Non-lethal Estimation of Energy Content of Yukon River Chinook Salmon

Final Report

to

Arctic-Yukon-Kuskokwim Sustainable Salmon Initiative
705 Christensen Drive
Anchorage, AK 99501

By

F. Joseph Margraf, Principal Investigator

Kyle J. Hartman, Co-Principal Investigator

M. Keith Cox, Post-Doctoral Research Associate

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Alaska Cooperative Fish and Wildlife Research Unit
Institute of Arctic Biology, University of Alaska Fairbanks
216 Irving I Building
Fairbanks, AK 99775-7020

Executive Summary

Because of the importance of Chinook salmon to commercial and subsistence fisheries on the Yukon River, further study of the factors that may affect the success of this species and our ability to manage the fisheries is warranted. Critical to these studies is the determination of the amount of lipids (fat) stored and available to the fish as its primary energy source for migration and spawning. Recent developments of Bioelectrical Impedance Analysis (BIA) promise a simple, non-lethal means of estimating proximate composition (e.g. fat, protein, water content) for field applications with fish. The goal of the project was to develop BIA models for Chinook salmon from the Yukon River watershed that would permit the non-lethal estimation of body proximate composition for use in field studies.

Our results clearly demonstrated that BIA can be used to estimate proximate composition and energy density of salmon. While some minor refinements were suggested, the methodology can be used in a wide variety of field applications. For instance, application of the BIA models to predict energy levels of fish during their migration will allow evaluation of management programs, while also yielding data that can be used to evaluate energy use along the migratory path. Correlations of energy level with ongoing tagging, radio-tracking, and genetic studies also have the potential to allow managers and scientists to understand the relationship between fat content and distance to spawning location. These models have the potential for application to this species in other river systems. They also provide tools for a variety of other scientific investigation such as: 1) differences in energy stores in spawning and recruitment success; 2) effects of global warming on migratory salmonid stocks; and 3) differences in annual flow and temperature regiments upon migratory energy costs and resulting recruitment success.

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Introduction

Because of the importance of Chinook salmon (*Oncorhynchus tshawytscha*) to commercial and subsistence fisheries on the Yukon River, further study of the factors that may affect the success of this species and our ability to manage the fisheries is warranted. Critical to these studies is the determination of the amount of lipids (fat) stored and available to the fish as its primary energy source for migration and spawning. The most direct means of determining accurate measures of fat content and other body components has been through proximate analysis. Unfortunately, in addition to laboratory facilities and personnel capable of conducting the analysis, standard measures of proximate analysis requires sacrifice of the fish. Clearly, what is needed is a simple, non-lethal means of estimating proximate composition. Recent developments of Bioelectrical Impedance Analysis (BIA) promise such a method for field applications with fish.

Bioelectrical impedance analysis has permitted a non-invasive, non-lethal means of estimating proximate composition components in humans since the 1940's with fairly strong coefficient of determination scores ($r^2 > 0.7$). BIA measures consist of measuring the resistance and reactance of a current (800μA, AC, and 50 kHz) while it is passed through a subject. Resistance and reactance are sensitive to the impedance of a current as it relates to the cross-sectional area, conductor length and signal frequency (Lukaski 1987). A cell membrane consists of a non-conductive lipid bi-layer sandwiched between two conductive protein layers. When exposed to a very low voltage alternating current, cells become capacitive and therefore reflective of cell membrane volume. A non-lethal method of estimating the proximate composition of fish and other animals is possible by developing regression models that relate BIA measures to the amounts of water, fat, protein, ash and other components in their bodies (as measured with traditional laboratory extractions). The use of BIA in humans is thoroughly noted, but exploration of BIA in fish could result in a technology that will provide accurate proximate body composition estimations. The potential to use BIA in fish was demonstrated by Cox and Hartman (2005) for brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*).

Their results suggested a strong relationship between the predicted and the observed body compositions.

The objectives of this study were to develop BIA models for Chinook salmon that will permit the non-lethal estimation of body proximate composition (e.g. fat, protein, water content) for use in future field applications. This work serves as a feasibility and pilot project that will permit development of much broader studies relating to the understanding of Chinook salmon ecology and the ability to wisely manage the fisheries based on ecological principles.

Methods

Field collection—Development of BIA models for Chinook salmon required measurements of resistance and reactance, and subsequent sacrifice and proximate analysis of fish. Models were to be developed by sampling wild populations to obtain fish with different energy levels, of both sexes, and spanning the size ranges of fish for which the models will be applied. A target total of 80 fish were to be sacrificed and included in development of the model for Chinook salmon. This total was to include 40 males and 40 females, 20 of each sex from near the mouth of the Yukon River (Emmonak to Russian Mission) and 20 of each sex from as far upstream as practical in the Yukon Territory, Canada (Dawson to Teslin). The exact locations were to depend on the availability of fish and the efforts of the cooperating agencies: Alaska Department of Fish and Game (ADFG), Department of Fisheries and Oceans -Canada (DFO), and US Fish and Wildlife Service (FWS).

BIA measurements—Once collected, live fish were given a cranial concussion in preparation for BIA measurements, blotted dried on absorbent pads and placed on a non-conductive board in a left lateral recumbent position. Electrical impedance (resistance and reactance) was measured with a tetrapolar bioelectrical impedance analyzer (RJL Systems, Detroit, MI.). The analyzer had two sets of needle electrodes (stainless, 28 gauge, 12 mm) each consisting of one signal and one detecting electrode, with the

detecting electrodes of each set placed 1.0 cm proximal to the signal electrodes. Two different locations were used for BIA measurements to ensure maximum precision in model development. Electrodes were placed laterally on the fish, one set in the anteriad dorsad region and the second set in the caudle peduncle region of the fish. A second measure was made on each fish with the electrodes placed ventrally from a position beneath the opercal to an area anterior of the anal fin. Once the electrodes were in place, the BIA analyzer sent a current (800 μA, AC and 50 kHz) through the signal electrodes and the proximal detecting electrodes measured the voltage drop. These resistance and reactance measures, as well as the distance between anterior and posterior electrode placement, were recorded along with measures of the fish length (mid-eye to fork length in mm) and weight (to nearest 0.1 Kg). The body temperature of the fish was also recorded to the nearest 0.1º C. The fish were then labeled and iced or frozen for return to the laboratory for analysis.

Proximate analysis—In the laboratory, fish were homogenized using an industrial grinder, and three 100-ml subsamples were refrozen and retained for analysis. The remaining carcass material was discarded according to local ordinances. One subsample from each fish was sent to a contracted analytical laboratory for proximate analysis of tissue for percent water content and fat, protein, and ash content expressed as percent dry weight following standard techniques of the AOAC (1990). Laboratory quality control consisted of taking two independent measures for each proximate component. If any two samples exceeded a predetermined percent difference, another measure was taken. The final proximate value consisted of an average of the repeated measures. Of the remaining two subsamples from each fish, one set was provided to FWS for an unrelated study and the other set was retained as a backup.

Model development—Proximate analysis estimates were then related to BIA measures of resistance, reactance, and distance between electrodes in predictive models for each proximate component. Because BIA measures vary with temperature, we corrected resistance and reactance values to a 10º C standard temperature according to formulas determined for chum salmon (Table 1). Independent models for each body composition

parameter were then developed from seven electrical properties equations (Table 1; Lukaski 1987) in a linear regression model using an optimum model development algorithm in PROC REG with SAS® 8.1 software (Table 2).

Results

Field collection—Because of the late award date of the grant and the perception of a relatively small Chinook run, we were unable to secure permission in time to collect fish from the lower Yukon River in Emmonak. As a substitute, 46 (19 females, 27 males) Chinook salmon were collected from the Yukon River at the FWS tagging wheel at Rapids Camp (RM 730, RKM 1176) on June 22-25, 2004 (Table 3). Males had a wide range of sizes (541-956 mm, 2.2-11.6 Kg), while females were on average larger than males (797-998 mm, 6.1-14.0 Kg; Figure 1).

Arrangements were made with DFO and First Nations representatives to collect Chinook from spawning grounds near Faro, YT, Canada in fall 2004. However, the small run size resulted in the First Nations reducing the number of fish that they were willing to allow us to collect so that we were not able to attain a sufficient sample from near the spawning grounds. Fortunately, we were also conducting a companion study of chum salmon (*Oncorhynchus keta*) in which we were able to collect adequate numbers of fish from both Emmonak and near spawning grounds (Delta River, near its confluence with the Tanana River) to develop BIA models.

Proximate analysis—We obtained laboratory proximate composition analysis on the 46 Chinook salmon from the University of Idaho Hagerman Fish Laboratory (Table 3). Water content of the fish ranged between 58% and 69%, with one outlier at 49% (Figure 2A). Fat content ranged from 11% to 20%, with one outlier at 26% (Figure 2B). Protein ranged from 16% to 22% (Figure 2C). Ash ranged from 1.5% to 3% (Figure 2D). Based on regressions of proximate composition values against fish length, we found no significant relationships ($p \geq 0.05$) between composition and size of fish. Similarly, using Analysis of Variance, we found no significant relationships ($p \geq 0.05$) between

composition and sex of fish. Proximate composition values for Chinook salmon were similar to those found for chum salmon at Emmonak (Figure 3A-D).

Model development—The 46 Chinook salmon were combined with 86 chum salmon to develop a single set of models for both species (Table 4). Percent water was predicted with a coefficient of determination (r^2) of 0.80, but there were two outliers (one Chinook and one chum) from the laboratory data, which if removed would have increased the accuracy somewhat (Figure 3A). BIA predicted fat and protein, expressed as percent dry weight, with coefficients of determination of 0.88 and 0.87, respectively (Figure 3A,B). The ability to predict ash was relatively low with a coefficient of determination of 0.66, which was not surprising given the small variation in measured values (Figure 3D). The models using Chinook and chum combined were equivalent to models developed using chum only in their predictive capability (r^2 = 0.80 to 0.88 for both sets of models for water, fat, and protein, and ash was low for both, $r^2 \approx 0.65$). Energy density (joules per gram) had a coefficient of determination of 0.84, but again two outliers (the same two fish) were included in the model (Figure 3E).

Discussion

Despite some problems obtaining Chinook from the planned locations, we were able to successfully develop usable BIA models of proximate composition and energy density by combining the Chinook data with similar data from chum salmon. Our ability to predict proximate composition of salmon exceeds that obtained for and used on humans in a medical context (Lukaski et al. 1985, 1986; Segal et al. 1985; Settle et al. 1980). Energetically important fat and protein components and energy density are predicted by BIA with accuracies that equal or exceed those obtained in far more sophisticated laboratory settings for small mammals (Personal Communication: B. Barnes, Professor of Biology and Director of the Institute of Arctic Biology, University of Alaska Fairbanks).

Much of the inaccuracy in the BIA modeling actually comes from the laboratory proximate analysis. Some of this inaccuracy can be reduced; some is inherent in the

laboratory procedures. A major source of potential error that can be moderated involves our ability to adequately homogenize a whole body sample of large fish like adult salmon. We used a large grinder at a local commercial fish processing plant. While this allowed us to adequately handle the large fish, time constraints placed on us because we were monopolizing a critical piece of machinery at a commercial facility were such that we could not remix and rerun the fish through multiple times. The presence of two major outliers in the percent water analysis suggests that perhaps other handling issues could be indicated as well. One solution in addition to more careful handling might be to take and analyze multiple independent subsample aliquots from each fish. However, given that we were able to equal or better similar efforts on mammals, we may already be operating near the current theoretical best of the lab science.

Some improvements are also suggested for the BIA measurements. Temperature affects the readings, as both resistance and reactance change with body temperature. This effect has not been a major issue in mammalian studies as their body temperatures remain relatively constant. To address the temperature issue, we performed some preliminary experiments with chum salmon to provide the temperature adjustment equations we used in this study. While this approach apparently worked well enough to provide usable results, further refinement is still required. We also performed some repeatability experiments with chum, where several fish were repeatedly measured for BIA parameters by multiple investigators. The results of this experiment suggest that some refinement of technique and equipment should aid repeatability and accuracy. Finally, additional Chinook, particularly those with high and low fat content, would greatly help to expand the models.

Conclusions— Our results clearly demonstrated that BIA can be used to estimate proximate composition and energy density of salmon. While some minor refinements were suggested, the methodology can be used in a wide variety of field applications. For instance, application of the BIA models to predict energy levels of fish during their migration will allow evaluation of management programs, while yielding data that can be used to evaluate energy use along the migratory path. Correlations of energy level with

ongoing tagging, radio-tracking, and genetic studies also have the potential to allow managers and scientists to elucidate the relationship between fat content and distance to spawning location. These models have the potential for application to this species in other river systems. They also provide tools for a variety of other scientific investigation such as: 1) differences in energy stores in spawning and recruitment success; 2) effects of global warming on migratory salmonid stocks; and 3) differences in annual flow and temperature regiments upon migratory energy costs and resulting recruitment success.

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Table 1. Temperature correction equations and seven electrical properties equations used to develop BIA models for estimating proximate composition of Chinook salmon.

Temperature correction equations:

Corrected lateral resistance =
measured lateral resistance((-10.866temperature+460.41)/(-10.866*10+460.41))

Corrected lateral reactance =
measured lateral reactance((-3.1723temperature+160.78)/(-3.1723*10+160.78))

Corrected Ventral resistance =
measured ventral resistance((-10.243temperature+447.25)/(-10.243*10+447.25))

Ventral reactance was not corrected

Electrical properties equations:

Equation 1 = (detector length^2)/resistance

Equation 2 = (detector length^2)/(resistance+(reactance^2/resistance))

Equation 3 = (detector length^2)/reactance

Equation 4 = (detector length^2)/(reactance+(resistance^2/reactance))

Equation 5 = (detector length^2)/(3.1831E-18/(reactance+resistance^2/reactance))

Equation 6 = (detector length^2)/((resistanc^2+reactance^2)^0.5)

Equation 7 = arctangent(reactance/resistance)

Table 2. SAS program to develop optimal BIA models for estimating proximate composition of Chinook salmon.

```
*First import field and proximate analysis dataset from Excel spreadsheet;
data two;
set one;
*Drops dummy variables and 'dead' lines from spreadsheet;
drop F20-F198;
if lres=. then delete;
*Calculates temperature corrections for resistance and reactance;
lrest = lres* ((-10.866*itemp+460.41)/(-10.866*10+460.41));
lreat = lrea* ((-3.1723*itemp+160.78)/(-3.1723*10+160.78));
vrest = vres* ((-10.243*itemp+447.25)/(-10.243*10+447.25));
vreat = vrea;
*Calculates the 7 electrical properties from resistance, reactance, and detector length;
Rp = lrest+(lreat**2/lrest);
Xcp = lreat+(lrest**2/lreat);
Cpf = 3.1831E-18/Xcp;
Z = (lrest**2+lreat**2)**0.5;
LE1 = ldl**2/lrest;
LE2 = ldl**2/Rp;
LE3 = ldl**2/lreat;
LE4 = ldl**2/Xcp;
LE5 = ldl**2/Cpf;
LE6 = ldl**2/Z;
LE7 = atan(lreat/lrest);
VRp = vrest+(vreat**2/vrest);
VXcp = vreat+(vrest**2/vreat);
VCpf = 3.1831E-18/VXcp;
VZ = (vrest**2+vreat**2)**0.5;
VE1 = vdl**2/vrest;
VE2 = vdl**2/VRp;
VE3 = vdl**2/vreat;
VE4 = vdl**2/VXcp;
VE5 = vdl**2/VCpf;
VE6 = vdl**2/VZ;
VE7 = atan(vreat/vrest);
ED =9450*pwfat+5650*pwprot;
ods html;
*Estimates the 'best' predictive model based on lateral only and lateral & ventral
measurements;
proc reg data=two outest=est;
        model ED pwater pdfat pdprot pdash = LE1 LE2 LE3 LE4 LE5 LE6 LE7
flen/selection = rsquare cp b best=1;
        model ED pwater pdfat pdprot pdash = LE1 LE2 LE3 LE4 LE5 LE6 LE7 VE1
VE2 VE3 VE4 VE5 VE6 VE7 flen/selection = rsquare cp b best=1;
```

Table 2. Continued.

```
Proc print data=est;
run;
ods html close;
run;
*Remember to export model parameters (data=est) to Excel spreadsheet;
```

Table 3. Field measurements and laboratory proximate analysis results for 46 Chinook salmon collected from the Yukon River at Rapids Camp on June 22-25, 2004. ID was assigned as the date and unique two digit number for tracking each fish (MMDDYY##), Weight was measured in Kg, Length was measured from mid-eye to fork of tail in mm, Temp is temperature in degrees centigrade, Lres is the lateral resistance, Lreac is the lateral reactance, Ldetlen is the lateral distance between BIA probes, Vres, Vreac, and Vdetlen are the ventral BIA measurements, Water is the percent water measured in the laboratory proximate analysis, Fat is the lipid proximate analysis value expressed as percent dry weight, Protein and Ash are proximate analysis values expressed as percent dry weight.

ID	Sex	Weight	Length	Temp	Lres	Lreac	Ldetlen	Vres	Vreac	Vdetlen	Water	Fat	Protein	Ash
06220404	F	6.1	795	21.0	334	115	456	365	63	295	60.06	41.47	51.34	5.17
06240416	F	6.7	797	21.0	354	143	461	398	56	303	63.29	43.52	52.70	6.78
06250443	F	6.8	817	21.5	290	103	474	404	67	290	61.64	45.31	50.00	6.69
06240423	F	7.1	814	20.9	322	119	491	426	68	300	60.93	42.09	50.68	6.38
06240422	F	7.2	834	21.1	287	111	497	308	68	314	63.26	39.27	54.89	6.08
06240418	F	8.2	867	20.8	292	105	511	333	63	311	65.89	33.62	59.98	7.94
06250439	F	8.8	865	21.3	354	115	516	439	87	332	57.90	46.59	47.87	3.81
06240420	F	9.3	859	21.0	300	105	496	333	64	311	61.90	41.93	51.13	5.38
06230413	F	9.4	925	21.0	368	122	532	405	54	337	65.03	42.16	52.84	6.90
06250435	F	9.6	895	21.1	309	108	560	321	71	342	65.31	44.76	49.32	5.86
06250446	F	9.7	895	21.4	291	102	520	343	70	341	63.92	42.56	51.51	6.15
06250437	F	9.8	890	21.6	328	118	527	367	82	339	59.98	51.02	43.98	4.34
06240419	F	9.8	895	20.8	279	97	535	306	57	337	62.28	40.17	51.81	6.40
06250438	F	10.0	897	21.2	368	129	524	421	92	330	49.26*	50.81	44.34	5.25
06220405	F	10.1	905	21.0	335	107	545	363	61	340	58.85	46.83	46.68	4.19
06240429	F	10.7	910	21.4	322	103	550	379	50	346	59.20	47.77	47.15	5.04
06240432	F	10.8	923	21.1	336	119	546	400	53	347	61.58	45.01	51.32	5.15
06240430	F	12.0	956	21.7	361	125	576	412	86	385	63.54	44.48	50.71	5.90
06250434	F	14.0	998	20.8	289	106	603	335	71	373	65.09	40.18	54.66	8.42
06240433	M	2.2	541	21.1	338	150	294	435	122	185	65.84	41.25	53.88	8.17
06220403	M	2.4	565	21.0	322	118	317	354	90	195	68.61	40.72	54.39	6.19
06230414	M	2.5	600	21.0	296	121	342	333	96	217	59.90	36.70	51.16	7.30
06240425	M	2.6	586	21.1	358	149	340	423	95	221	63.03	45.97	50.81	5.45
06250441	M	3.2	620	21.5	376	155	362	443	119	227	62.64	44.90	44.64	5.74
06240424	M	4.2	680	20.7	308	119	394	353	65	252	65.40	37.11	54.35	5.54
06230410	M	4.3	673	21.0	316	115	380	346	81	242	64.70	41.17	54.86	6.45
06230406	M	4.4	695	21.0	311	126	416	363	102	266	66.21	37.62	55.93	6.29

Table 3. Continued.

ID	Sex	Weight	Length	Temp	Lres	Lreac	Ldetlen	Vres	Vreac	Vdetlen	Water	Fat	Protein	Ash
06230411	M	4.4	710	21.0	379	130	406	391	82	251	65.42	36.14	50.90	7.73
06230409	M	4.5	684	21.0	278	99	386	325	68	244	64.28	40.01	53.45	7.47
06230408	M	5.0	742	21.0	331	117	432	381	79	280	63.82	41.40	52.54	6.49
06220402	M	5.1	727	21.0	340	121	421	371	85	273	60.39	41.08	54.59	5.83
06210401	M	5.3	730	21.0	287	102	420	320	58	257	60.98	43.21	51.50	6.56
06250442	M	5.5	754	21.2	372	139	431	425	83	275	60.10	46.19	49.57	5.55
06230415	M	5.8	765	21.0	315	114	446	350	70	295	65.22	38.54	55.31	7.36
06240417	M	5.9	765	21.0	304	123	458	313	61	285	66.23	36.46	57.31	7.15
06240426	M	6.0	745	21.3	365	120	432	442	82	282	60.28	48.37	47.88	4.62
06240428	M	6.1	762	21.5	376	113	465	406	59	293	61.68	48.51	48.04	6.11
06250444	M	6.3	773	21.3	323	111	446	330	60	273	61.92	45.65	49.39	6.79
06230412	M	6.3	800	21.0	318	127	472	366	79	295	63.47	43.14	51.77	6.36
06230407	M	7.5	825	21.0	343	117	480	411	81	311	62.19	43.25	52.20	5.30
06240431	M	8.2	865	21.6	367	113	505	393	80	323	63.02	47.00	45.42	6.35
06250445	M	9.4	903	21.3	270	98	522	322	50	322	60.86	43.81	54.37	7.82
06250440	M	9.6	904	21.7	293	108	523	370	63	321	62.45	45.75	50.49	5.62
06250436	M	9.8	896	21.1	275	98	532	319	55	327	62.93	44.27	51.91	4.31
06240427	M	10.9	946	21.3	235	86	555	242	51	360	68.43	35.77	59.04	5.77
06240421	M	11.6	956	21.1	233	82	562	236	42	351	65.55	34.63	59.74	5.59

* This value is an outlier, relative to other measurements. It was included in the analyses, but dropping it would improve predictive capability.

Table 4. Coefficients for BIA predictive models based on seven electrical properties as measured laterally and ventrally on Chinook salmon. LE1 is the parameter based on lateral equation 1, VE1 is the parameter based on ventral equation 1, and so on, and length is mid-eye to fork length of the fish expressed in mm. (VE2 and VE6 were not significant predictors in any of the five models.) Water is percent water content, and fat, protein, and ash are expressed as percent dry weight. ED is energy density expressed as joules per gram dry weight.

Equation parameter	water	fat	protein	ash	ED
Intercept	125.534	-91.364	176.077	21.210	-329536.511
LE1	-	-	-	-	-14410.931
LE2	-3.146	9.220	-7.541	-1.306	8597.746
LE3	-0.081	0.233	-0.184	-0.035	803.675
LE4	-1.692	5.006	-4.025	-0.721	14424.914
LE5	2.395E-25	-3.702E-25	2.812E-25	3.567E-26	-2.201E-21
LE6	3.771	-11.044	8.992	1.571	-
LE7	-81.691	226.848	-200.142	-26.791	826090.438
VE1	-	-	-	-0.035	-
VE3	0.005	-0.012	0.010	0.006	-57.014
VE4	-	-	-	0.066	-
VE5	-2.315E-25	4.429E-25	-4.026E-25	-1.054E-25	2.054E-21
VE7	-14.359	20.660	-31.929	-8.824	86742.129
Length	-0.052	0.073	-0.067		472.560
r-square	0.83	0.90	0.89	0.70	0.86

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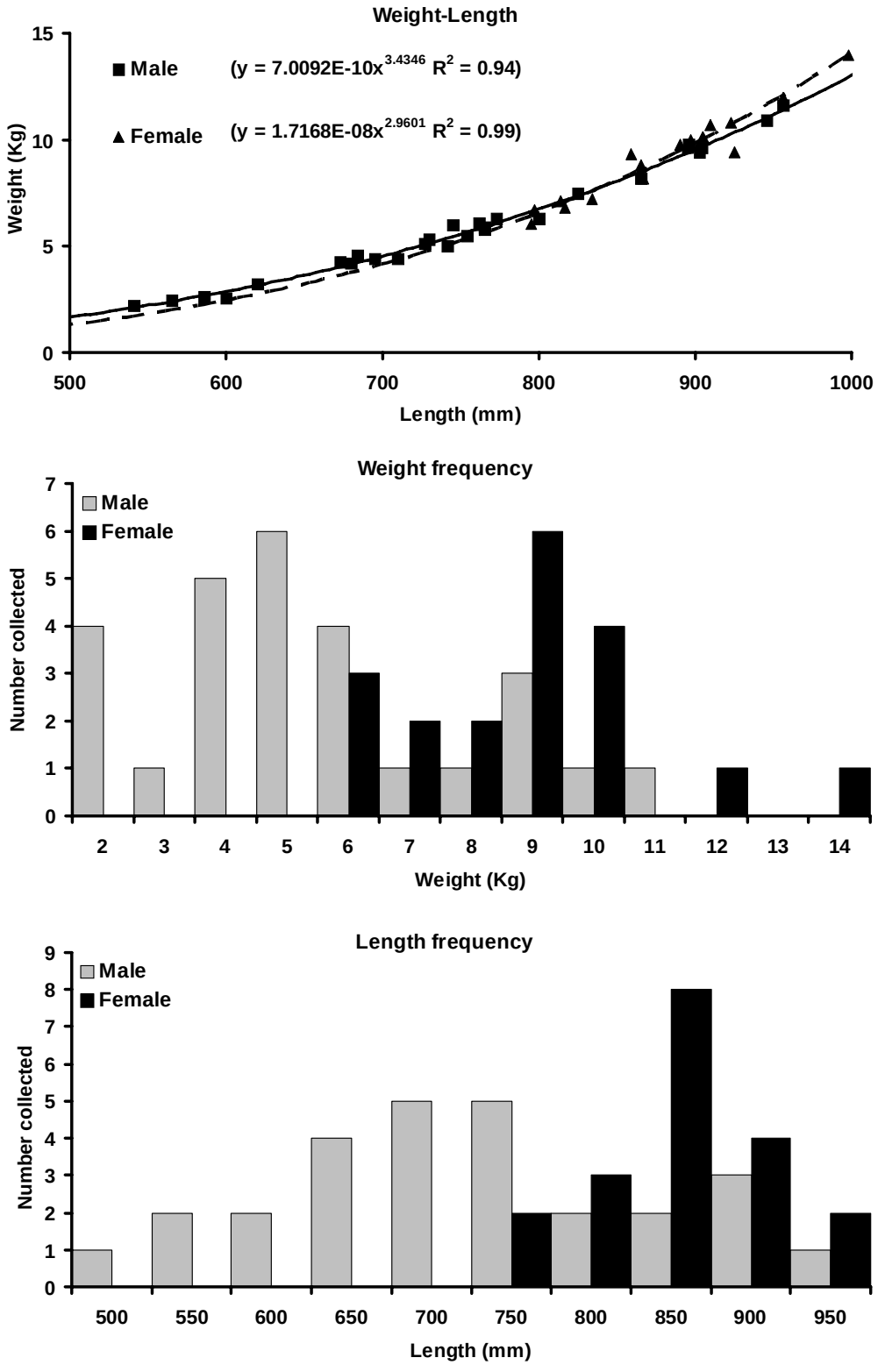
Weight-Length
Male (y = 7.0092E-10x^3.4346 R^2 = 0.94)
Female (y = 1.7168E-08x^2.9601 R^2 = 0.99)
Weight (Kg)
Length (mm)
Weight frequency
Male
Female
Number collected
Weight (Kg)
Length frequency
Male
Female
Number collected
Length (mm)

Figure 1.

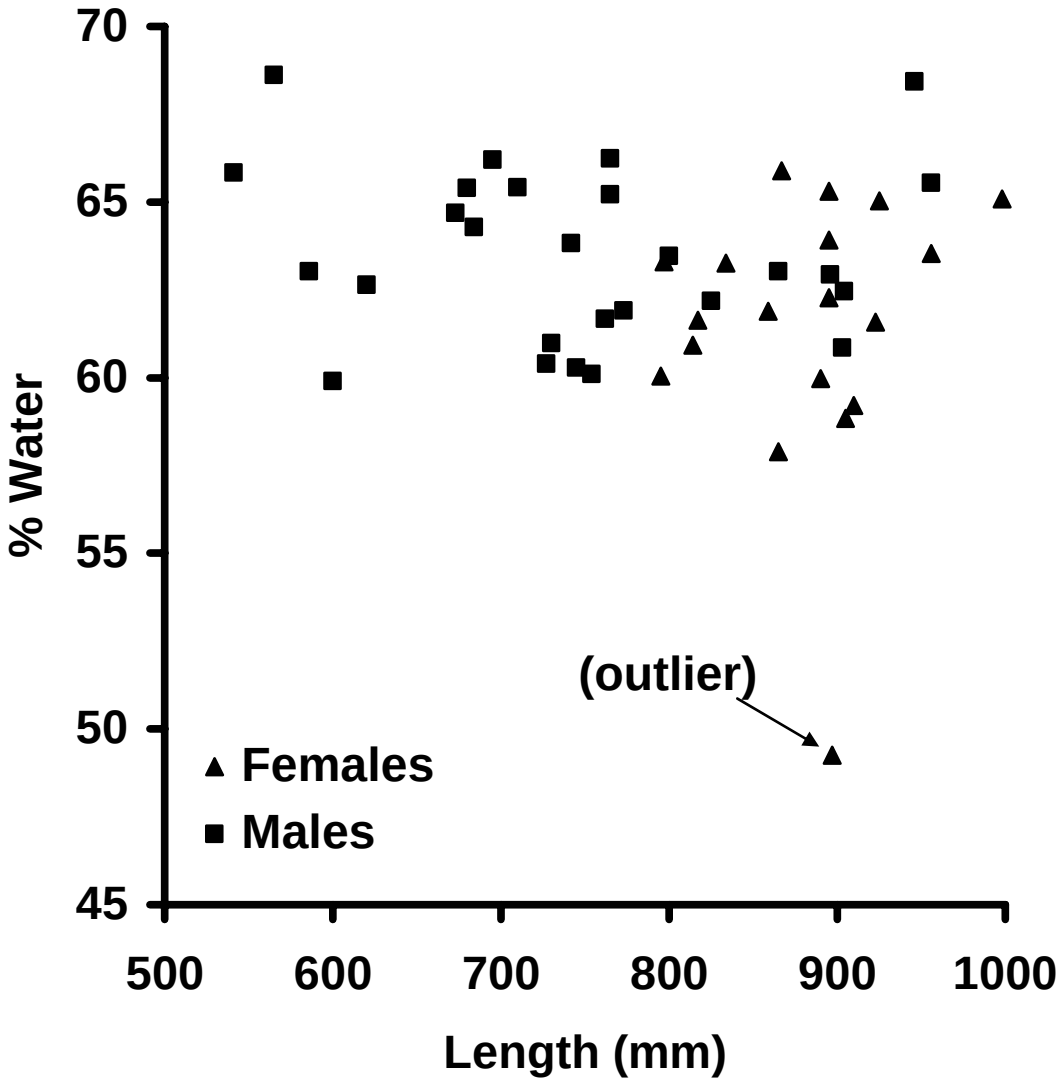
70
65
60
55
50
45
% Water
(outlier)
Females
Males
500
600
700
800
900
1000
Length (mm)

Figure 2 (A).

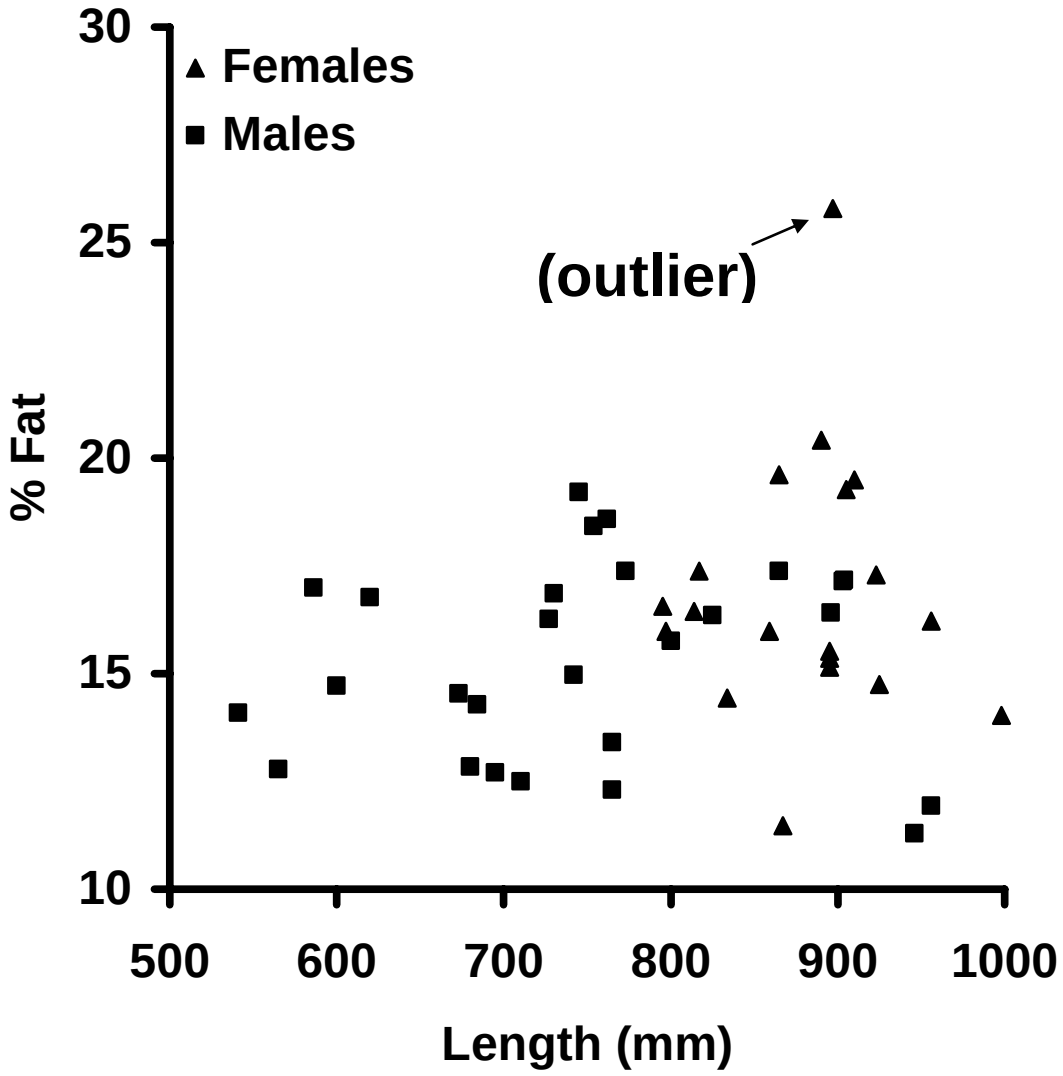
30
25
20
15
10
% Fat
Females
Males
(outlier)
500
600
700
800
900
1000
Length (mm)

Figure 2 (B).

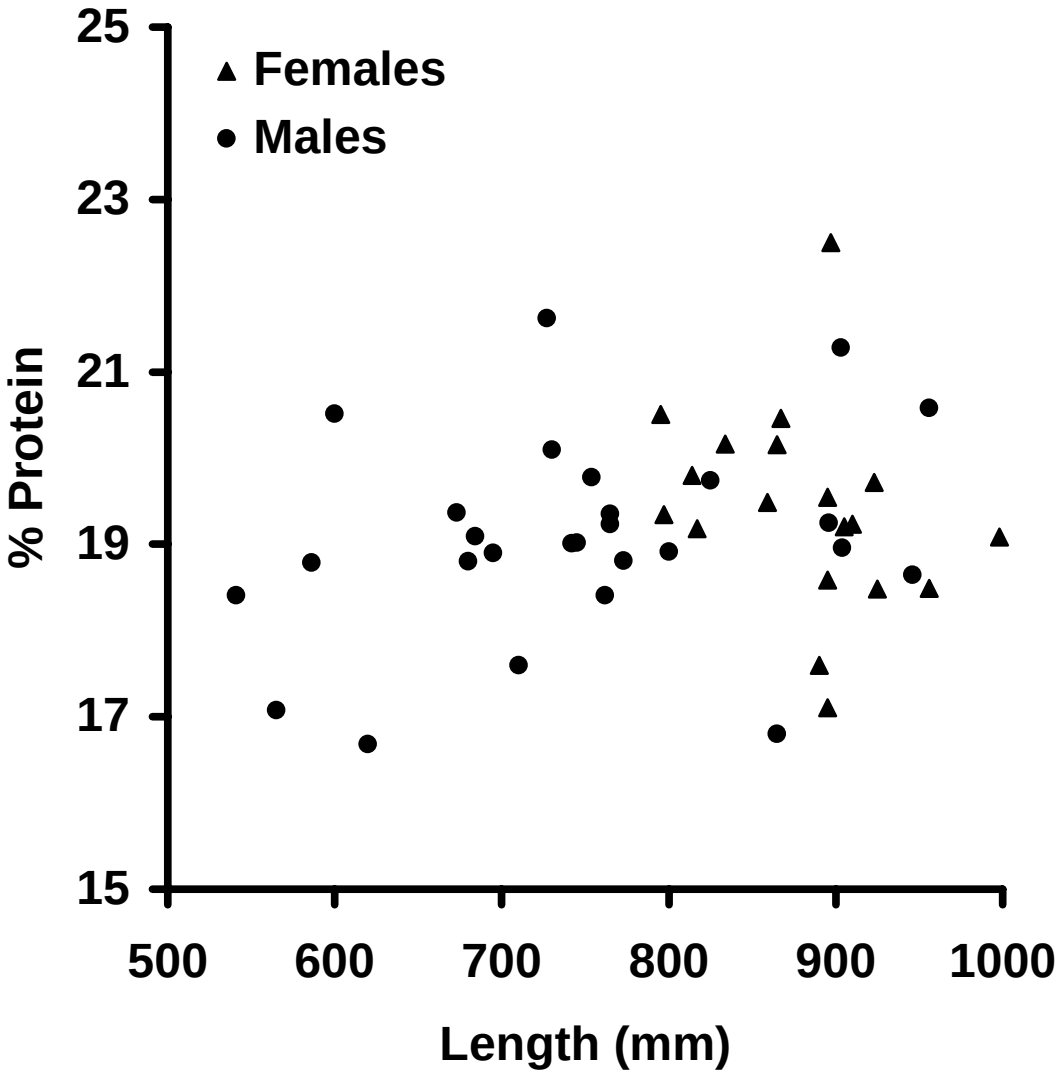
25
23
21
19
17
15
% Protein
Females
Males
500
600
700
800
900
1000
Length (mm)

Figure 2 (C).

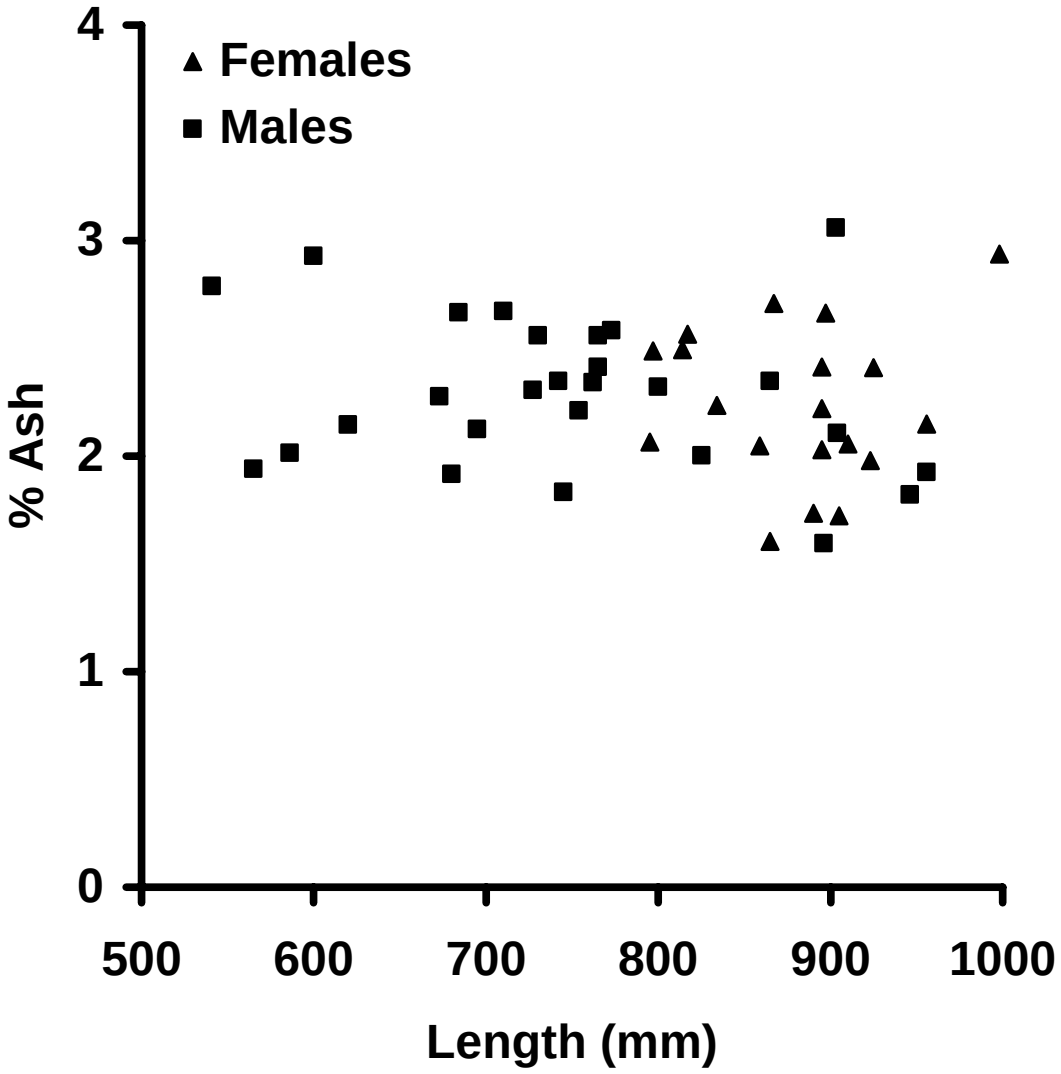

Figure 2 (D).

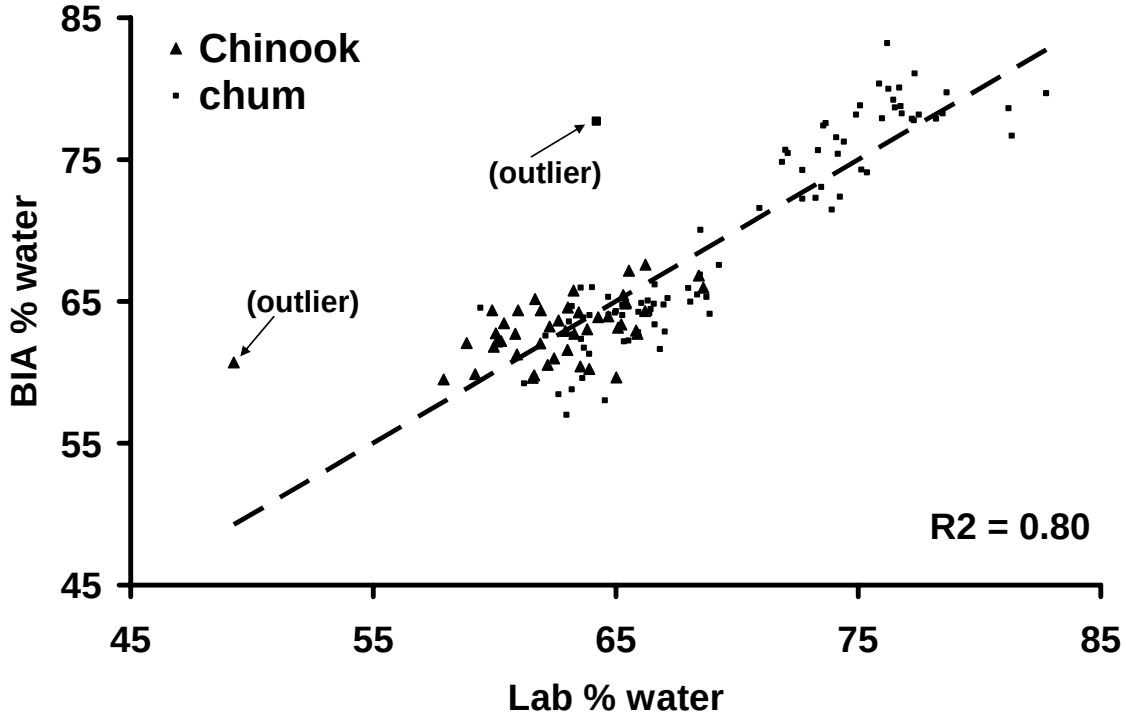

Figure 3 (A).

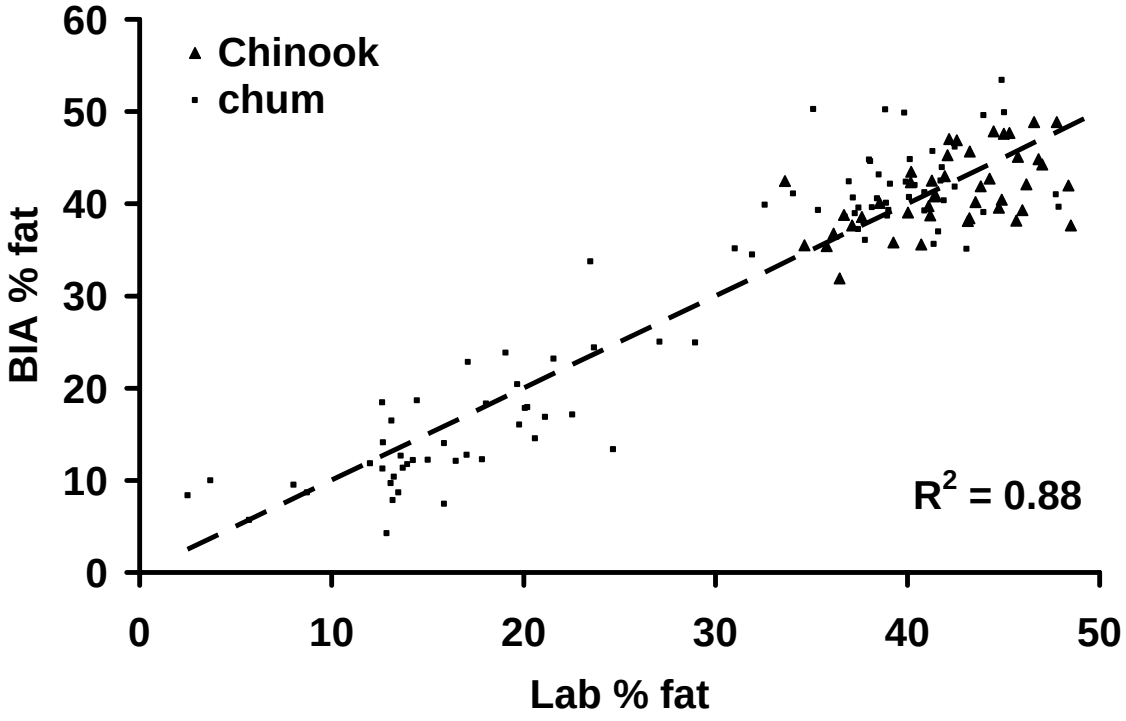

Figure 3 (B).

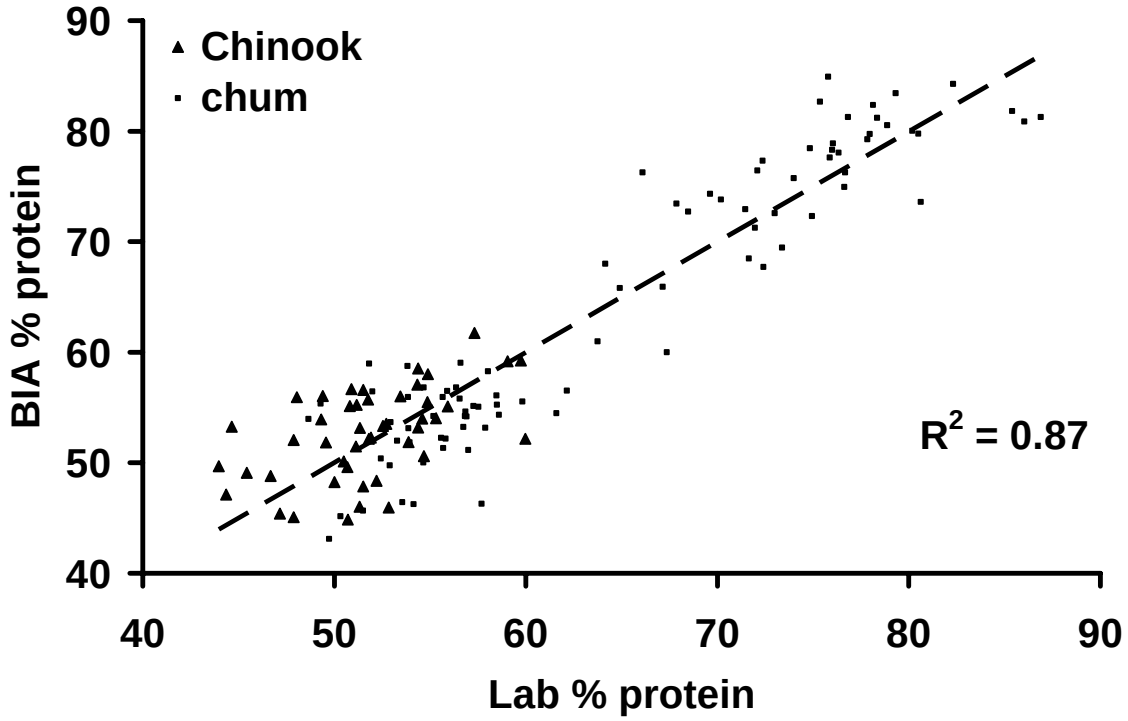

Figure 3 (C).

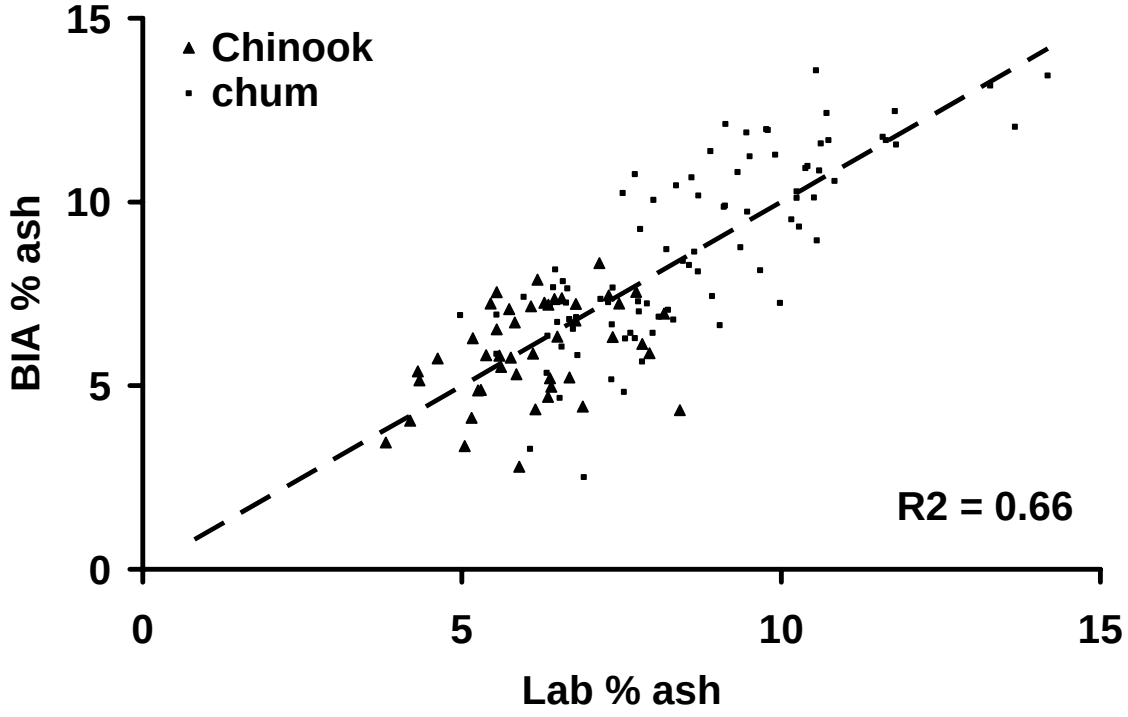

Figure 3 (D).

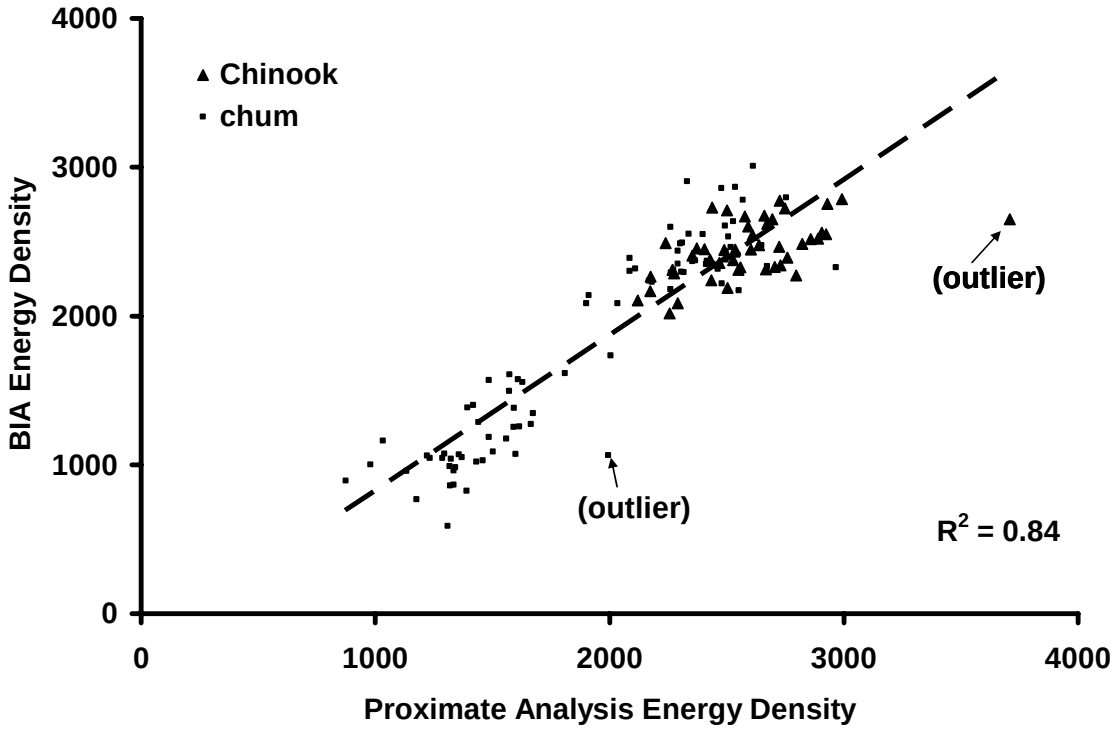
4000
3000
2000
1000
0
BIA Energy Density
Chinook
chum
(outlier)
(outlier)
R2 = 0.84
0
1000
2000
3000
4000
Proximate Analysis Energy Density

Figure 3 (E).