality index, severity index, feeding index, and HAI between 1996-97 and 2000-01. Although hematocrit was greater in 1996-97 than in 2000-01, all values were within normal ranges published for rainbow trout. In general, total protein levels were lower in 1996-97 than in 2000-01; however, the lower 1996-97 levels may be unrelated to the test because both sizes and sites were significantly lower in October 1996 (before the low flow began) than in October 2000. Percent muscle lipid showed no trend among size classes or sites within either sample collection. The low mesentery fat reserves and percent muscle lipids observed in adults in October 2000 are unexplained, but may be due to a disruption in the food source.

We conclude the health of the rainbow trout population did not appear to be negatively impacted by the 1996-97 low-flow test. However, potential chronic effects of extended low flows cannot be adequately assessed from the data collected in 1996-97 and in 2000-01. Based on the results presented in this report, a 4-month low-flow test and a one-year baseline study do not provide sufficient data to fully interpret the impact of multiple variables (both inherent and anthropogenic) on fish health. We recommend implementation of a multi-year baseline study in conjunction with monitoring future low flows to further assess seasonal versus low-flow effects on the long-term health of the rainbow trout population in the San Juan River.

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## INTRODUCTION

The tailwaters of Navajo Dam on the San Juan River in northwest New Mexico contain a world-class rainbow trout (Oncorhynchus mykiss) fishery. In addition, the San Juan River is home to the endangered Colorado pikeminnow (Ptchocheilus lucius) and the razorback sucker (Xyrauchen texamus). A reduction in winter flow releases from Navajo Dam was proposed by the San Juan River Basin Recovery Implementation Program (Holden 1999) to investigate responses of the native fish populations to manipulations of the river's flow regime (USFWS 1996). The altered flow regime was designed to mimic the historic hydrograph for the endangered fishes. Winter releases are also reduced to store sufficient water for high flows in spring, as well as to meet current and future downstream water needs. To determine the effects of long-term reduced release of Navajo Dam, a 4-month winter low-flow test was conducted October 1996 through March 1997, in which the flow was reduced from approximately 600 cfs (cubic feet per second) to about 300 cfs with a minimum release of 250 cfs . The purpose of the $1996-97$ investigation was to evaluate effects of the reduced flow on the trout fishery within the tailwaters of Navajo Dam. Specific objectives of the monitoring plan provided in a report by U.S. Bureau of Reclamation (USBOR 1998) were to determine if the reduced flow resulted in chronic stress as measured by a Health Condition Profile (Goede 1993), and physiological changes in the rainbow trout population. The results of the health condition profile in 1996-97 were inconclusive and indicated the effects of reduced flow on the health of the fish population may have been confounded by seasonal changes in food resources and metabolic demands (Sutton et al. 1999).

As a result, an additional study was conducted from October 2000 through March 2001 in which the health and condition of the fish population was monitored, but without the reduced flow. A sampling date in August was included to complete a full-year study period for the analysis of seasonal effects on the condition of the fish population. Reported are analyses of the results from the 2000-01 fish health condition profile and physiological indices of metabolic responses and a comparison of those results to the 1996-97 data.

## METHODS

## Sample Sites and Collections

Two sites representative of distinctly different flow regimes and aquatic habitat within the quality trout fishery were selected. The upper site (site 1; approximately 2.1 km long) was between Navajo Dam and Texas Hole, and the lower site (site 2 ; approximately 4.3 km long) was between Texas Hole and the end of the special regulation water. Site 1 was characterized by shallow depth ( $1-2 \mathrm{~m}$ ), narrow river margin ( $20-30 \mathrm{~m}$ ), frequent intermittent riffle areas and few pools. In contrast, site 2 was deeper ( $2-6 \mathrm{~m}$ ) having wider river margins ( $30-50 \mathrm{~m}$ ), infrequent riffle areas and frequent pools. Approximately 30 each of juvenile (155.7-197.2 mm) and adult fish ( $414.4-441.1 \mathrm{~mm}$ ) were collected at each site on each of five sample dates from October 2000 to August 2001.

Fish were collected using an electrofishing boat equipped with a $220-\mathrm{V}$ Smith-Root unit on 24-25 October 2000, 7-8 December 2000, 30-31 January 2001, 12-13 March 2001, and 28-29 August 2001. Immediately upon collection, fish were anesthetized in a buffered solution of Finquel ${ }^{\text {TM }}\left(200 \mathrm{mg} / \mathrm{L} \mathrm{Finquel}^{\mathrm{TM}}: 200 \mathrm{mg} / \mathrm{L} \mathrm{NaCO}_{3}\right)$, and whole blood was collected from the hemal arch at the base of the caudal peduncle using a heparinized 3-ce syringe and a 21-gauge needle. Two hematocrit tubes were filled with whole blood and centrifuged ( $1,500 \times \mathrm{g}, 5 \mathrm{~min}$ ) using a hematocrit centrifuge. The remaining whole blood was immediately placed on ice and centrifuged ( $5,000 \times \mathrm{g}, 10 \mathrm{~min}$ ) within 8 h to obtain plasma for total protein analysis. After centrifugation, the plasma was removed and frozen until analysis for total protein within 2 weeks.

## Health Condition Profile

After lengths ( mm ) and weights ( g ) were recorded, a necropsy-based fish health assessment was conducted. The method evaluates the whole organ appearance and provides a suite of indices including normality, degree of severity, feeding, and condition factor (Goede 1993; see Appendix A Summary of Necropsies and Fish Necropsies Data Sheets). A modification of this method was performed that substitutes numerical values for abnormal ratings and provides a quantitative health assessment index (HAI) for each fish that can be compared
statistically (Adams et al. 1993). After the necropsy, approximately 2 grams of dorsal epaxial muscle were removed for analysis of percent muscle lipids and placed in a cryovial. The muscle samples were frozen until analysis within 8 weeks.

## Physiological Indices

Changes in protein content were analyzed similar to that reported in the 1996-97 winter flow test (USBOR 1998; see Appendix B Total Protein Methods). For every 35 samples analyzed for total protein, a standard curve (serial dilutions of a standard reference- see Methods in Appendix B), a certified reference obtained from Sigma Chemical Co., and pooled fish serum (O. mykiss) were included in each assay. An assay was considered acceptable if all three of the following criteria were observed: (1) the linearity of the standard curve was $\mathrm{r}^{2}=0.97$ or greater; (2) the reference was within the certified range listed by the manufacturer $(5.3-6.7 \mathrm{~g} / \mathrm{dL}$; $\overline{\mathrm{X}}=$ $6.0 \mathrm{~g} / \mathrm{dL}$ ); and (3) the intra- and inter-assay coefficient of variation ( \{standard deviation $\div$ mean) x 100 ) were $\leq 10 \%$ (see Appendix B Quality Assurance - Quality Control). The interassay coefficient of variation was $2.6 \%$ for the certified reference ( $n=13$ ) and the intra-assay coefficient of variation ranged from 0.035 to $7.15 \%(n=12)$.

The procedure to determine total lipid content (percent wet weight) in muscle was determined gravimetrically following extraction and evaporation of methylene chloride (see Appendix C Percent Muscle Lipid Extraction). The method was slightly modified from the version developed for muscle lipid extraction of the 1996-97 Winter Flow Test (USBOR 1998) to include percent moisture.

## Statistical Analysis

Statistical analysis was performed using SAS (SAS, 1999) with a probability level of $\alpha=$ 0.05 applied to all analyses. Data from 2000-01 were analyzed initially without the August sampling period for statistical comparison with 1996-97. Differences between months (October, December, January, March) for condition factor, total protein, and percent muscle lipid were analyzed by analysis of variance in adult and juvenile fish at each site (site 1, site 2). Residuals were graphically displayed on a probability plot and tested for normality using the Shapiro-Wilk
test. If assumptions of normality were not met, the data were $\log$ transformed. A multivariate analysis (MANOVA) was then performed with condition factor, total protein, and muscle lipid as the dependent variables and month as the independent variable. Where the MANOVA results indicated a significant difference among means, Bonferroni multiple comparison test was applied. HAI data were rank transformed, analyzed by analysis of variance, and significant differences observed between months were tested with Tukey's Studentized Range Test. The same tests were applied to the 2000-01 data with the August sampling period included for within-year comparisons.

To compare October through March, 1996-97 and October through March, 2000-01 data, differences between given months were analyzed using MANOVA in adult and juvenile fish at site 1 and 2 with condition factor, total protein, and muscle lipid as the dependent variables and year as the independent variable. Where significant differences were indicated, Tukey's Test was performed on each variable. HAI data were subjected to the Wilcoxon rank sum method to determine differences between comparable months of both collection periods. Normality, severity, and feeding indices and hematocrits were compared between collection periods across all months using t-tests. Data are presented as arithmetic means and standard error (nontransformed) for each of the variables.

## RESULTS AND DISCUSSION

## Fish Health Assessment: October to March 2000-01

## Health Condition Profile

Throughout the study, mean lengths of adult rainbow trout in site 1 ranged from 431.8 to 437.5 mm and juvenile fish ranged from 169.7 to 197.2 mm (Table 1). Mean lengths of adults from site 2 ranged from 415.0 to 439.6 mm and juveniles ranged from 166.9 to 182.7 mm (Table 2). The sex ratios were slightly skewed with a greater percentage of adults identified as females from sites 1 and 2 in October ( $67 \%, 73 \%$ ), December ( $70 \%, 57 \%$ ), and January $(80 \%, 57 \%)$. Of these fish, from 46 to $86 \%$ were observed gravid or in post-spawning condition.
Although the percentage of adult female fish was lower in March for both sites 1 and $2(47 \%$, $47 \%$ ), over $50 \%$ of the fish were gravid or in post-spawning condition (Appendix A).

Table 1. Comparison of 1996-97 and 2000-01 results from the fish health condition profile on rainbow trout in the San Juan River tailwater between Navajo Dam and Texas Hole (site 1). Means are presented for length, condition factor and hematocrit with minimum and maximum in parenthesis.

|  | Sample <br> Size | Length (mm) | Condition <br> Factor | Hematocrit (\%) | Normality Index (\%) | Severity Index | Feeding <br> Index |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Adult Fish: |  |  |  |  |  |  |  |
| October 1996 | 24 | 397.4 (310,460) | $1.17(0.93,1.54)$ | $47(35,58)$ | 85.4 | 0.0 | 76.4 |
| October 2000 | 30 | $437.4(385,500)$ | $1.07(0.73,1.39)$ | $34(10,50)$ | 76.3 | 6.3 | 72.2 |
| December 1996 | 30 | 418.4 (350, 470) | 1.09 (0.92, 1.36) | - | 76.0 | 3.3 | 64.4 |
| December 2000 | 30 | $433.7(365,491)$ | $1.09(0.95,1.31)$ | $41(18,68)$ | 71.3 | 12.1 | 61.1 |
| February 1997 | 30 | 410.6 (351, 462) | 1.06 (0.73, 1.31) | $46(27,56)$ | 83.3 | 7.1 | 83.3 |
| January 2001 | 30 | $437.5(358,485)$ | 1.01 (0.77, 1.20) | $38(25,54)$ | 81.3 | 5.1 | 59.5 |
| March 1997 | 30 | $410.0(350,466)$ | 1.07 (0.65, 1.31) | $46(32,59)$ | 79.7 | 12.5 | 87.8 |
| March 2001 | 30 | $431.8(343,488)$ | $1.00(0.69,1.35)$ | 34 (10.49) | 80.3 | 4.6 | 86.7 |
| August 2001 | 30 | 414.4 (310, 480) | 1.16 (0.84, 1.68) | $45(25,68)$ | 77.0 | 10.0 | 60.0 |
| Juvenile Fish: |  |  |  |  |  |  |  |
| October 1996 | 28 | 186.1 (156, 248) | 1.18 (0.96, 1.55) | $53(38,72)$ | 92.9 | 0.0 | 90.5 |
| October 2000 | 21 | 169.7 (138.226) | 1.18 (0.95, 1.47) | $48(37,63)$ | 91.9 | 7.1 | 74.6 |
| December 1996 | 30 | 178.3 (117, 225) | $1.05(0.72,1.31)$ | - | 95.3 | 2.5 | 70.0 |
| December 2000 | 30 | $170.2(131,220)$ | 1.15 (0.95, 1.38) | $41(29,51)$ | 87.7 | 10.0 | 67.8 |
| February 1997 | 30 | 200.7 (159, 241) | 0.99 (0.87, 1.13) | $51(46,57)$ | 91.7 | 3.8 | 70.0 |
| January 2001 | 30 | 192.5 (146, 239) | 1.09 (0.84, 1.40) | $41(31,54)$ | 92.3 | 7.1 | 73.3 |
| March 1997 | 30 | 177.2 (110, 239) | 0.98 (0.83, 1.34) | $50(38,66)$ | 84.3 | 10.4 | 70.0 |
| March 2001 | 17 | 197.2 (132, 258) | 1.06 (0.91, 1.19) | $36(19.47)$ | 88.2 | 9.6 | 82.4 |
| August 2001 | 30 | $155.7(120,205)$ | 1.21 (0.89, 1.50) | $43(28.55)$ | 88.3 | 11.7 | 90.0 |

Table 2. Comparisons of 1996-97 and 2000-01 results from the fish health condition profile on rainbow trout in the San Juan River tailwater between Texas Hole and the end of the special regulation water (site 2). Means are presented for length, condition factor, and hematocrit with minimum and maximum in parenthesis.

|  | Sample <br> Size | Length <br> (mm) | Condition Factor | $\begin{aligned} & \text { Hematocrit } \\ & \text { (\%) } \end{aligned}$ | Normality Index (\%) | Severity Index | Feeding <br> Index |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Adult Fish: |  |  |  |  |  |  |  |
| October 1996 | 30 | $415.5(309,480)$ | 1.14 (0.89, 1.44) | $46(32,56)$ | 80.7 | 4.6 | 64.4 |
| October 2000 | 30 | 438.6 (389, 475) | 1.08 (0.63, 1.36) | $44(7,69)$ | 77.0 | 7.1 | 72.2 |
| December 1996 | 30 | $410.8(358,464)$ | $1.09(0.79,1.36)$ | $42(22,61)$ | 81.0 | 6.3 | 62.2 |
| December 2000 | 30 | $439.4(378,525)$ | 1.08 (0.80, 1.28) | $38(24,50)$ | 78.0 | 7.9 | 81.1 |
| February 1997 | 30 | $400.2(347,442)$ | 1.05 (0.87, 1.34) | $43(33,54)$ | 83.7 | 6.3 | 66.7 |
| January 2001 | 30 | $415.0(359,495)$ | 1.11 (0.89, 1.30) | $40(28,53)$ | 86.3 | 4.7 | 72.2 |
| March 1997 | 30 | $393.8(343,446)$ | 1.01 (0.82, 1.17) | $49(38,57)$ | 74.0 | 14.6 | 71.1 |
| March 2001 | 30 | $427.1(375,475)$ | 1.02 (0.80, 1.23) | $39(15,49)$ | 76.7 | 6.3 | 72.2 |
| August 2001 | 30 | $441.1(400,485)$ | $1.09(0.70 .1 .34)$ | $41(15,58)$ | 79.3 | 4.17 | 84.4 |
| Juvenile Fish: |  |  |  |  |  |  |  |
| October 1996 | 15 | 219.7 (155, 257) | $1.10(0.96,1.26)$ | $51(43,65)$ | 92.0 | 0.8 | 77.8 |
| October 2000 | 23 | 176.2 (122, 289) | 1.17 (0.37, 1.40) | $43(33,60)$ | 90.4 | 8.2 | 71.0 |
| December 1996 | 30 | 165.4 (131, 212) | 1.07 (0.89, 1.33) | $52(42,68)$ | 86.7 | 7.9 | 66.7 |
| December 2000 | 26 | $166.9(120,243)$ | 1.16 (0.93, 1.48) | $40(21,61)$ | 90.4 | 8.7 | 84.6 |
| February 1997 | 30 | 185.0 (130, 226) | 0.91 (0.76, 1.37) | $50(33,61)$ | 83.0 | 10.4 | 71.1 |
| January 2001 | 30 | 175.8 (125, 260) | $1.04(0.84,1.18)$ | $38(29,52)$ | 93.3 | 5.0 | 73.3 |
| March 1997 | 30 | $181.9(123,224)$ | 0.93 (0.73, 1.08) | $51(34,66)$ | 88.3 | 8.8 | 60.0 |
| March 2001 | 28 | $182.7(122,228)$ | 0.99 (0.83, 1.16) | $41(29,53)$ | 90.7 | 7.6 | 77.4 |
| August 2001 | 30 | 176.7 (130, 230) | 1.21 (0.98, 1.59) | $43(33,54)$ | 89.0 | 13.3 | 98.9 |

A decline in condition factors from October to March, believed to reflect seasonal effects, was observed for both adult and juvenile fish. The decreases observed in adults ( $6.5 \%$ at site 1 , $5.6 \%$ at site 2 ) were not significant (Figure IA). However, the $10.2 \%$ and $15.4 \%$ declines in juveniles at sites 1 and 2, respectively, were significant (Figure 1B).

A series of indices (normality, severity, and feeding) have been developed from the Health Condition Profile. The normality index reflects the percent normal ratings assigned to: eyes, gills, pseudobranchs, kidney, thymus, spleen, hindgut, liver, fins, and opercles. In general, the higher the normality index, the healthier the population. Although no general trend was observed in adults or juveniles at either site, average values for the index at sites 1 and 2 were greater in juveniles $(90 \%, 91.2 \%)$ than adults $(77.3 \%, 79.5 \%)$ (Tables 1 and 2 ). An acceptable range for normality index (with $100 \%$ being normal or indicative of a healthy population) is 90 $100 \%$ (Goede 1993). This criterion indicates that the juvenile fish are within the accepted or normal range while the adult fish are below the acceptable range. The lower normality index for the adults at both sites was influenced by the predominance of abnormal ratings for clubbed and marginate gills, swollen pseudobranchs, and blindness due to cataracts.

The severity index is computed from ratings or level of severity of thymus, hindgut, fin and opercles. The higher the index, the greater the level of severity combined in the four variables. An acceptable range for severity index (with $0 \%$ being normal or indicative of a healthy population) is $0-10 \%$ (Goede 1993). The severity index increased sharply (from $6.3 \%$ in October to $12.1 \%$ in December) in adult fish at site 1 and subsequently decreased to $5.1 \%$ in January (Table 1). The main contributing factor to the increase was fin erosion which may be explained by increased spawning activity due to a higher percentage of sexually mature adults observed in December ( $60 \%$ at site 1 and $53 \%$ at site 2 ). Except for the increase in adults in December, severity indices fluctuated but remained at or below $10 \%$ in both size classes at both sites throughout the study (Tables 1 and 2).

The feeding index is based on the fullness and color of bile in the gallbladder at the time of necropsy and provides an excellent indicator of time to last feeding. The higher the feeding index, the greater the feeding activity. An acceptable range for feeding index (with $100 \%$ being indicative of active feeding) is greater than $67 \%$ (Goede 1993). The index varied for both adults

## 2000-01 Condition Factor



Figure 1. Mean 2000-01condition factor of adult (A) and juvenile (B) rainbow trout collected on four sample dates from site 1 (Navajo Dam to Texas Hole) and site 2 (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a site, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.
and juveniles throughout the study at both sites (Tables 1 and 2). In general, average feeding indices were slightly lower in adults at site $1(69.9 \%)$ and site $2(74.4 \%)$ compared to juveniles at sites $1(74.5 \%)$ and $2(76.6 \%)$. Except for adults at site 1 when the index decreased to $61.1 \%$ in December and $59.5 \%$ in January, both size classes at both sites were within the acceptable range for a population with adequate resources.

The Health Assessment Index (HAI) is calculated by assigning a numerical rating to the values given in the Health Condition Profile to the pseudobranchs, thymus, eyes, gills, spleen, hindgut, kidney, liver, opercles, and fins (Adams et al. 1993). A rating of 0 is given for normal values, 10 for mild abnormalities, 20 for moderate, and 30 for severe. The ratings are summed for each fish and then the means are calculated for each group. The higher the index, the greater the level of abnormalities within that group. Adult fish exhibited higher HAI indices than juveniles at both sites (Figures 2A and B) due to higher levels of abnormalities in the eyes, gills, pseudobranchs, thymus, kidneys, and fins. No significant difference was detected among the months for adults except in January when a decline in the ratings (or improvement in health) was observed at both sites in the pseudobranchs, kidneys, and fins (site $1 P=0.088$, site $2 P=0.012$ ). Juveniles also exhibited a decline in January, although not significant, due to an improvement in pseudobranchs and thymus.

## Physiological Indices

Hematocrit reflects the percent red blood cells to total blood volume and is evaluated in the Health Condition Profile as a broad indicator of population health. It is assumed that elevated levels of hematocrit may represent a population under stress while low levels indicate the presence of disease (Goede and Barton 1990). There was no general trend in hematocrit for adults at both sites or juveniles at site 2 , and even though juveniles at site 1 experienced a $25 \%$ decrease from October to March, both sites and size classes were within normal ranges for rainbow trout (34-57\%, Denton and Yousef 1975; 22-44\%, Miller et al. 1983) (Tables 1 and 2).

Changes in total plasma protein concentrations are considered a measure of sustainable growth (Brett and Groves 1979). Adults and juveniles at site 1 exhibited significant decreases ( $22.4 \%$ and $21.4 \%$, respectively) in protein concentrations from October to March (Figures 3A

2000-01 Health Assessment Index


Figure 2. Mean 2000-01 health assessment index of adult (A) and juvenile (B) rainbow trout collected on four sample dates from site 1 (Navajo Dam to Texas Hole) and site 2 (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a site, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

2000-01 Total Plasma Protein (g/dL)
A


B


Figure 3. Mean 2000-01 total plasma protein (g/dL) in adult (A) and juvenile (B) rainbow trout collected on four sample dates from site I (Navajo Dam to Texas Hole) and site 2 (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a site, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.
and B). Adults at site 2 also experienced a significant decrease ( $26.3 \%$ ), however, juvenile protein concentrations were varied and decreased by only $3.7 \%$ (Figures 3 A and B). In both mature and immature salmonids from Canadian streams, Cunjak (1988) observed decreases in plasma protein levels from peak concentrations in summer to the lowest at the end of winter. Thus, decreases in total protein concentrations observed in this study may reflect seasonal changes.

Lipids are an important source of potential chemical energy, and their presence or absence reflects the performance capacity of fish. No general trend in muscle lipids was observed for adults at site 1 or 2 throughout the study (Figure 4A). In contrast, juveniles in October at both sites had twice the lipid levels of adults but experienced a significant decrease from October to December of $51.5 \%$ at site 1 and $61.9 \%$ at site 2 (Figure 4B). Muscle lipids in both size groups at both sites increased slightly in January possibly reflecting an increase in food resources.

## Comparative Fish Health Assessments: October to March 1996-97 and 2000-01

## Health Condition Profile

Condition factors decreased significantly from October 1996 to March 1997 in both size classes and at both sites ( $P=0.06$ for adults at site 1) (Figures 5A and B). Condition factors also decreased in 2000-01 in both size classes and at both sites; however, only juveniles exhibited a statistically significant decrease (Figures 1A and B). Juveniles had consistently higher condition factors in 2000-01 than 1996-97 with significant differences in all months at both sites except October at both sites and March at site 2 (Figure 6B). In contrast, condition factors in adults in 1996-97 generally were greater than or equal to 2000-01 condition factors (Figures 7A and B); however, only adults at site I in October 1996 had a significantly higher condition factor. The decrease from October to March seen across both sites and size classes in both collection periods appears biologically relevant with respect to changes in seasonal energy requirements. This overwinter loss in condition has been reported in other populations of salmonids, including rainbow trout in the Glen Canyon Dam tailwater (Valdez and Ryel 1995). Also, Cunjak and Power (1987) observed a decline in condition factor in salmonids from late summer through

## 2000-01 Muscle Lipid (\%)



Figure 4. Mean 2000-0t percent muscle lipid (wet weight) in adult (A) and juvenile (B) rainbow trout collected on four sample dates from site I (Navajo Dam to Texas Hole) and site 2 (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a site, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.


Figure 5. Mean 1996-97condition factor of adult (A) and juvenile (B) rainbow trout collected on four sample dates from site 1 (Navajo Dam to Texas Hole) and site 2 (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a site, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

## Comparison of 1996-97 and 2000-01 Condition Factor of Juveniles

A


B


Figure 6. Comparison of 1996-97 and 2000-01 mean condition factor of juvenile rainbow trout collected on four sample dates each collection year from site 1 (A) (Navajo Dam to Texas Hole) and site 2 (B) (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a month, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

Comparison of 1996-97 and 2000-01 Condition Factor of Adults


Figure 7. Comparison of 1996-97 and 2000-01 mean condition factor of adult rainbow trout collected on four sample dates each collection year from site 1 (A) (Navajo Dam to Texas Hole) and site 2 (B) (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a month, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.
early winter as a result of metabolic costs being higher than energy intake when food resources were limiting.

Normality indices of adults at site 1 in 2000-01 were lower, although not significantly, in all collection months compared to indices of adults at the same site in 1996-97 (Table 1). Normality indices for juveniles at site 1 and for adults and juveniles at site 2 were varied for both collection periods (Tables 1 and 2) with no significant difference. In 2000-01 adults received higher abnormality ratings for eyes, kidneys, and fins than adults in 1996-97, whereas in 1996-97 the higher abnormality ratings occurred mainly in the thymus. In contrast, the juveniles in 200001 received higher ratings for the thymus, while in 1996-97 the abnormal ratings were highest in gills, liver and opercles.

Overall, severity indices were within the recommended $10 \%$ for "normal" or healthy fish populations throughout the 1996-97 and 2000-01 studies. In 1996-97, the exceptions were adults in March at sites 1 (12.5\%) and 2 (14.6\%) and juveniles in March at site 1 ( $10.4 \%$ ) and in February at site $2(10.4 \%$ ) (Tables 1 and 2). The higher indices were due mainly to the degree of hemorrhaging in the thymus and shortening of the opercles. The only exception in the 2000-01 study was in December when the index was $12.1 \%$ for adults at site 1 (Tables I and 2). It is important to note that evaluation of the thymus weighs heavily in the severity index; however, the rating of the condition of the thymus has questionable interpretation due to broad and generalized effects of a multitude of stressors in wild populations. In an unpublished stress study, Barton observed a higher incident of thymic hemorrhaging in healthy juvenile brook trout (Salvelinus fontinalis) than in a diseased population (Goede and Barton 1990). Thus, the severity index should be interpreted with caution.

No general trends were observed for feeding indices from October to March for either site or size class within 1996-97 and 2000-01; however, differences were observed between sample collections (Tables 1 and 2). Although not statistically significant, adults at site 1 in 1996-97 had higher feeding indices than adults in 2000-01. In contrast, adults at site 2 had significantly lower feeding indices in 1996-97 than in 2000-01. The average feeding index for juveniles at site 1 was the same for 1996-97 and 2000-01. Although not significantly different, juveniles at site 2 in 1996-97 had a lower average feeding index than in 2000-01. An acceptable range for feeding
index is greater than $67 \%$ with indices below the threshold indicating reduced feeding activity. In 1996-97, half of feeding indices were below the acceptable range for adults while $25 \%$ of feeding indices were below the acceptable range for juveniles. In contrast, $\mathbf{2 5 \%}$ of feeding indices for adults in 2000-01 were below the acceptable range, while none of the indices were below the acceptable range for juveniles.

A Health Assessment Index (HAI) was calculated in 1996-97 from the necropsy ratings in the Health Condition Profile (USBOR 1998). There were no temporal or spatial trends for HAI for either adults or juveniles; however, adults consistently received more abnormal ratings than juveniles (Figures 8A and B). Likewise, there was no general trend for HAI in 2000-01 (Figures 2A and B). Adults in 2000-01 also exhibited higher HAI indices than juveniles and followed the same fluctuating pattern at each site as adults in 1996-97. The majority of abnormal ratings in 1996-97 were observed in gills, pseudobranchs, and thymus, while the majority of abnormal ratings in 2000-01 were observed in gills, pseudobranchs, and eyes. Only adults at site 1 in October 2000 had a significantly higher HAI index than adults in 1996 (Figure 9A) due to increased abnormalities in fins, opercles, kidney, and the hindgut. Adults at site 1 in December had the greatest level of abnormalities for both collection periods with a subsequent improvement in February 1997 and January 2001. Adults at site 2 had the lowest level of abnormalities in February 1997 and January 2001 (Figure 9B). Juveniles at both sites in 1996-97 had consistently higher HAI indices than juveniles in 2000-01 (except for site 1 in December). However, only site 1 in February 1997 exhibited a statistically higher index (Figures 10A and B).

Little is known about the physiological response of the fish pseudobranch and thymus to environmental stressors. Goede and Barton (1990) suggest the swelling of pseudobranchs may indicate a change in the partial pressure of oxygen and carbon dioxide. Increases in salinity levels may also cause pseudobranchial cell disruption (King et al. 1993). In the thymus, seasonal changes may cause visible physiological alterations. Alvarez et al. (1994) described a decrease in intrathymic erythropoiesis activity during winter, as well as a decrease in thymic size from winter to spring (1998). Further studies of environmental factors that affect these organs need to be conducted before implications of abnormalities observed in wild fish populations can be properly addressed.

1996-97 Health Assessment Index


Figure 8. Mean 1996-97 health assessment index of adult (A) and juvenile (B) rainbow trout collected on four sample dates from site 1 (Navajo Dam to Texas Hole) and site 2 (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a site, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

Comparison of 1996-97 and 2000-01
Health Assessment Index of Adults


Figure 9. Comparison of 1996-97 and 2000-01 mean health assessment index of adult rainbow trout collected on four sample dates each collection year from site 1 (A) (Navajo Dam to Texas Hole) and site 2 (B) (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a month, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

Comparison of 1996-97 and 2000-01
Health Assessment Index of Juveniles


Figure 10. Comparison of $1996-97$ and 2000-01 mean health assessment index of juvenile rainbow trout collected on four sample dates each collection year from site 1 (A) (Navajo Dam to Texas Hole) and site 2 (B) (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a month, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

## Physiological Indices

Although there was no general trend from October to March for hematocrit within each collection period, values for both size classes at both sites were significantly lower ( $P=0.06$ for adults at site 2) in 2000-01 than in 1996-97 (Tables 1 and 2). Despite the significant difference between collection periods, the range of mean hematocrit for each period ( $42-53 \%$ in 1996-97 and $34-48 \%$ in 2000-01) falls within the levels of normality identified for rainbow trout ( $34-57 \%$. Denton and Yousef 1975; 22-44\%, Miller et al. 1983). It is important to point out that hematocrit may vary with season (Denton and Yousef 1975), age (Barnhart 1969), and acute stress prior to blood collection (Fletcher 1975); i.e., hematocrit levels could inerease as a result of handling stress. Thus, hematocrit should be interpreted with caution.

Concentrations of total plasma protein in adults at site 1 in 1996-97 and 2000-01 decreased similarly from October to March by $22.7 \%$ and $22.4 \%$, respectively (Figure 11A). However, concentrations in October, December, and March 1996-97 were significantly lower in adults at site 1 than the same months in 2000-01 (February 1996 was also lower but not significantly). At site 2 , results varied between collection periods for adults with a slight increase from October to March in 1996-97 ( $2.4 \%$ ) while concentrations decreased by $26.3 \%$ in 2000-01 (Figure 11B). Total protein concentrations were significantly lower in adults at site 2 in 1996-97 than 2000-01 in October and February/January.

Protein concentrations in juveniles at site 1 decreased from October to March in 1996-97 and $2000-01$ by $15.2 \%$ and $21.4 \%$, respectively (Figure 12A). Between collection periods, bowever, protein levels were highly variable with October 1996 and March 1997 levels significantly lower than October 2000 and March 2001; December 1996 and 2000 levels were equal; and February 1997 levels were significantly higher than January 2001. Slight decreases were observed in plasma protein concentrations in juveniles at site 2 for both 1996-97 (6.4\%) and 2000-01 (3.7\%) with concentrations in 1996-97 significantly lower than 2000-01 in October, December, and March (Figure 12B).

The trends in 2000-01 observed for plasma protein concentrations in adults are similar to those observed by Cunjak (1988) in salmonids (which were related to seasonal changes). That trend was not as evident for this study in 1996-97 because of the highly variable pattern exhibited

## Comparison of 1996-97 and 2000-01 Total Plasma Protein (g/dL) in Adults



Figure 11. Comparison of 1996-97 and 2000-01 mean total plasma protein (g/dL) in adult rainbow trout collected on four sample dates each collection year from site 1 (A) (Navajo Dam to Texas Hole) and site 2 (B) (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a month, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

Comparison of 1996-97 and 2000-01
Total Plasma Protein ( $\mathrm{g} / \mathrm{dL}$ ) in Juveniles


Figure 12. Comparison of $1996-97$ and $2000-01$ mean total plasma protein ( $\mathrm{g} / \mathrm{dL}$ ) in juvenile rainbow trout collected on four sample dates each collection year from site 1 (A) (Navajo Dam to Texas Hole) and site 2 (B) (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a month, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.
by adults and juveniles at both sites (Figures 13A and B). However, both size classes in 1996-97 had significantly lower protein concentrations in October before the low flow test began than their counterparts in 2000-01 (Figures 11A,B and 12A,B). Consequently, inherent sample and physiological variation between collections must also be taken into consideration when comparing results of 1996-97 and 2000-01. Thus, interpretation of low-flow effects should be made with caution.

From October to March of 1996-97, percent muscle lipids in adults exhibited a significant decline of $47.8 \%$ at site 1 and $45.8 \%$ at site 2 (Figures 14A and B). However, 2000-01 lipid levels in adults declined by only $3.2 \%$ and $15.7 \%$ at sites 1 and 2 , respectively (Figure 4 A ). Between sample collections, lipids in adults at site I were consistently lower in 2000-01 than 1996-97 with significant differences observed in October and December (Figure 15A). At site 2, lipid levels were lower in 2000-01 than in 1996-97 in all months except March, although no significant differences were observed (Figure 15B).

In 1996-97, percent muscle lipids in juveniles at site 1 declined significantly from October to March by $65.2 \%$ while a non-significant decrease ( $32 \%$ ) was observed at site 2 (Figure 14B). In 2000-01, juveniles at both sites exhibited significant declines in lipid levels from October to March ( $48.4 \%$ at site 1 and $53.5 \%$ at site 2 ) (Figure 4 B ). Lipid levels in juveniles between sample collections were varied at site 1 with October and December 1996 slightly higher than 2000, but February and March 1997 significantly lower than 2001 (Figure 16A). At site 2 , juvenile lipids were consistently higher in 2000-01 than in 1996-97 with significant differences between February/January and March (Figure 16B).

Depletion of energy stores through autumn and winter in salmonids has been documented by others (Cunjak and Power 1986; Cunjak 1988). Cunjak and Power (1987) observed fish were unable to effectively assimilate ingested foods in winter, resulting in lower energy intake while metabolic costs remained the same. Adults at site 1 in 1996-97 exhibited a seasonal trend whereas adults in 2000-01 showed little change throughout the collection year. Lipids in juveniles at site 1 in 1996-97 also followed a seasonal pattern while juveniles in 2000-01 had fluctuating levels throughout the collection year. Adults and juveniles at site 2 in both sample collections exhibited varying lipid levels among the four sampling periods with no trends

1996-97 Total Plasma Protein (g/dL)


Figure 13. Mean 1996-97 total plasma protein (g/dL) in adult (A) and juvenile (B) rainbow trout collected on four sample dates from site 1 (Navajo Dam to Texas Hole) and site 2 (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a site, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

## 1996-97 Muscle Lipid (\%)



Figure 14. Mean 1996-97 percent muscle lipid (wet weight) in adult (A) and juvenile (B) rainbow trout collected on four sample dates from site I (Navajo Dam to Texas Hole) and site 2 (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a site, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

Comparison of 1996-97 and 2000-01 Muscle Lipid (\%) in Adults


Figure 15. Comparison of $1996-97$ and 2000-01 mean pereent muscle lipid (wet weight) in adult rainbow trout collected on four sample dates each collection year from site 1 (A) (Navajo Dam to Texas Hole) and site 2 (B) (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a month, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

## Comparison of 1996-97 and 2000-01 Muscle Lipid (\%) in Juveniles



B


Figure 16. Comparison of $1996-97$ and 2000-01 mean pereent muscle lipid (wet weight) in juvenile rainbow trout collected on four sample dates each collection year from site 1 (A) (Navajo Dam to Texas Hole) and site 2 (B) (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represem standard error of the mean. Within a month, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.
observed except for the overall decrease from October to March. The absence of a distinguishable pattern between 1996-97 and 2000-01 precludes an accurate interpretation of seasonal versus low-flow effects.

## Fish Health Assessment: August 2001

## Health Condition Profile

For the August 2001 collection, mean lengths of adults and juveniles at site 1 were 414.4 mm and 155.7 mm , respectively (Table 1). Mean lengths of adults and juveniles at site 2 were 441.1 mm and 176.7 mm , respectively (Table 2). Sex ratios once again were slightly skewed with $53 \%$ of adults identified as females from site 1 and $60 \%$ from site 2 . Of the adult females, $56 \%$ from site 1 and $72 \%$ from site 2 were gravid (Appendix A). Condition factors increased significantly from March to August for adults at site 1 and for juveniles at both sites (Figures 17A and B). The $6.4 \%$ increase for adults at site 2 was not statistically significant but may be biologically significant in reflecting a seasonal pattern of increased fitness through the summer months across both sites and sizes (Figure 17A).

Normality indices for both sites and size classes were below the accepted $90 \%$ range for the month of August; however, this represented little change from the March indices (Tables 1 and 2). Although severity indices decreased from $6.3 \%$ in March to $4.2 \%$ in August for adults at site 2, adults at site 1 increased from $4.6 \%$ to $10.0 \%$. Juveniles increased at both sites in August to the highest levels of the collection period ( $11.7 \%$ at site 1 and $13.3 \%$ at site 2 ) (Tables 1 and 2). Hemorrhaging in the thymus was again the main contributing factor. Generally, feeding indices increased in August for both sites and sizes to the highest levels of the collection period, except for adults at site 1 which decreased to $60.0 \%$ (Tables 1 and 2). No significant difference was detected in the health assessment index for the month of August for either site or size class (Figures 18A and B).

## Physiological Indices

Hematocrit levels in August for adults and juveniles at both sites remained in the range observed in the previous 2000-01 sampling periods (Tables 1 and 2). Although the difference

2000-01 Condition Factor (Including August Data)


Figure 17. Mean 2000-01 (including August data) condition factor of adult (A) and juvenile (B) rainbow trout collected on five sample dates from site 1 (Navajo Dam to Texas Hole) and site 2 (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of mean. Within a site, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

2000-01 Health Assessment Index (Including August Data)


Figure 18. Mean $2000-01$ (including August data) health assessment index of adult (A) and juvenile (B) rainbow trout collected on five sample dates from site I (Navajo Dam to Texas Hole) and site 2 (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a site, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.
was not statistically significant, total plasma protein in adults at site 1 and 2 reflected a seasonal increase from March to August (Figures 19A). Protein levels in juveniles at both sites also increased slightly in August (Figure 19B). A seasonal pattern was observed over the 2000-01 collection year in both adults and juveniles as protein levels decreased from October to January and then began increasing in March and August. With the addition of the August data, muscle lipids in juveniles from both sites also exhibited a seasonal pattern with a significant decrease in lipid levels from October to March and a subsequent significant increase from March to August (Figure 20B). Lipid levels in adults at both sites also increased significantly from March to August; however, no seasonal trend was observed due to the low levels measured in October (Figure 20A).

## CONCLUSIONS AND RECOMMENDATIONS

Analysis of the data between the low-flow test (1996-97) and baseline study (2000-01) revealed relatively few significant differences. No relevant differences were observed in condition factor, normality index, severity index, feeding index, and HAI between 1996-97 and 2000-01. Although hematocrit was greater in 1996-97 than in 2000-01, all values were within normal ranges published for rainbow trout. Total plasma protein exhibited a seasonal trend of decreasing concentrations for both age classes at site 1 (Navajo Dam to Texas Hole) while results varied at site 2 (Texas Hole to the end of the special regulation water) in both sample collections. Despite this general similarity, protein levels were generally lower in 1996-97 than in 2000-01. However, total protein in both size classes and sites were statistically lower in October 1996 (before the low flow began) than in October 2000, indicating that the lower 1996-97 levels may be unrelated to the test. Percent muscle lipid levels showed no trend among size classes or sites within either sample collection. The lower mesentery fat reserves and percent muscle lipids observed in adults in October 2000 are unexplained. October was the only month in the 2000-01 sampling period in which lipid levels of adults and juveniles were not similar. When considering the expected seasonal increase in lipid levels (as seen in August 2001 for both size classes), the low levels recorded for adults in October 2000 may be due to a disruption in the food source.

2000-01 Total Plasma Protein (g/dL) (Including August Data)


Figure 19. Mean 2000-01 (including August data) total plasma protein ( $\mathrm{g} / \mathrm{dL}$ ) in adult (A) and juvenile (B) rainbow trout collected on five sample dates from site 1 (Navajo Dam to Texas Hole) and site 2 (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a site, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

2000-01 Muscle Lipid (\%) (Including August Data)


Figure 20. Mean 2000-01 (including August data) pereent muscle lipid (wet weight) in adult (A) and juvenile (B) rainbow trout collected on five sample dates from site I (Navajo Dam to Texas Hole) and site 2 (Texas Hole to the end of the special regulation water) on the San Juan River. Vertical bars represent standard error of the mean. Within a site, values having the same letter are not significantly different from each other. Sample sizes are in parentheses.

The presence of such an anomaly as well as high variability among the health condition parameters confounds the interpretation of baseline data collected from only a one-year study.

Therefore, two important questions arise that cannot be adequately addressed by the lowflow test and baseline study: 1) are data from the 2000-01 collection period an accurate baseline for the San Juan River rainbow trout population, and 2) are the differences observed between the low-flow test and baseline study an artifact of the low-flow or because of inherent variability within the San Juan River system (i.e., attributable to differences in annual rainfall, diurnal and seasonal temperature fluctuations, invertebrate biomass, degrees of fishing pressure). If flow was reduced to 250 cfs when rainbow trout have lower energy reserves (as was observed in October 2000), the effects on the overall health of the population may be different than observed in 1996-97 (when the population began the winter season with higher energy reserves). Also the effect of habitat type and food resources within the San Juan River on adult versus juvenile health warrant further study to provide possible explanations for differences observed between the two size classes and the two sites in the various health condition parameters.

We conclude the health of the rainbow trout population did not appear to be negatively impacted by the 1996-97 low-flow test. However, potential chronic effects of extended low flows cannot be adequately assessed from the data collected in 1996-97 and in 2000-01. Based on results presented in this report, a 4-month low-flow test and a one-year baseline study do not provide sufficient data to fully interpret the impact of multiple variables (both inherent and anthropogenic) on fish health. We recommend implementation of a multi-year baseline study in conjunction with monitoring future low flows to further assess seasonal versus low-flow effects on the long-term health of the rainbow trout population in the San Juan River.

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ATTACHMENT A

## Summary of Necropsies




General Remarks
$\qquad$ Gonads mary temulet were gavid. DuA notabons were not made
Skin


## Summary of Necropsies



Fix. 15y1B


General Remarks


## Summary of Necropsies




General Remarks

Fins
Skin one fash wiesion on abdomen

Gonads several fenales were gravid, but notations were not made
Other 8 fish wet damage from anglers

## Summary of Necropsies




General Remarks

## Summary of Necropsies



Ficezsifis


General Remarks
Fins 2 fah wturga, 1 Soh missing pectoral in Gonads 13 gravid. 5 posi-spamin lemales, 1 male ativing mil
Skin $\qquad$ Other 11 fint weth gamage from angiens

## Summary of Necropsies





General Remarks
Fins
Skin one fish wlevion on abdomen Gonads Other one Bah melewctrahock bruse

## Surnmary of Necropsies




General Remarks
Fins 2 figh missing 1 pedionalin, 1 delomed pect Gonads lemales - 6 gavid, 10 post-spawned, males - 3 estuding mit Skin one fain wlesion on abdomen Other A Asth weth damage from anglers

## Summary of Necropsies




General Remarks
$\qquad$ Gonads


## Summary of Necropsies




Genetal Remarks
Fins one deformed pectonal 2 wturgus on fine
Gonads females - 5 gravid, 5 post-Apawn, males -1 evinuaing milt Skin Other 6 fieh wth darnage from angens

## Summary of Necropsies




General Remarks

## Summary of Necropsies




## General Remarks

Fins one finh miasing a pectoral in

## Summary of Necropsies




General Remarks


## Summary of Necropsies




General Remarks
Fins 2 frss minsing. 2 lins wlungus, intection Skin Bealiod losion on apercie

Gonads temales -3 gravis. 3 post-spam
Other 16 lish wdianage from angleri, one whicoiotis

## Summary of Necropsies




## Summary of Necropsies




## General Pemarks

Fins one caudar ton weth lesion
Skin one with apen wound on side

Gonads females - 4 grawd, 4 posi-spewn Other 14 和h whth damage from angers

## Summary of Necropsies




General Remarks

## Fins

 Gonads[^0] Other

## Summary of Necropsies




General Remarks
Fins one fish wfurgas on cuutal fin Skin teot faht wath entemal lesions

## Summary of Necropsies





General Remarks
Fins Gonads
Skin Other $\qquad$

## Summary of Necropsies




General Remarks
Fins one fith misieg win polvic in Skin $\qquad$ Gonads 3 spmened oue, 8 absorting eggs, 2 rpe females Other one wicobigis, 16 wlaw damage from anglers

## Summary of Necropsies




General Remarks
Fins one fish missing iet pectoral fin Skin $\qquad$
$\qquad$

ATTACHMENT B

# Total Protein Determination (Phenol Reagent Method for Biological Fluids) Sigma Procedure No. 690 

The procedure is based on the combined methods of the biuret and Lowry for determination of protein in plasma. The two methods were combined to improve stability of the reagents, and provide better sensitivity. Since the method is very sensitive, the plasma or sera sample is diluted so that the final protein concentration is between 15 and $100 \mathrm{mg} / \mathrm{dl}$. The diluted protein is further diluted with the biuret reagent, and later with Folin and Ciocalteu's Phenol reagent. The color formed is read at a wavelength between 700 and 750 nm $(725 \mathrm{~nm})$. Protein concentrations are determined from the calibration curve.

## Equipment, Materials, and Supplies

Adjustable pipets and tips ( $100-1000 \mathrm{uL} ; 10-100 \mathrm{uL})$, repeater pipettor, laboratory vortex, borosilicate glass tubes ( 5 and 10 mL ) and test tube rack, spectrophotometer and cuvettes, timer. Sufficient pooled fish plasma and or a certified reference to serve as a control for assays (Sigma "Accutrol" Certified Standard Reference Material- prepare according to instructions). The Accutrol solution is stable 10 days at 4 C . Follow instructions for the preparation of the Accutrol Reference. Maintain a log for recording the Accutrol and the pooled fish sera. Sodium Chloride Solution ( $0.85 \% ; 8.5 \mathrm{~g} \mathrm{NaCl}$ dissolved in 1 liter of deionized water).

## Set-Up Procedure

A. The sample must be diluted to obtain total protein in the range of the standard curve. A range-finding test may be necessary depending upon the level of total protein in the sample. This could vary between species or within species subjected to various environmental factors. The final dilution factor for the following assay is $101(50 \mathrm{uL}$ of the sample was diluted with 5.0 mL of NaCl solution). Treat the unknowns similarly to the Pooled Fish Sample and the Accutrol Reference Sample.

In large test tubes ( 10 mL ), pipet 5.0 mL of NaCl to all test tubes; pipet 50 uL of the sample to its respective tube and vortex.
B. Dilute protein standard $=0.05 \mathrm{~mL}$ standard in 5 mL NaCl .
C. In a second rack of test tubes ( 5 mL ) label the tubes accordingly:

| Test <br> tube No. | Tube label | Contents of tube |
| :---: | :---: | :--- |
| 1 | Blank $(0.0 \mathrm{mg} / \mathrm{dL})$ | $(0.10 \mathrm{~mL} \mathrm{NaCl})$ |
| 2 | Standard $25 \mathrm{mg} / \mathrm{dL}$ | $(0.025 \mathrm{~mL}$ diluted Protein Standard $+0.075 \mathrm{~mL} \mathrm{NaCl})$ |
| 3 | Standard $50 \mathrm{mg} / \mathrm{dL}$ | $(0.05 \mathrm{~mL}$ diluted Protein Standard $+0.05 \mathrm{~mL} \mathrm{NaCl})$ |
| 4 | Standard $75 \mathrm{mg} / \mathrm{dL}$ | $(0.075 \mathrm{~mL}$ diluted Protein Standard $+0.025 \mathrm{~mL} \mathrm{NaCl})$ |
| 5 | Standard $100 \mathrm{mg} / \mathrm{dL}$ | $(0.10 \mathrm{~mL}$ diluted Protein Standard) |


| Test <br> tube No. | Tube label |  |
| :---: | :--- | :--- |
| 6 | Accutrol Reference | Contents of tube |
| 7 | Pooled Fish Sample | $(0.10 \mathrm{~mL}$ diluted Reterence) |
| 8 | Unknown Fish Sample | $(0.10 \mathrm{~mL}$ diluted Pooled Fish Sample) |
| 9 | And so on... | Repeat step for tube \#8 for each unknown. |

## Test Procedure

1. The Biuret Reagent is already prepared. There is sufficient amount of the Reagent to run 50 test tubes (including standards, references, and unknowns). Using the repeater pipettor, pipet 1.1 mL of the Reagent to all tubes. Vortex each tube immediately after addition of Reagent, and allow the tubes to incubate at room temperature for the 10 minutes. Begin timing the 10 minute incubation period with the first tube*.
2. After the 10 minutes, use the repeater pipettor to add to each tube 0.05 mL of the Folin and Ciocalteu's Phenol Reagent (this has also been prepared for you by the manufacture. There is sufficient sample to run 50 test tubes). Vortex each tube immediately after addition of Folin, and allow the tubes to incubate at room temperature for 30 minutes. Begin timing the 30 minute incubation period with the first tube*.
3. While the tubes are incubating, turn on the spectrophotometer and allow to warm up. Set the wavelength to 725 nm .
4. Plot the absorbance values ( Y axis) versus the total protein concentration ( x axis). From the standard curve, read the absorbance for the unknowns to get the diluted protein concentrations in $\mathrm{mg} / \mathrm{dL}(\mathrm{mg} / 100 \mathrm{~mL}$ ). Multiply the diluted concentration by 101 (dilution factor: $5.05 \div 0.05$ ) to get the actual protein concentrations in $\mathrm{mg} / \mathrm{dL}$, then divide by 1000 to get $\mathrm{g} / \mathrm{dL}$. Total protein in reported as $\mathrm{g} / \mathrm{dL}$.

* For reproducibility of results, the timing of the $10-$ minute and $30-$ minute incubation periods as well as reading on the spectrophotometer should be consistent with each tube. When adding the Reagent and Folins from one tube to the next, allow the same amount of time required to read a sample on the spectrophotometer. This keeps the reading of each sample at 40 minutes from the time of the incubation of the Reagent ( 10 min ) and the incubation of the Folins ( 30 min ).

Quality Assurance - Quality Control
San Juan River Protein Determination

Accutrol Inter-assay Controls

| Accutrol <br> Control | Diluted Concn <br> mg/dL | Actual Concn <br> $\mathrm{g} / \mathrm{dL}$ | Mean <br> Concentration |
| :---: | ---: | ---: | :---: |
| $12 / 4 / 2000$ | 64.685 | 6.533 |  |
| $12 / 4 / 2000$ | 64.131 | 6.477 |  |
| $12 / 4 / 2000$ | 62.406 | 6.303 | 6.266 |
| $1 / 22 / 2001$ | 60.511 | 6.112 |  |
| $1 / 15 / 2001$ | 64.228 | 6.487 | Standard Deviation |
| $1 / 17 / 2001$ | 62.512 | 6.314 | 0.30883207 |
| $1 / 17 / 2001$ | 64.164 | 6.481 |  |
| $2 / 13 / 2001$ | 52.590 | 5.312 | n=16 |
| $2 / 12 / 2001$ | 63.642 | 6.428 |  |
| $2 / 12 / 2001$ | 63.527 | 6.416 |  |
| $4 / 19 / 2001$ | 60.099 | 6.070 |  |
| $4 / 19 / 2001$ | 59.863 | 6.046 |  |
| $4 / 19 / 2001$ | 59.469 | 6.006 |  |
| $10 / 10 / 2001$ | 63.438 | 6.407 |  |
| $10 / 10 / 2001$ | 64.164 | 6.481 |  |
| $10 / 10 / 2001$ | 63.145 | 6.378 |  |

Rainbow Trout Intra-assay Control
$\left.\begin{array}{|c|r|r|c|c|}\hline \begin{array}{c}\text { RBT } \\ \text { Contro1 }\end{array} & \begin{array}{c}\text { Diluted Concn } \\ \text { mg/dL }\end{array} & \begin{array}{c}\text { Actual Concn } \\ \text { g/dL }\end{array} & \begin{array}{c}\text { Nean } \\ \text { Standard Deviation }\end{array} & \begin{array}{c}r^{2} \text { values } \\ \text { Std. Curves }\end{array} \\ \hline 12 / 4 / 2000 & 45.861 & 4.632 & 4.511 & 1.00 \\ 12 / 4 / 2000 & 43.238 & 4.367 & 0.13395585 & \\ 12 / 4 / 2000 & 44.890 & 4.534 & & \\ \hline \hline & & 4.579 & 4.628 & \\ 12 / 4 / 2000 & 45.333 & 4.309 & & 0.06967019\end{array}\right]$

| $\begin{aligned} & 2 / 13 / 2001 \\ & 2 / 13 / 2001 \end{aligned}$ | $\begin{aligned} & 35.211 \\ & 34.551 \end{aligned}$ | $\begin{aligned} & 3.556 \\ & 3.490 \end{aligned}$ | $\begin{gathered} 3.523 \\ 0.04714217 \\ \hline \end{gathered}$ | 1.00 |
| :---: | :---: | :---: | :---: | :---: |
| $\begin{aligned} & 2 / 12 / 2001 \\ & 2 / 12 / 2001 \end{aligned}$ | $\begin{aligned} & 45.485 \\ & 47.487 \\ & \hline \end{aligned}$ | $\begin{aligned} & 4.594 \\ & 4.796 \\ & \hline \end{aligned}$ | $\begin{gathered} 4.695 \\ 0.14299412 \\ \hline \end{gathered}$ | 1.00 |
| $\begin{aligned} & 2 / 12 / 2001 \\ & 2 / 12 / 2001 \end{aligned}$ | $\begin{aligned} & 46.581 \\ & 45.798 \end{aligned}$ | $\begin{aligned} & 4.705 \\ & 4.626 \end{aligned}$ | $\begin{gathered} 4.665 \\ 0.05595798 \end{gathered}$ | 1.00 |
| $\begin{aligned} & 4 / 19 / 2001 \\ & 4 / 19 / 2001 \end{aligned}$ | $\begin{aligned} & 35.335 \\ & 36.529 \end{aligned}$ | $\begin{aligned} & 3.569 \\ & 3.689 \end{aligned}$ | $\begin{gathered} 3.629 \\ 0.08525855 \end{gathered}$ | 1.00 |
| $\begin{aligned} & 4 / 19 / 2001 \\ & 4 / 19 / 2001 \end{aligned}$ | $\begin{aligned} & 34.765 \\ & 35.447 \\ & \hline \end{aligned}$ | $\begin{aligned} & 3.511 \\ & 3.580 \end{aligned}$ | $\begin{gathered} 3.546 \\ 0.04866908 \end{gathered}$ | 1.00 |
| $\begin{aligned} & 4 / 19 / 2001 \\ & 4 / 19 / 2001 \end{aligned}$ | $\begin{aligned} & 35.757 \\ & 35.787 \end{aligned}$ | $\begin{aligned} & 3.611 \\ & 3.614 \end{aligned}$ | $\begin{gathered} 3.613 \\ 0.00209468 \end{gathered}$ | 1.00 |
| $\begin{aligned} & 10 / 10 / 2001 \\ & 10 / 10 / 2001 \end{aligned}$ | $\begin{aligned} & 67.636 \\ & 69.395 \end{aligned}$ | $\begin{aligned} & 6.831 \\ & 7.009 \end{aligned}$ | $\begin{gathered} 6.920 \\ 0.12562388 \end{gathered}$ | 1.00 |
| $\begin{aligned} & 10 / 10 / 2001 \\ & 10 / 10 / 2001 \end{aligned}$ | $\begin{aligned} & 67.848 \\ & 71.134 \end{aligned}$ | $\begin{aligned} & 6.853 \\ & 7.185 \\ & \hline \end{aligned}$ | $\begin{gathered} 7.019 \\ 0.23467884 \end{gathered}$ | 1.00 |
| $\begin{aligned} & 10 / 10 / 2001 \\ & 10 / 10 / 2001 \end{aligned}$ | $\begin{aligned} & 68.699 \\ & 70.854 \end{aligned}$ | $\begin{aligned} & 6.939 \\ & 7.156 \end{aligned}$ | $\begin{gathered} 7.047 \\ 0.15390533 \\ \hline \end{gathered}$ | 1.00 |

## Protein Determination - San Juan River

October, 2000

| Sample <br> Number | $\begin{gathered} \text { Diluted Conen } \\ \mathrm{mg} / \mathrm{dL} \end{gathered}$ | Actual Conen $\mathrm{g} / \mathrm{dL}$ | Comments | Mean Concn for each size/site group |
| :---: | :---: | :---: | :---: | :---: |
| 1 A 01 | 40.755 | 4.116 |  |  |
| 1 A 02 | 49.150 | 4.964 |  | Site 1/Adult Mean |
| 1 A 03 | 59.441 | 6.004 |  | 5.857 |
| 1 A 04 | 76.790 | 7.756 |  |  |
| 1 A 05 | 45.749 | 4.621 |  | Standard Deviation |
| 1 106 | 46.035 | 4.650 |  | 2.203541435 |
| 1 A07 | 105.005 | 10.606 |  |  |
| 1 108 | 48.789 | 4.928 |  | Standard Error |
| 1 A 09 | 49.478 | 4.997 |  | 0.4023 |
| 1 A10 | 81.264 | 8.208 |  |  |
| 1 A11 | 42.439 | 4.286 |  | $n=30$ |
| 1 A12 | 75.424 | 7.618 |  |  |
| 1 A13 | 39.322 | 3.972 |  |  |
| 1 A 14 | 42.123 | 4.254 |  |  |
| 1A15 | 55.183 | 5.573 |  |  |
| 1 A16 | 13.457 | 1.359 | plasma too clear?? |  |
| 1 A17 | 49.464 | 4.996 |  |  |
| 1 A 18 | 66.166 | 6.683 |  |  |
| 1 A19 | 64.593 | 6.524 |  |  |
| 1A20 | 64.431 | 6.508 |  |  |
| 1 A 21 | 70.726 | 7.143 |  |  |
| 1 A 22 | 41.024 | 4.143 |  |  |
| 1A23 | 68.364 | 6.905 |  |  |
| 1 1.24 | 72.362 | 7.309 |  |  |
| 1 A 25 | 104.104 | 10.515 |  |  |
| 1 A26 | 97.088 | 9.806 |  |  |
| 1 127 | 57.984 | 5.856 |  |  |
| 1 A28 | 14.182 | 1.432 |  |  |
| 1 A 29 | 53.042 | 5.357 |  |  |
| 1A30 | 45.893 | 4.635 |  |  |
| 1801 | 61.726 | 6.234 |  | Site 1/Juvnl Mean |
| 1802 | 48.581 | 4.907 |  | 5.006 |
| 1803 | 47.556 | 4.803 |  |  |
| 1804 | 53.948 | 5.449 |  | Standard Deviation |
| 1805 | 60.965 | 6.157 | light hemolysis | 0.719086159 |
| 1806 | 45.274 | 4.573 | 1 t . Hemo. - 25 uL |  |
| 1807 | 37.162 | 3.753 | light hemolysis | Standard Error |
| 1808 | 51.192 | 5.170 | 1 Ight hemolysis | 0.1569 |
| 1809 | 49.905 | 5.040 | hemolysis - 25 uL |  |
| 1810 | 43.057 | 4.349 | light hemolysis | $\mathrm{n}=21$ |
| 1811 | 46.127 | 4.659 | light hemolysis |  |


| 1812 | 40.613 | 4.102 | light hemolysis |  |
| :---: | :---: | :---: | :---: | :---: |
| 1813 | 47.873 | 4.835 | light hemo. - 25 uL |  |
| 1814 | 41.896 | 4.231 |  |  |
| $1 \mathrm{B15}$ | 51.617 | 5.213 | light hemolysis |  |
| 1816 | 55.164 | 5.572 |  |  |
| $1 \mathrm{B17}$ | 48.870 | 4.936 | dark red - 25 uL |  |
| $1 \mathrm{B18}$ | 48.769 | 4.926 | light hemolysis |  |
| $1 \mathrm{B19}$ | 66.382 | 6.705 |  |  |
| $1 \mathrm{B20}$ | 49.313 | 4.981 | hemolysis |  |
| $1 \mathrm{B21}$ | 44.963 | 4.541 |  |  |
| 2 A 01 | 54.919 | 5.547 |  | Site 2/Adult Mean |
| 2A02 | 41.591 | 4.201 |  | 5.965 |
| 2A03 | 48.808 | 4.930 |  |  |
| 2A04 | 42.865 | 4.329 |  | Standard Deviation |
| 2A05 | 87.748 | 8.863 |  | 1.530422645 |
| 2A06 | 99.689 | 10.069 |  |  |
| 2A07 | 82.949 | 8.378 |  | Standard Error |
| 2A08 | 57.797 | 5.837 | hemolys is | 0.2794 |
| 2A09 | 60.708 | 6.132 |  |  |
| 2 AlO | 39.237 | 3.963 |  | $\mathrm{n}=30$ |
| 2A11 | 33.508 | 3.384 | Ifght hemolysis |  |
| $2 \mathrm{Al2}$ | 43.558 | 4.399 |  |  |
| 2A13 | 66.489 | 6.715 |  |  |
| 2 A 14 | 51.981 | 5.250 |  |  |
| 2 A 15 | 51.281 | 5.179 | light hemolysis |  |
| 2 Al 6 | 46.874 | 4.734 |  |  |
| 2 A 17 | 61.629 | 6.225 |  |  |
| 2A18 | 65.480 | 6.613 | hemolysis |  |
| $2 \mathrm{A19}$ | 60.394 | 6.100 |  |  |
| 2A20 | 83.862 | 8.470 |  |  |
| 2A21 | 50.758 | 5.127 |  |  |
| 2 A 22 | 51.506 | 5.202 |  |  |
| 2 A 23 | 65.943 | 6.660 | light hemolysis |  |
| 2 A 24 | 66.648 | 6.731 |  |  |
| 2A25 | 57.029 | 5.760 | hemolysis |  |
| 2A26 | 56.515 | 5.708 |  |  |
| 2A27 | 60.221 | 6.082 | light hemolysis |  |
| 2A28 | 46.543 | 4.701 |  |  |
| 2A29 | 74.995 | 7.574 |  |  |
| 2A30 | 60.248 | 6.085 |  |  |
| 2B01 | 56.246 | 5.681 | light hemolysis | Site 2/Juvnl Mean |
| $2 \mathrm{B02}$ | 26.016 | 2.628 | dark red/brwn - 25 uL | $4.573$ |
| 2 BO 3 | 54.183 | 5.472 | light hemo. - 25 uL |  |
| $2 \mathrm{B04}$ | 48.157 | 4.864 | light hemolysis | Standard Deviation |
| 2805 | 40.615 | 4.102 |  | 0.77582677 |
| 2806 | 56.394 | 5.696 |  |  |


| 2 B 07 | 37.460 | 3.783 |  | Standard Error |
| :--- | :--- | :--- | :--- | :--- |
| 2 B 08 | 48.542 | 4.903 |  | 0.1618 |
| 2809 | 55.555 | 5.611 | light hemolysis |  |
| 2 B 10 | 48.168 | 4.865 |  |  |
| 2 B 11 | 45.688 | 4.614 | light hemolysis |  |
| 2 B 12 | 43.624 | 4.406 | light hemolysis |  |
| 2 B 13 | 46.027 | 4.649 |  |  |
| 2 B 14 | 44.587 | 4.503 |  |  |
| 2 B 15 | 37.689 | 3.807 |  |  |
| 2 B 16 | 35.525 | 3.588 | light hemolysis |  |
| 2 B 17 | 40.775 | 4.118 | not enough plasma?? |  |
| 2 B 18 | 47.150 | 4.762 |  |  |
| 2 B 19 | 48.516 | 4.900 |  |  |
| 2 B 20 | 53.819 | 5.436 |  |  |
| 2 B 21 | 38.688 | 3.907 |  |  |
| 2 B 22 | 50.225 | 5.073 |  |  |
| 2 B 23 | 37.770 | 3.815 | hemolysis |  |

## Protein Determination - San Juan River

 December, 2000| Sample <br> Number | Diluted Concn mg/dL | Actual Conen $\mathrm{g} / \mathrm{dL}$ | Comments | Nean Concn for each size/site group |
| :---: | :---: | :---: | :---: | :---: |
| 1A31 | 94.413 | 9.536 | some hemolysis |  |
| 1 A32 | 81.249 | 8.206 |  | Site 1/Adult Mean |
| 1833 | 44.462 | 4. 491 |  | 5.731 |
| 1 A34 | 44.727 | 4.517 |  |  |
| 1 A35 | 52.008 | 5.253 |  | Standard Deviation |
| 1 A36 | 43.071 | 4.350 |  | 1.564774395 |
| 1 A37 | 46.627 | 4.709 |  |  |
| 1 A38 | 65.538 | 6.619 |  | Standard Error |
| 1 A39 | 93.285 | 9.422 |  | 0.2857 |
| 1 A 40 | 61.226 | 6.184 |  |  |
| 1 A 41 | 55.629 | 5.619 |  | $n=30$ |
| 1 A 42 | 53.802 | 5.434 |  |  |
| 1 A 43 | 35.920 | 3.628 |  |  |
| 1 A 44 | 57.508 | 5.808 | some hemolysis |  |
| 1A45 | 52.982 | 5.351 |  |  |
| 1 146 | 45.369 | 4.582 |  |  |
| 1847 | 68.662 | 6.935 |  |  |
| 1 A 48 | 62.330 | 6.295 |  |  |
| 1 A 49 | 41.887 | 4.231 |  |  |
| 1 A 50 | 48.724 | 4.921 |  |  |
| 1 A 51 | 49.774 | 5.027 | hemolysis |  |
| 1 A52 | 54.918 | 5.547 |  |  |
| 1 A53 | $58+108$ | 5.869 |  |  |
| 1 1.54 | 44.874 | 4.532 |  |  |
| 1A55 | 73.783 | 7.452 |  |  |
| 1 1.56 | 33.594 | 3.393 |  |  |
| 1857 | 62.232 | 6.285 |  |  |
| 1A58 | 49.294 | 4.979 |  |  |
| 1A59 | 81.408 | 8.222 |  |  |
| 1 A 60 | 44.875 | 4.532 |  |  |
| 1B31 | 43.890 | 4.433 |  | Site 1/Juvnl Mean |
| 1832 | 47.170 | 4.764 |  | 4.143 |
| 1833 | 52.674 | 5.320 | some hemolysis |  |
| 1834 | 40.271 | 4.067 |  | Standard Deviation |
| 1835 | 40.842 | 4.125 |  | 0.72604381 |
| 1836 | 41.278 | 4.169 |  |  |
| 1837 | 43.272 | 4.370 |  | Standard Error |
| 1B38 | 35.462 | 3.582 | hemolysis | 0.1372 |
| 1839 | 34.141 | 3.448 |  |  |
| 1840 | 48.484 | 4.897 |  | $\mathrm{n}=28$ |
| 1841 | 47.529 | 4.800 |  |  |


| $1 \mathrm{B42}$ | 36.510 | 3.688 | hemolysis |  |
| :---: | :---: | :---: | :---: | :---: |
| $1 \mathrm{B43}$ | 47.166 | 4.764 |  |  |
| $1 \mathrm{B44}$ | 34.846 | 3.519 |  |  |
| $1 \mathrm{B45}$ | 32.914 | 3.324 |  |  |
| 1846 |  |  | not enough plasma |  |
| $1 \mathrm{B47}$ | 63.549 | 6.418 |  |  |
| $1 \mathrm{B48}$ | 46.679 | 4.715 |  |  |
| $1 \mathrm{B49}$ | 36.499 | 3.686 |  |  |
| $1 \mathrm{B50}$ | 39.141 | 3.953 |  |  |
| 1851 | 35.021 | 3.537 |  |  |
| $1 \mathrm{B5} 2$ | 34.415 | 3.476 |  |  |
| 1853 | 27.739 | 2.802 | hemolysis |  |
| $1 \mathrm{B54}$ | 40.826 | 4.123 |  |  |
| $1 \mathrm{B55}$ | 39.453 | 3.985 |  |  |
| 1856 | 44.864 | 4.531 |  |  |
| 1857 | 39.572 | 3.997 | some hemolysis |  |
| 1858 | 36.834 | 3.720 |  |  |
| $\begin{aligned} & 1859 \\ & 1860 \\ & \hline \end{aligned}$ | 37.420 | 3.779 | some hemolysis <br> not enough plasma |  |
| 2A31 | 66.007 | 6.667 |  | Site 2/Adult Mean |
| 2 2.32 | 50.724 | 5.123 |  | 5.101 |
| 2A33 | 48.361 | 4.884 |  |  |
| 2A34 | 54.356 | 5.490 | some hemolysis | Standard Deviation |
| 2A35 | 45.998 | 4.646 |  | 1.157493072 |
| 2 A36 | 68.495 | 6.918 |  |  |
| 2 A 37 | 36.857 | 3.723 |  | Standard Error |
| 2A38 | 50.355 | 5.086 |  | 0.2113 |
| 2 239 | 40.595 | 4.100 |  |  |
| 2A40 | 50.811 | 5.132 |  | $n=30$ |
| 2A41 | 52.568 | 5.309 |  |  |
| 2A42 | 81.577 | 8.239 |  |  |
| 2A43 | 56.514 | 5.708 | some hemolysis |  |
| 2A44 | 77.859 | 7.864 |  |  |
| 2A45 | 55.830 | 5.639 |  |  |
| 2A46 | 40.046 | 4.045 |  |  |
| 2447 | 45.306 | 4.576 |  |  |
| 2A48 | 38.407 | 3.879 |  |  |
| 2A49 | 44.965 | 4.542 |  |  |
| 2.250 | 48.889 | 4.938 |  |  |
| 2A51 | 54.254 | 5.480 |  |  |
| 2A52 | 39.919 | 4.032 |  |  |
| 2 2A3 | 56.899 | 5.747 |  |  |
| 2A54 | 47.418 | 4.789 |  |  |
| 2A55 | 46.641 | 4.711 |  |  |
| 2A56 | 42.751 | 4.318 |  |  |
| 2A57 | 46.577 | 4.704 |  |  |


| $\begin{aligned} & 2 A 58 \\ & 2 A 59 \\ & 2 A 60 \end{aligned}$ | $\begin{aligned} & 56.475 \\ & 30.997 \\ & 38.725 \end{aligned}$ | $\begin{aligned} & 5.704 \\ & 3.131 \\ & 3.911 \end{aligned}$ |  |  |
| :---: | :---: | :---: | :---: | :---: |
| 2831 2832 | 48.310 54.775 | 4.879 5.532 |  | Site 2/Juvnl Mean 4.814 |
| 2833 2834 2835 | 49.099 47.776 48.623 | 4.959 4.825 4.911 | hemolysis | $\begin{gathered} \text { Standard Deviation } \\ 0.842600277 \end{gathered}$ |
| 2836 2837 $2 B 38$ | 44.127 52.989 43.913 | 4.457 5.352 4.435 |  | $\begin{gathered} \text { Standard Error } \\ 0.1652 \end{gathered}$ |
| 2B39 | 52.297 | 5.282 |  |  |
| 2B40 | 45.349 | 4.580 |  | $\mathrm{n}=26$ |
| 2B41 | 75.671 | 7.643 |  |  |
| $2 \mathrm{B42}$ | 59.266 | 5.986 |  |  |
| $2 \mathrm{B43}$ | 40.501 | 4.091 |  |  |
| 2B44 | 47.230 | 4.770 |  |  |
| 2B45 | 47.990 | 4.847 |  |  |
| 2846 | 47.119 | 4.759 | some hemolysis |  |
| 2B47 | 51.866 | 5.238 |  |  |
| 2B48 | 40.062 | 4.046 | some hemolysis |  |
| 2B49 | 42.033 | 4.245 | some hemolysis |  |
| 2850 | 27.608 | 2.788 | hemolysis - 25 uL |  |
| $2 \mathrm{B51}$ | 44.217 | 4.466 | hemolysis |  |
| 2B52 | 51.045 | 5.156 | some hemolysis |  |
| 2B53 | 48.729 | 4.922 | some hemolysis |  |
| 2B54 | 44.165 | 4.461 | hemolysis - 25 uL |  |
| 2855 | 46.068 | 4.653 |  |  |
| 2856 | 38.461 | 3.885 | hemolysis - 25 uL |  |

Protein Determination - San Juan River January, 2001

| Sample <br> Number | Diluted Conen mg/dt | Actual Concn g/dL | Comments | Mean Concn for each size/site group |
| :---: | :---: | :---: | :---: | :---: |
| 1 1461 | 39.494 | 3.989 |  |  |
| 1 1.62 | 45.519 | 4. 597 |  | Site 1/Adult Mean |
| 1 1A63 | 43.123 | 4.355 |  | 4.359 |
| 1A64 | 46.838 | 4.731 |  |  |
| 1 A65 | 57.221 | 5.779 |  | Standard Deviation |
| 1 A66 | 80.750 | 8.156 |  | 1.102 |
| 1 1467 | 26.491 | 2.676 |  |  |
| 1A68 | 41.609 | 4.202 |  | Standard Error |
| 1A69 | 38.007 | 3.839 |  | 0.2013 |
| 1 A70 | 57.717 | 5.829 |  |  |
| 1 A71 | 48.851 | 4.934 |  | $n=30$ |
| 1 A72 | 46.799 | 4.727 |  |  |
| $1 \times 73$ | 41.731 | 4.215 |  |  |
| $1 \times 74$ | 13.679 | 1.382 |  |  |
| 1A75 | 31.199 | 3.151 |  |  |
| 1 1476 | 47.556 | 4.803 |  |  |
| $1 \times 77$ | 41.698 | 4.211 |  |  |
| $1 \times 78$ | 39.700 | 4.010 |  |  |
| 1 A79 | 48.704 | 4.919 |  |  |
| 1 A80 | 41.682 | 4.210 |  |  |
| 1 181 | 45.878 | 4.634 |  |  |
| 1 A82 | 44.117 | 4.456 |  |  |
| 1483 | 49.644 | 5.014 |  |  |
| 1 184 | 40.941 | 4.135 |  |  |
| 1 185 | 40.037 | 4.044 |  |  |
| 1 186 | 39.556 | 3.995 | clear |  |
| 1 1887 | 40.156 | 4.056 |  |  |
| 1 188 | 40.671 | 4.108 |  |  |
| 1A89 | 40.279 | 4.068 |  |  |
| 1490 | 35.063 | 3.541 |  |  |
| $1 \mathrm{B61}$ | 32.026 | 3.235 |  | Site 1/Juvnl Mean |
| $1 \mathrm{B62}$ | 40.747 | 4.115 |  | 3.803 |
| $1 \mathrm{B63}$ | 29.638 | 2.993 |  |  |
| 1B64 | 41.433 | 4.185 | some hemolysis | Standard Deviation |
| 1B65 | 47.776 | 4.825 |  | 0.658 |
| $1 \mathrm{B66}$ | 45.659 | 4.612 |  |  |
| $1 \mathrm{B67}$ | 43.978 | 4.442 | some hemolysis | Standard Error |
| 1868 | 48.694 | 4.918 |  | 0.1202 |
| $1 \mathrm{B69}$ | 31.500 | 3.182 |  |  |
| 1870 | 32.604 | 3.293 |  | $\mathrm{n}=30$ |
| 1871 | 41.423 | 4.184 |  |  |


| 1872 | 28.856 | 2.915 | some hemolysis |  |
| :---: | :---: | :---: | :---: | :---: |
| 1873 | 40.210 | 4.061 |  |  |
| 1B74 | 31.750 | 3.207 | hemolysis |  |
| 1B75 | 34.141 | 3.448 |  |  |
| 1876 | 52.992 | 5.352 |  |  |
| 1877 | 33.299 | 3.363 |  |  |
| 1878 | 39.154 | 3.955 |  |  |
| $1 \mathrm{B79}$ | 37.539 | 3.791 |  |  |
| 1880 | 45.470 | 4.592 |  |  |
| $1 \mathrm{B81}$ | 33.606 | 3.394 |  |  |
| 1882 | 42.309 | 4.273 |  |  |
| $1 \mathrm{B83}$ | 30.692 | 3.100 |  |  |
| 1884 | 34.933 | 3.528 |  |  |
| 1885 | 41.572 | 4.199 |  |  |
| 1886 | 35.218 | 3.557 | some hemolysis |  |
| 1887 | 28.757 | 2.904 | hemolysis |  |
| $1 \mathrm{B88}$ | 33.526 | 3.386 | some hemolysis |  |
| $1 \mathrm{B89}$ | 31.022 | 3.133 | hemolysis |  |
| 1890 | 39.083 | 3.947 | some hemolysis |  |
| 2A61 | 55.655 | 5.621 |  | Site 2/Adult Mean |
| 2A62 | 44.246 | 4.469 |  | 4.466 |
| 2A63 | 58.257 | 5.884 |  |  |
| 2A64 | 45.636 | 4.609 |  | Standard Deviation |
| 2A65 | 75.279 | 7.603 |  | 1.142 |
| 2A66 | 50.347 | 5.085 |  |  |
| 2A67 | 70.659 | 7.137 |  | Standard Error |
| 2A68 | 43.609 | 4.405 |  | 0.2085 |
| 2A69 | 38.135 | 3.852 |  |  |
| 2A70 | 37.846 | 3.822 |  | $n=30$ |
| 2A71 | 35.216 | 3.557 | clear |  |
| 2A72 | 39.003 | 3.939 |  |  |
| $2 \times 73$ | 42.983 | 4.341 |  |  |
| 2A74 | 40.679 | 4.109 | some hemolysis |  |
| 2A75 | 32.138 | 3.246 |  |  |
| 2A76 | 19.443 | 1.964 |  |  |
| 2A77 | 49.868 | 5.037 |  |  |
| 2A78 | 49.771 | 5.027 |  |  |
| 2A79 | 44.116 | 4.456 |  |  |
| 2A80 | 46.073 | 4.653 |  |  |
| 2A81 | 29.932 | 3.023 |  |  |
| 2 A 82 | 45.355 | 4.581 | some hemolysis |  |
| $2 \mathrm{AB3}$ | 43.985 | 4.442 |  |  |
| 2A84 | 37.563 | 3.794 |  |  |
| $2 A 85$ | 44.281 | 4.472 |  |  |
| 2A86 | 26.153 | 2.641 |  |  |
| 2A87 | 40.567 | 4.097 |  |  |


| $\begin{aligned} & 2 A 88 \\ & 2 A 89 \\ & 2 A 90 \end{aligned}$ | $\begin{aligned} & 42.047 \\ & 51.562 \\ & 46.082 \end{aligned}$ | $\begin{aligned} & 4.247 \\ & 5.208 \\ & 4.654 \end{aligned}$ | hemolysis |  |
| :---: | :---: | :---: | :---: | :---: |
| 2B61 | 33.116 32.668 | 3.345 3.299 |  | Site $2 /$ Juvnl Mean 4.047 |
| 2B63 | 41.375 40.661 44.431 | 4.179 4.107 4.488 | hemolysis | Standard Deviation $0.659$ |
| 2866 2867 $2 B 68$ | 45.909 42.948 36.772 | 4.637 4.338 3.714 |  | $\begin{gathered} \text { Standard Error } \\ 0.1224 \end{gathered}$ |
| 2869 | 39.149 | 3.954 |  |  |
| 2870 | 38.860 | 3.925 | some hemolysis | $n=29$ |
| $2 \mathrm{B71}$ |  |  | no plasma |  |
| 2B72 | 43.927 | 4.437 | hemolysis |  |
| $2 \mathrm{B73}$ | 31.958 | 3.228 |  |  |
| $2 \mathrm{B74}$ | 28.566 | 2.885 | some hemolysis |  |
| 2B75 | 36.284 | 3.665 | hemolysis |  |
| 2876 | 39.864 | 4.026 | some hemolysis |  |
| 2B77 | 47.440 | 4.791 | hemolysis |  |
| 2878 | 47.647 | 4.812 | hemolysis |  |
| 2B79 | 32.596 | 3.292 | some hemolysis |  |
| 2B80 | 39.197 | 3.959 |  |  |
| $2 \mathrm{B81}$ | 43.045 | 4.348 |  |  |
| 2B82 | 53.565 | 5.410 |  |  |
| 2B83 | 35.107 | 3.546 | some hemolysis |  |
| 2B84 | 56.930 | 5.750 | some hemolysis |  |
| 2B85 | 34.638 | 3.498 | some hemolysis |  |
| 2B86 | 34.545 | 3.489 | hemolysis |  |
| 2887 | 41.060 | 4.147 | hemolysis |  |
| 2888 | 45.768 | 4.623 | hemolys is |  |
| 2B89 | 35.446 | 3.580 | hemolys is |  |
| 2890 | 38.598 | 3.898 |  |  |

Protein Determination - San Juan River March, 2001

| Sample <br> Number | Diluted Concn mg/dL | Actual Conen $\mathrm{g} / \mathrm{dL}$ | Comments | Mean Concn for each size/site group |
| :---: | :---: | :---: | :---: | :---: |
| 1 A91 | 46.602 | 4.707 |  |  |
| 1492 | 21.751 | 2.197 |  |  |
| 1 A93 | 37.344 | 3.772 |  | Site 1/Adult Mean |
| 1 A94 | 42.174 | 4.260 |  | 4.552 |
| 1 1995 | 46.837 | 4.731 |  |  |
| 1 A96 | 48.032 | 4.851 |  | Standard Deviation |
| 1497 | 59.471 | 6.007 |  | 1.131551754 |
| 1 198 | 65.419 | 6.607 |  |  |
| 1 A99 | 73.167 | 7.390 |  | Standard Error |
| 1 A 100 | 42.994 | 4.342 |  | 0.2066 |
| 1 1.101 | 35.421 | 3.578 |  |  |
| 1 1.102 | 33.167 | 3.350 |  | $n=30$ |
| 1 A 103 | 46.627 | 4.709 |  |  |
| 1A104 | 63.753 | 6.439 |  |  |
| 1 A 105 | 32.250 | 3.257 |  |  |
| 1 1106 | 21.305 | 2.152 |  |  |
| 1 A 107 | 44.514 | 4.496 |  |  |
| 1A108 | 40.276 | 4.068 |  |  |
| 1 A 109 | 40.966 | 4.138 |  |  |
| 1A110 | 44.741 | 4.519 |  |  |
| $1 \mathrm{Al11}$ | 50.833 | 5.134 |  |  |
| 1 A 112 | 44.436 | 4.488 |  |  |
| 1 A113 | 47.516 | 4.799 |  |  |
| 18114 | 37.893 | 3.827 |  |  |
| 1 A115 | 49.302 | 4.979 |  |  |
| 1 1116 | 40.601 | 4.101 |  |  |
| $1 \times 117$ | 50.135 | 5.064 |  |  |
| 1 A118 | 42.209 | 4.263 |  |  |
| 1 A119 | 49.319 | 4.981 |  |  |
| 1 A120 | 52.955 | 5.348 |  |  |
| $1 \mathrm{B91}$ | 33.773 | 3.411 |  | Site 1/Juvnl Nean |
| 1892 | 44.936 | 4.539 |  | 3.942 |
| 1893 | 50.529 | 5.103 |  |  |
| 1894 | 43.154 | 4.359 |  | Standard Deviation |
| 1895 | 42.927 | 4.336 |  | 0.637773712 |
| 1896 | 25.777 | 2.603 |  |  |
| 1897 | 31.488 | 3.180 |  | Standard Error |
| 1898 | 40.847 | 4.126 |  | 0.1594 |
| 1899 | 42.467 | 4.289 |  |  |
| 18100 | 37.870 | 3.825 |  | $\mathrm{n}=16$ |
| 18101 | 37.276 | 3.765 |  |  |


| 18102 | 44.855 | 4.530 |  |  |
| :---: | :---: | :---: | :---: | :---: |
| 18103 | 34.205 | 3.455 |  |  |
| 18104 | 41.990 | 4.241 |  |  |
| 18105 |  |  | no plasma |  |
| 18106 | 31.886 | 3.220 |  |  |
| 18107 | 40.479 | 4.08B |  |  |
| 2891 | 25.796 | 2.605 |  | Site 2/Adult Mean |
| 2 292 | 50.479 | 5.098 |  | 4.401 |
| 2A93 | 27.204 | 2.748 |  |  |
| 2A94 | 33.692 | 3.403 |  | Standard Deviation |
| 2A95 | 48.925 | 4.941 |  | 0.957620946 |
| 2896 | 37.248 | 3.762 |  |  |
| 2 A97 | 40.454 | 4.086 |  | Standard Error |
| 2A98 | 53.001 | 5.353 |  | 0.1748 |
| 2A99 | 48.211 | 4.869 |  |  |
| 2 A 100 | 27.890 | 2.817 |  | $\mathrm{n}=30$ |
| 2 A 101 | 35.398 | 3.575 |  |  |
| 2 A 102 | 48.386 | 4.887 |  |  |
| 2 A103 | 31.368 | 3.168 |  |  |
| 2A104 | 52.482 | 5.301 |  |  |
| 2 A 105 | 56.636 | 5.720 |  |  |
| 2 A 106 | 43.705 | 4.414 |  |  |
| 2 A 107 | 47.045 | 4.752 |  |  |
| 2A108 | 22.914 | 2.314 |  |  |
| 2A109 | 53.083 | 5.361 |  |  |
| 2A110 | 44.663 | 4.511 |  |  |
| 2A111 | 37.919 | 3.830 |  |  |
| 2 A112 | 47.964 | 4.844 |  |  |
| 2 A 113 | 46.511 | 4.698 |  |  |
| 2 A 114 | 42.906 | 4.334 |  |  |
| $2 . A 115$ | 48.412 | 4.890 |  |  |
| 2 A116 | 49.723 | 5.022 |  |  |
| 2 A 117 | 45.240 | 4.569 |  |  |
| $2 \mathrm{Al18}$ | 53.002 | 5.353 |  |  |
| 2 A119 | 50.918 | 5.143 |  |  |
| 2 A 120 | 56.074 | 5.663 |  |  |
| $2 \mathrm{B91}$ | 46.506 | 4.697 |  | Site 2/Juvn 1 Mean |
| $2 \mathrm{B92}$ | 40.597 | 4.100 |  | 4.403 |
| $2 \mathrm{B93}$ | 50.747 | 5.125 |  |  |
| 2B94 | 50.685 | 5.119 |  | Standard Deviation |
| $2 \mathrm{B95}$ | 40.615 | 4.102 |  | 0.658640296 |
| 2B96 | 57.456 | 5.803 |  |  |
| 2897 | 44.230 | 4.467 |  | Standard Error |
| 2B98 | 41.895 | 4.231 |  | 0.1268 |
| $2 \mathrm{B99}$ | 45.032 | 4.548 |  |  |
| 2B100 |  |  | no plasma | $\mathrm{n}=27$ |



Protein Determination - San Juan River August 2001

| Sample <br> Number | Diluted Conen mg/dL | Actual Concn g/dL | Comments | Mean Concn for each size/site group |
| :---: | :---: | :---: | :---: | :---: |
| 1 12121 | 62.493 | 6,312 |  |  |
| 1 A122 | 67.695 | $6+837$ |  | Site 1/Adult Mean |
| 1 A123 | 56.647 | 5.721 |  | 5.643 |
| 18124 | 55.371 | 5.592 |  |  |
| 1A125 | 66.748 | 6.742 |  | Standard Deviation |
| 1 A126 | 40.000 | 4.040 |  | 1.414048508 |
| 1 A127 | 46.832 | 4.730 |  |  |
| 1 A128 | 49.286 | 4.978 |  | Standard Error |
| 1A129 | 55.772 | 5.633 |  | 0.2582 |
| $1 \mathrm{A130}$ | 55.245 | 5.580 |  |  |
| 1 1231 | 88.839 | 8.973 |  | $n=30$ |
| 1 1132 | 43.783 | 4.422 |  |  |
| 1 1.133 | 67.432 | 6.811 |  |  |
| 1A134 | 39.171 | 3.956 |  |  |
| 1A135 | 45.512 | 4.597 | light hemolysis |  |
| 18136 | 46.197 | 4.666 | light hemolysis |  |
| 1 1.137 | 74.081 | 7.482 |  |  |
| 1A138 | 48.447 | 4.893 |  |  |
| 1A139 | 45.526 | 4.598 |  |  |
| $1 \mathrm{A140}$ | 49.014 | 4.950 |  |  |
| 1 1.141 | 96.549 | 9.751 |  |  |
| $1 \mathrm{A142}$ | 49.532 | 5.003 |  |  |
| 1 1.143 | 55.167 | 5.572 |  |  |
| $1 \mathrm{A144}$ | 56.621 | 5.719 |  |  |
| 1 1a145 | 38.331 | 3.871 |  |  |
| 1 A 146 | 41.058 | 4.147 |  |  |
| 1 A 147 | 51.393 | 5.191 |  |  |
| 1 1248 | 47.646 | 4.812 |  |  |
| 1 A149 | 71.034 | 7.174 |  |  |
| 1.A150 | 64.610 | 6.526 |  |  |
| 18121 | 50.829 | 5.134 |  | Site 1/Juvnl Mean |
| $1 \mathrm{B122}$ | 46.580 | 4.705 | hemolysis | 4.192 |
| 18123 | 43.352 | 4.379 |  |  |
| 18124 | 47.540 | 4.802 | light hemolysis | Standard Deviation |
| 18125 | 40.579 | 4.098 | 25 uL. Light hemo | 0.669230717 |
| 18126 | 34.481 | 3.483 |  |  |
| 18127 | 40.147 | 4.055 |  | Standard Error |
| $1 \mathrm{B1} 28$ | 43.379 | 4.381 | hemolysis | 0.1243 |
| 18129 | 37.812 | 3.819 | 25 uL. light hemo |  |
| 18130 | 45.709 | 4.617 |  | n=29 |
| 18131 | 36.091 | $3+645$ | 25 uL |  |


| 18132 | 44.490 | 4.493 |  |  |
| :---: | :---: | :---: | :---: | :---: |
| 18133 | 39.358 | 3.975 | 25 uL, light hemo |  |
| 18134 | 45.705 | 4.616 | 25 uL. light hemo |  |
| 18135 | 42.099 | 4.252 |  |  |
| 18136 | 45.503 | 4.596 | 25 uL. light hemo |  |
| 1 B137 | 37.523 | 3.790 |  |  |
| 1 B138 | 40.565 | 4.097 | 25 ut, light hemo |  |
| 18139 | 32.521 | 3.285 | 25 uL. light hemo |  |
| $1 \mathrm{B140}$ | 54.649 | 5.520 |  |  |
| 18141 | 41.586 | 4.200 | clotted |  |
| 18142 | 55.953 | 5.651 |  |  |
| 1B143 |  |  | not enough plasma |  |
| 18144 | 25.589 | 2.584 |  |  |
| 18145 | 45.264 | 4.572 |  |  |
| $1 \mathrm{B146}$ | 42.261 | 4.268 |  |  |
| $1 \mathrm{B147}$ | 34.004 | 3.434 |  |  |
| $1 \mathrm{B148}$ | 41.324 | 4.174 |  |  |
| 18149 | 36.053 | 3.641 |  |  |
| $1 \mathrm{B150}$ | 32.554 | 3.288 |  |  |
| $2 \mathrm{Al21}$ | 33.109 | 3.344 |  | Site 2/Adult Mean |
| 2A122 | 51.898 | 5.242 |  | 5.417 |
| 2A123 | 56.670 | 5.724 |  |  |
| $2 \mathrm{Al24}$ | 39.718 | 4.012 |  | Standard Deviation |
| 2A125 | 48.841 | 4.933 |  | 1.544122911 |
| 2A126 | 50.936 | 5.145 |  |  |
| 2A127 | 60.052 | 6.065 |  | Standard Error |
| 2A128 | 48.222 | 4.870 |  | 0.2819 |
| 2A129 | 45.624 | 4.608 | hemolysis |  |
| 2A130 | 81.177 | 8.199 | light hemolysis | $\mathrm{n}=30$ |
| 2A131 | 77.625 | 7.840 |  |  |
| 2A132 | 60.479 | 6.108 |  |  |
| 2A133 | 39.486 | 3.988 |  |  |
| 2A134 | 64.344 | 6.499 |  |  |
| 2 2135 | 63.153 | 6.378 |  |  |
| 2A136 | 59.273 | 5.987 |  |  |
| 2A137 | 66.954 | 6.762 |  |  |
| 2A138 | 76.948 | 7.772 |  |  |
| 2A139 | 66.231 | 6.689 |  |  |
| 2A140 | 38.035 | 3.842 |  |  |
| 2A141 | 77.853 | 7.863 |  |  |
| 2 A 142 | 32.545 | 3.287 |  |  |
| 2 A 143 | 52.496 | 5.302 |  |  |
| 2A144 | 15.106 | 1.526 |  |  |
| 2 A 145 | 38.111 | 3.849 |  |  |
| 2 A 146 | 46.754 | 4.722 |  |  |
| 2A147 | 46.487 | 4.695 |  |  |


| $\begin{aligned} & 2 A 148 \\ & 2 A 149 \\ & 2 A 150 \end{aligned}$ | 46.822 62.099 62.029 | 4.729 6.272 6.265 |  |  |
| :---: | :---: | :---: | :---: | :---: |
| $\begin{aligned} & 2 B 121 \\ & 2 B 122 \end{aligned}$ | 38.757 41.761 | 3.914 4.218 | 25 uL, heavy partic | Site 2/Juvnl Mean $4.612$ |
| $\begin{aligned} & 2 \mathrm{~B} 123 \\ & 2 \mathrm{~B} 124 \\ & 2 \mathrm{~B} 125 \end{aligned}$ | 41.285 46.412 37.983 | 4.170 4.688 3.836 | 25 uL, light hemo | $\begin{gathered} \text { Standard Deviation } \\ 0.616095768 \\ \hline \end{gathered}$ |
| $2 B 126$ 2 B127 2 B128 | 43.053 43.205 43.383 | 4.348 4.364 4.382 | light hemolysis hemolysis 25 uL, light hemo | $\begin{gathered} \text { Standard Error } \\ 0.1125 \\ \hline \end{gathered}$ |
| 2B129 | 44.659 | 4.511 | light hemolysis |  |
| 2B130 | 38.859 | 3.925 | 25 uL, light hemo | $\mathrm{n}=30$ |
| 2 B131 | 54.218 | 5.476 | hemolysis |  |
| 2 B132 | 43.664 | 4.410 | 25 uL, hemolysis |  |
| 2B133 | 45.071 | 4.552 |  |  |
| 28134 | 45.645 | 4.610 |  |  |
| 2 B 135 | 44.602 | 4.505 |  |  |
| 2B136 | 41.806 | 4.222 |  |  |
| $2 \mathrm{B137}$ | 53.049 | 5.358 |  |  |
| 2B138 | 57.302 | 5.788 |  |  |
| 2B139 | 40.594 | 4.100 |  |  |
| 2B140 | 45.462 | 4.592 |  |  |
| 2B141 | 40.665 | 4.107 |  |  |
| 2B142 | 35.305 | 3.566 |  |  |
| 2 B143 | 58.704 | 5.929 |  |  |
| 2B144 | 44.744 | 4.519 |  |  |
| 2B145 | 54.299 | 5.484 |  |  |
| 2B146 | 43.817 | 4.426 | hemolysis |  |
| 2B147 | 57.296 | 5.787 |  |  |
| 2B148 | 50.954 | 5.146 |  |  |
| $2 \mathrm{B149}$ | 49.531 | 5.003 |  |  |
| 2B150 | 43.833 | 4.427 | hemolysis |  |

## ATTACHMENT C

## Percent Muscle Lipid Extraction Procedure (Wet Weight)

## Procedure

Epaxial fish muscle is dried and muscle lipids are extracted with methylene chloride and determined gravimetrically.

## Materials Needed

50 mL beakers (prelabeled), 50 mL burets and teflon stopcocks, buret stands and clamps, glass wool, heavy duty aluminum foil, pestle, funnel, methylene chloride (approximately 50 mL per sample), sodium sulfate (approximately 2 g per sample), drying oven, fume hood, and scale.

## Set-Up

Prior to lipid extraction procedure, take one 50 mL beaker for each sample and heat for 20-30 minutes at $90^{\circ} \mathrm{C}$ then cool in desiccator for 20 minutes. Record the weights for each beaker to the nearest 0.5 mg . Repeat this procedure until the difference between successive weighing is less than 0.5 mg .

1. Thaw the muscle tissue until it is at room temperature. Weigh aluminum foil (doubled with shiny side inside and marked with specimen I.D.). The weight of the clean foil needs to be noted for later calculations. Tare scale, remove tissue from cryovial, place on foil and weigh to nearest 0.5 mg (mass of wet tissue). Care should be taken to eliminate bone, blood, scales and skin. Dry tissue for 12 hours at $60^{\circ} \mathrm{C}$.
2. After drying, cool tissue and weigh dry tissue and foil. Subtract original clean foil weight to determine mass of dry tissue. Fold all four sides of the foil around the tissue and pulverize the tissue with a pestle.
3. Add approximately 2 cm of glass wool at the base of the buret nearest the stopcock, and setup burets on buret stands. Using a funnel, pour approximately 1 cm of sodium sulfate into the buret above the glass wool. This will act as an additional dehydrant to water remaining in the tissue.
4. Add the dry tissue to the buret. Add approximately 1 cm of sodium sulfate above the tissue layer. Then rinse the foil and inside of funnel with approximately 5.0 mL methylene chloride into buret to remove all remaining tissue (do not rinse before placing the sodium sulfate into buret as this causes the tissue to bubble up on top of the sodium sulfate). Place a labeled 50 mL beaker under each buret.
5. Record the beaker number used for each muscle specimen.
6. Open the stopcock carefully to allow the methylene chloride to run through the tissue and sodium sulfate layers and into the glass wool, then close stopcock. Allow the methylene chloride to soak the tissue for 1 hour.
7. After soaking, pour methylene chloride into the buret up to the 45 mL mark. Open stopcock and allow methylene chloride to drip at approximately 1 mL per minute into the 50 mL beaker. The lipids will be collected in the beaker in the solvent phase.
8. Allow the beakers containing the solvent to evaporate in a fume hood ( $12-15$ hours).
9. After all solvent has evaporated, place beakers in the drying oven at $90^{\circ} \mathrm{C}$ for 2 hours. Allow beakers to cool in desiccator for 25 minutes, then weigh and record the weights to the nearest 0.5 mg .
10. Repeat step 10 until the difference between successive weighing is less than 0.5 mg . Subtract the clean beaker weight from the lipid beaker weight for lipid mass after extraction.
11. Calculation for percent muscle lipid (wet weight): (Lipid mass after extraction/Mass of wet tissue) $\times 100$.
12. Calculation for percent moisture: (Mass of wet tissue - Mass of dry tissue/Mass of wet tissue) $\times 100$.

| Adult - site 1 |  |  | Adult - site 2 |  |  | Juvenile - Site 1 |  |  | Juvenile - Site 2 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent <br> Moisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent <br> Moisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent <br> Moisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent <br> Moisture |
| 1A01 | 0.1257 | 82.77 | 2A01 | 0.5713 | 76.12 | 1B01 | 3.3491 | 75.62 | 2B01 | 4.7012 | 72.37 |
| 1 A02 | 1.4110 | 74.48 | 2A02 | 0.1511 | 78.88 | 1 BO 2 | 0.8770 | 75.88 | 2B02 | 3.2577 | 74.41 |
| 1A03 | 0.9385 | 74.57 | 2A03 | 0.1241 | 78.40 | $1 \mathrm{B03}$ | 0.7211 | 77.15 | $2 \mathrm{B03}$ | 3.9651 | 73.65 |
| 1 A 04 | 1.0911 | 76.77 | 2 A 04 | 0.6665 | 73.37 | 1804 | 0.8618 | 77.46 | $2 \mathrm{B04}$ | 0.4103 | 78.95 |
| 1 A 05 | 0.1735 | 78.94 | 2A05 | 1.2406 | 75.58 | $1 \mathrm{B05}$ | 2.0669 | 76.11 | 2B05 | 2.1876 | 77.13 |
| 1 1206 | 1.9969 | 74.77 | 2A06 | 1.7766 | 75.38 | 1806 | 0.8505 | 77.35 | 2B06 | 0.6999 | 77.62 |
| 1 1.07 | 0.9352 | 75.83 | 2A07 | 0.5343 | 75.02 | 1807 | 0.7024 | 78.89 | 2B07 | 0.4784 | 76.69 |
| $1 \times 08$ | 0.6307 | 76.00 | 2A08 | 1.0179 | 77.33 | $1 \mathrm{B08}$ | 1.4670 | 76.41 | 2B08 | 2.6192 | 75.43 |
| 1 109 | 1.0648 | 75.88 | 2A09 | 0.7443 | 77.64 | $1 \mathrm{B09}$ | 1.0159 | 78.36 | 2B09 | 3.6846 | 74.20 |
| 1A10 | 0.4379 | 75.94 | 2A10 | 0.2191 | 77.33 | $1 \mathrm{B10}$ | 1.4882 | 76.99 | 2B10 | 0.5033 | 77.23 |
| 1 A11 | 0.1851 | 77.08 | 2 A11 | 0.7261 | 77.54 | $1 \mathrm{B11}$ | 1.0139 | 77.11 | 2B11 | 0.9723 | 76.12 |
| 1 A12 | 0.6254 | 77.32 | 2 A 12 | 0.4851 | 76.62 | $1 \mathrm{B12}$ | 1.4113 | 77.76 | 2B12 | 0.2618 | 86.63 |
| 1813 | 0.3357 | 76.15 | 2A13 | 0.8211 | 75.52 | $1 \mathrm{B13}$ | 0.8205 | 76.80 | 2B13 | 0.6550 | 75.36 |
| 1 1.14 | 0.1266 | 78.20 | 2A14 | 0.1989 | 78.89 | 1 B14 | 1.4125 | 76.18 | 2B14 | 1.1901 | 77.47 |
| 1A15 | 0.1505 | 76.80 | 2A15 | 0.3907 | 76.17 | $1 \mathrm{B15}$ | 1.6101 | 74.98 | 2B15 | 0.7214 | 76.56 |
| 1 A16 | 0.0968 | 81.21 | 2A16 | 0.7978 | 77.33 | 1816 | 2.0243 | 76.18 | 2B16 | 0.4336 | 78.52 |
| 1 147 | 0.1975 | 86.05 | 2A17 | 0.2163 | 75.73 | 1B17 | 0.9436 | 78.25 | 2B17 | 2.5874 | 76.01 |
| 1A18 | 0.3276 | 76.18 | 2A18 | 0.9072 | 76.17 | $1 \mathrm{B18}$ | 1.4413 | 75.75 | 2B18 | 3.2382 | 73.35 |
| 1A19 | 0.3718 | 76.46 | 2A19 | 0.1681 | 77.38 | $1 \mathrm{B19}$ | 1.1311 | 76.27 | 2B19 | 2.6456 | 74.12 |
| 1 A 20 | 0.1691 | 75.98 | 2A20 | 1.1390 | 75.81 | 1820 | 1.0804 | 75.31 | 2B20 | 0.8949 | 76.22 |
| 1 A21 | 0.6925 | 75.93 | 2A21 | 0.1102 | 77.53 | 1B21 | 0.6441 | 77.67 | 2 B 21 | 0.9151 | 76.57 |
| 1 122 | 0.1924 | 76.33 | 2 A 22 | 2.6032 | 73.74 |  |  |  | 2B22 | 1.1337 | 75.16 |
| 1 A23 | 1.3886 | 74.21 | 2A23 | 0.4832 | 74.70 |  |  |  | 2B23 | 0.5822 | 77.88 |
| 1 A 24 | 1.1245 | 76.11 | 2A24 | 0.1828 | 78.02 |  |  |  |  |  |  |
| 1 A25 | 1.7632 | 74.87 | 2 A 25 | 1.4111 | 75.31 |  |  |  |  |  |  |
| 1 126 | 0.3340 | 76.78 | 2 2. 26 | 2.0944 | 75.81 |  |  |  |  |  |  |
| 1 127 | 0.6138 | 75.60 | 2 A27 | 3.4839 | 74.34 |  |  |  |  |  |  |
| 1 128 | 0.0945 | 83.10 | 2 A28 | 0.4336 | 76.95 |  |  |  |  |  |  |
| 1 A29 | 0.5581 | 73.79 | 2A29 | 0.8138 | 74.18 |  |  |  |  |  |  |
| 1 A 30 | 0.2879 | 76.71 | 2A30 | 2.1556 | 74.52 |  |  |  |  |  |  |
| 1A Mean | 0.6147 | 77.0267 | 2A Mean | 0.8889 | 76.2438 | 18 Mean | 1.2825 | 76.7837 | 28 Mean | 1.6843 | 76.4199 |
| Std Err | 0.0957 | 0.5112 |  | 0.1488 | 0.2732 |  | 0.1363 | 0.2295 |  | 0.2831 | 0.5841 |
| Sta Dev. | 0.52 | 2.80 |  | 0.81 | 1.50 |  | 0.62 | 1.05 |  | 1.36 | 2.80 |


| Adult - site 1 |  |  | Adult - site 2 |  |  | Juvenile - Site 1 |  |  | Juvenile - sito 2 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent <br> Lipid | Percent <br> Moisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \\ \hline \end{gathered}$ | Percent Lipid | Percent <br> Moisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \\ \hline \end{gathered}$ | Percent Lipid | Percent Moisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent Moisture |
| 1A32 | 1.6054 | 73.77 | 2A31 | 0.7320 | 75.58 | 1831 | 0.2679 | 77.79 | 2B31 | 0.4516 | 76.20 |
| 1A32 | 0.5482 | 76.42 | 2A32 | 0.6888 | 76.14 | 1832 | 0.6758 | 77.26 | $2 \mathrm{B32}$ | 0.1697 | 76.40 |
| 1 A33 | 0.4382 | 78.35 | 2A33 | 0.1323 | 77.84 | 1 B 33 | 2.2290 | 74.59 | 2 B 33 | 0.6640 | 76.32 |
| 1 1.34 | 0.6038 | 77.29 | 2A34 | 1.2126 | 76.26 | 1834 | 0.4863 | 78.07 | 2 B 34 | 0.3824 | 76.47 |
| 1A35 | 0.2261 | 77.47 | 2 A35 | 0.4349 | 76.47 | 1835 | 0.2661 | 78.35 | 2 B 35 | 0.6260 | 76.72 |
| 1 A36 | 0.7430 | 76.85 | 2 A 36 | 0.8611 | 76.08 | 1836 | 0.7522 | 76.74 | 2 B 36 | 0.3872 | 77.36 |
| 1 A37 | 0.3206 | 76.43 | 2 237 | 0.4141 | 78.45 | 1837 | 0.4175 | 77.62 | 2 B 37 | 1.3876 | 76.00 |
| 1 A38 | 0.4840 | 77.33 | 2A38 | 0.9860 | 74.66 | 1838 | 0.3763 | 76.48 | 2B38 | 0.4269 | 76.18 |
| 1839 | 0.7770 | 75.99 | 2A39 | 0.1314 | 79.96 | 1839 | 0.1382 | 77.34 | 2 B 39 | 0.2960 | 77.97 |
| 1A40 | 0.7472 | 75.21 | 2A40 | 0.1738 | 78.16 | 1B40 | 1.1441 | 75.77 | 2B40 | 0.4351 | 75.59 |
| 1 A 41 | 0.4936 | 67.28 | 2A41 | 0.4112 | 78.01 | $1 \mathrm{B41}$ | 0.6490 | 75.25 | 2B41 | 0.2976 | 76.37 |
| 1 A 42 | 2.7024 | 75.07 | 2A42 | 0.4036 | 75.91 | 1842 | 0.2466 | 80.77 | 2B42 | 0.8445 | 76.67 |
| 1 A43 | 0.1698 | 79.37 | 2A43 | 1.0705 | 76.24 | 1843 | 1.1179 | 76.52 | 2B43 | 0.5548 | 76.28 |
| 1 A44 | 0.7165 | 76.29 | 2A44 | 0.3565 | 75.23 | 1844 | 0.4393 | 77.08 | $2 \mathrm{B44}$ | 0.4861 | 76.37 |
| 1 A 45 | 0.9817 | 74.74 | 2A45 | 0.6647 | 75.49 | 1845 | 0.2797 | 78.20 | 2B45 | 0.6282 | 75.94 |
| 1846 | 0.8703 | 83.80 | 2A46 | 1.8604 | 74.36 | 1846 | 0.3112 | 78.11 | $2 \mathrm{B4} 6$ | 0.7322 | 77.41 |
| 1847 | 0.8900 | 75.60 | 2A47 | 0.7872 | 74.99 | 1847 | 0.4322 | 76.58 | 2B47 | 0.8444 | 77.21 |
| 1 A48 | 1.1175 | 75.00 | 2A48 | 0.3037 | 78.77 | $1 \mathrm{B48}$ | 0.4655 | 75.98 | 2B48 | 0.8894 | 77.31 |
| 1.49 | 0.8994 | 75.10 | 2A49 | 0.5074 | 77.10 | 1849 | 0.4224 | 77.46 | 2B49 | 0.3610 | 77,09 |
| $1 \mathrm{A50}$ | 0.5058 | 75.26 | 2A50 | 0.5408 | 77.40 | 1850 | 0.7911 | 77.33 | 2B50 | 0.2979 | 77.91 |
| 1 1851 | 0.2909 | 76.45 | 2A51 | 0.5766 | 75.95 | 1851 | 0.4100 | 77.00 | 2B51 | 0.5417 | 77.19 |
| 1452 | 0.8967 | 76.81 | 2A52 | 0.1211 | 80.59 | 1852 | 0.3944 | 78.43 | 2B52 | 0.3898 | 77.84 |
| 1853 | 0.8092 | 75.44 | 2A53 | 0.6036 | 75.30 | $1 \mathrm{B5} 3$ | 0.5894 | 75.00 | 2 B 53 | 0.8798 | 77.13 |
| 1A54 | 1.2216 | 75.35 | 2A54 | 0.6003 | 75.70 | 1B54 | 0.2452 | 77.77 | 2B54 | 0.6012 | 77.81 |
| 1A55 | 0.2275 | 76.45 | 2A55 | 1.1307 | 74.86 | 1B55 | 0.6936 | 77.41 | 2B55 | 1.5868 | $76+84$ |
| 1A56 | 0.1549 | 78.21 | 2A56 | 1.0715 | 73.94 | 1856 | 1.1556 | 76.90 | 2B56 | 1.3702 | 76.69 |
| 1857 | 0.4979 | 75.39 | 2A57 | 0.7280 | 78.38 | 1B57 | 0.4702 | 76.08 |  |  |  |
| 1A58 | 1.2359 | 75.49 | 2A58 | 0.3667 | 75.80 | 1858 | 0.7745 | 76.65 |  |  |  |
| 1 A59 | 0.1005 | 77.69 | 2A59 | 0.1876 | 84.15 | 1859 | 0.9554 | 77.58 |  |  |  |
| 1A60 | 0.3236 | 76.50 | 2A60 | 0.6490 | 77.18 | 1860 | 1.1110 | 77.46 |  |  |  |
| 12 Moan | 0.7200 | 76.21 | 2A Mean | 0.6236 | 76.83 | 18 Mean | 0.6236 | 77.12 | 2B Mean | 0.6358 | 76.82 |
| Std Err | 0.0953 | 0.4551 |  | 0.0707 | 0.3886 |  | 0.0771 | 0.2183 |  | 0.0654 | 0.1209 |
| Std Dev. | 0.52 | 2.49 |  | 0.39 | 2.13 |  | 0.42 | 1.20 |  | 0.36 | 0.66 |

January 2001

| Adult - Site 1 |  |  | Adult - site 2 |  |  | Juvenile - Site 1 |  |  | Juvenile - Site 2 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent <br> Moisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent <br> Moisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent <br> Moisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent <br> Moisture |
| 1A61 | 0.5649 | 76.98 | 2A61 | 0.6536 | 76.36 | 1B61 | 1.0409 | 75.66 | 2B61 | 0.6068 | 75.70 |
| 1A62 | 1.5294 | 74.73 | 2A62 | 0.8486 | 75.80 | 1862 | 0.3921 | 76.98 | 2B62 | 0.9056 | 72.28 |
| 1 A63 | 0.8698 | 75.32 | 2A63 | 0.7477 | 77.43 | $1 \mathrm{B6} 3$ | 0.9135 | 77.48 | $2 \mathrm{B63}$ | 0.4981 | 77.55 |
| 1 A 64 | 0.7245 | 76.07 | 2A64 | 0.9655 | 75.96 | $1 \mathrm{B64}$ | 1.2226 | 77.09 | 2B64 | 0.3700 | 75.46 |
| 1 A65 | 2.3063 | 75.34 | 2A65 | 0.8812 | 76.85 | 1865 | 0.9283 | 77.01 | 2B65 | 0.8760 | 76.72 |
| 1A66 | 0.8267 | 76.99 | 2A66 | 2.9729 | 73.62 | $1 \mathrm{B66}$ | 0.5145 | 76.66 | 2B66 | 0.6853 | 75.74 |
| 1A67 | 0.1782 | 79.59 | 2 2. 67 | 1.3578 | 74.83 | 1867 | 0.4486 | 75.82 | 2B67 | 0.5785 | 77.08 |
| 1A68 | 0.3164 | 77.10 | 2A68 | 1.5182 | 74.54 | 1868 | 0.4030 | 76.58 | 2B68 | 1.0728 | 76.08 |
| 1269 | 0.7676 | 76.98 | 2A69 | 0.3370 | 77.09 | 1869 | 1.1281 | 75.88 | 2B69 | 0.5739 | 76.96 |
| 1 A70 | 0.9648 | 76.29 | 2A70 | 0.5119 | 77.05 | 1870 | 0.6715 | 77.13 | 2B70 | 0.8053 | 76.63 |
| 1 171 | 2.4799 | 73.00 | 2 A71 | 0.2775 | 77.34 | 1871 | 0.6979 | 75.03 | 2B71 | 0.7013 | 76.98 |
| 1 A72 | 0.4230 | 76.90 | 2A72 | 0.2568 | 76.85 | 1872 | 1.2895 | 76.40 | 2B72 | 0.5856 | 77.51 |
| 1 1873 | 0.2567 | 77.13 | 2A73 | 0.7431 | 76.20 | 1873 | 0.3986 | 75,22 | 2B73 | 0.8951 | 77.28 |
| 1 174 | 0.2617 | 87.49 | 2A74 | 1.9249 | 74, 38 | 1874 | 0.5720 | 77.52 | 2B74 | 0.8229 | 77.82 |
| 1A75 | 0.2240 | 78.16 | 2A75 | 0.2938 | 77.37 | 1875 | 0.8943 | 77.12 | 2 7 75 | 0.6359 | 78.31 |
| 1 1.76 | 0.3638 | 74.29 | 2A76 | 0.2559 | 79.38 | 1876 | 0.5580 | 73.54 | $2 \mathrm{B76}$ | 0.8898 | 76.85 |
| 1 1.77 | 2.5652 | 74.81 | 2A77 | 1.0143 | 75.41 | 1877 | 1.0789 | 76.50 | $2 \mathrm{B77}$ | 1.1372 | 77.24 |
| 1 1278 | 0.5036 | 76.45 | 2A78 | 0.9196 | 75.37 | 1878 | 2.2369 | 76.37 | 2B78 | 1.0835 | 75.76 |
| 1279 | 0.6125 | 75.64 | 2A79 | 0.5992 | 75.23 | 1879 | 1.7609 | 76.49 | 2B79 | 0.4908 | 77.58 |
| 1A80 | 0.3954 | 76.52 | 2A80 | 0.9441 | 74.36 | 1880 | 0.7711 | 73.00 | 2B80 | 1.2099 | 75.86 |
| 1 A81 | 1.5032 | 74, 86 | 2A81 | 0.5636 | 76,46 | 1881 | 1.0622 | 76.72 | 2B81 | 1.7747 | 74.16 |
| $1 \times 82$ | 0.5295 | 75.54 | 2AB2 | 1.9085 | 75.46 | 1882 | 0.9841 | 76.11 | 2B82 | 0.9820 | 74.28 |
| $1{ }^{\text {A } 83}$ | 0.6565 | 74.56 | $2 \mathrm{AB3}$ |  |  | 1883 | 0.5225 | 76.91 | $2 \mathrm{B83}$ |  |  |
| 1 A84 | 0.2864 | 77.87 | 2A84 | 0.7269 | 75.32 | 1884 | 0.5481 | 77.32 | $2 \mathrm{B84}$ | 1.3988 | 75.86 |
| 1 A85 | 0.5068 | 72.12 | 2A85 | 0.4170 | 74.64 | 1885 | 0.9417 | 75.15 | 2B85 | 1.3119 | 76.92 |
| 1 A86 | 0.6375 | 75.16 | 2A86 | 0.2173 | 78.50 | $1 \mathrm{B86}$ | 0.8176 | 77.22 | $2 \mathrm{B86}$ | 0.9372 | 77.38 |
| 1 187 | 0.9144 | 79.88 | 2A87 | 1.4354 | 75.08 | 1887 | 0.4241 | 77,86 | $2 \mathrm{B87}$ | 1.9644 | 76.61 |
| 1A88 | 0.5440 | 75.24 | 2A88 | 1.4694 | 73.86 | 1888 | 0.4469 | 77.70 | 2B88 | 0.6101 | 74.93 |
| 1A89 | 0.4569 | 75.66 | 2A89 | 1.7995 | 75.43 | 1889 | 0.5760 | 77.74 | $2 \mathrm{B89}$ | 0.7255 | 78.37 |
| 1 A 90 | 0.2009 | 78.70 | 2A90 | 0.7037 | 75.34 | 1890 |  |  | 2B90 | 0.9171 | 75.07 |
| 18 Mean | 0.7790 | 76.51 | 2A Mean | 0.9402 | 75.91 | 18 Mean | 0.8360 | 76.42 | 2B Mean | 0.8981 | 76.38 |
| sta Err. | 0.1198 | 0.4915 |  | 0.1169 | 0.2485 |  | 0.0776 | 0.2129 |  | 0.0675 | 0.2465 |
| Std Dev. | 0.66 | 2.69 |  | 0.64 | 1.36 |  | 0.43 | 1.17 |  | 0.37 | 1.35 |

San Juan River Fish Health Assessment -- Percent Muscle Lipid and Moisture
March 2001

| Adult - site 1 |  |  | Adult - site 2 |  |  | Juvenile - site 1 |  |  | Juvenile - site 2 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent <br> Moisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent <br> Moisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent Moisture | $\begin{aligned} & \text { Specimen } \\ & \text { I.D. } \end{aligned}$ | Percent Lipid | Percent Moisture |
| 1 A091 | 1.1092 | 75.02 | 2A091 | 0.2127 | 79.23 | 1B091 | 0.4608 | 76.63 | 2B091 | 0.6539 | 76.90 |
| 1A092 | 0.1994 | 86.59 | 2A092 | 0.7507 | 75.18 | 1B092 | 0.6333 | 75.52 | 2B092 | 0.5988 | 77.95 |
| 1A093 | 0.5785 | 76.68 | 2A093 | 0.4155 | 79.71 | 18093 | 0.6242 | 76.86 | $2 \mathrm{B093}$ | 0.8211 | 78.00 |
| 1A094 | 0.4845 | 77.96 | 2A094 | 0.5455 | 77.22 | $1 \mathrm{B094}$ | 0.5543 | 75.31 | 2B094 | 0.8819 | 76.77 |
| 1A095 | 0.3296 | 75.80 | 2A095 | 1.0055 | 74.95 | 18095 | 0.3225 | 76.61 | 2B095 | 0.6273 | 78.41 |
| 1A096 | 0.7075 | 76.09 | 2A096 | 0.2548 | 79.58 | 18096 | 0.7999 | 77.78 | 28096 | 1.8864 | 76.81 |
| 1A097 | 0.8342 | 75.13 | 2A097 | 1.0608 | 76.95 | 18097 |  |  | 28097 | 0.5581 | 76.68 |
| 1A098 | 0.4802 | 75.00 | 2A098 | 2.2899 | 74.52 | 1B098 | 0.3998 | 76.62 | 28098 | 0.6898 | 76.23 |
| 1A099 | 0.6974 | 76.71 | 2 A 099 | 0.4800 | 77.32 | 1B099 |  |  | 2B099 | 1.1911 | 75.82 |
| 1A100 | 0.3899 | 76.07 | 2A100 | 0.1852 | 79.18 | $1 \mathrm{B100}$ | 0.5066 | 76.83 | 2B100 | 1.2051 | 77.42 |
| 1A101 | 0.3275 | 76.57 | 2A101 | 0.2668 | 78.19 | $1 \mathrm{B101}$ | 0.4928 | 77.80 | $2 \mathrm{B1} 01$ | 0.4169 | 77.59 |
| 1A102 | 0.9719 | 76.64 | 2A102 | 0.5595 | 76.14 | $1 \mathrm{B102}$ | 0.8102 | 74.65 | $2 \mathrm{B1} 02$ | 0.8285 | 75.81 |
| 1A103 | 0.4336 | 74.73 | 2 A 103 | 0.0563 | 78.65 | $1 \mathrm{B103}$ | 1.6494 | 75.60 | $2 \mathrm{B103}$ | 0.8780 | 75.61 |
| 1A104 | 0.7902 | 75.13 | 2A104 | 1.0711 | 74.85 | 1B104 | 1.1287 | 77.52 | $2 \mathrm{B1} 04$ | 0.6801 | 76.73 |
| 1A105 | 0.3384 | 74.88 | 2A105 | 1.3025 | 74.58 | $1 \mathrm{B1} 05$ | 0.4303 | 78.68 | 2B105 | 0.5546 | 78.12 |
| 1 1.106 | 0.2226 | 80.62 | 2A106 | 0.6335 | 77.75 | 18106 | 0.2462 | 77.91 | 2 B 106 | 0.9904 | 78.18 |
| 1 A107 | 0.6010 | 75.91 | 2A107 | 0.4676 | 75.49 | 18107 | 1.2798 | 75.73 | $2 \mathrm{B107}$ | 0.4585 | 77.61 |
| 1 A 108 | 0.7279 | 77.87 | 2 A 108 | 0.1993 | 81.03 |  |  |  | $2 \mathrm{B108}$ | 0.5503 | 77.13 |
| 14.09 | 0.3923 | 75.30 | 2 A109 | 0.6189 | 75.53 |  |  |  | 28109 | 0.3245 | 78.28 |
| 1A110 | 0.6292 | 75.93 | 2A110 | 0.9023 | 76.79 |  |  |  | $2 \mathrm{B110}$ | 0.5795 | 77.30 |
| 1 12111 | 0.8629 | 75.42 | 2 A111 | 0.8007 | 77.39 |  |  |  | 2B111 | 0.7256 | 78.29 |
| 1 1.112 | 0.7898 | 75.24 | 2 A 112 | 0.6070 | 74.89 |  |  |  | $2 \mathrm{B112}$ | 0.4624 | 78.43 |
| 1 A113 | 0.3988 | 77.65 | 2A113 | 0.7835 | 73.91 |  |  |  | $2 \mathrm{B113}$ | 0.6009 | 77.65 |
| 1 1214 | 0.2136 | 78.63 | 2A114 | 1.5746 | 73.63 |  |  |  | 2B114 | 0.4407 | 77.12 |
| 1 A115 | 1.0077 | 75.08 | 2 A 115 | 0.9320 | 75.40 |  |  |  | 2B115 | 0.8285 | 78.11 |
| 1 A116 | 0.3277 | 76.78 | $2 \mathrm{Al16}$ | 0.5152 | 75.32 |  |  |  | $2 \mathrm{B116}$ | 1.4833 | 76.59 |
| 1 A117 | 1.3850 | 74.48 | $2 \mathrm{A117}$ | 0.5041 | 75.70 |  |  |  | $2 \mathrm{B117}$ | 1.2147 | 77.07 |
| 1 A118 | 0.5767 | 78.43 | 2 A 118 | 0.6883 | 74.50 |  |  |  | 2B118 | 0.6967 | 77.78 |
| 1 1.119 | 0.8877 | 75.68 | 2 A 119 | 0.5161 | 75.87 |  |  |  |  |  |  |
| 1A120 | 0.1619 | 77.49 | 2A120 | 2.3787 | 74.11 |  |  |  |  |  |  |
| 1a Mean | 0.5952 | 76.65 | 2A Mean | 0.7526 | 76.45 | 18 Mean | 0.6893 | 76.67 | 2B Mean | 0.7796 | 77.30 |
| std. Err | 0.0548 | 0.4278 |  | 0.1004 | 0.3616 |  | 0.0973 | 0.2844 |  | 0.0662 | 0.1562 |
| Std Dev. | 0.30 | 2.34 |  | 0.55 | 1.98 |  | 0.39 | 1.14 |  | 0.35 | 0.83 |

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| Adult - site 1 |  |  | Adult - Site 2 |  |  | Juvenile - site 1 |  |  | Juvenile - Site 2 |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent <br> Lipid | Percent <br> Noisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent <br> Moisture | $\begin{gathered} \text { Specimen } \\ \text { I.D. } \end{gathered}$ | Percent Lipid | Percent Moisture | Specimen I.D. | Percent <br> Lipid | Percent <br> Moisture |
| 1A121 | 2.7859 | 73.61 | 2A121 | 0.2636 | 85.59 | 18121 | 0.8480 | 76.17 | 2B121 | 1.0642 | 78.46 |
| 18122 | 1.6934 | 76.58 | 2 A122 | 1.1938 | 75.77 | $1 \mathrm{B122}$ | 0.5228 | 85.36 | 2B122 | 1.8884 | 75.26 |
| 1 12123 | 1.7629 | 74.48 | 2A123 | 1.8000 | 75.70 | 18123 |  |  | 2B123 | 2.9277 | 77.69 |
| 1 A124 | 1.1967 | 74.11 | 2 A 124 | 0.9368 | 75.87 | 18124 | 1.6524 | 78.19 | 2B124 | 0.9450 | 83.92 |
| 1A125 | 2.3948 | 73.99 | 2A125 | 2.1534 | 82.91 | 18125 | 0.8188 | 75.86 | $2 \mathrm{B125}$ | 0.9617 | 78.97 |
| 1 12126 | 0.3761 | 77.70 | 2A126 | 1.3598 | 74.13 | 1B126 | 1.1528 | 78.33 | 2B126 | 2.7510 | 77.37 |
| 1 A 127 | 1.8422 | 74.24 | 2A127 | 1.4906 | 76.17 | 18127 | 1.0560 | 77.41 | 28127 | 1.0656 | 76.52 |
| 1 A 128 | 1.7199 | 74.26 | 2A128 | 2.4916 | 73.80 | 18128 | 0.9908 | 79.33 | 2B128 | 1.2861 | 78.99 |
| 1 A129 | 1.1692 | 75.09 | 2A129 | 1.0649 | 76.51 | 18129 | 0.8151 | 83.25 | 2B129 | 1.8902 | 77.85 |
| 18130 | 0.8472 | 76.16 | 2A130 | 0.9164 | 74.51 | $1 \mathrm{B130}$ | 1.2706 | 76.65 | 2B130 | 1.5013 | 78.35 |
| 1 1.131 | 1.3115 | 73.96 | 2A131 | 0.7688 | 75.76 | 1B131 | 0.9040 | 78.38 | 2B131 | 1.0676 | 78.12 |
| 1 1432 | 1.3544 | 75.53 | 2A132 | 1.2923 | 84.79 | 1B132 | 1.0372 | 77.37 | 2B132 | 0.9640 | 86.03 |
| 1 12133 | 1.2640 | 73.84 | 2A133 | 1.2760 | 76.20 | 18133 | 1.3674 | 78.28 | 2 E 133 | 1.4513 | 77.48 |
| 1A134 | 0.4427 | 77.25 | 2A134 | 1.0680 | 77.76 | 18134 | 1.9854 | 77.99 | 2B134 | 2.3414 | 77.96 |
| 1 A135 | 2.5571 | 73.71 | 2A135 | 2.0057 | 77.43 | 18135 | 0.9460 | 77.71 | 2B135 | 1.8138 | 77.17 |
| 1 A136 | 2.4311 | 70.43 | 2A136 | 1.3980 | 77.09 | $1 \mathrm{B136}$ | 1.5208 | 78.06 | 2B136 | 0.6729 | 78.69 |
| 1 A 137 | 2.2333 | 74.98 | 2A137 | 0.9878 | 87.87 | 18137 | 2.2546 | 76.25 | 2B137 | 1.7683 | 76.73 |
| 1 A138 | 0.9050 | 76.40 | 2A138 | 0.6359 | 78.16 | 18138 | 0.5547 | 78.93 | 2B138 | 1.7235 | 76.65 |
| 1 A139 | 4.4047 | 72.96 | 2A139 | 1.7415 | 75.32 | 18139 | 1.1163 | 78.18 | 2B139 | 0.8965 | 86.08 |
| 18140 | 0.8600 | 77.15 | 2A140 | 1.0483 | 75.77 | 1B140 | 1.5461 | 76.49 | 2B140 | 0.9933 | 85.67 |
| 1 A 141 | 3.1205 | 74.11 | 2A141 | 2.4633 | 75.84 | $1 \mathrm{B141}$ | 1.5072 | 77.34 | 2B141 | 1.1825 | 77.21 |
| 1 A142 | 0.4294 | 73.68 | 2A142 | 0.2839 | 82.20 | 18142 | 1.1463 | 76.36 | $2 \mathrm{B142}$ | 0.5365 | 78.31 |
| 1 A143 | 1.1581 | 75.96 | 2 A143 | 3.3142 | 74.52 | 18143 | 2.3791 | 77.20 | $2 \mathrm{B143}$ | 1.5328 | 76.01 |
| 1 1414 | 1.3665 | 74.36 | 2A144 | 0.3546 | 83.63 | 1B144 | 1.3067 | 79.33 | 2B144 | 1.4046 | 76.12 |
| 1A145 | 0.6790 | 77.51 | 2A145 | 1.3879 | 75.56 | 13145 | 1.5936 | 77.35 | 2B145 | 0.6879 | 84.37 |
| 1A146 | 0.2223 | 78.66 | 2A146 | 0.6343 | 76.70 | 18146 | 1.2992 | 78.32 | $2 \mathrm{B146}$ | 2.2521 | 75.94 |
| 1 A 147 | 0.6166 | 73.72 | 2A147 | 2.6902 | 76.21 | $1 \mathrm{B147}$ | 1.9943 | 76.26 | 2B147 | 2.3970 | 75.23 |
| $1 \mathrm{A148}$ | 0.5326 | 83.87 | 2A148 | 0.3720 | 79.27 | 18148 | 1.5010 | 77.06 | 23148 | 1.1697 | 75.91 |
| 1 A 149 | 1.4805 | 75.60 | 2A149 | 1.1662 | 76.55 | 1B149 | 1.1956 | 78.45 | $2 \mathrm{B149}$ | 2.6667 | 75.25 |
| 1A150 | 1.3761 | 67.29 | 2A150 | 2.5753 | 74.79 | 18150 | 0.9796 | 78.51 | 2B150 | 1.3678 | 78.90 |
| 12 Mean | 1.4845 | 75.04 | 2N Mean | 1.3712 | 77.75 | 18 Mean | 1.2849 | 78.08 | 2B Mean | 1.5057 | 78.57 |
| Std Err | 0.1712 | 0.5039 |  | 0.1421 | 0.6793 |  | 0.0857 | 0.3697 |  | 0.1184 | 0.5916 |
| Std Dev. | 0.94 | 2.76 |  | 0.78 | 3.72 |  | 0.46 | 1.99 |  | 0.65 | 3.24 |


[^0]:    Skin 4 fist with lesions

