Morphometric analysis for nonlethal sex determination in brook trout: a new tool for research and management

A Final Independent Project Report

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Introduction

Brook trout (*Salvelinus fontinalis*) are an iconic species that has been considered recreationally and aesthetically important throughout its native range for centuries. Brightly-colored and charismatic, brook trout are also ecologically important - they are recognized as a bioindicators for water temperature and quality in the southern regions of their historical range (Waco and Taylor 2009). Brook trout require water that is

relatively cold (seldom exceeding 25°C) and well-oxygenated. As such this species typically inhabits streams that are surrounded by forests.

Brook trout face many threats and have already been extirpated from many of their native streams in Maryland (Stranko et al. 2008). The only native trout species found in Maryland, they have decreased from a population of millions to a few hundred thousand (MD DNR 2005). Habitat threats include water temperature increases due to climate change, land use changes, run-off (urban, agricultural and mining) and habitat fragmentation (Heft 2006, Letcher et al 2007). Brook trout also face competition and predation by stocking of non-native brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*). The western Appalachian region, with its cool streams in less disturbed, mostly forested watersheds, contains most of the state's extant brook trout. Brook trout can be found in other streams throughout Maryland, but those populations are considered greatly reduced (Hudy et al. 2005). However, even strong populations are in danger of decline.

As a species of "Greatest Conservation Need' in Maryland (MD DNR 2005), conservation efforts and management plans for the species have been instituted, including fishing restrictions, conservation assessments, and life history research. Sex-specific life history differences are not known, but are potentially important to effective management. However, no reliable non-lethal methods for sexing brook trout have been established outside of the spawning season when adults are full of eggs or milt. A non-lethal approach to distinguishing male and female brook trout is essential to reduce stress on the threatened native populations of brook trout while obtaining important life

history information. Anecdotally, there are distinctive physical differences between male and female brook trout within populations, though this has not been quantified to-date.

Sexual dimorphism has been studied extensively in fish and, typically, becomes more pronounced with size in sexually mature fishes (Beacham and Murray 1986). In species such as threespine sticklebacks (*Gasterosteus aculeatus*) and Mediterranean blennies (*Blenniidae* sp.), body size has shown significant relationship to dimorphism (Cooper et al 2011, Lengkeek et al. 2008). The relationship between head size and sex was examined in threespine sticklebacks (*Gasterosteus aculeatus*), using a geometric morphometric approach based on anatomical landmarks, most of which were located in the head region of the fish. Through photo-analysis, males could be distinguished from females through larger head, eye and mouth size (Cooper et al 2011). For some species, such as the California Sheephead (*Semicossyphus pulcher*), whose life history involves transition from female to male, morphometrics do not provide adequate basis for sex determination (Loke-Smith et al. 2010).

Although sexual dimorphism has not been rigorously examined in brook trout, other salmonids have been studied for morphological differences. Jahunen et al. (2009) conducted photo-analysis of Arctic charr based on a truss network of twenty-eight measurements and found that mature males have more robust (greater length and depth) bodies and heads than females and juveniles. Pacific salmon species were shown to exhibit differences in kype presence and adipose fin size compared between sexes (Beacham and Murray 1986); length and height of adipose fin was on average 31%-48% (variable between species) larger in males than females and suggesting that

adipose fin size could be used to externally determine sex in Pacific salmon species. Further work with *Oncorhynchus* species has found that 87-97% (variable between species) of individuals could be correctly sexed using adipose fin size and/or jaw length (Beacham and Murray 1986). Similarly, Merz and Merz (2004) found that ratios of snout length to fork length in chinook salmon (*Oncorhynchus tshawytscha*) led to 96% accuracy in determining sex in handled fish, and ratios of adipose fin length to fork length led to 86% accuracy for fish measured from video images at a fish passage facility. When combining these two ratios with head length measurements, accuracy increased to 92% in determining sex of fish measured through video images.

While nonlethal approaches to sex determination via morphology have been successfully developed for other salmonids (Beacham and Murray 1986, Merz and Merz 2004), no such techniques exist for brook trout. Thus, biologists lack effective tools to determine sex for brook trout. To address this concern, I measured a suite of morphological characters in hopes of identifying distinct and quantifiable differences between male and female brook trout that could allow for non-lethal determination of sex. Here I present those metrics most effective at determining sex and which of those metrics may be applied to rapidly sex brook trout in the field.

Although brook trout are exhibiting widespread declines and are the focus of many conservation efforts, we still lack basic management tools. Developing a nonlethal approach to determine sex via morphology will open new doors to brook trout research and has the potential to improve the biological information on which management decisions are based. Metrics for determining sexual dimorphism could be used to

examine sex-specific vital rates, including growth, survival, and movement. Sex ratios may also be evaluated using this approach. This information may have considerable implications for population dynamics, but is currently overlooked by managers because it is too difficult to obtain. Although this tool was developed based on individuals collected in a single watershed, two distinct geographic morphotypes were determined, and the results show that this tool should be applicable across a much broader geographic area. The development of this non-lethal tool for sex identification in brook trout will further basic and applied science and, consequently, aid in the conservation of the species.

Methods

Data Collection

In October 2011, backpack electrofishing was used to collect brook trout in tributaries within the Savage River watershed in western Maryland (Figure 1). This work was conducted in close collaboration with MD DNR and the UMCES Appalachian Laboratory, in support of an ongoing collaborative research project. The collection took place during spawning season when sex of brook trout is most evident and can be accurately determined. The fish were lightly anesthetized with tricaine methanesulfonate (ms-222) buffered with 0.2 mM NaHCO3, pH = 7. The total length (mm) of the fish was measured, and the width of head (mm) was measured with calipers. The fish were assessed through manual gamete expression to accurately determine sex. They were then placed on a light-colored background and photographed on their left side with a digital camera attached to a tripod at a specified height. The target sample size of 25 fish of each sex was calculated based on a two-tailed powered analysis for a moderate

effect size. We collected 111 individuals - 36 adult males, 29 adult females, 46 adults of unknown sex (15 only of which full measurements were taken). Fish were collected from the upper reaches of the Big Run system and from the lower portion of Big Run (Figure 1). Individuals <100 mm total length were not used for analysis, as these fish were assumed to be sexually immature and we could not determine their sex.

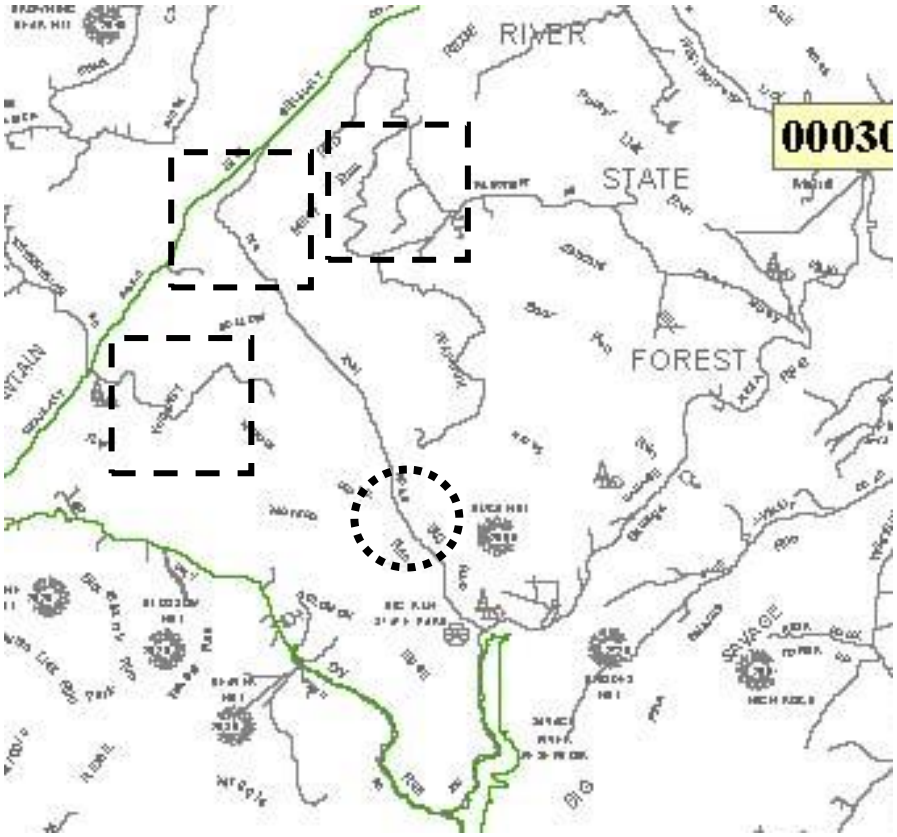

Figure 1. Sample sites in Savage River watershed. The squares represent presence of Morphotype 1 and the circle represents presence of Morphotype 2.

Image Analysis

I analyzed brook trout images of known sex using ImageJ, an open-source imaging software. I measured twenty-seven distances between anatomical features in form of a truss network (Figure 2a, b). This method has been successfully used to examine

morphologic variation in other salmonids (Janhunen 2009). A suite of metrics were derived from the truss network (Table 1).

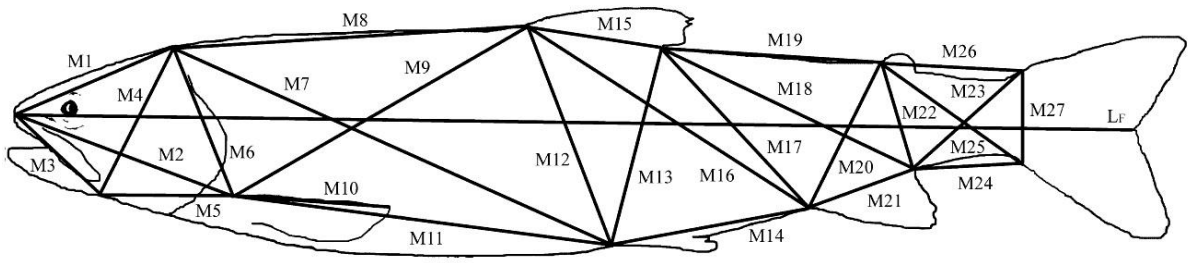

Figure 2a. Truss network of morphometrics applied to Arctic charr (*Salvelinus alpinus*) (Janhunen et al. 2009)

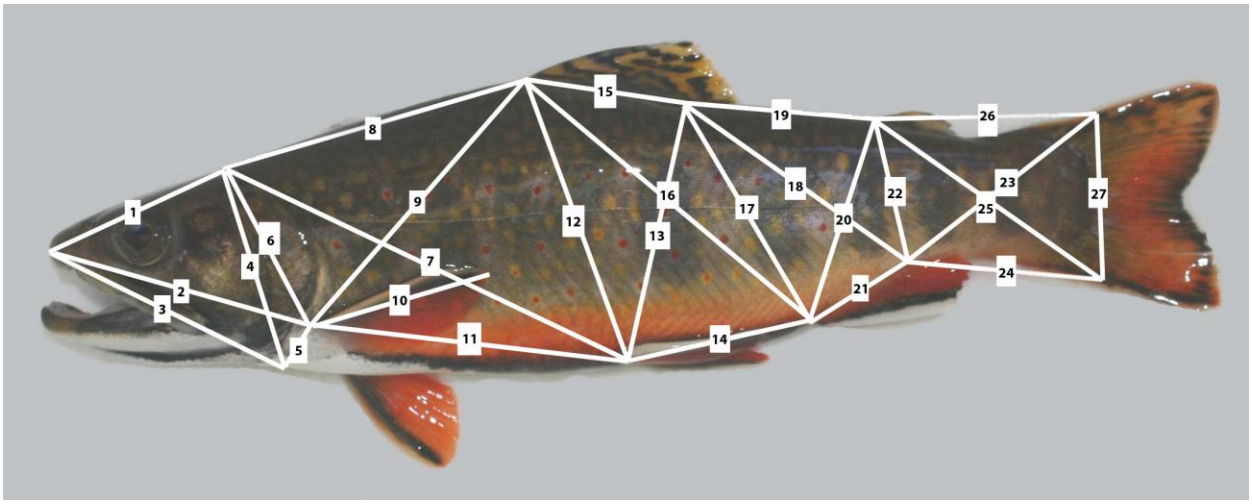

Figure 2b. Truss network of morphometrics applied to brook trout.

Table 1. Measurement descriptions modeled after truss network measurements used by Jahunen et al.

Metric	Description
M1	crown to snout tip
M2	snout tip to anterior pectoral fin
M3	snout tip to gill juncture
M4	gill juncture to crown
M5	gill juncture to anterior pectoral fin

M6	crown to anterior pectoral fin
M7	crown to anterior pelvic fin
M8	crown to anterior dorsal fin
M9	anterior pectoral fin to anterior dorsal fin
M10	total pectoral width
M11	anterior pectoral fin to anterior pelvic fin
M12	anterior dorsal fin to anterior pelvic fin
M13	anterior pelvic fin to posterior dorsal fin
M14	anterior pelvic fin to anterior anal fin
M15	anterior dorsal fin to posterior dorsal fin
M16	anterior dorsal fin to anterior anal fin
M17	posterior dorsal fin to anterior anal fin
M18	posterior dorsal fin to posterior anal fin
M19	posterior dorsal fin to anterior adipose fin
M20	anterior anal fin to anterior adipose fin
M21	anterior anal fin to posterior anal fin
M22	posterior anal fin to anterior adipose fin
M23	posterior anal fin to dorsal insertion of caudal fin
M24	posterior anal fin to ventral insertion of caudal fin
M25	anterior adipose fin to ventral insertion of caudal fin
M26	anterior adipose fin to dorsal insertion of caudal fin
M27	dorsal insertion of caudal fin to ventral insertion of caudal fin

Statistical Analysis

After processing the images, each measurement was regressed against fish length in order to determine if the raw data or log10 transformed data were most appropriate (Janhunen et al. 2009) for analysis. The standardized residuals were calculated and used in Analysis of Variance (ANOVA) to test for differences between sexes for each measurement. All variables used in the analyses met the homogeneity of variances assumptions of regression and ANOVA. Results were considered statistically significant at $P<0.05$. Finally, I used discriminant functions analysis to predict sex from the morphological measurements and used jackknifing to produce a cross-classified error rate on the predictions. As utilizing the full suite of 27 measurements is not practical for field application, I re-ran the discriminant functions analysis on the seven

measurements with the highest separations to determine if fewer variables could be used to accurately assess sex. All analyses were conducted using the software program R (a free software environment for statistical computing and graphics).

Results

All morphometric measurements were significantly related to length, regardless of whether the raw or log10 transformed data were used (Table 2). Except for measurements M3 and M5, two distinct morphotypes were present with each forming a distinct group as demonstrated in Figure 3. Morphotype 1 consisted of fish captured from the upper portions of the Big Run system, while morphotype 2 consisted of those captured in the lower portion of Big Run. Fish from the two morphotypes were separated by approximately 4 km of stream (Figure 1). Truss network measures that showed strong separation of the morphotypes were measurements M1,M 6, M7, M8, M9, M16, M17, M18, and M23 (Appendix A). As there were two distinct groups that emerged, I ran regressions for each morphological measurement on each morphotype where it was obvious from regression plots that the two morphotypes were present. This was necessary because the standardized residuals used in the ANOVA analysis would be biased if they were calculated on both morphotypes combined.

Table 2. Regression table of results for log10 Transformed Measurements

metric	slope	intercept	df	t-value	p-value
M1	0.0030082	1.996221	90	17.34	<2e-16
M2	0.0033577	2.081042	90	21.13	<2e-16
M3	0.003266	2.028842	90	15.77	<2e-16
M4	0.0031836	1.96218	90	19.5	<2e-16
M5	0.0035024	1.409687	90	10.62	<2e-16
M6	0.0033132	1.9134	90	18.48	<2e-16

M7	0.003093	2.33764	90	17.52	<2e-16
M8	0.0030887	2.199012	90	17.01	<2e-16
M9	0.0031684	2.191284	90	17.16	<2e-16
M10	0.003005	1.949583	90	12.95	<2e-16
M11	0.0029966	2.217496	90	15.16	<2e-16
M12	0.0032837	2.079204	90	17.67	<2e-16
M13	0.003205	2.047015	90	16.79	<2e-16
M14	0.0029282	2.058806	90	13.06	<2e-16
M15	0.0027448	1.96071	90	13.05	<2e-16
M16	0.003087	2.261037	90	17.54	<2e-16
M17	0.0031859	2.054343	90	17.61	<2e-16
M18	0.002941	2.174958	90	15.98	<2e-16
M19	0.0029095	2.041283	90	12.59	<2e-16
M20	0.002956	2.011721	90	17.27	<2e-16
M21	0.002612	1.855225	90	11.93	<2e-16
M22	0.0029306	1.869091	90	17.03	<2e-16
M23	0.0030088	2.077689	90	17.27	<2e-16
M24	0.0029804	1.995624	90	17.16	<2e-16
M25	0.002712	2.19559	90	14.98	<2e-16
M26	0.002601	2.122275	90	13	<2e-16
M27	0.0029142	1.939445	90	18.43	<2e-16

Significant differences in the standardized residuals existed between sexes for the measures M2, M3, M4 and M6 (Table 3). All four measurements (M2, M3, M4, and M6) represent the geometric relationship of anatomical landmarks in the head of the fish (Table 1, Figure 2). All four measures tend to be longer for males, at a given length, than females, suggesting that males have longer snouts and deeper, more robust heads than females. Specifically, the significance of M3 suggests that the distance from snout along the top jaw line could provide accurate determination of sex that is conserved across morphotypes.

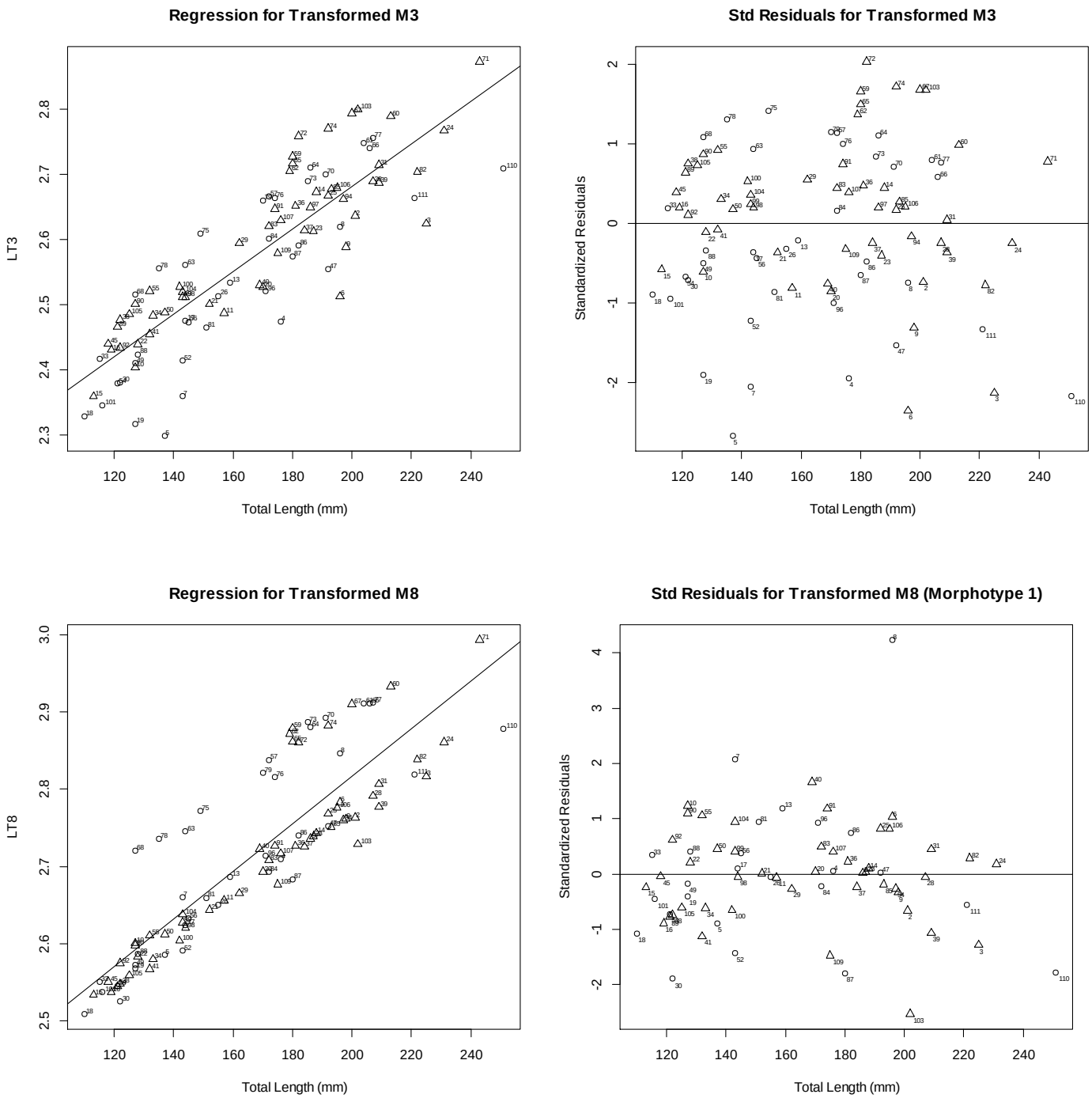

Figure 3. a) Linear regression for the log-transformed values of M3 shows no clear distinction between individuals caught in the lower portion of Big Run and individuals caught in upper reaches of Big Run. b) Standard residuals for the log-transformed values of M7 shows no clear distinction between individuals caught in the lower portion of Big Run and individuals caught in upper reaches of Big Run.
c) Linear regression for the log-transformed values of M8. Points 57-80 represent the group of individuals caught in the lower portion of Big Run while the rest represent the individuals caught in upper reaches of Big Run. d) Standard residuals for the log-transformed values of M8 of individuals that fall into morphotype 1.

Table 3. ANOVA table of results comparing the standardized residuals of the log10 transformed measurements in brook trout of known sex.

metric	morphotype	df	f-value	p-value	metric	morphotype	df	f-value	p-value
M1	1	1, 90	3.0875	0.08333	M15	1	1, 90	2.6768	0.1064
M1	2	1, 90	4.8126	0.04089	M15	2	1, 90	2.1045	0.1632
M2	***1***	***1, 90***	***8.5704***	***0.004624***	M16	1	1, 90	1.5245	0.2211
M2	***2***	***1, 90***	***11.346***	***0.003227***	M16	2	1, 90	3.2075	0.08925
M3	***combined***	***1, 90***	***5.1858***	***0.02514***	M17	1	1, 90	0.8781	0.352
M4	***1***	***1, 90***	***5.6696***	***0.02003***	M17	2	1, 90	3.3135	0.0845
M4	***2***	***1, 90***	***5.6696***	***0.02003***	M18	1	1, 90	0.1371	0.7123
M5	combined	1, 90	0.18	0.6724	M18	2	1, 90	2.9727	0.1009
M6	***1***	***1, 90***	***5.9372***	***0.01741***	M19	1	1, 90	0.281	0.5978
M6	2	1, 90	7.2669	0.01432	M19	2	1, 90	1.8065	0.1948
M7	1	1, 90	1.7507	0.1902	M20	1	1, 90	2.4865	0.1194
M7	2	1, 90	4.465	0.04805	M20	2	1, 90	5.1412	0.03522
M8	1	1, 90	1.4692	0.2296	M21	1	1, 90	0.1156	0.7349
M8	2	1, 90	5.2526	0.03351	M21	2	1, 90	1.5857	0.2232
M9	1	1, 90	2.1013	0.1517	M22	1	1, 90	3.9344	0.05129
M9	2	1, 90	3.6009	0.07305	M22	2	1, 90	5.8058	0.02628
M10	1	1, 90	3.5417	0.06406	M23	1	1, 90	3.1223	0.08165
M10	2	1, 90	3.2475	0.08742	M23	2	1, 90	5.3112	0.03264
M11	1	1, 90	0.118	0.7322	M24	1	1, 90	2.9997	0.08775
M11	2	1, 90	1.2437	0.2787	M24	2	1, 90	5.5614	0.02923
M12	1	1, 90	7.1919	0.009155	M25	1	1, 90	2.2181	0.141
M12	2	1, 90	5.7852	0.02651	M25	2	1, 90	4.9012	0.03927
M13	1	1, 90	5.1397	0.02652	M26	1	1, 90	2.9503	0.9035
M13	2	1, 90	4.2523	0.05314	M26	2	1, 90	4.2602	0.05294
M14	1	1, 90	0.0015	0.9695	M27	1	1, 90	0.6202	0.4337
M14	2	1, 90	0.1966	0.6625	M27	2	1, 90	2.78	0.1118

The discriminant functions analysis provided a reasonable method to predict sex of individuals from the morphological measurements. Good separation existed between sexes (Figure 4), with relatively low misclassification error rates (Table 4); classification accuracy for the full dataset was 85% (Table 4). Accuracy decreased only slightly with jackknifing. The discriminant functions analysis ran on the jackknifed data had an accuracy rate of 85% with 5 individuals randomly removed and 82% with 10 individuals removed from the full data set (Table 4). Simplifying the model from the full

27 variables to the best seven variables (Table 5) reduced classification accuracy from 85% to 84% (Table 4). Classification accuracy remained high on the reduced variable dataset after performing cross-classification error analysis with jackknifing based on leaving out 5 individuals (82% accuracy) or 10 individuals (84% accuracy). Variables with the highest separation between sexes were in decreasing order: M3, M2, M19, M14, M12, M21, and M6). Variables were related to the head(M2, M3, M6) and body depth(M12), which are typically longer in males, and posterior length(M14, M19, and M21), which are typically longer in females. Regardless of the specific model implementation, all errors were females that were misclassified as males (Table 4).

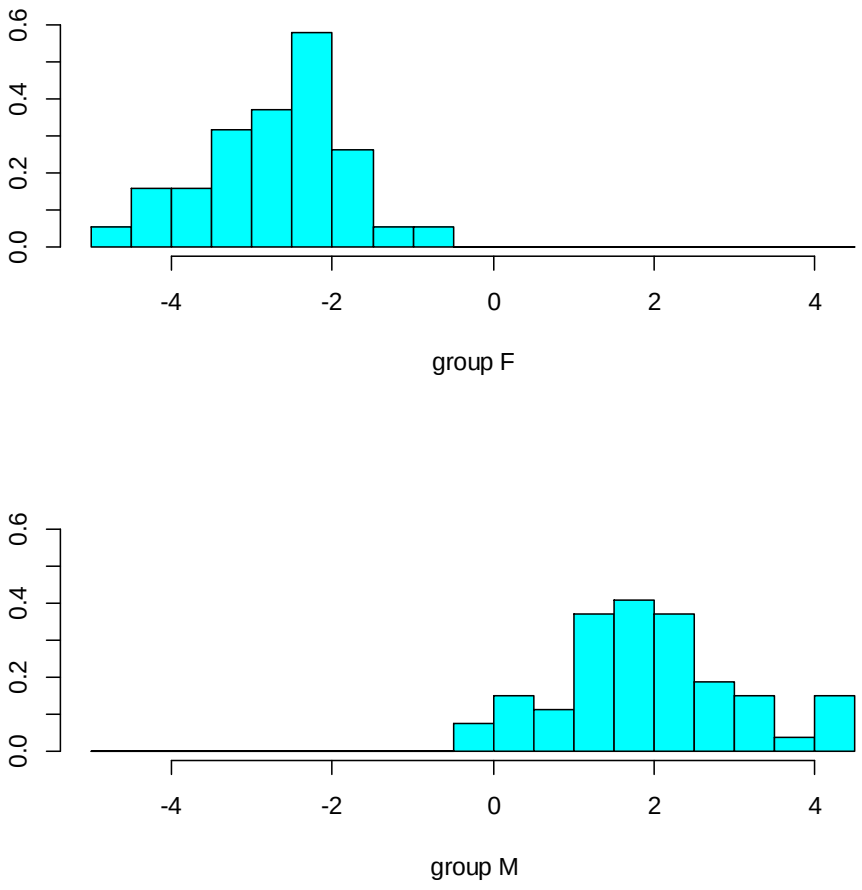

Figure 4. Histogram depicting separation between females and males across all fish collected in the Big Run system.

Table 4. Cross classified error assessment of the full model containing 27 measurement variables and a reduced model containing the seven most discriminating measurement variables. Accuracy is shown for the full dataset as well as jackknifing where 5 individuals were withheld or with 10 individuals withheld.

	Predicted female	Predicted male	% accuracy
Full dataset, full model			
Female	24	14	
Male	0	54	
			85
Jackknifed = 5, full model			
Female	24	14	
Male	0	54	
			85
Jackknifed = 10, full model			
Female	21	17	
Male	0	54	
			82

	Allocated to female	Allocated to males	% accuracy
Full dataset, reduced model			
Female	23	15	
Male	0	54	
			84
Jackknifed = 5, reduced model			
Female	21	17	
Male	0	54	
			82
Jackknifed = 10, reduced model			
Female	23	15	
Male	0	54	
			84

Table 5. Discriminant Function Analysis loadings for full model of 27 measurement variables and a reduced model of the seven most discriminating measurement variables.

metric	LD1 loadings	group means (female)	group means (male)
Full model			
M1	-22.88366711	2.490929	2.502699
M2	51.65118868	2.613321	2.660376
M3	15.43027415	2.537682	2.598625
M4	-34.75095005	2.478411	2.503353
M5	-0.081170412	1.985272	1.999642
M6	-27.83649984	2.449941	2.477104
M7	18.47884481	2.853197	2.853543
M8	-23.9935471	2.714681	2.713603
M9	55.58716658	2.719158	2.719942

M10	-0.002272917	290.8025	296.8336
M11	-34.36231401	2.73111	2.70738
M12	65.21217122	2.609129	2.63916
M13	-38.74489797	2.570168	2.589377
M14	-5.015257331	2.567879	2.532436
M15	6.167404176	2.418329	2.41845
M16	0.001454255	623.6509	616.6677
M17	-0.002685495	404.4089	398.0883
M18	-27.61226887	2.675573	2.658099
M19	3.766363909	2.548077	2.511231
M20	-15.35328773	2.501257	2.507006
M21	-0.017192892	209.3817	196.8706
M22	10.10387989	2.348964	2.363961
M23	5.52685327	2.573355	2.583673
M24	11.87373987	2.483711	2.498854
M25	-0.00609112	457.6997	459.1995
M26	0.013895082	371.8922	370.9906
M27	-0.023424126	276.8923	273.5961
Reduced model			
M3	9.06212215	2.537682	2.598625
M2	27.2913073	2.613321	2.660376
M19	-8.776314	2.548077	2.511231
M14	-12.410922	2.567879	2.532436
M12	11.15788638	2.609129	2.63916
M21	-0.02014005	209.381711	196.87063
M6	-18.22662649	2.449941	2.477104

Discussion

Distinct morphological differences existed between male and female brook trout. Of the 27 truss network measurements used, retaining only 7 that focused on the head, depth of body and the posterior length produced a model with good separation between sexes and minimal loss of accuracy (84% accurate). Males had longer snouts, more robust heads and deeper bodies, while females had longer post-dorsal lengths. Sexual dimorphism appears common in salmonids. Mature male Artic charr have more robust (greater length and depth) bodies and heads than females (Jahunen et al. 2009). Similarly, Pacific salmon were correctly sexed with 87-97% accuracy using jaw length

(Beacham and Murray 1986), whereas sex of chinook salmon was predicted with 92% accuracy by combining head length measurements with snout and adipose fin length to fork length ratios (Merz and Merz 2004). Although sex determination classification accuracy for Big Run brook trout was lower than the previously mentioned studies, it remains high enough to be a useful tool.

Sexual dimorphism in salmonids typically increases with an individual's age or size where males tend to develop more divergent head shape (Beacham and Murray 1986, Janhunen et al. 2009). Thus, most classification errors should comprise small males classified as females because they have not yet achieved secondary sexual characters. However, all misclassified fish in Big Run were females classified as males. Size did not appear to affect results as the lengths of misclassified females spanned almost the entire range from 110-206 mm and with weights ranging from 13-97 grams. Female salmonids can have greater variability in morphological measurements and ratios (Merz and Merz 2004), which could cause overlap with more male-like features for some female brook trout. Classification errors were distributed across both morphotypes, suggesting that the morphological variation in females can be widespread and is not restricted to a single subpopulation. Morphometric measurements on misclassified fish were checked and found to be error free.

Two distinct morphotypes were present in the Big Run system. One morphotype was present in the upper reaches of Big Run and the second was found exclusively in the lower portion of the system. The presence of two morphotypes allowed for better determination of measurements most strongly associated with sex and least related to

population variation. Sex-specific morphological differences are conserved in salmonids exhibiting morphological variation related to population divergence (Janhunen et al. 2009). Distinct separation between the sexes was apparent and sexual dimorphism can be determined empirically, regardless of morphotype.

Brook trout, and salmonids in general, exhibit great phenotypic plasticity and morphological variation. Multiple morphotypes may occur in the same system due to genetic or behavioral separation caused by physical or other barriers. Populations of brook trout separated between two lakes exhibited morphological variation both in groups that were genetically distinct and those that did not show significant genetic variation (Dynes et al. 1999). Similarly, populations of rainbow trout (*Oncorhynchus mykiss*) isolated by waterfalls have been found to be genetically and morphologically divergent (Currens et al. 1990). Genetic divergence is not a likely cause for the morphological variation in the Big Run population, as there are no physical barriers, and gene flow is probable. However, behavioral segregation could possibly occur if habitat or life history strategy influences body shape.

Life history strategies are frequently associated with phenotypic expression. Migratory and resident brook trout were correctly classified with 87% accuracy based on discriminant functions analysis of five traits, including measurements of body depth and pectoral and pelvic fin lengths (Morinville and Rasmussen 2008). Migratory brook trout exhibit more streamlined bodies and shorter fins than resident brook trout (Morinville and Rasmussen 2008). Similarly, body depth decreased in coho salmon (*Oncorhynchus*

kisutch) and sockeye salmon (*Oncorhynchus nerka*) with increased migration distances (Fleming and Gross 1989, Hendry and Quinn 1997).

Environmental variation, such as water depth or resource availability could explain morphological divergence in the Big Run system. One morphotype was found in the headwaters where there are multiple, small tributaries and shallower water, whereas the second was found in the lower portion of Big Run where the water is deeper and discharge greater. Salmonids, including coaster brook trout and sockeye salmon, that breed in deeper waters have deeper bodies than those who breed in shallower stream environments (Huckins et al. 2011, Hendry and Quinn 1997, Blair et al. 1993). Similarly, brook trout were assigned to geographic groups with 69%-78% accuracy based on fin shape, posterior body length and caudal peduncle height, with differences consistent to the respective foraging requirements of benthic and pelagic environments (Dynes et al. 1999). Morphotypic variation based on benthic and pelagic foraging types has also been found in Artic charr (S*alvelinus alpinus*) (Arbour et al. 2006, Snorrason et al. 1994).

Collection of morphological data required only a few additional seconds to fish processing. The tools used are basic making this an accessible method. Post processing with ImageJ was similarly straightforward, but does allow for human error. However, repeating measurements on the same fish resulted in very little inconsistency. The high classification accuracy rates indicate discriminant functions analysis is a reliable and repeatable method for determining sex. The truss network accounts for all

of the major anatomical landmarks on a fish and provides a thorough method to determine morphological variability.

Brook trout are an ecologically important, declining, and well studied species but significant gaps remain in our understanding of their life history. The morphological differences that I found between the sexes allow a quick, nonlethal way of determining the sex for each brook trout captured. The method is easily implemented with minimal additional handling or equipment, but is over 80% accurate. Although more validation is required for small, immature fish, the tool should be useful for researchers and managers alike. Determining sex-specific life history attributes such as growth rates, survival rates, and movement as well as sex ratios may provide important insights in more effective management of brook trout and the watersheds they inhabit.

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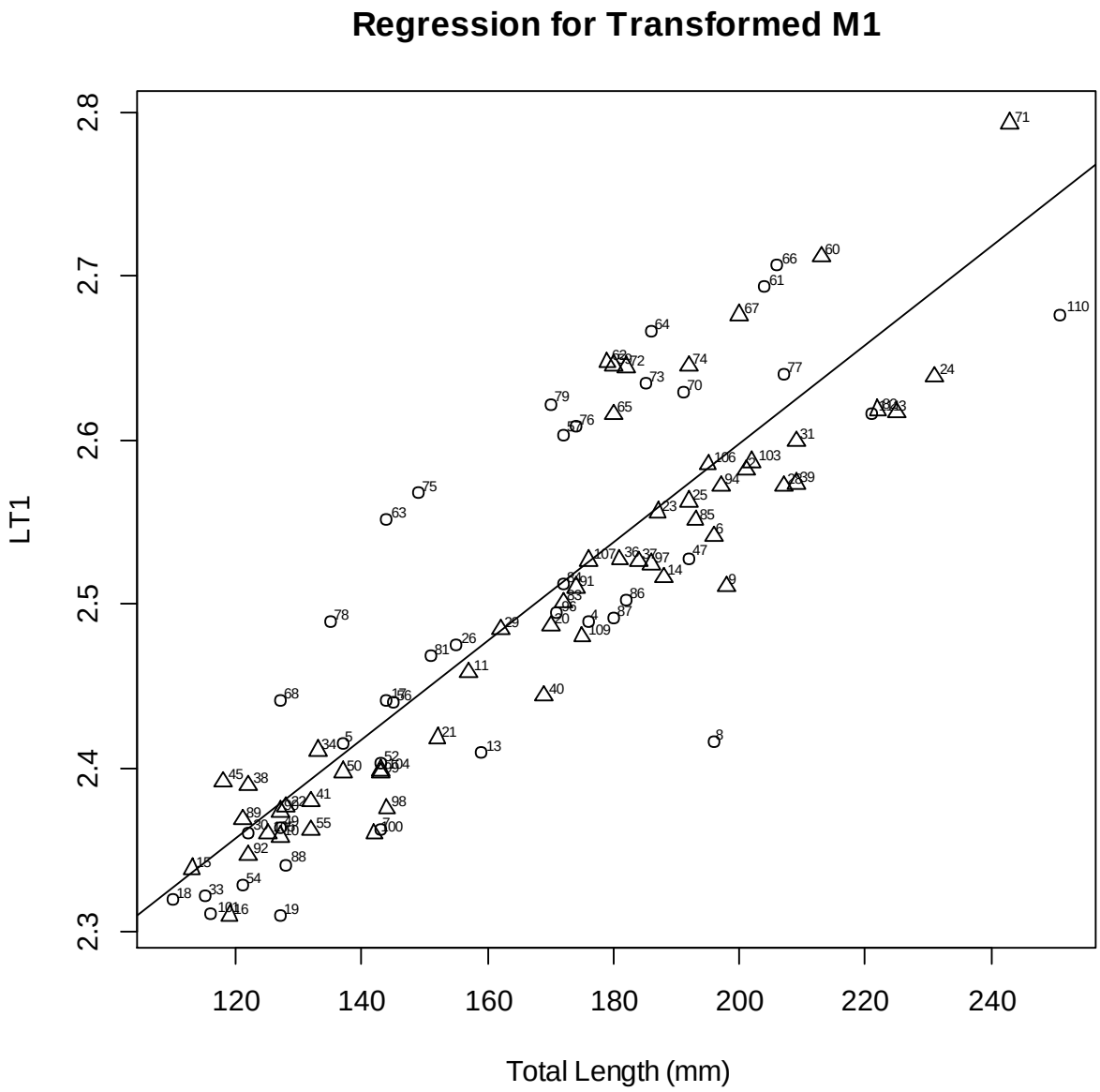
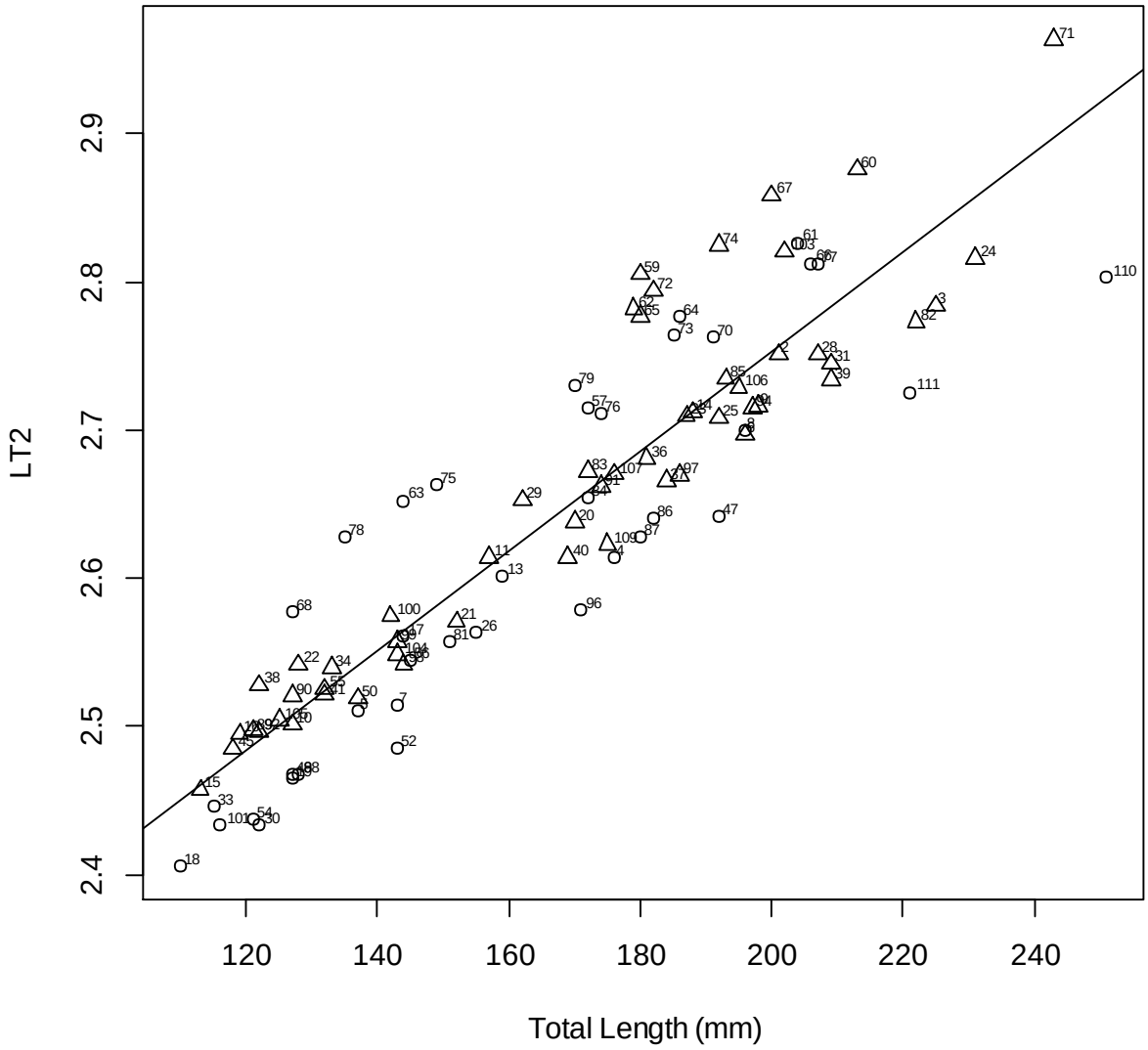
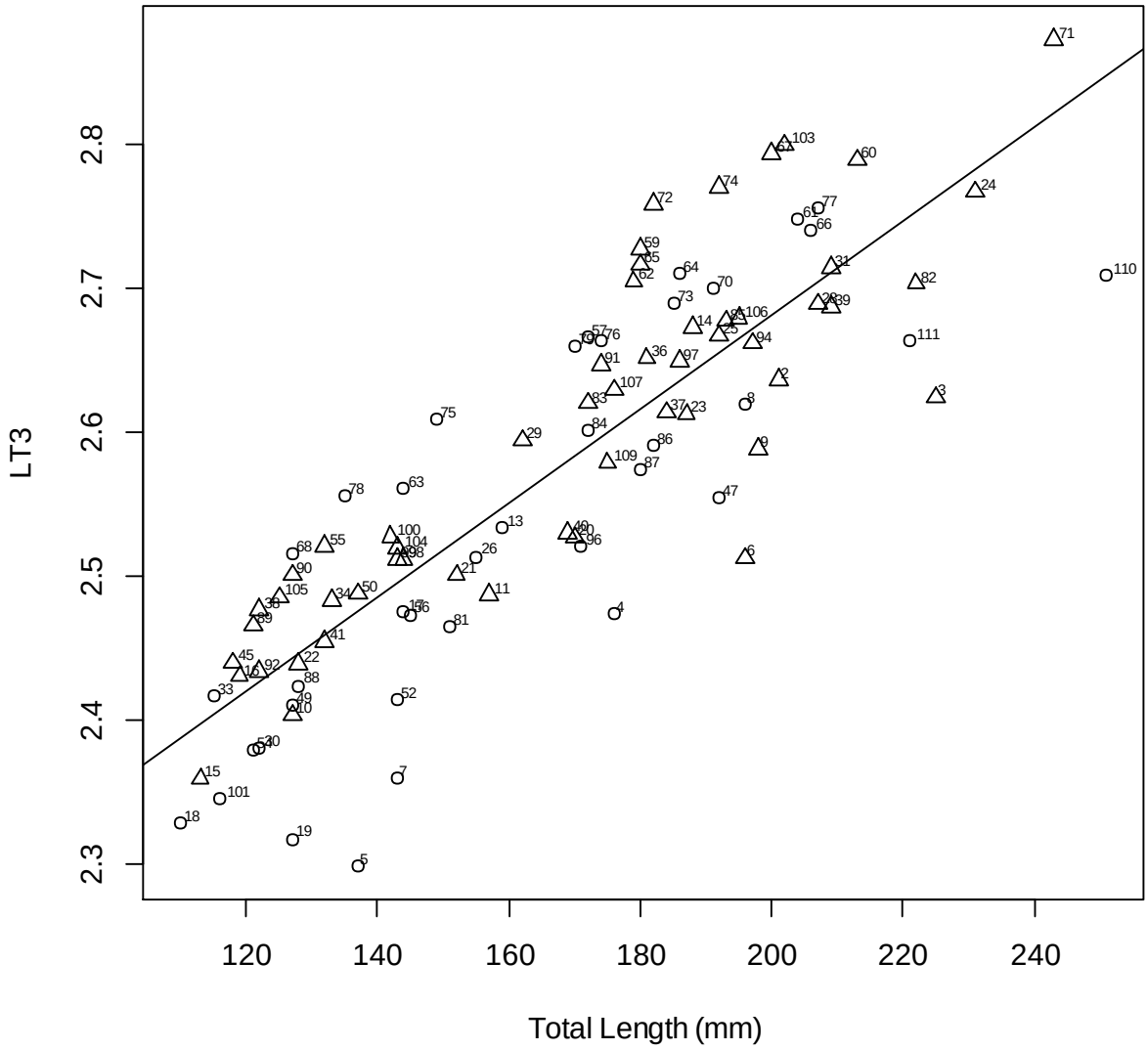
Appendix A. Linear regression plots for log10 transformed values of 27 measurement variables.



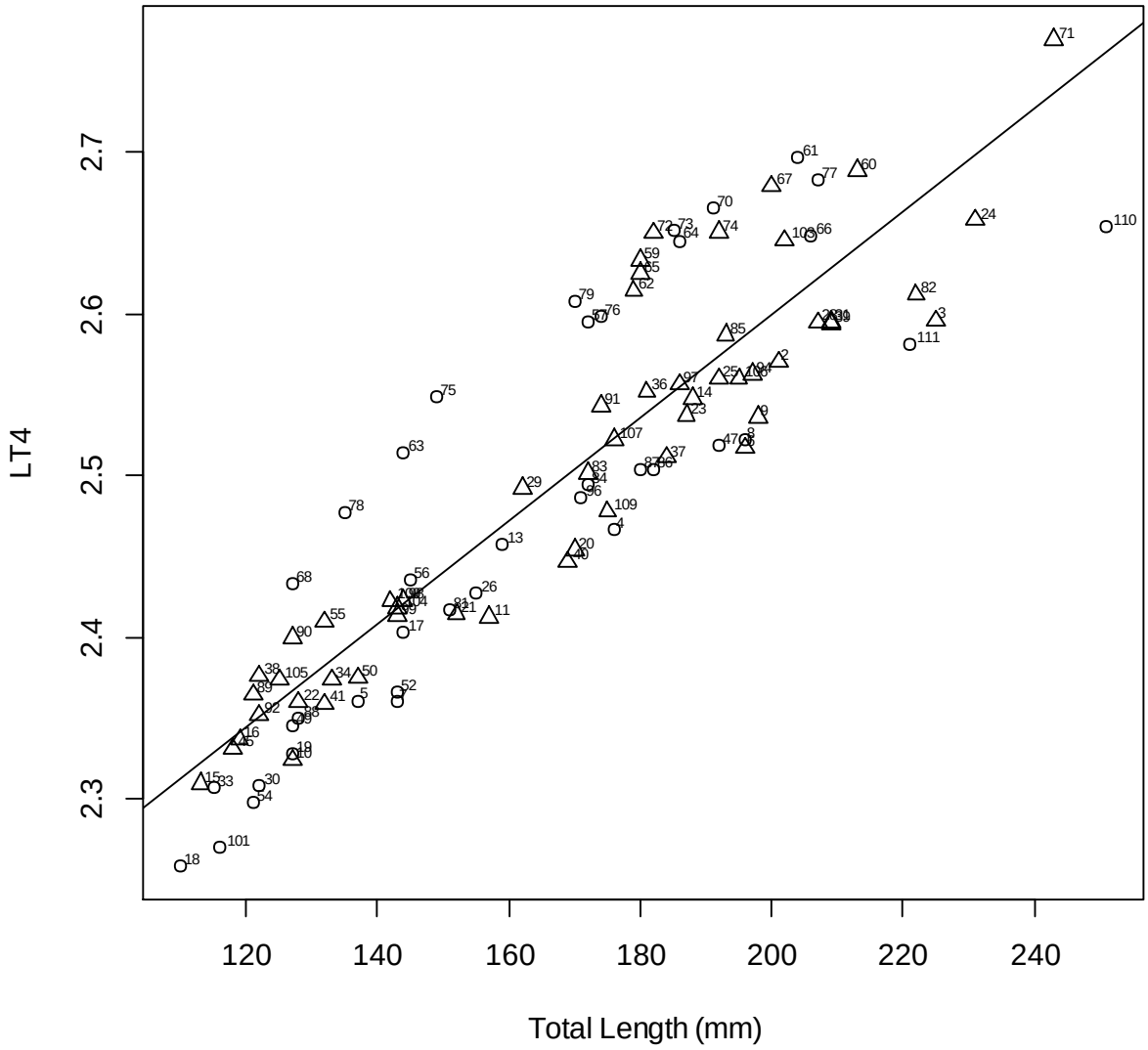
Regression for Transformed M2



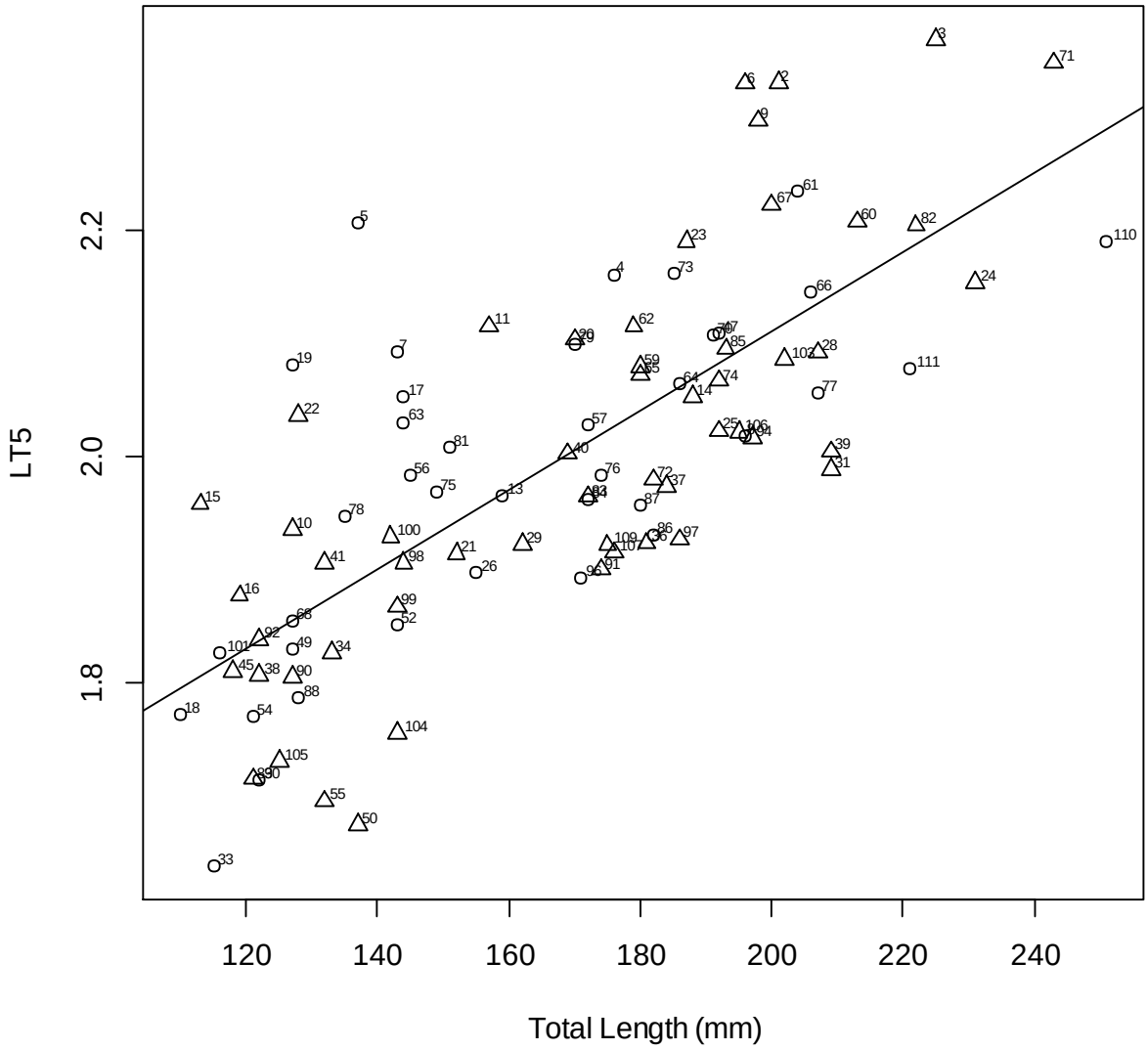
Regression for Transformed M3



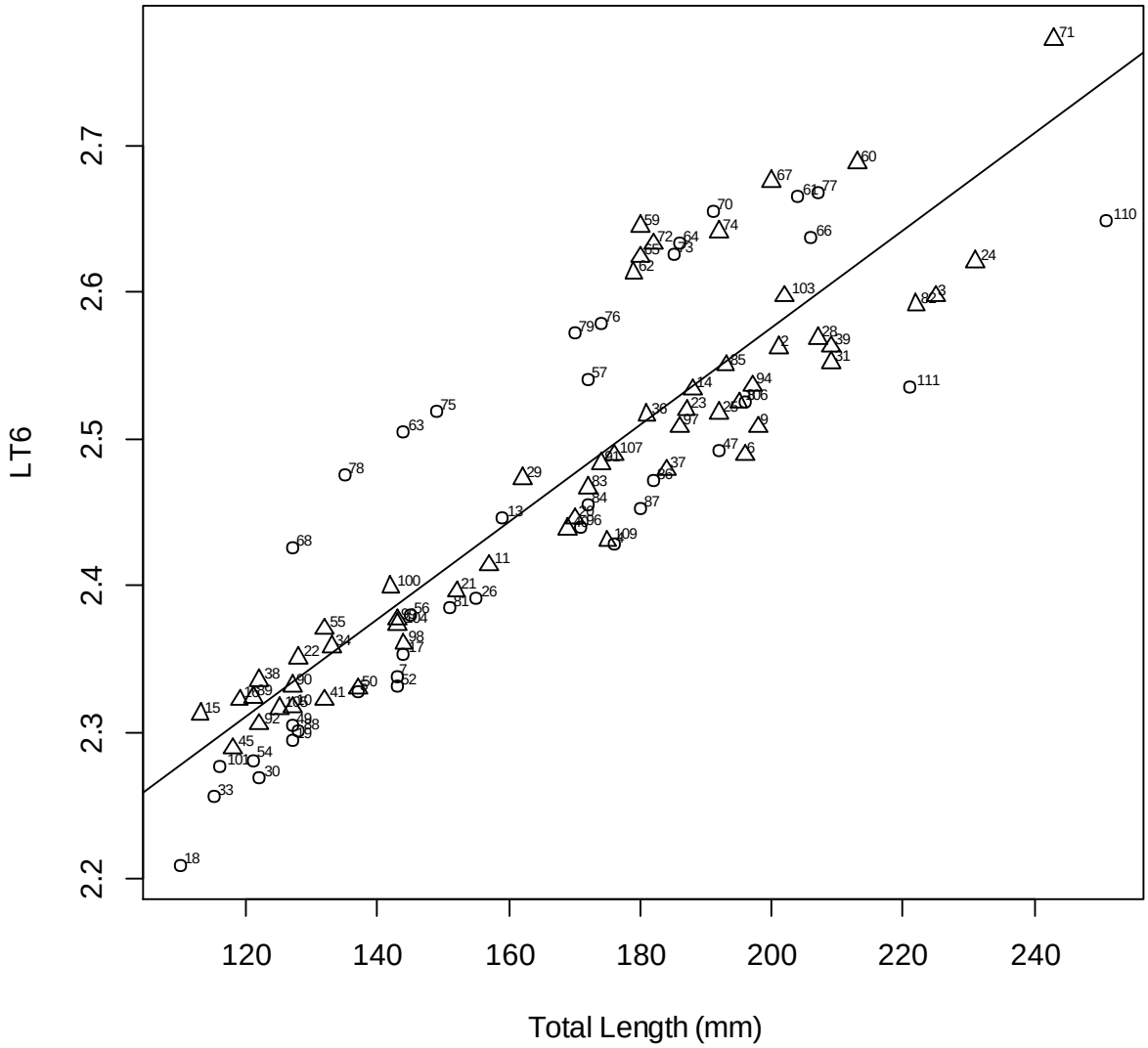
Regression for Transformed M4



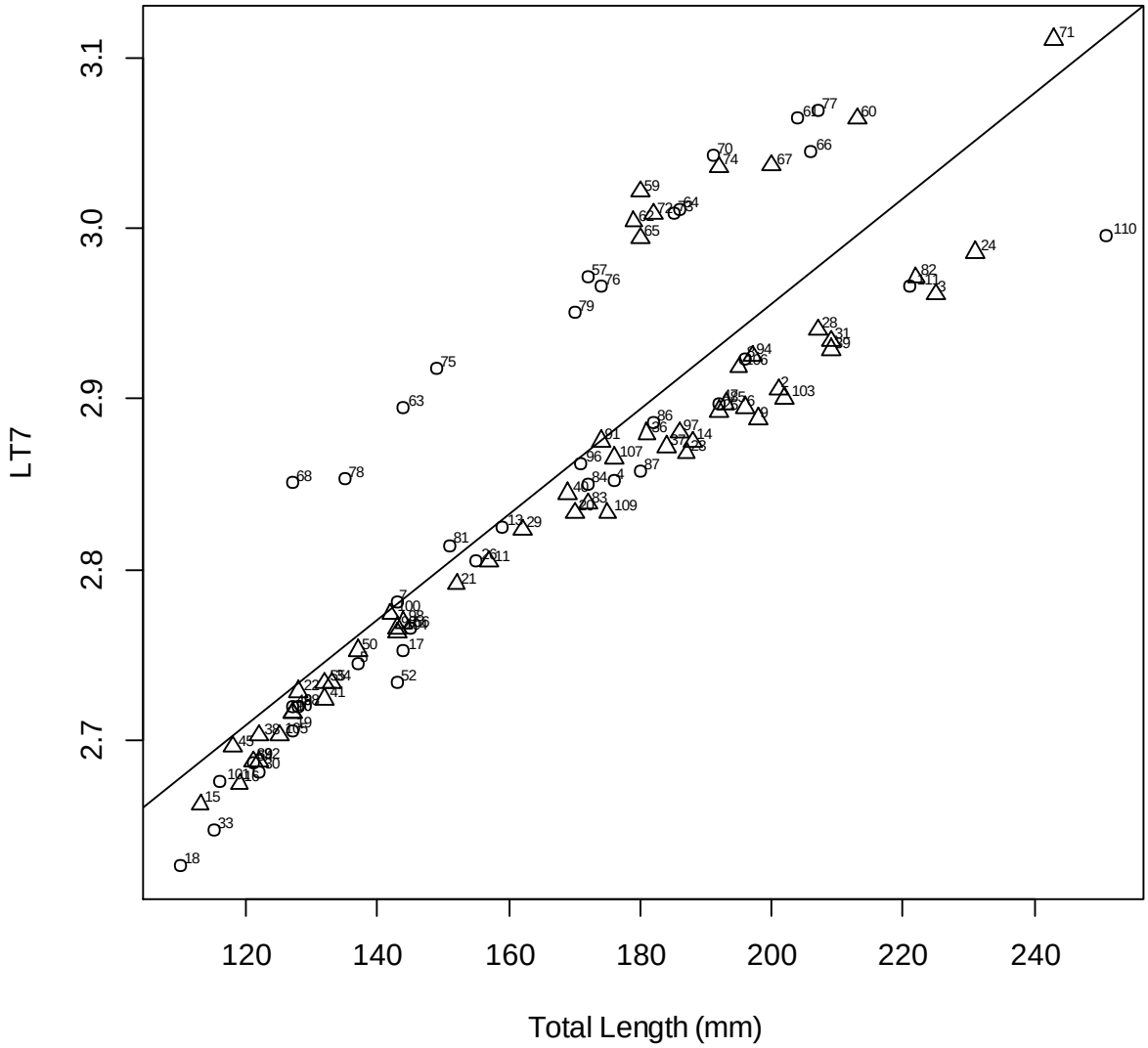
Regression for Transformed M5



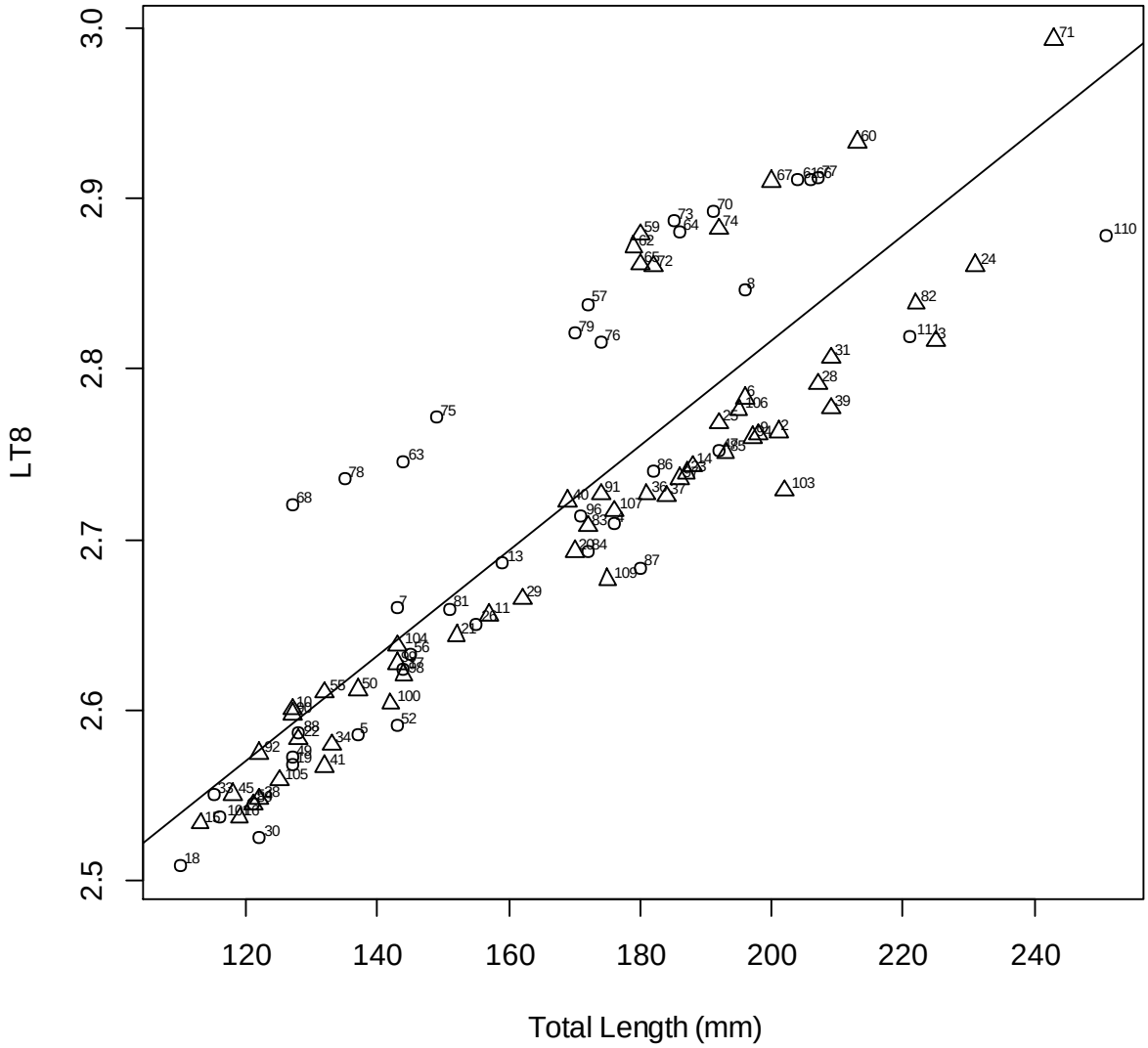
Regression for Transformed M6



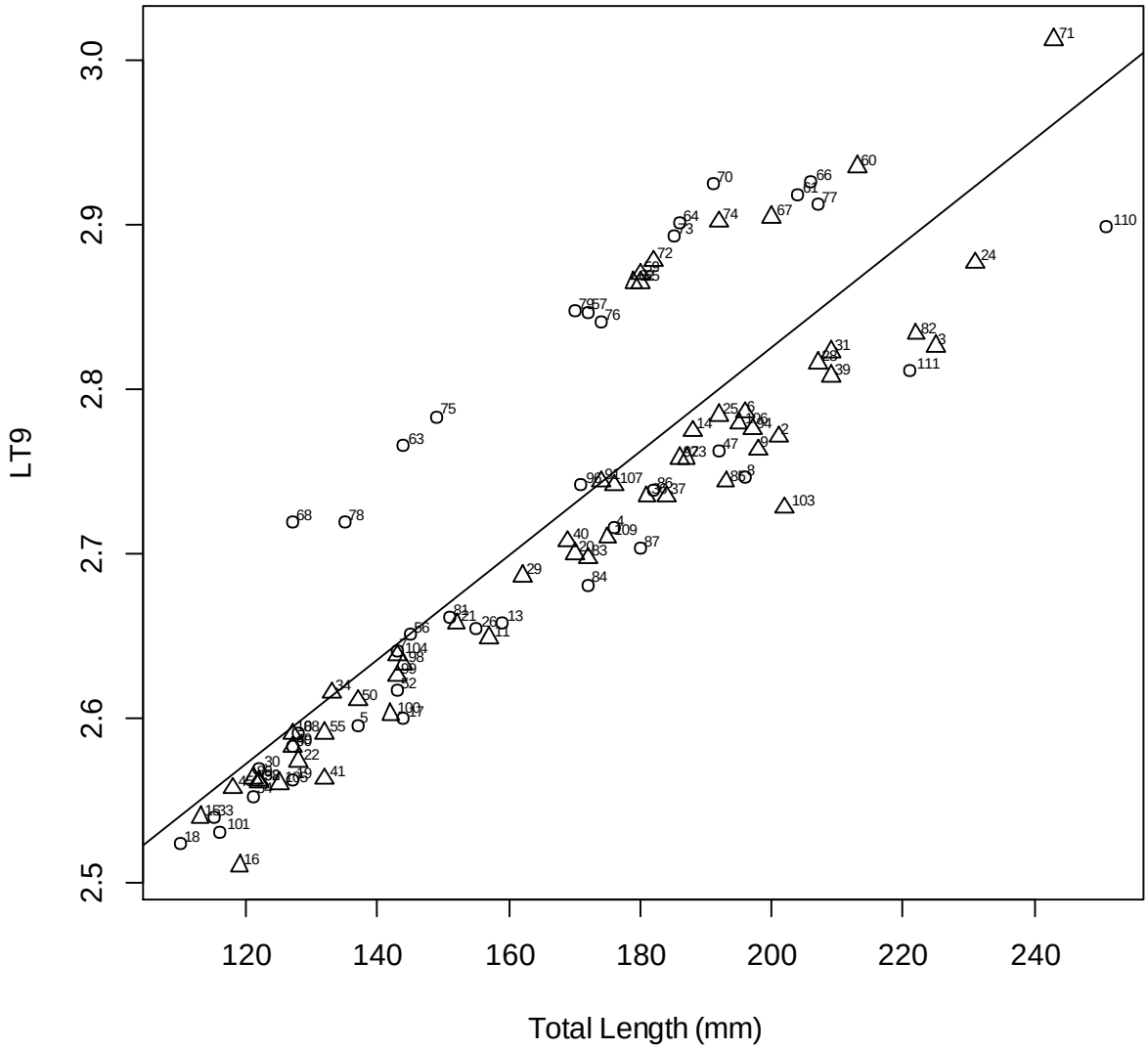
Regression for Transformed M7



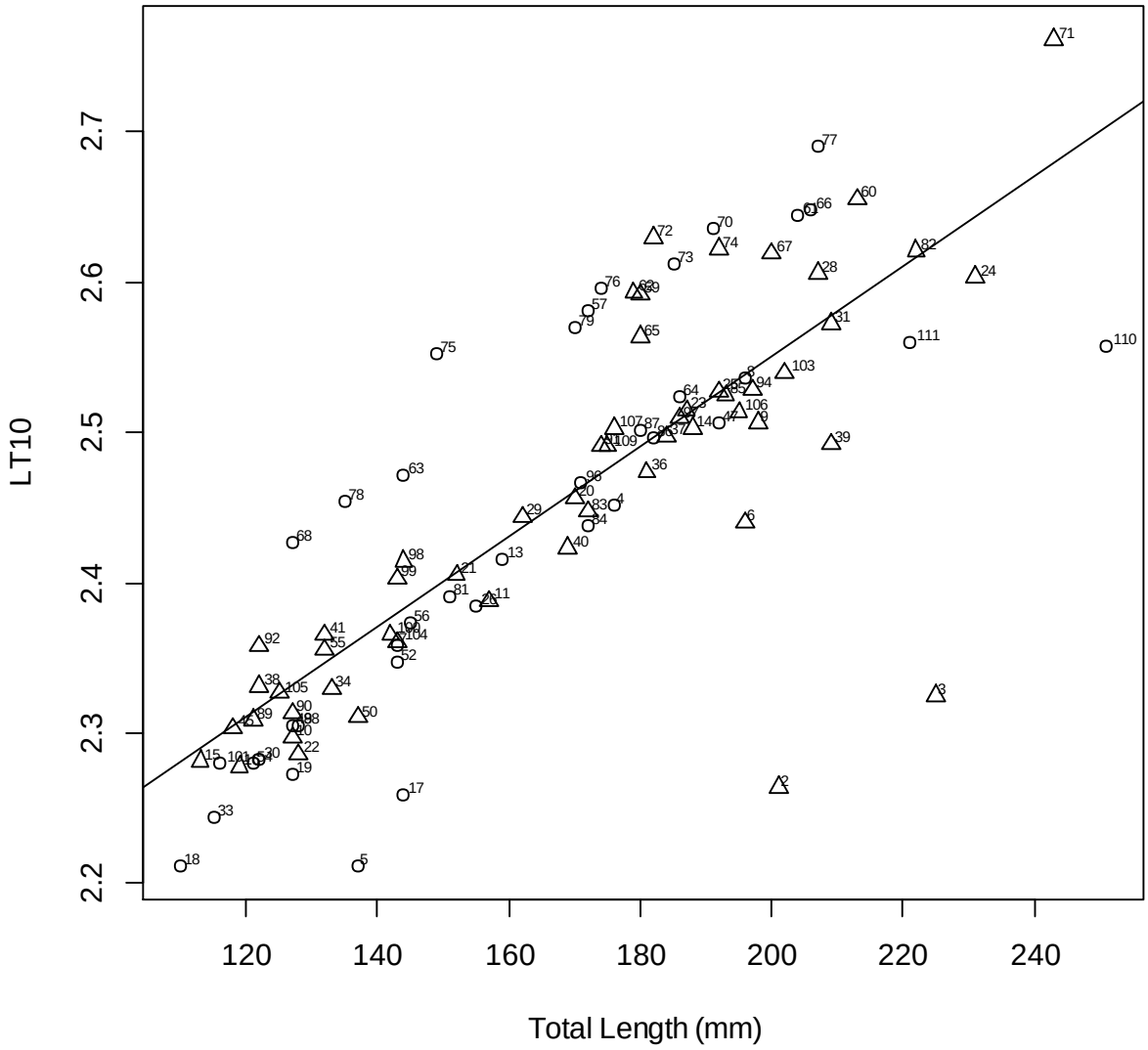
Regression for Transformed M8



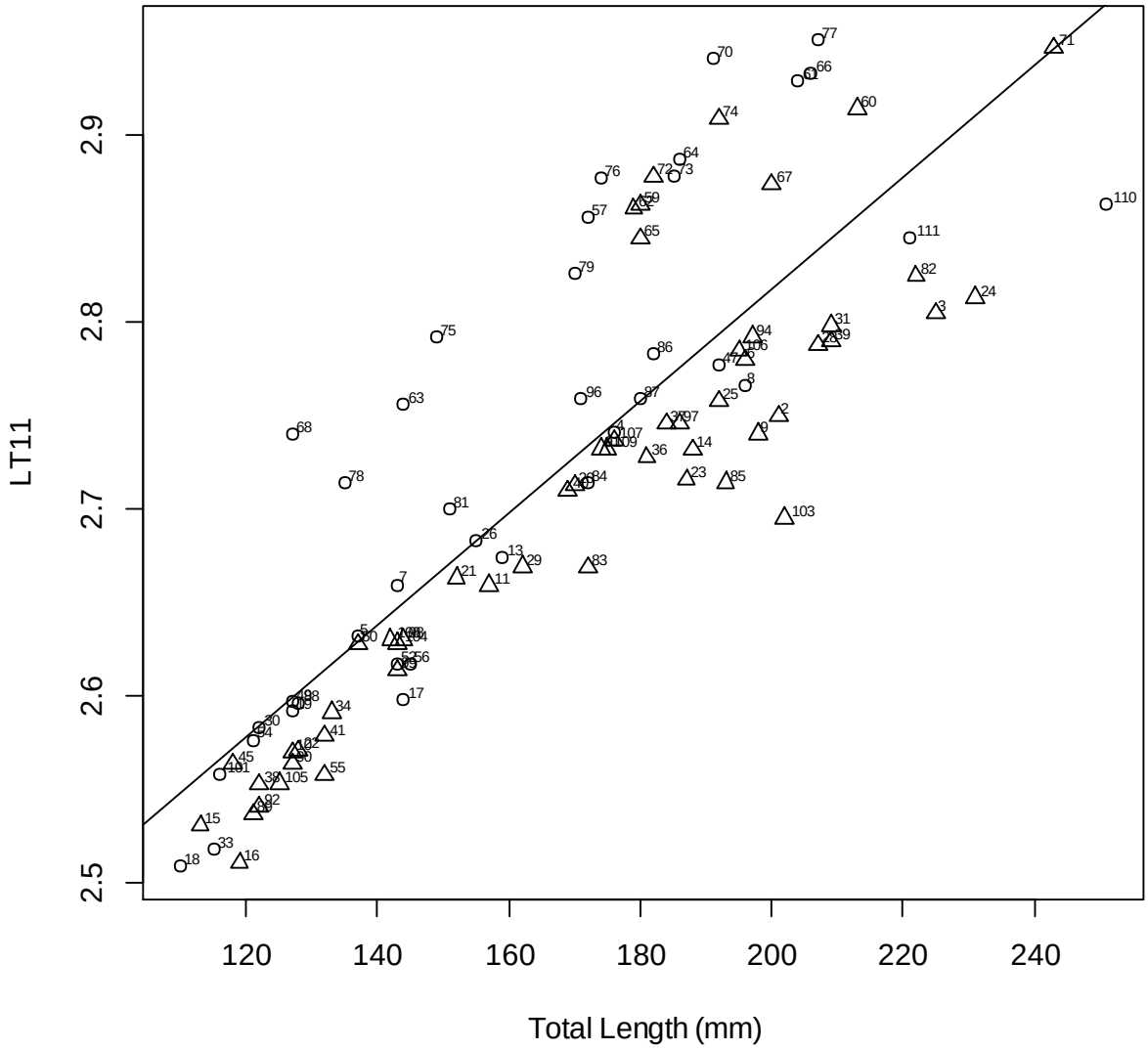
Regression for Transformed M9



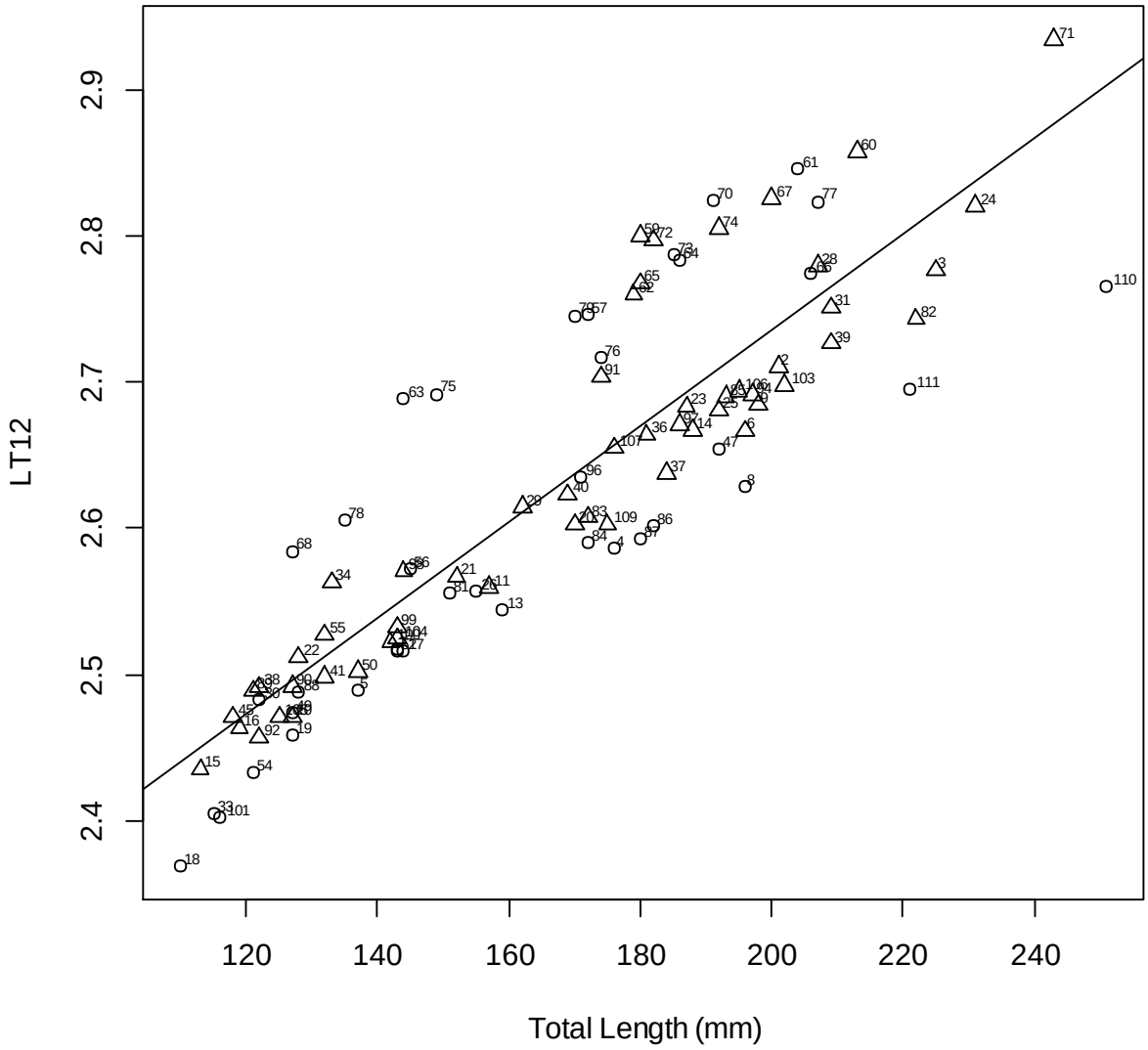
Regression for Transformed M10

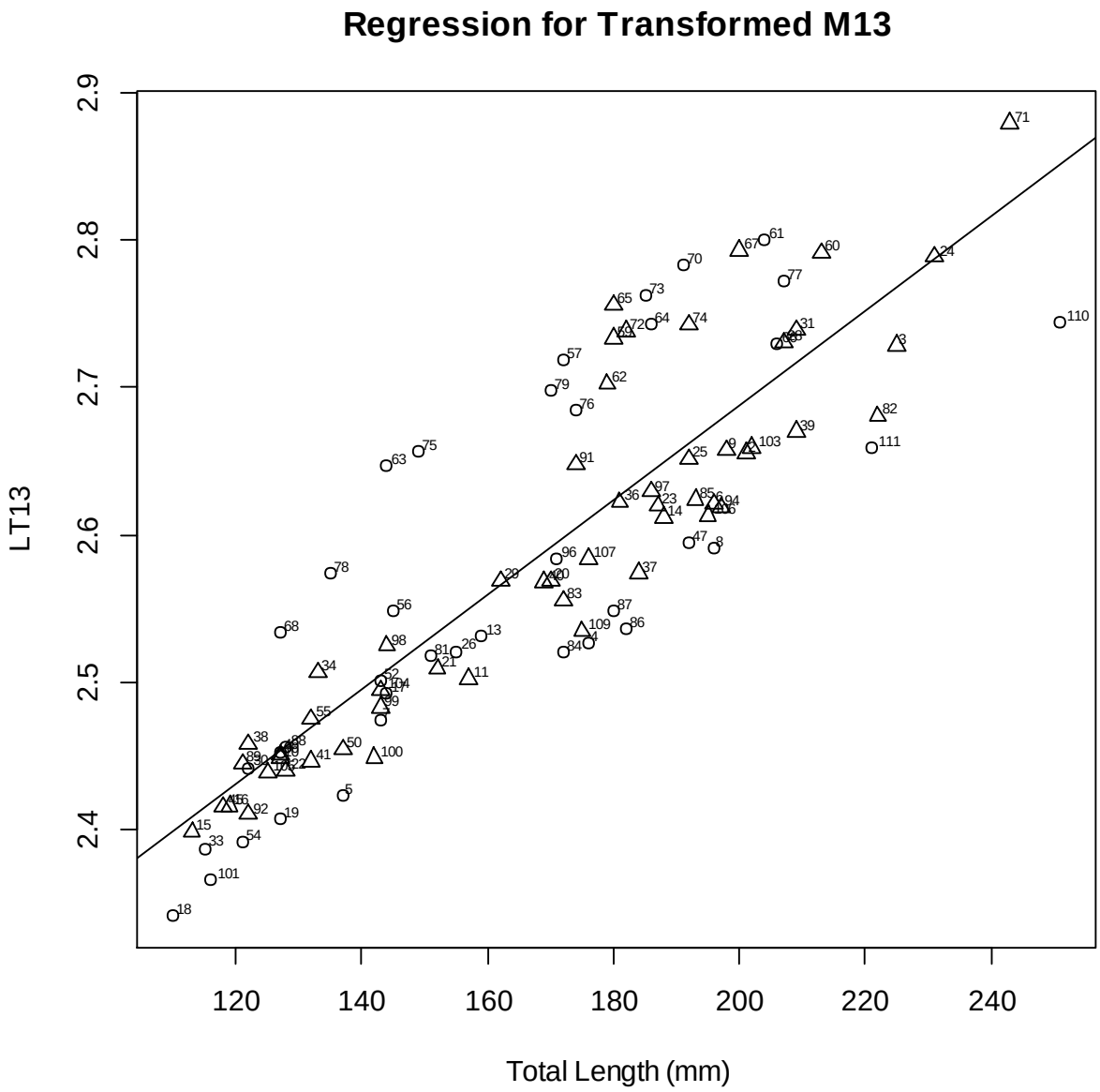


Regression for Transformed M11

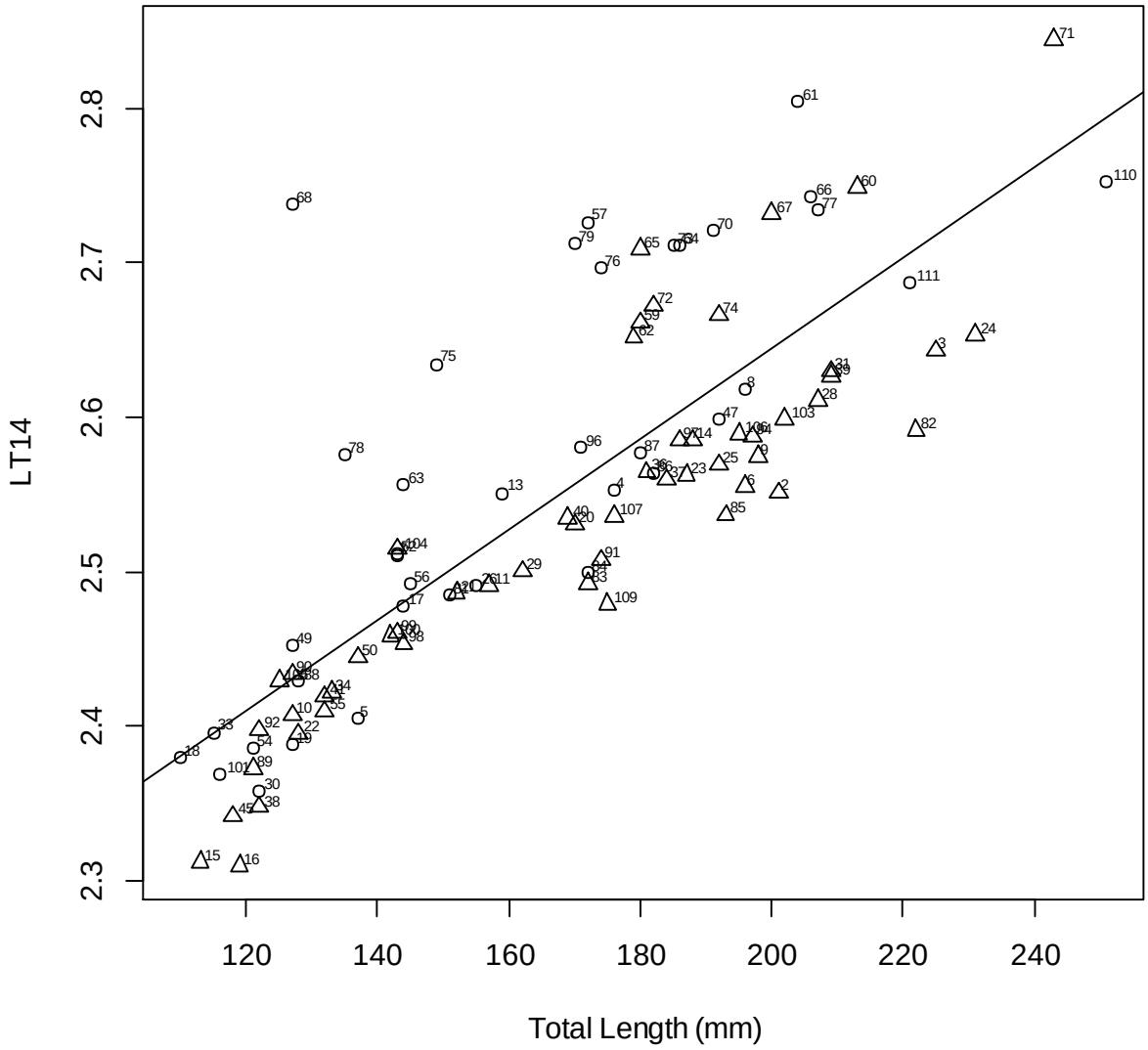


Regression for Transformed M12

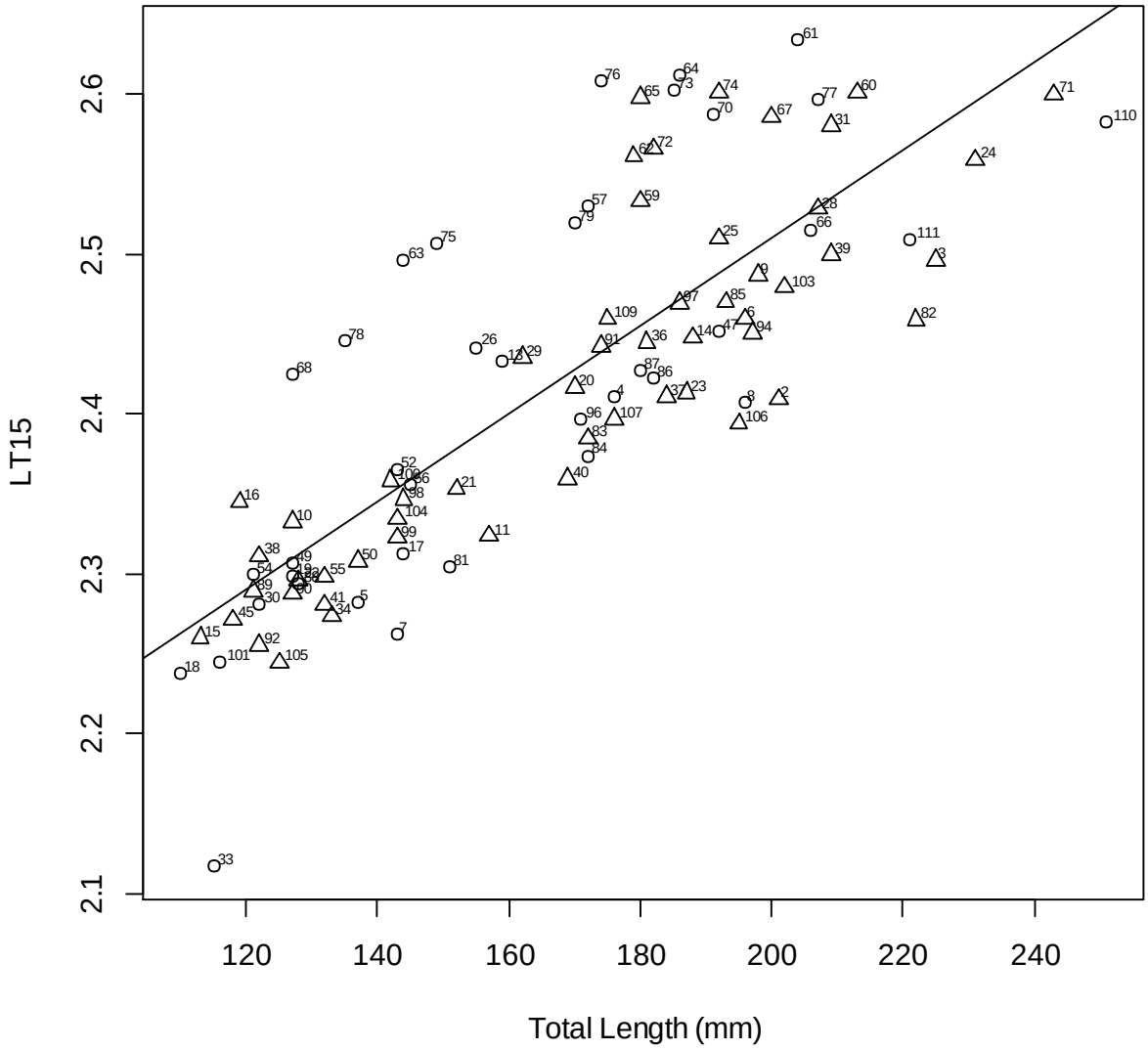



Regression for Transformed M13
LT13
Total Length (mm)
2.4
2.5
2.6
2.7
2.8
2.9
120
140
160
180
200
220
240

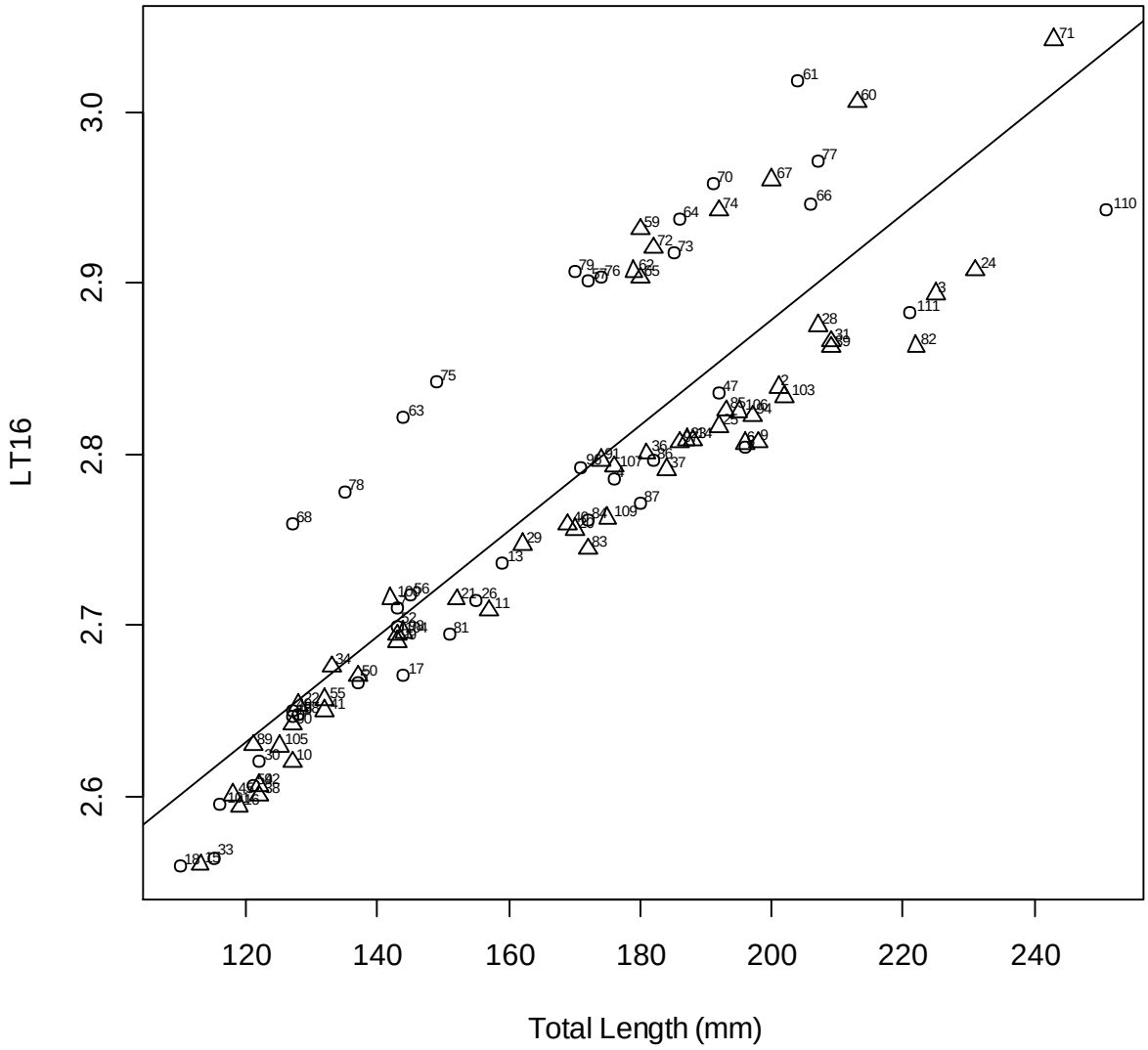
Regression for Transformed M14



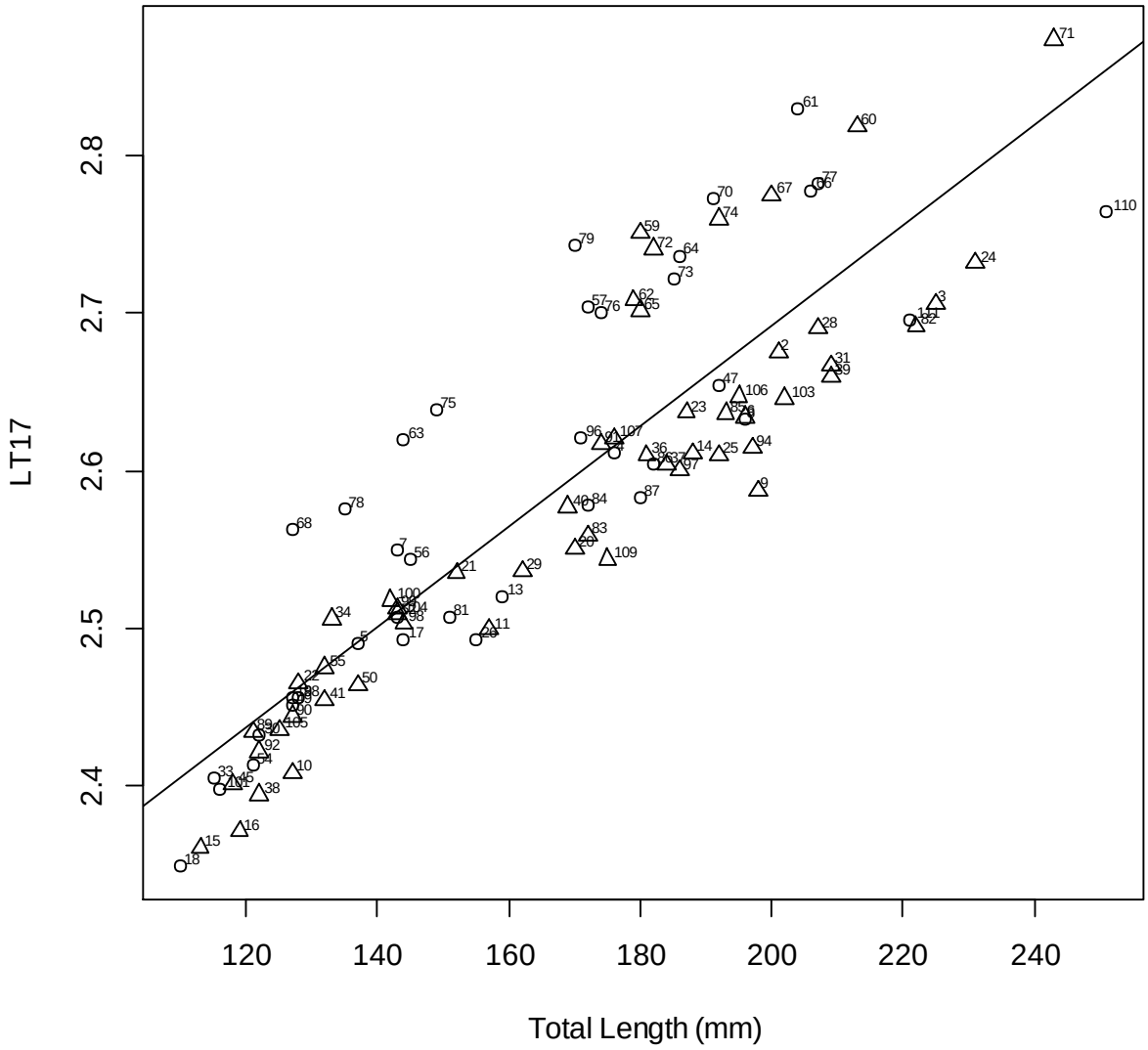
Regression for Transformed M15



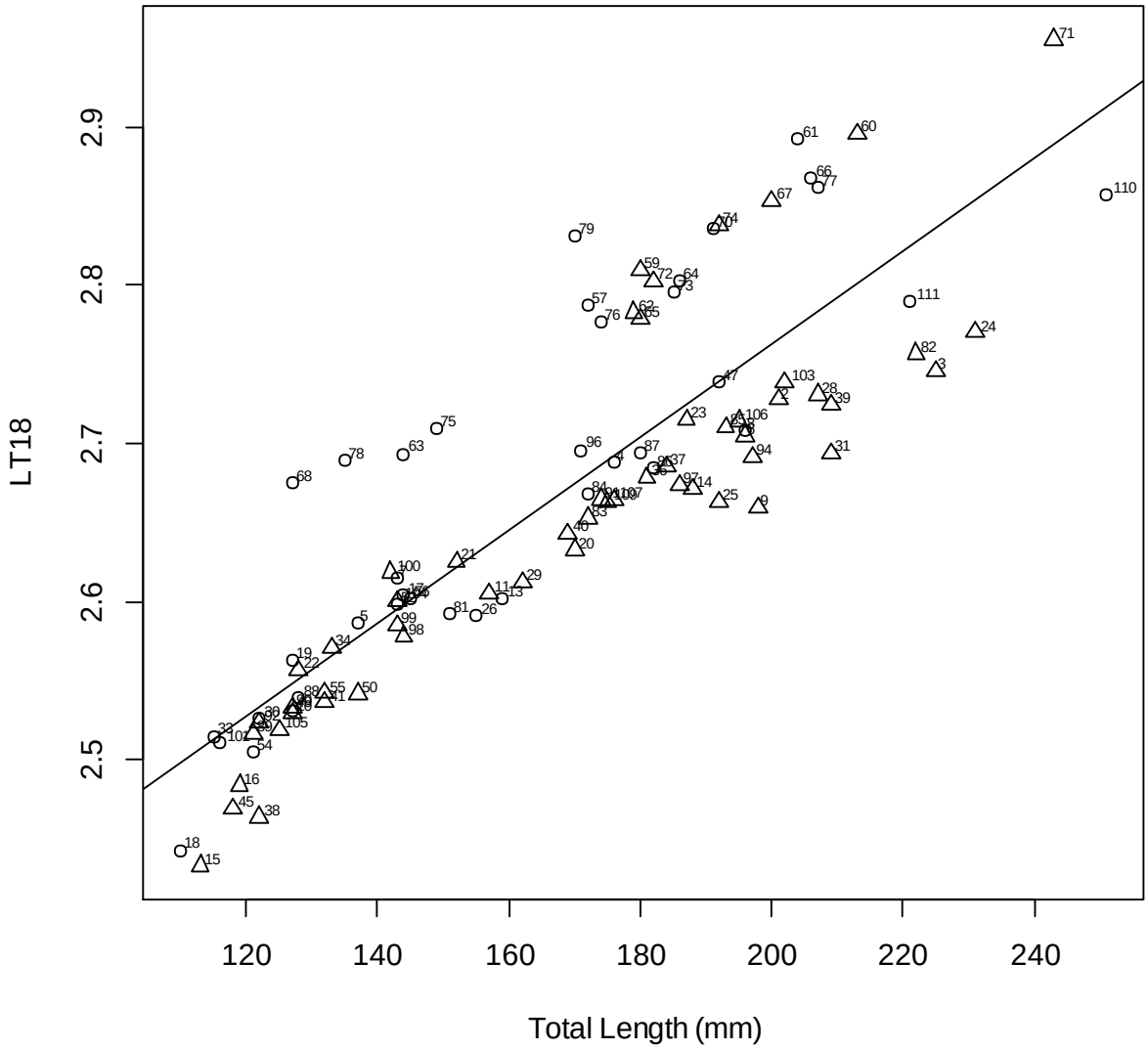
Regression for Transformed M16



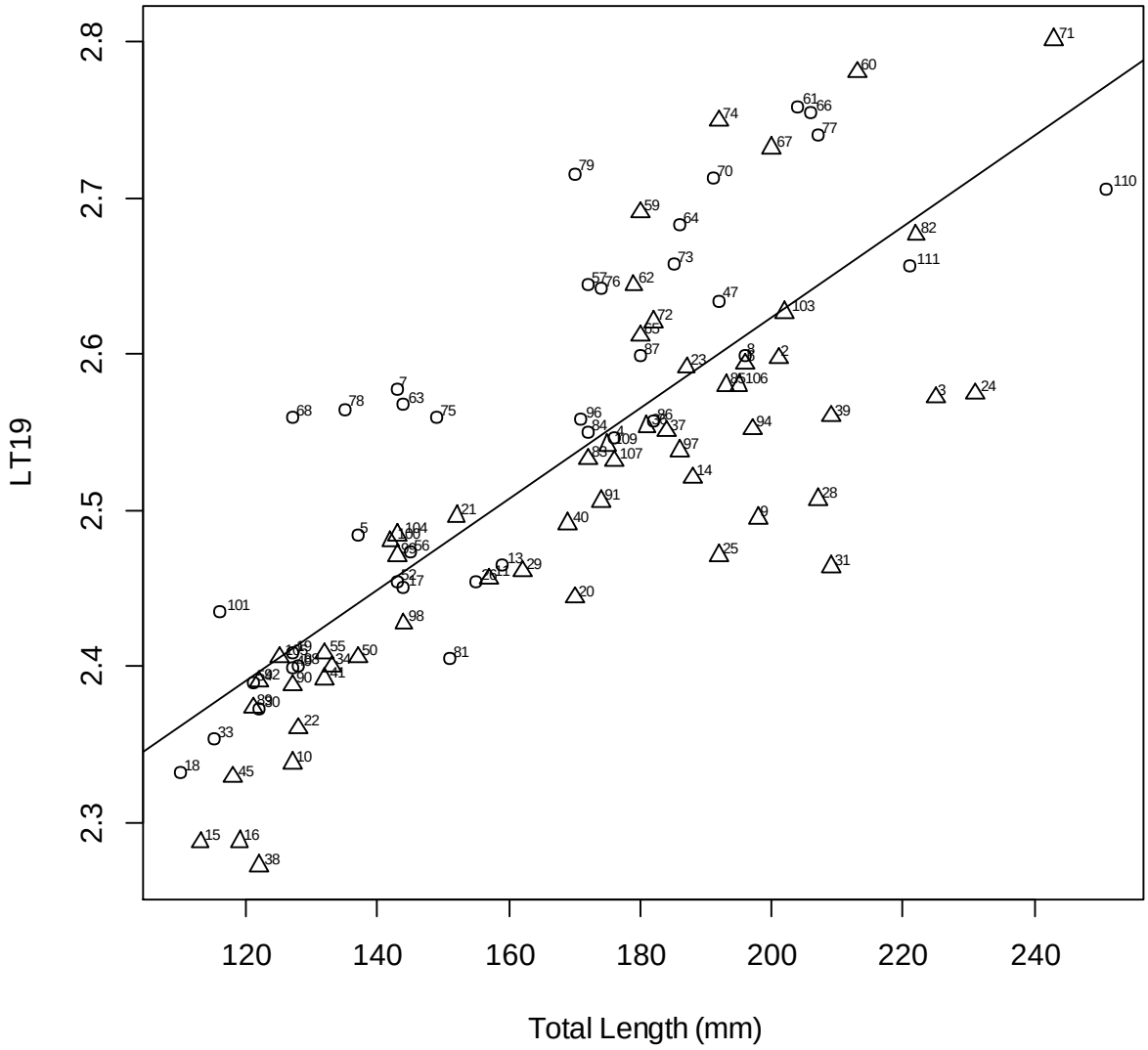
Regression for Transformed M17



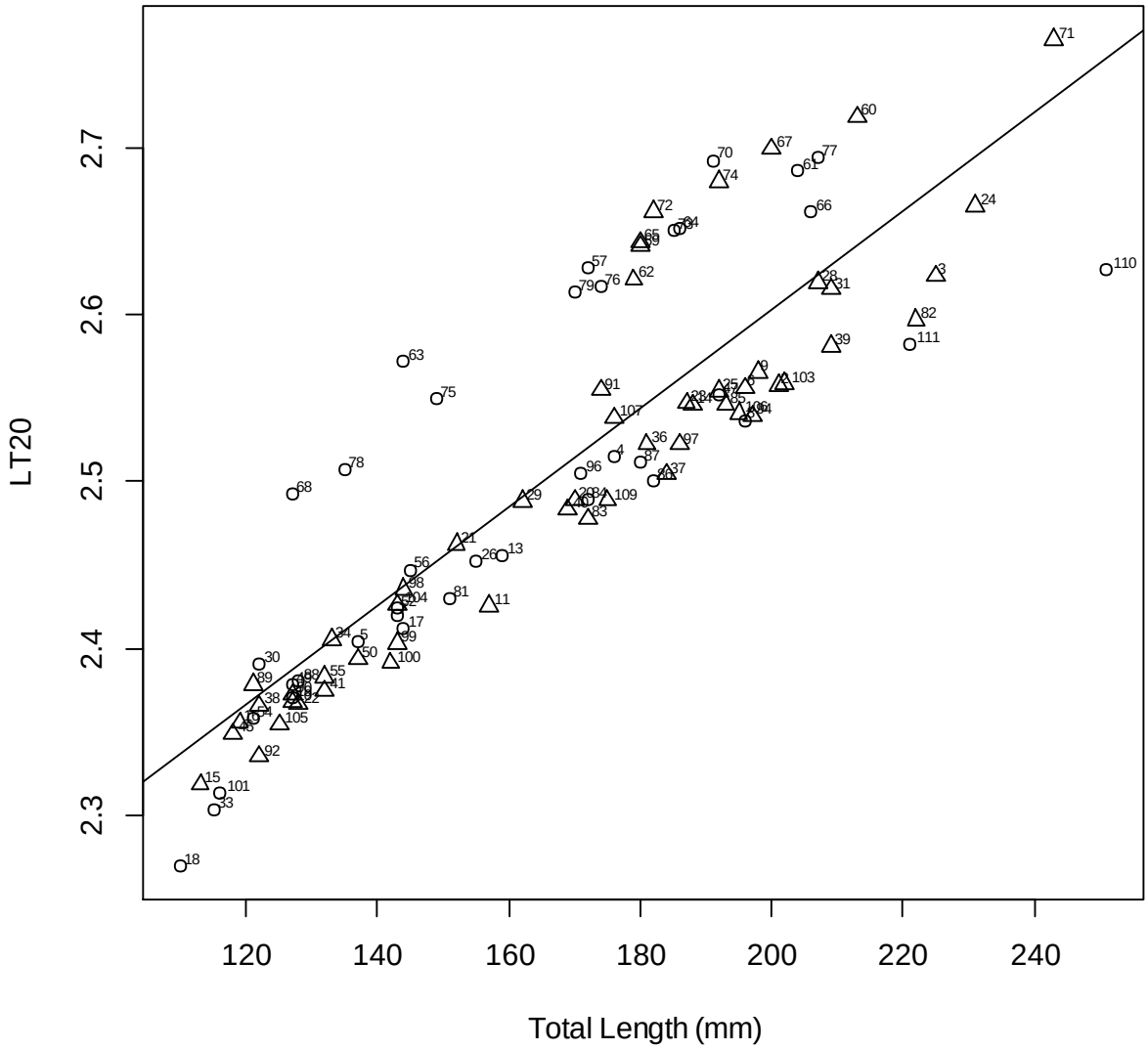
Regression for Transformed M18



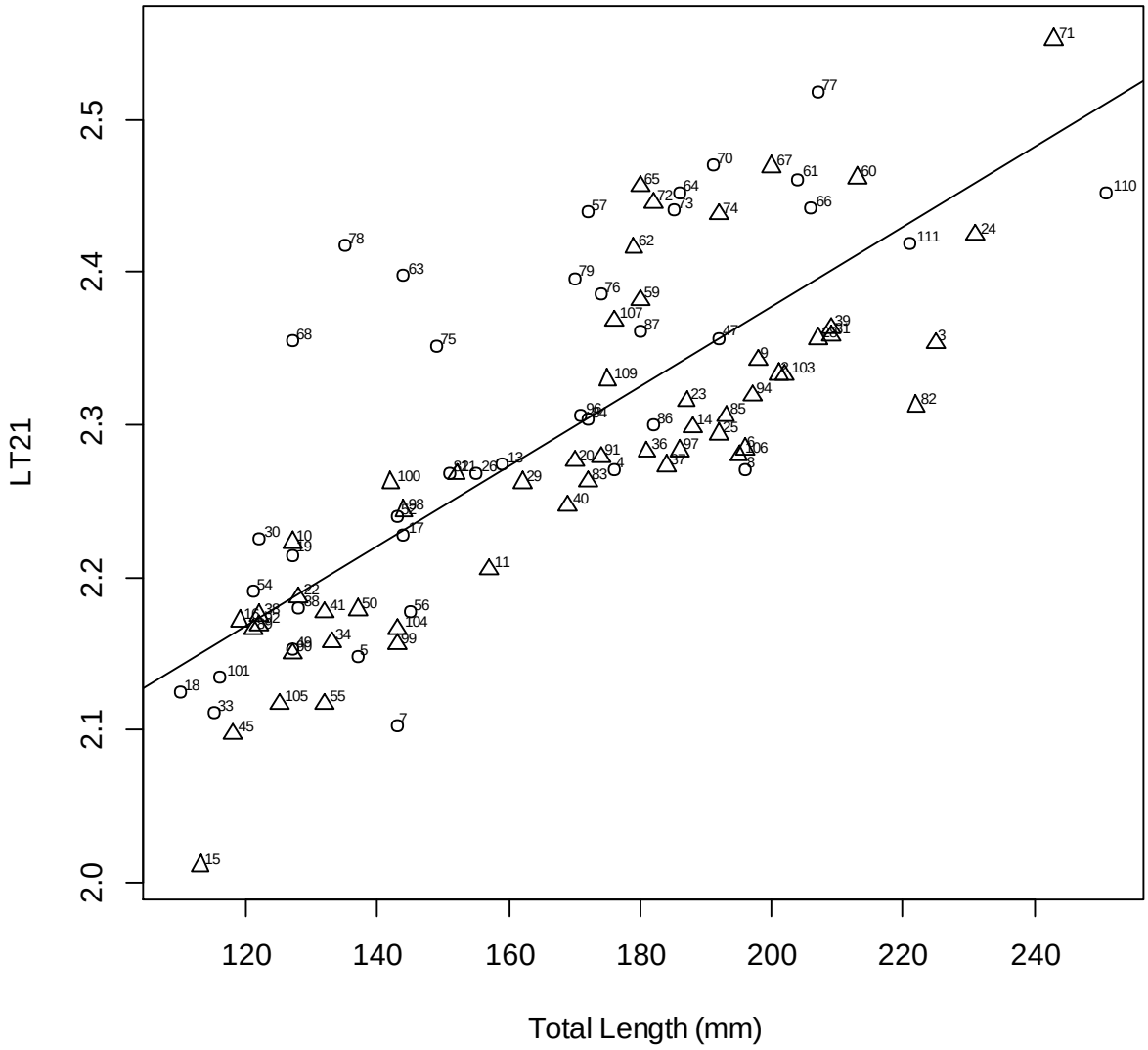
Regression for Transformed M19



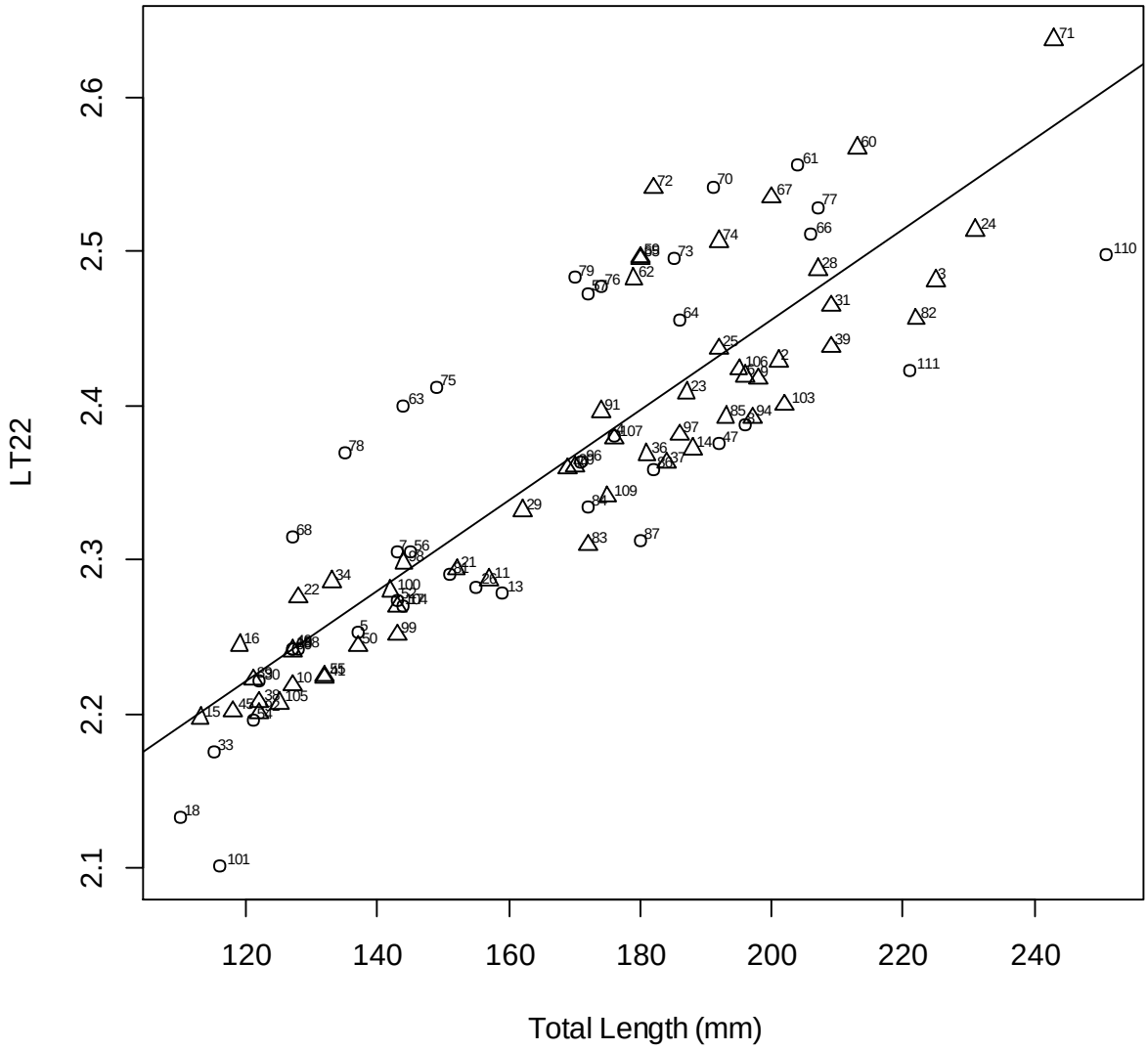
Regression for Transformed M20



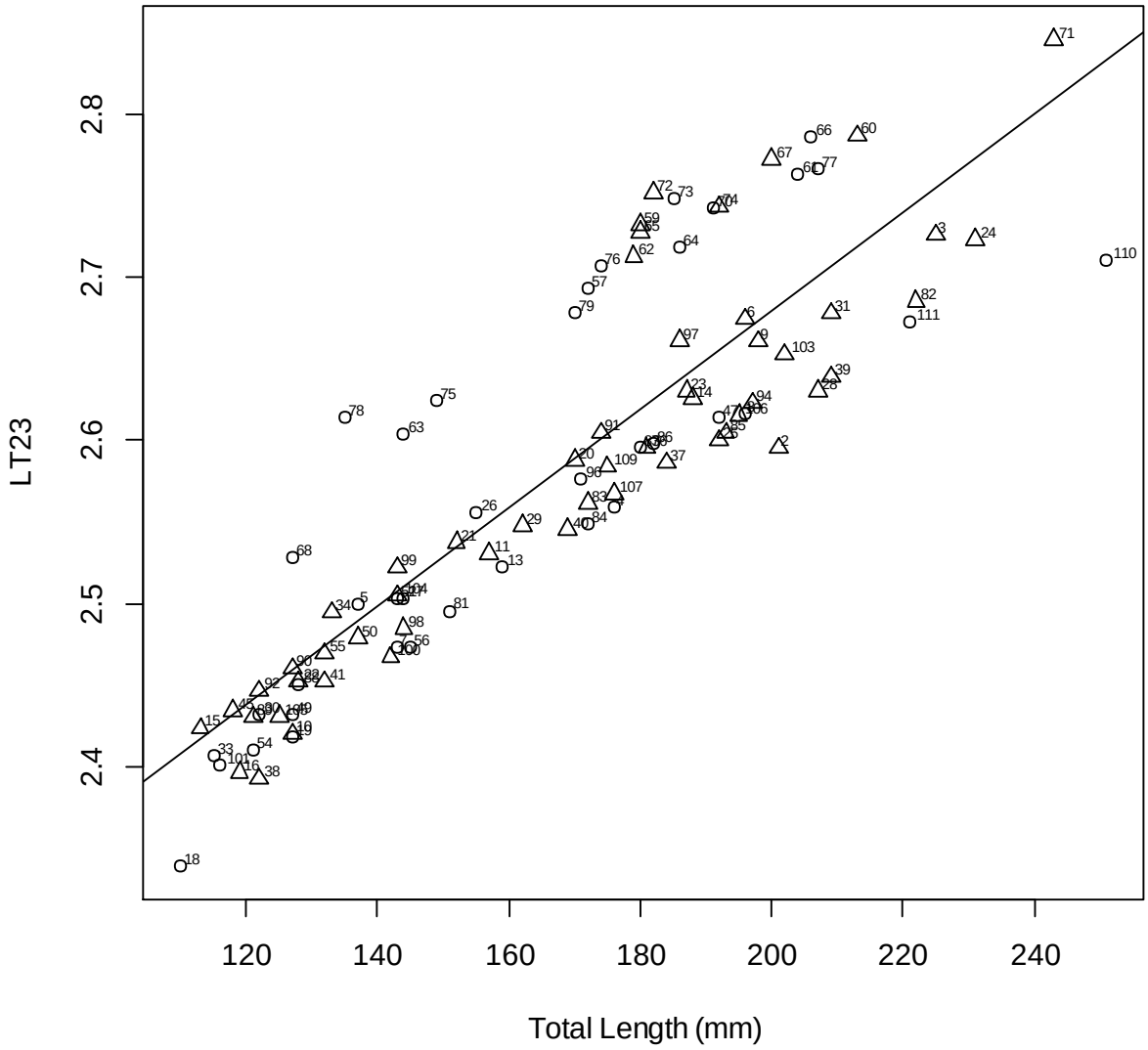
Regression for Transformed M21



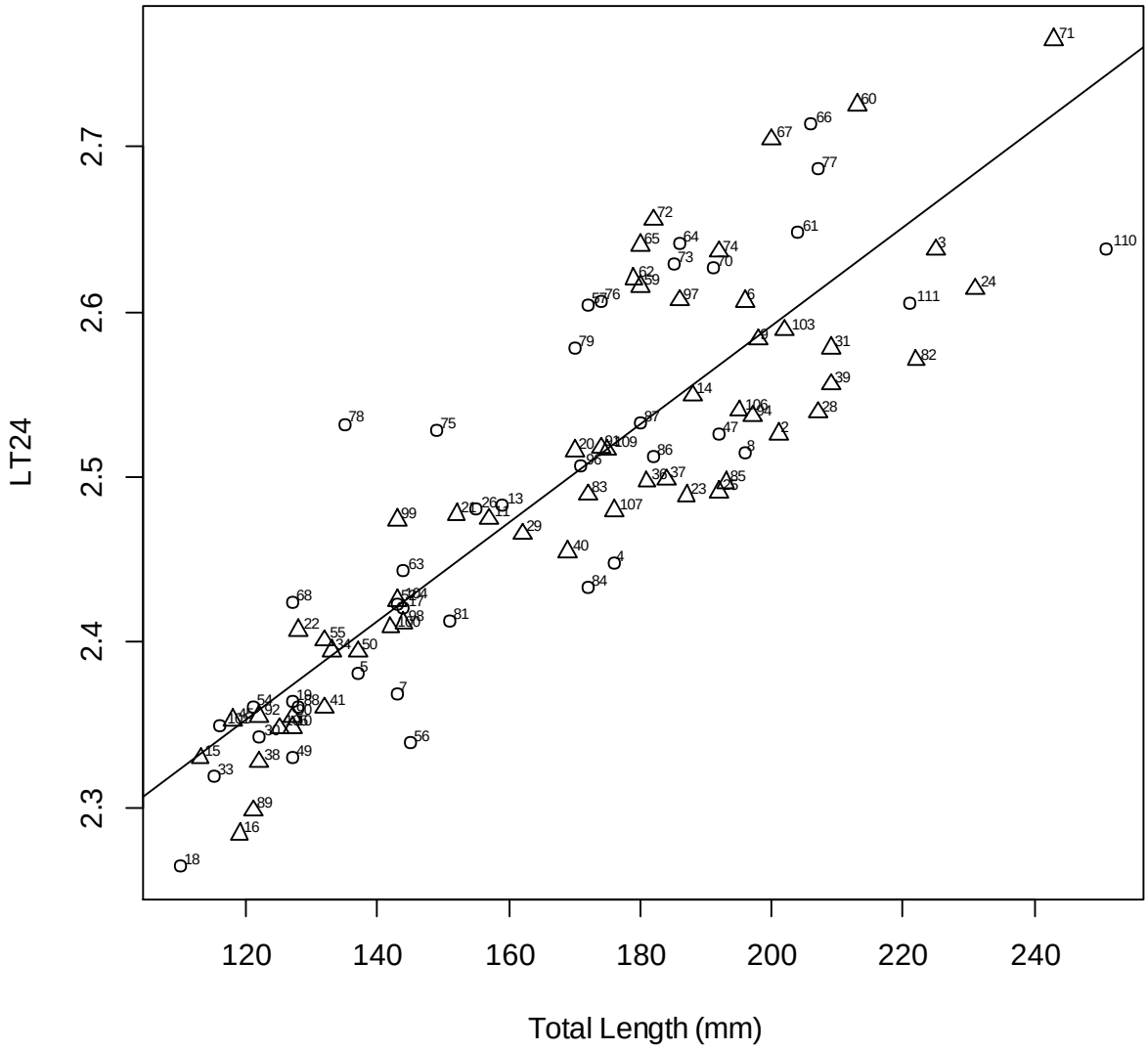
Regression for Transformed M22



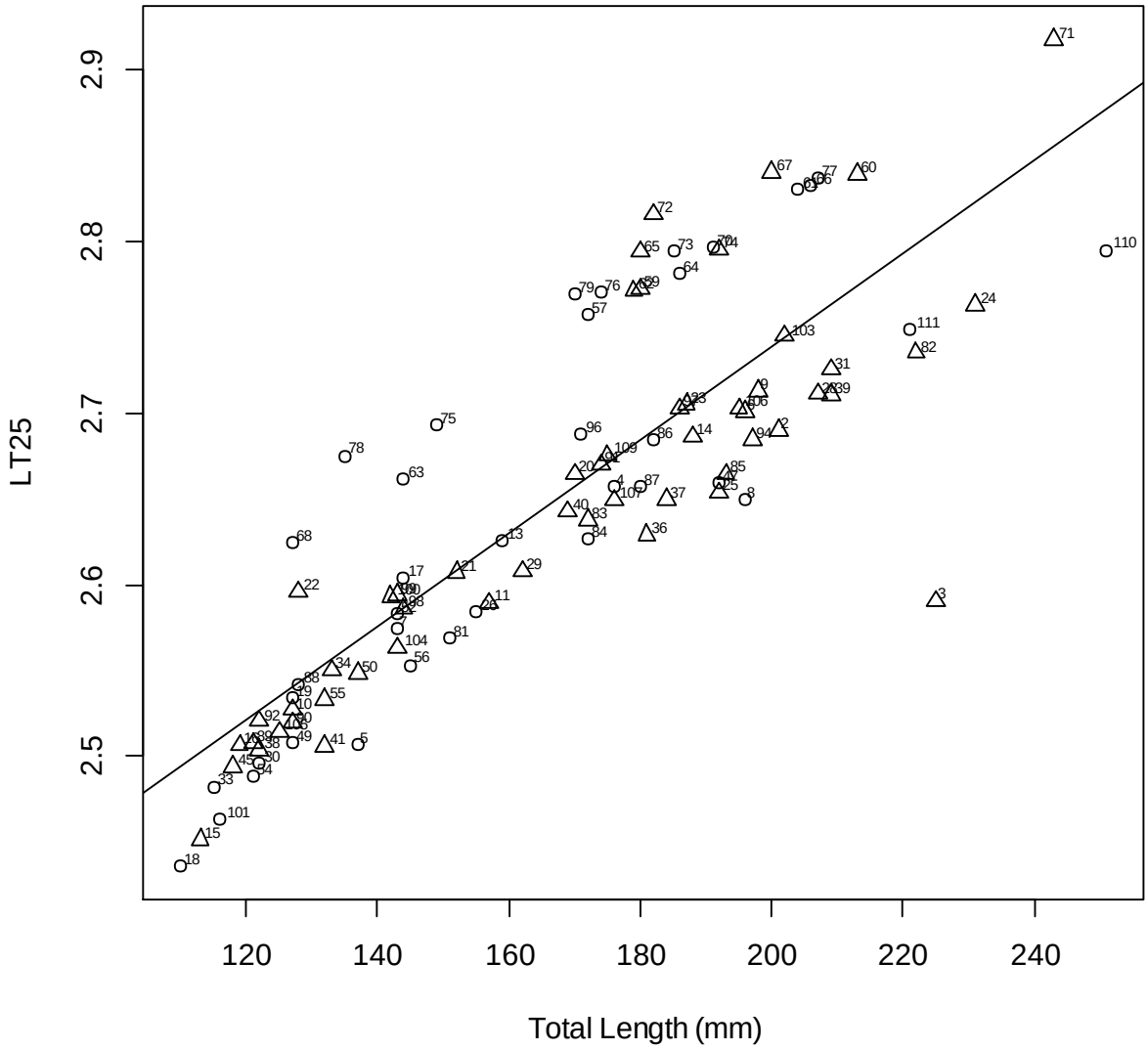
Regression for Transformed M23



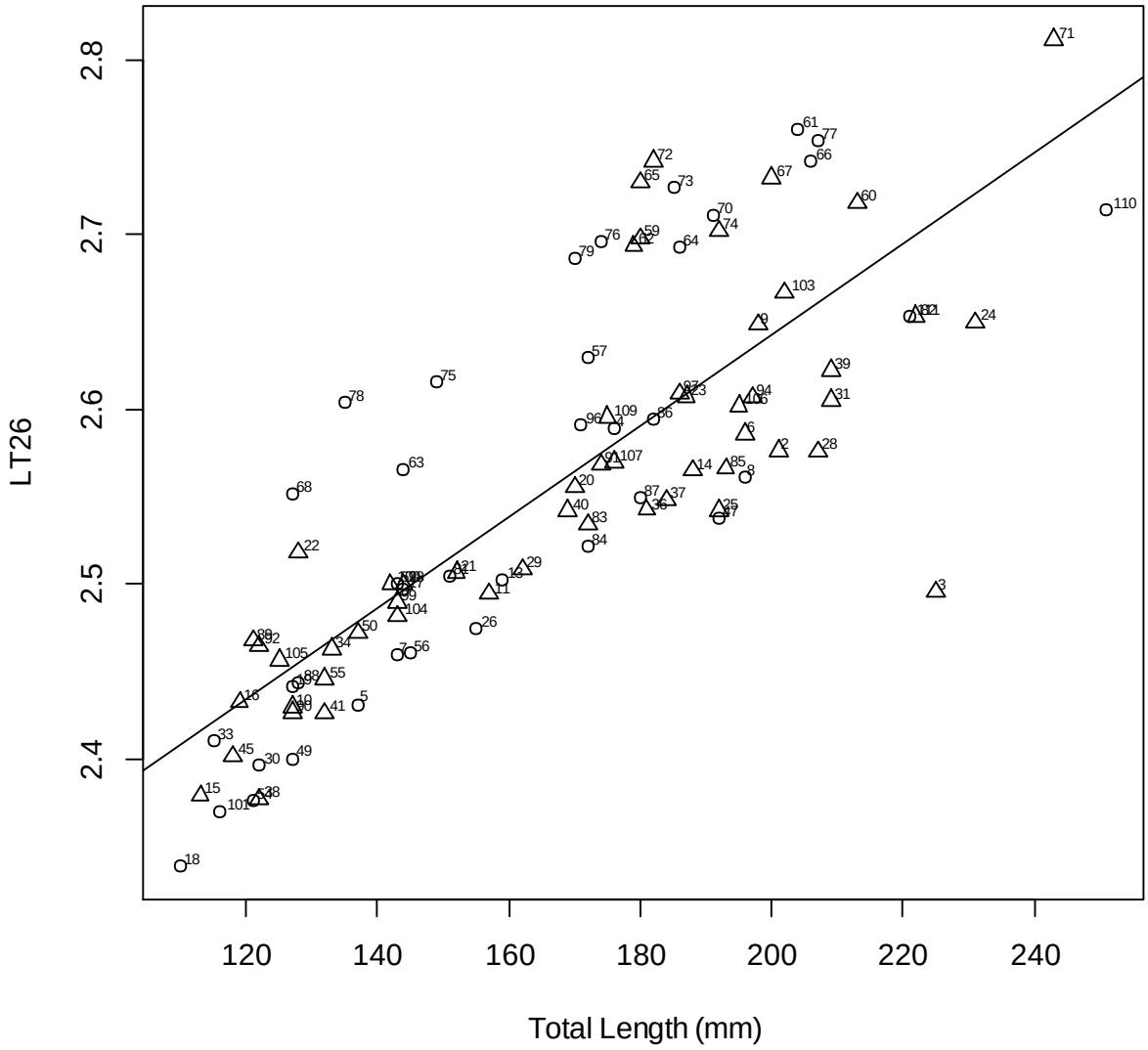
Regression for Transformed M24



Regression for Transformed M25



Regression for Transformed M26



Regression for Transformed M27