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MERCURY CONCENTRATIONS
IN THE MUSCLES OF
NORTH AMERICAN
BULLFROGS
(*Rana catesbeiana*)

BY
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Abstract

Many studies have been done on mercury in the environment. The studies though have focused on sport fish and migratory waterfowl. North American Bullfrog (*Rana catesbeiana*) has been one species neglected from studies. One study has shown that bullfrogs do accumulate significant amounts of mercury (Gerstenberger S., and Pearson R. 2002). Though, no study has been done to find out if hind limbs have more concentrations than front limbs. Also to see if there is a difference between skin-off and skin-on, to find out if skin accumulates mercury. This study found that there was no significant difference between front and hind limbs at low levels. From this study, skin-off to skin-on had no significant difference in mercury concentrations. Females have been found, in this study, to have significantly less mercury concentrations than males. These findings show that front limbs can be used to predict hind limb mercury concentrations.

Introduction

There have been many studies done on mercury in the environment. Almost all of the studies have pertained to sport fish and migratory waterfowl. These species are known to accumulate mercury, which are bioaccumulate through the food chain and can result in exposure to humans. With a wide geographical distribution due to human mediated introduction (Figure 1, in appendix) one aquatic species of concern is the North American bullfrog (*Rana catesbeiana*). With frog legs being a delicacy and with extremely, or no, restrictions on their harvesting mercury, consumption can be a problem.

Mercury has been well documented to be a toxic chemical to humans, with fetus being most susceptible. Infants may appear normal at birth but manifest effects of

mercury exposure later. Problems associated with mercury exposure are delays in reaching developmental milestones, such as the age of first walking and talking, to more severe effects including brain damage, mental retardation, non-coordination, and inability to move (EPA,1984). Other severe effects observed in children whose mothers were exposed to very toxic levels of mercury during pregnancy include blindness, involuntary muscle contractions and seizures, muscle weakness, and inability to speak (RTECS, 1998).

In adults mercury is a neurotoxin that causes demyelization to nerve cells, this may cause numbness in extremities and also a loss of neuromuscular control. Also, include personality changes (irritability, shyness, nervousness), tremors, changes in vision (constriction or narrowing of the visual field), deafness, loss of muscle coordination, loss of sensation, and difficulties with memory (RTECS. 1998).

Bullfrogs are predators; they usually feed on snakes, worms, insects, crustaceans, frogs, and tadpoles. They are cannibalistic and will not hesitate to eat their own kind. There have also been a few cases reported of bullfrogs eating bats (Conant, R. 1975). Bullfrogs are a good indicator species because their skeletal, muscle, digestive, and nervous systems are similar to those of higher animals (Bruening S., 2002). With frogs being close to the top of the food chain in their respective ecological areas, they will bioaccumulate mercury much the same way as predatory fish and birds. Studies that have been done have shown that there is significant amounts of Mercury in bullfrogs (Gerstenberger S., and Pearson R. 2002).

Bullfrogs accumulate mercury from the food they ingest. The skin may be a possible route of exposure and absorption of mercury. The bullfrog's skin is very permeable; it acts like a secondary set of lungs taking in oxygen and water from its environment. From other studies on mercury in bullfrogs, lungs had significant amounts of mercury (Gerstenberger S., and Pearson R. 2002). So the skin of the bullfrogs should also have concentrations of mercury.

As noted before the hind frog limbs being delicacy consumption could be a problem. Though, no studies have been done to see if mercury concentrations are higher in the desired edible portion of the frog, the hind limbs, compared to the front limbs. People consume mainly the hind limbs, because of this bullfrogs collected do not have their hind limbs. The hind limbs are of interest because there is more blood flow and more muscle concentrations compared to the front limbs. Bullfrogs front limbs serve as shock absorbers when the frog hits the ground after a jump. On the other hand, the hind limbs are used to jump and for other locomotion (Bruening, S. 2002.).

As seen in other mercury studies, female frogs should have less concentration of mercury than male frogs. Frogs with bigger size should have more concentration of mercury than smaller frogs. This study will also try to find out if front limbs to hind limbs, hind limb skin-off to hind limb skin-off are good prediction models can be made.

Methods

Thirty-one Bullfrogs were collected from Dos Palmas Preserve, California. The frogs were then placed on ice then taken to the Environmental Science laboratory. In the laboratory, frogs were placed in whirlpack bags and frozen. The frogs were thawed out at

room temperature, weighed in grams with the stomach removed. The frog's length was measured from snout to vent, then tissue was dissected, in the Environmental Science laboratory, from the front and the hind limbs for chemical analysis. The muscle tissue was taken from upper portion of the limbs near the joint. The weight of the tissues ranged from 1.1 ± 0.4 . Of the thirty one frogs, only 25 of them had enough front limb muscle for Mercury analysis.

Mercury analysis was performed on an AMA 245 Atomic Absorption Mercury Analyzer from Leo Corporation as previously published (Cizdziel J., Gerstenberger S., in press). Briefly, this instrument allows analysis of total mercury. Samples will be analyzed in nickel sample boat with drying, decomposition (550°C), and waiting times of 45:125:45 seconds for all tissues and certified reference materials. Ultra pure oxygen will be used as the carrier gas with an inlet pressure of 250 kPa and a flow rate of 200 ml/min. The AMA 254 has a detection limit of 0.01 ng Hg and a linear range from 0.05 to 40 ng. Quality assurances and Quality control (QA/QC) will be performed with certified reference material (CRM) dogfish muscle (Dorm-2) from AccuTrace, New Haven, CT, USA. Approximately 5 to 9 mg of CRM will be weighed and analyzed after every ten tissue samples. Followed by two blank runs (empty boat) to ensure all mercury will be removed.

Linear regression will be performed to examine the relationship between front limbs and hind limb concentration, and skin-off and skin-on concentrations of mercury. The frog's length, weight, and mercury concentrations in muscle tissue will also be examined by linear regression. Differences between the sexes fore and hind limbs and

skin off and skin on will be determined using a Mann-Whitney test. A significant level of $p=0.05$ was used for all samples.

Results

Out of the 31 bullfrogs twenty two were Female and nine were male. The mean lengths of the bullfrogs were 9.1 ± 2.7 cm. The mean weight was 66.0 ± 54.0 g. The mean length of female bullfrogs was 8.8 ± 2.6 cm and the female mean weight is 59.1 ± 54.2 g. The mean male lengths were 10.1 ± 2.8 cm and mean weight 82.8 ± 52.7 g. See table 1 for mercury concentrations between limbs and sex.

Table 1: Mercury Concentrations in North American Bullfrog

	Front Limb (ppb)	Hind limb skin off (ppb)	Hind Limb Skin on (ppb)	N
Male	23.70 ± 16.13	23.30 ± 17.94	20.25 ± 14.67	Front limb=8 Hind limbs=9
Female	16.53 ± 10.87	15.05 ± 9.97	13.03 ± 8.38	Front limb=17 Hind limbs=22
Total	19.60 ± 17.02	18.33 ± 12.31	18.33 ± 12.31	Front limb=25 Hind limbs=31

Table 2 shows the Coefficients of determents, or R^2 , for significant and not significant variables.

Table 2: Coefficient of determents for Bullfrog Muscle Tissue

Significant	R²	Mann-Whitney
Hind skin off to Hind skin on	0.9821	U= 420.5 p=0.398
Male to Female Front limbs	0.8954	U=49 p=0.268
Male to Female length	0.0651	U=68.5 p=0.184
Male to Female Weight	0.1417	U=73 p=0.258
Male to Female Hind limbs skin off	0.9240	U=71 p=0.223
Male to Female hind limbs skin on	0.8662	U=67 p=0.164
not significant		
Hind skin off to Front limb	0.7776	U=349.5 p=0.531
Female skin off to front limbs	0.9128	U=170.0 p=.0630
Female skin off to skin on	0.9615	U=204.5 p=0.379
Male skin off to skin on	0.9956	U=36.0 p=0.691

Discussion

From the sample it was determined that there was no significant difference in mercury concentrations between front limbs and hinds limbs of the bullfrogs (U=349.5 p=0.531). So for this study, front limbs are the same as hind limbs with low levels of mercury concentrations. So the edible portions of the frog would have the same mercury concentrations as non-edible samples. It seems that the usage of the muscle and amount of muscle plays no part in mercury concentrations at low levels. Front limbs and hind limbs do not differ, thus either can be used for mercury determination in wild frogs. Most frogs are collected by spearing. From this study we found that the unwanted portion of the frog, everything except the hind limbs can be used for mercury studies.

Bullfrog's skin had no significant mercury concentrations. The female bullfrogs skin had no significance mercury concentrations (U=204.5 p=0.379). The male bullfrogs

also had no significant mercury concentrations ($U=36.0$ $p=0.691$). So when testing for total mercury skin does not play a part in mercury concentrations. Since skin is made up of a by-lipid membrane, mercury does not accumulate in fat of the frog. This is important so if fats are in the sample it will not skew the results (Dellinger J., et al 1994).

The differences in the sexes played a role in mercury concentrations. There was significant mercury concentrations between males and females muscle ($U=71$ $p=0.223$). Males had higher mercury concentrations, mean of 24.33 ± 18.50 ppb, than females, 13.7 ± 8.4 ppb. This could be due to the fact that males were bigger could have played a part in the mercury concentrations. With males being bigger the males would have to eat more. For this, males would have more chances to bioaccumulate mercury. The females could have lower mercury concentrations because of when females give birth; eggs and live births, the toxic levels of the females go down. This is because the embryos take in most of the toxins in the female's bodies (WHO. 1990). So breeding season could play a part in mercury concentrations in female bullfrogs. A prediction model could be made that in the winter time female frogs should have more mercury concentrations than in the summer and spring time.

Conclusion

This study has found that in the sample tested that mercury concentrations between front limb and hind limbs are not significant. Models now can be used to predict that the hind limbs are the same as front limbs in low concentrations. So frogs harvested for consumption can be used for experimentation, as the hind legs are only removed from the frog's body. Since skin does not accumulate mercury in low levels. Though, we still

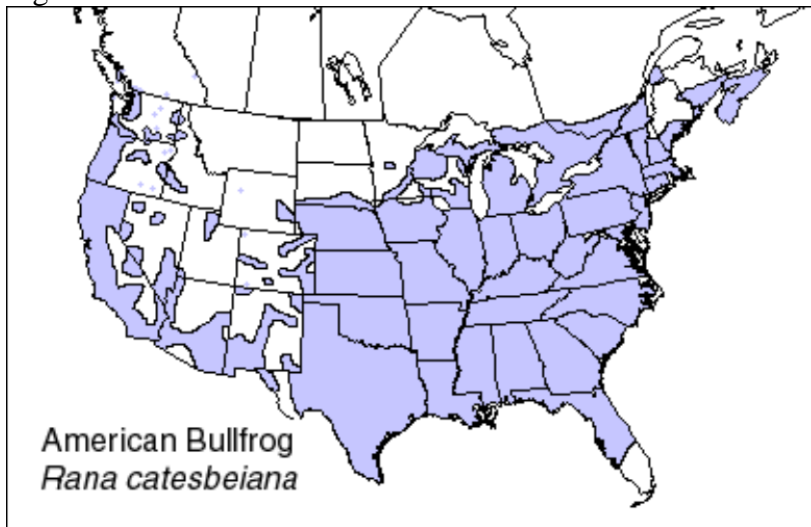
do not know if this is the case for higher concentrations of mercury. At low levels front and hind limbs have no significant difference. At high levels this may not be true, more testing is needed. More testing needs to be done to see if there is a difference in female due to mating seasons.

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Appendix

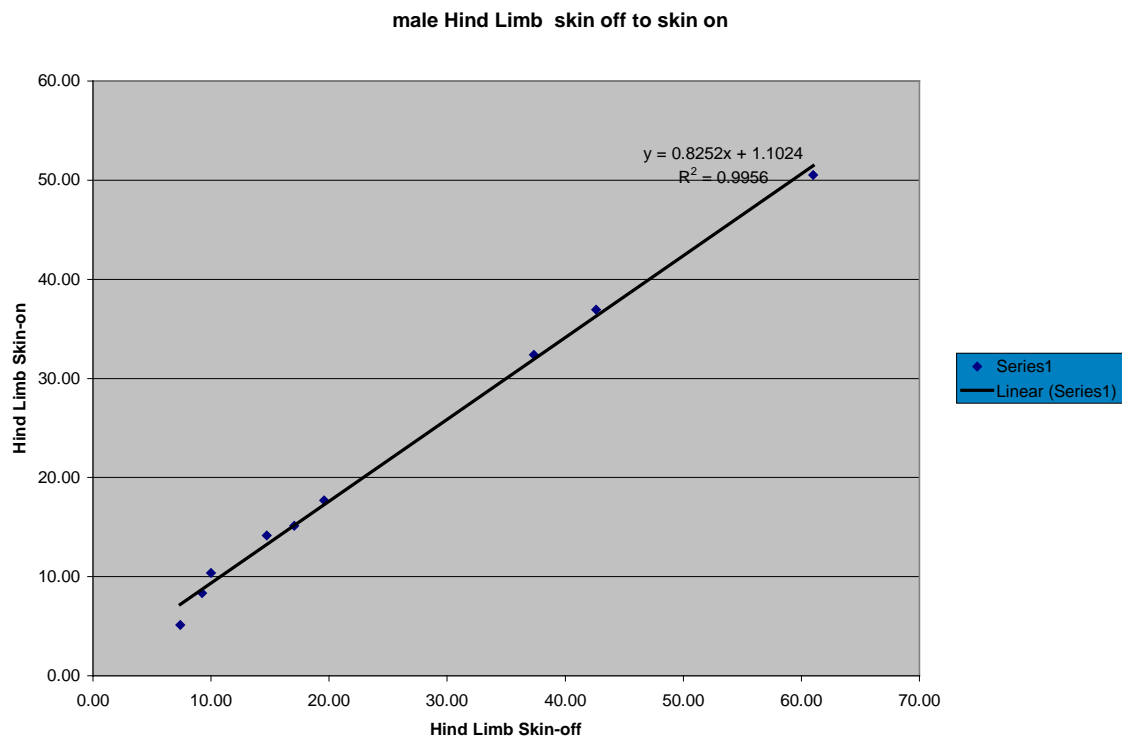
Figure 1:



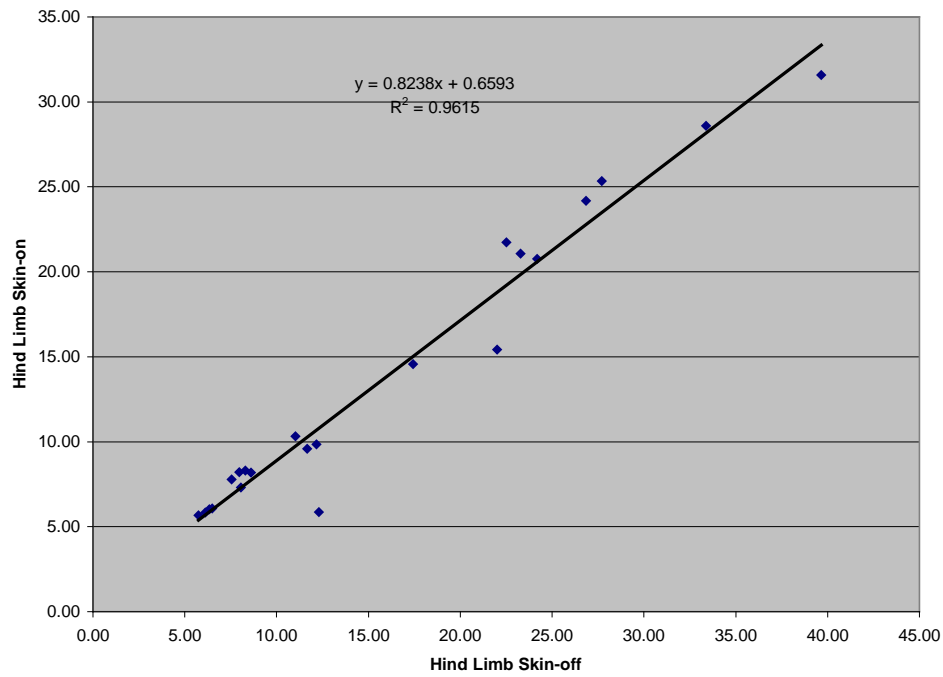
Introduced Species Summary Project North American Bullfrog (*Rana catesbeiana*)

http://www.columbia.edu/itc/cerc/danoff-urg/invasion_bio/inv_spp_summ/Rana_catesbeiana.htm

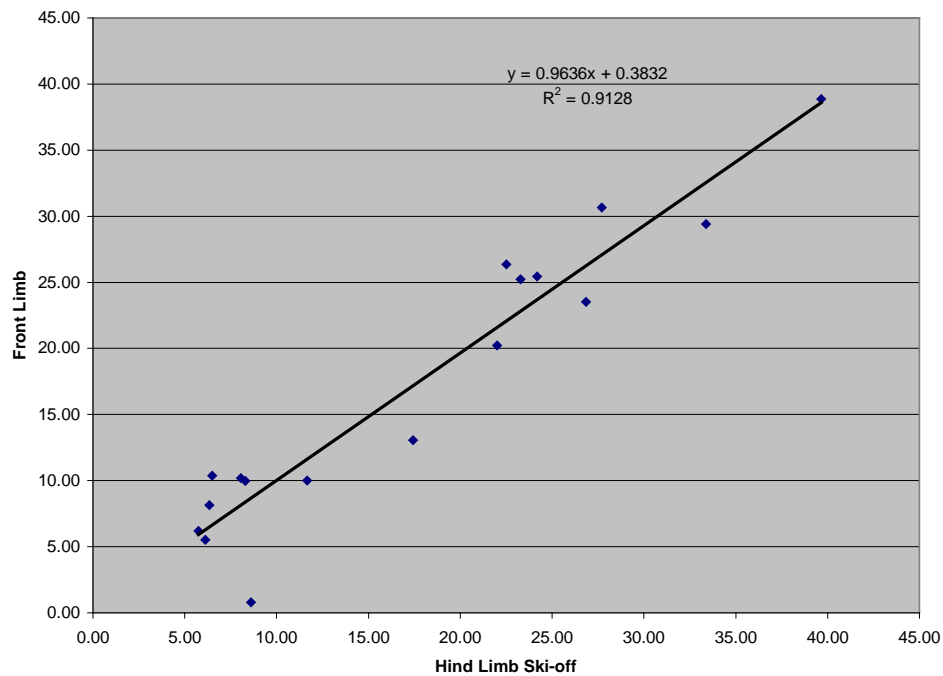
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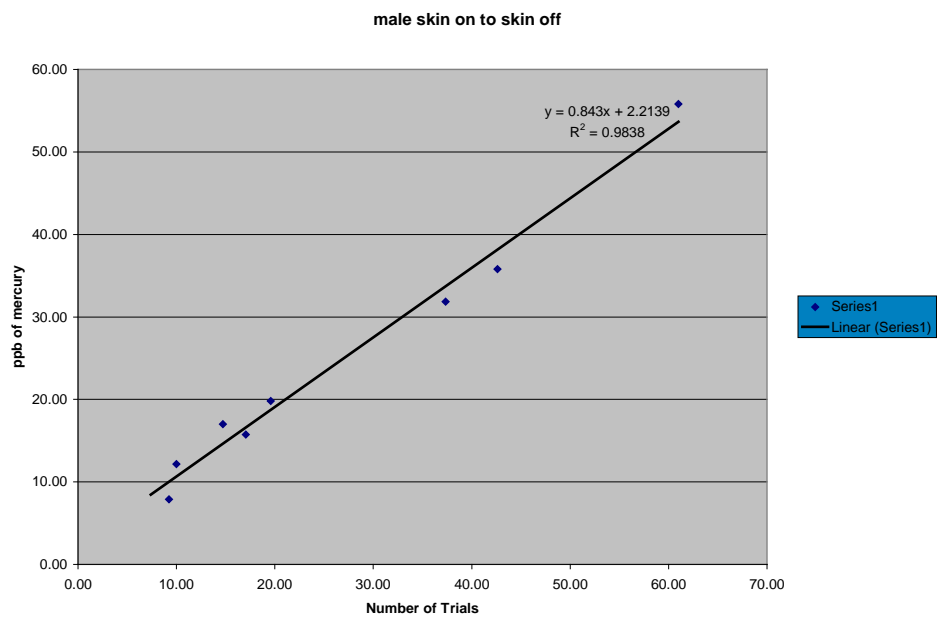
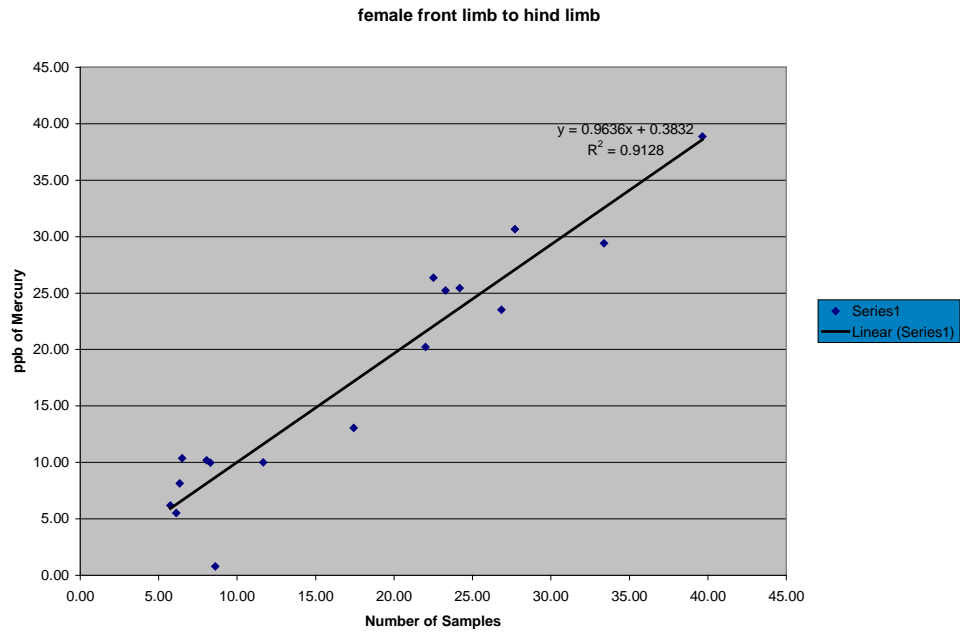


female Hind limb skin off to skin on

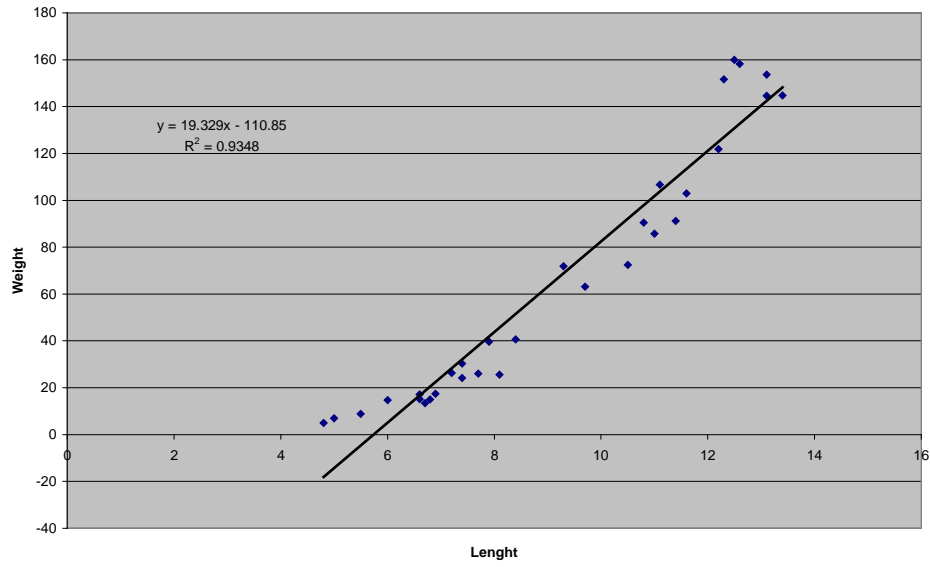


female skin off to front limbs

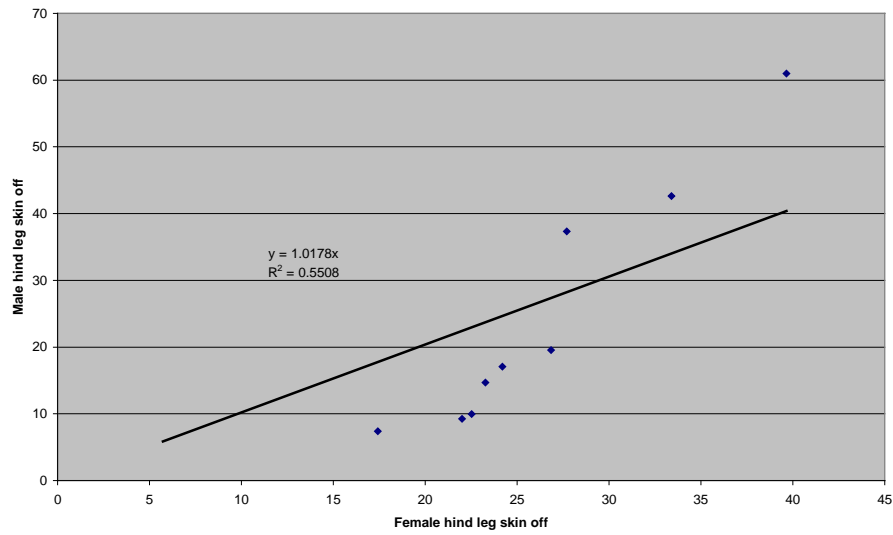




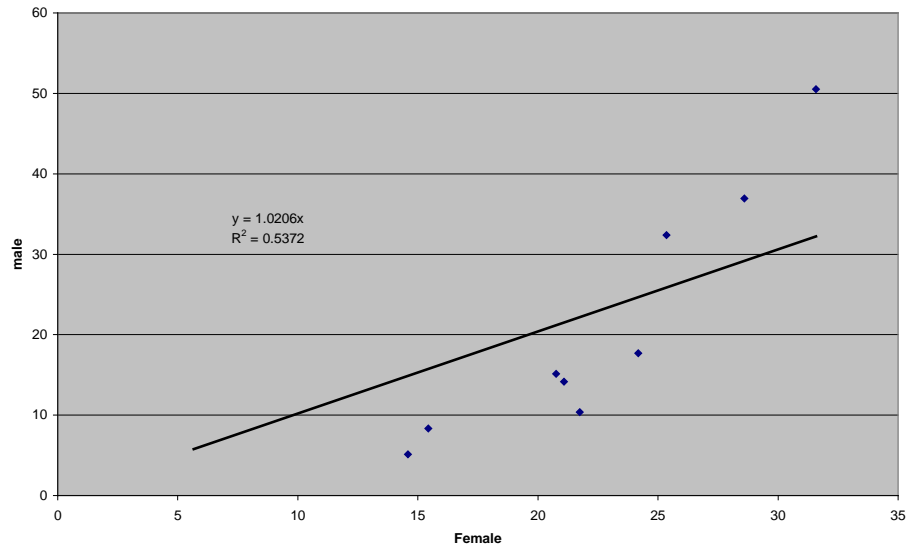
Lenght Vs. Weight of Bullfrogs



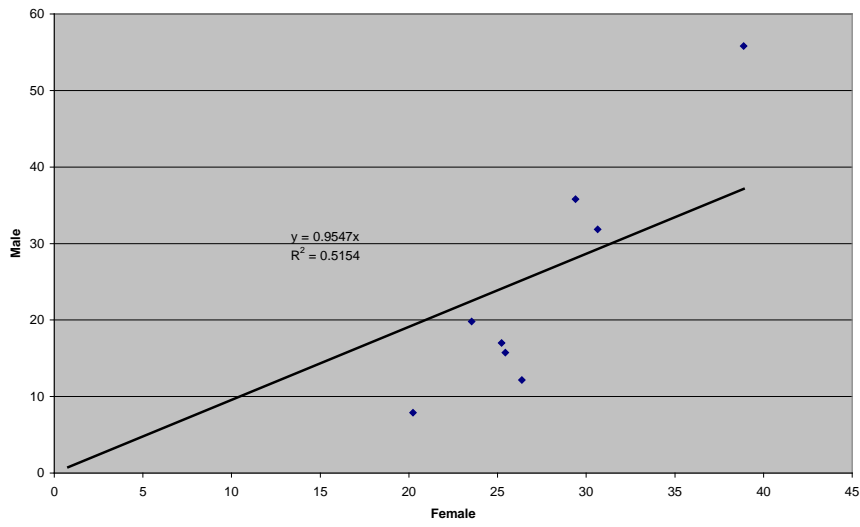
Male Vs Female hind leg skin off



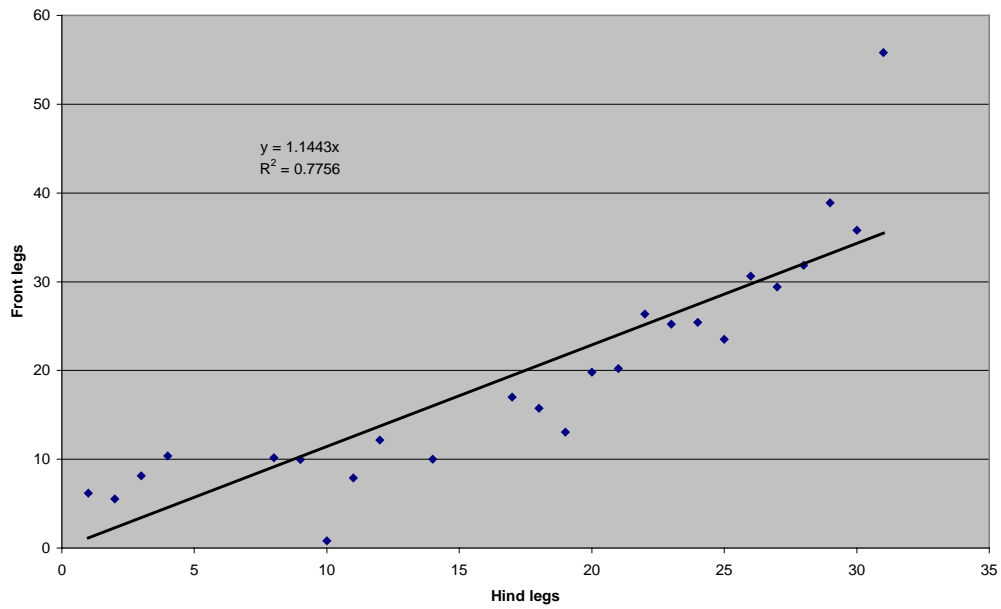
Male Vs Femal Hind leg skin on



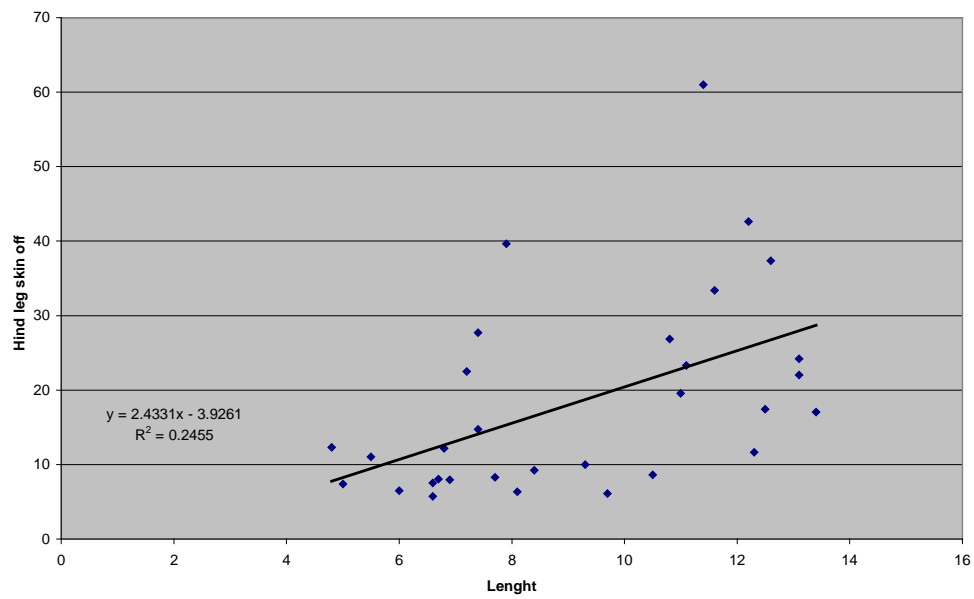
Male Vs. Female



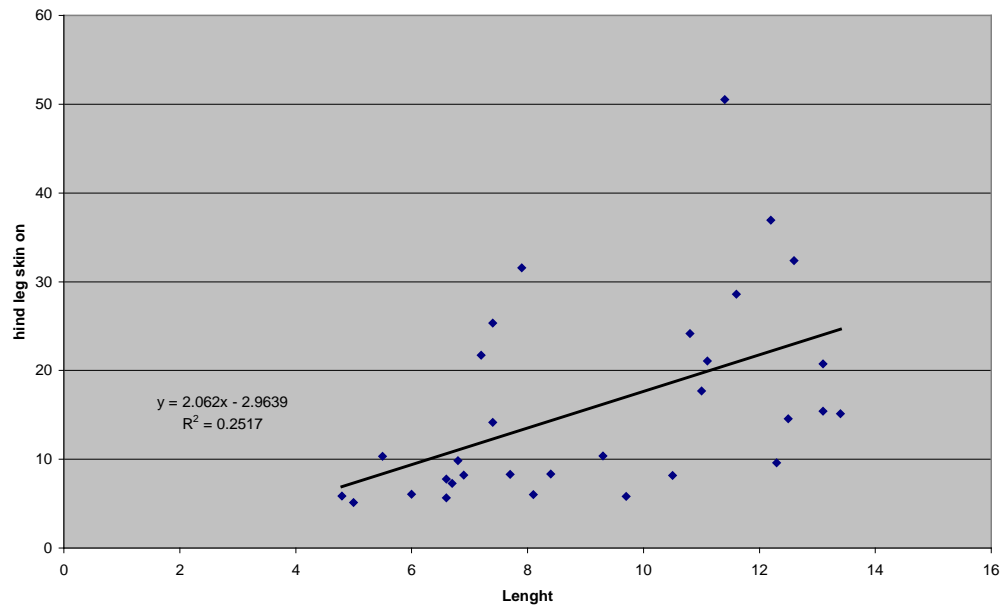
Hind legs Vs. Front legs



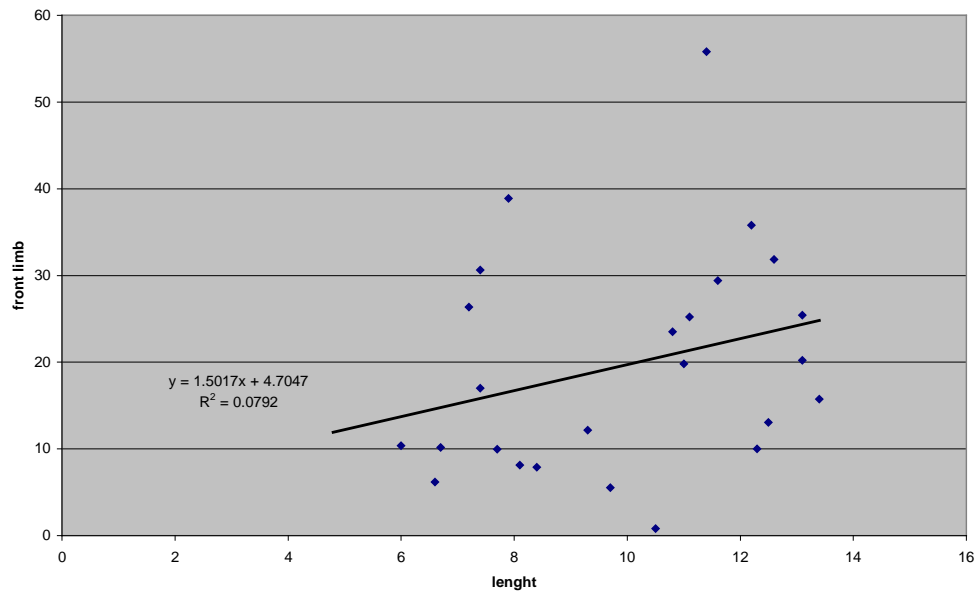
hind leg skin off vs lenght



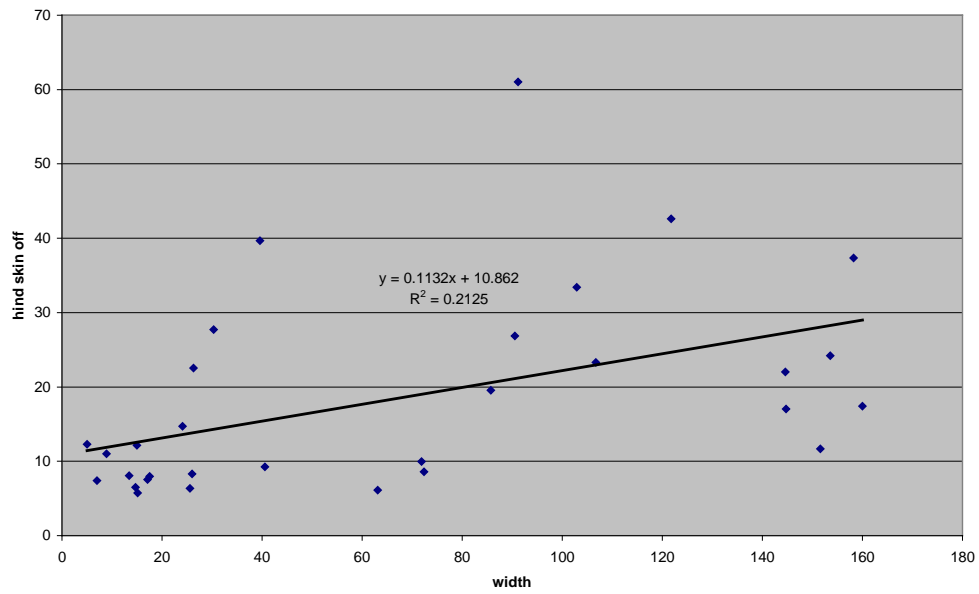
Hind leg skin on Vs. Lenght



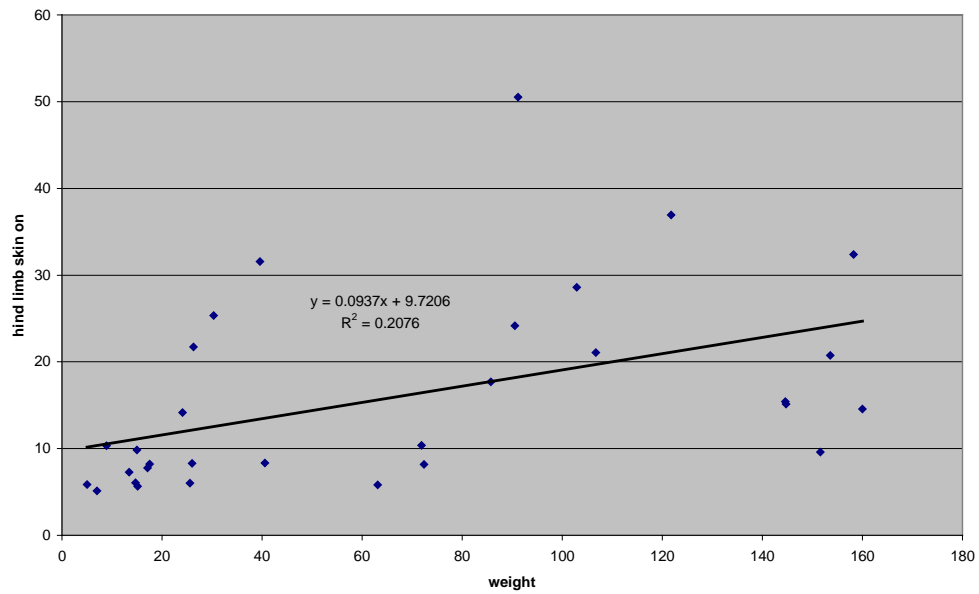
lenth Vs front limbs



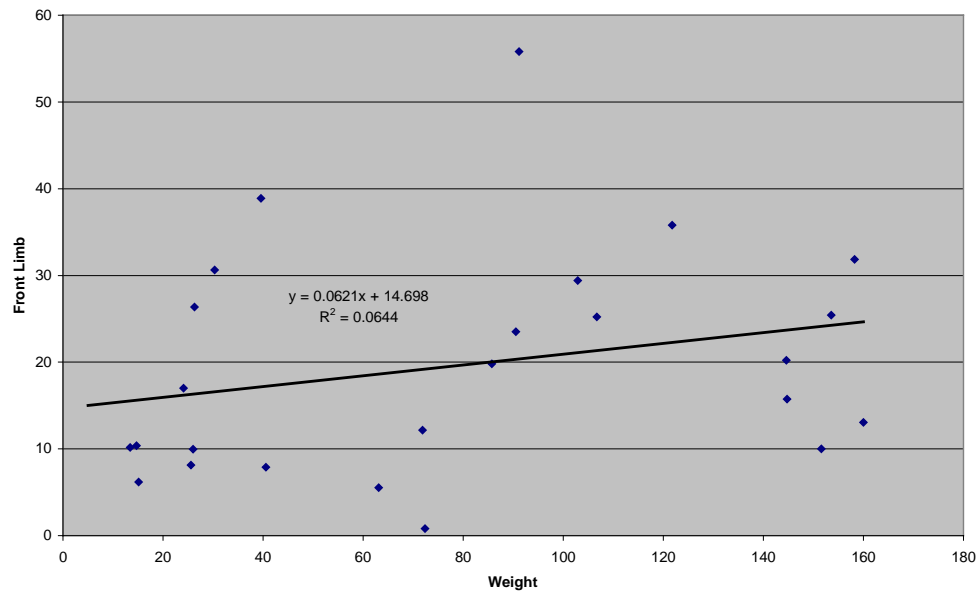
Weight Vs hind limb skin off



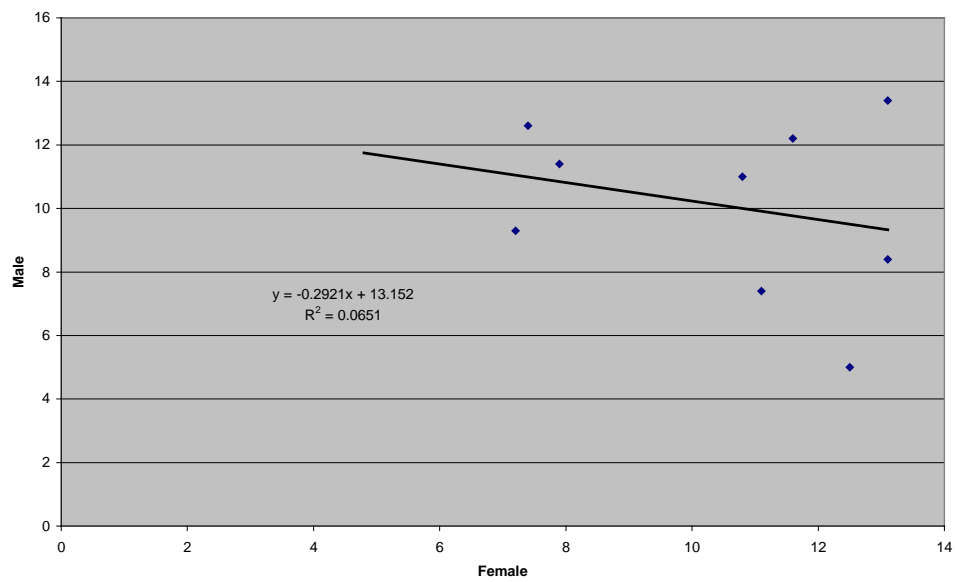
Weight Vs hind skin on



Weight Vs Front limb



Male Vs. Female lenght



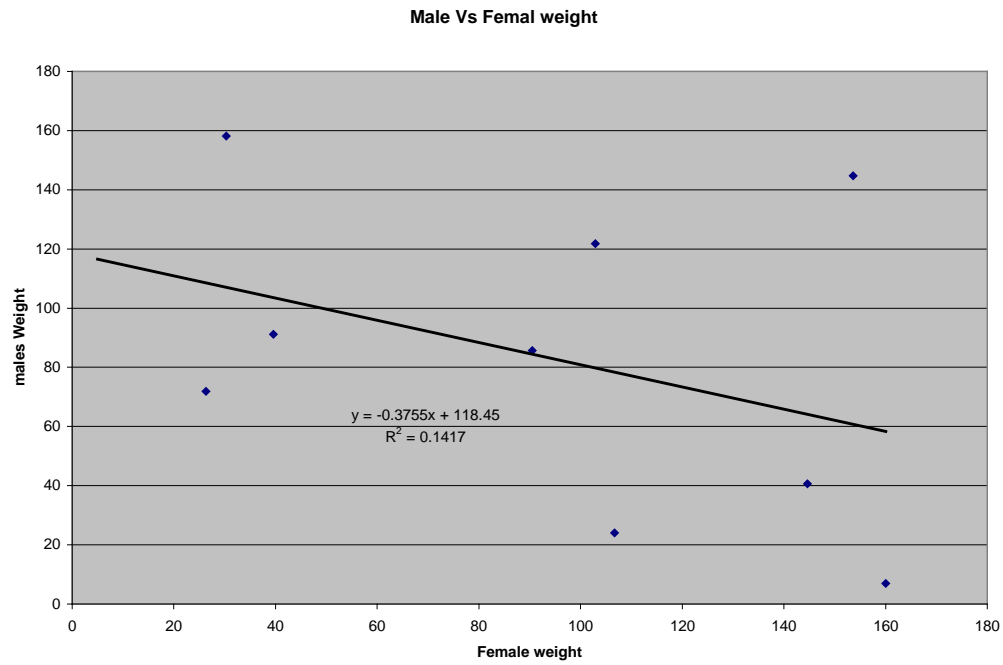
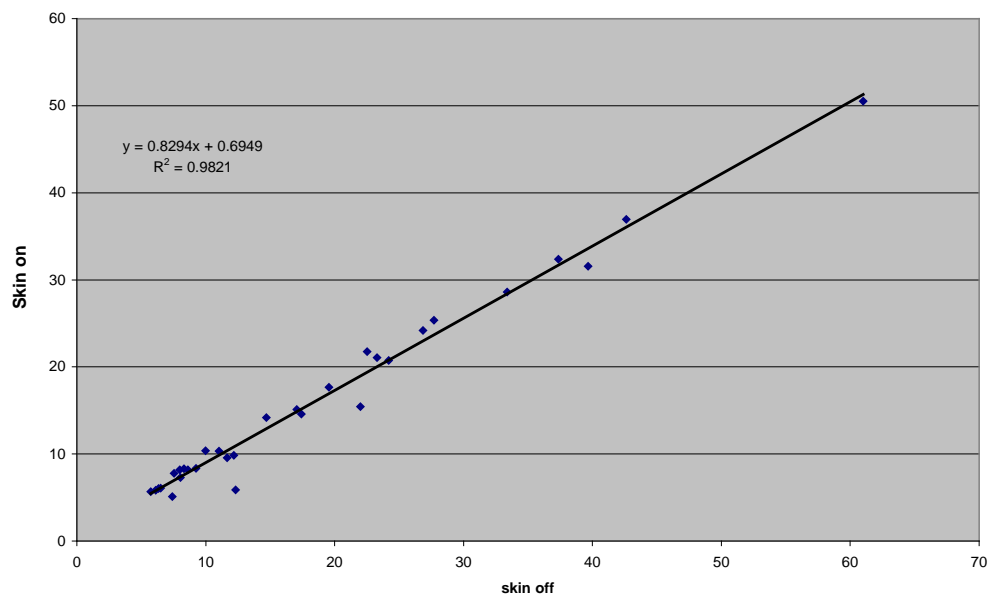
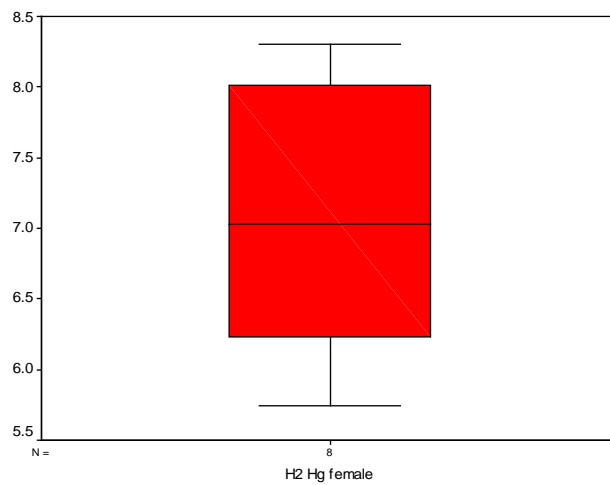
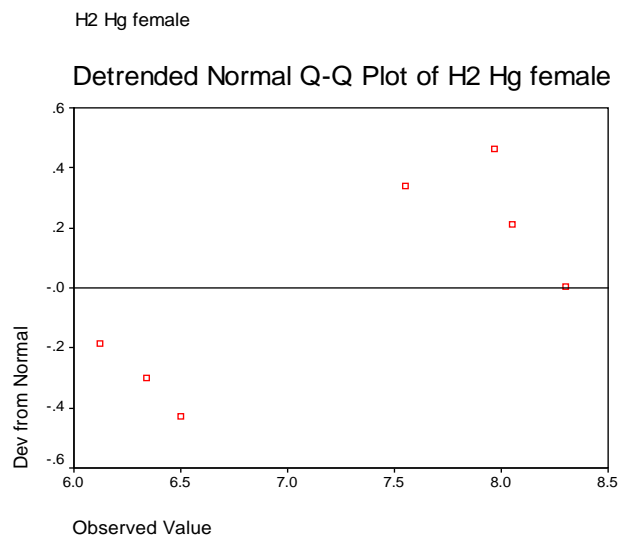
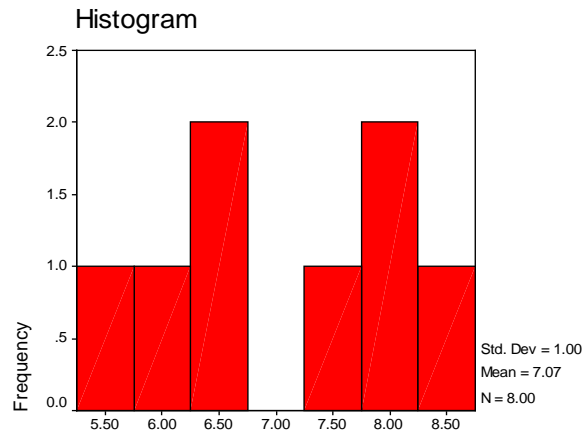


Figure 1: Skin on to skin off

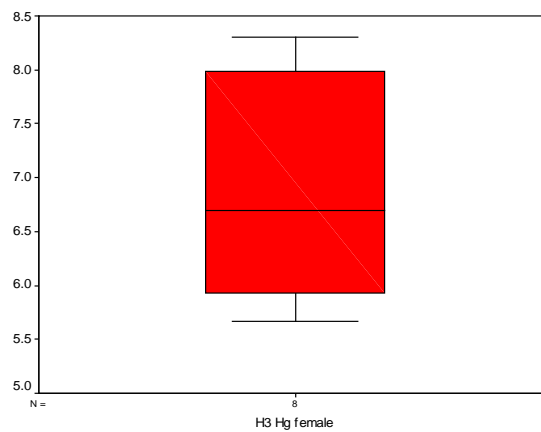
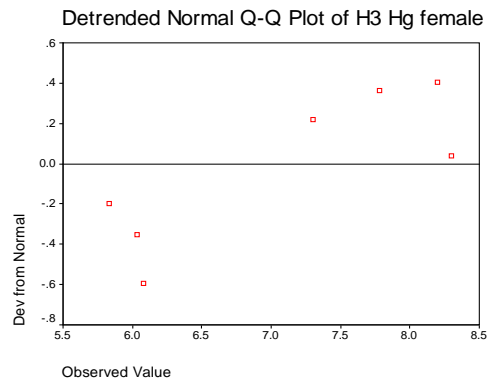
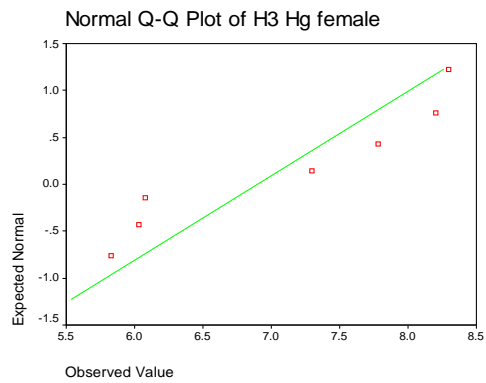
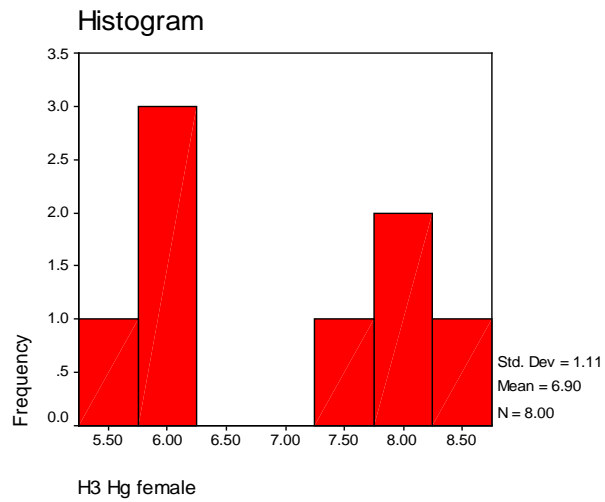


Histograms

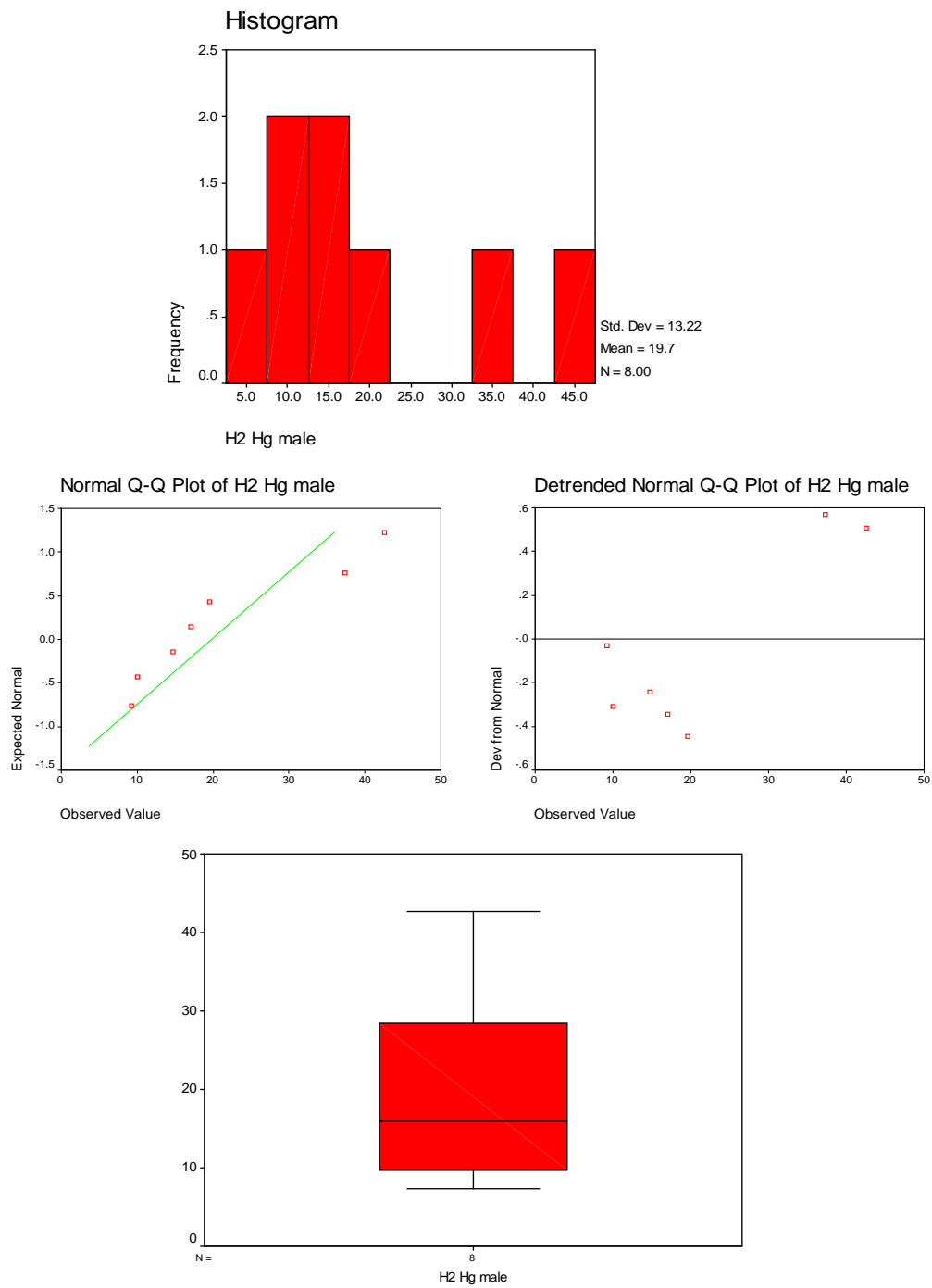
Hind limb skin-off Hg female



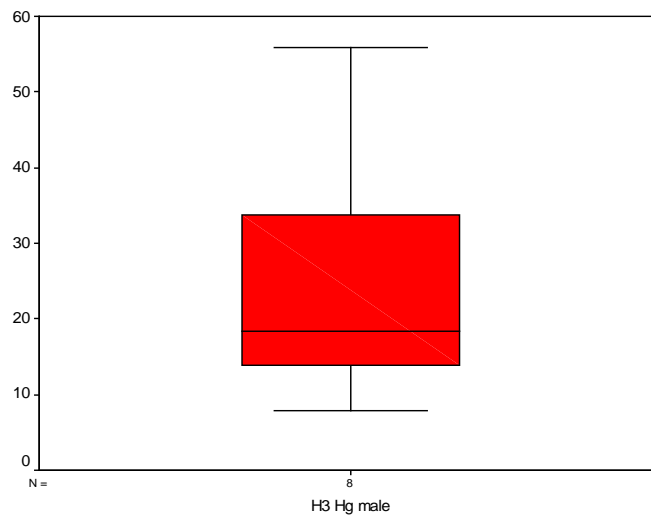
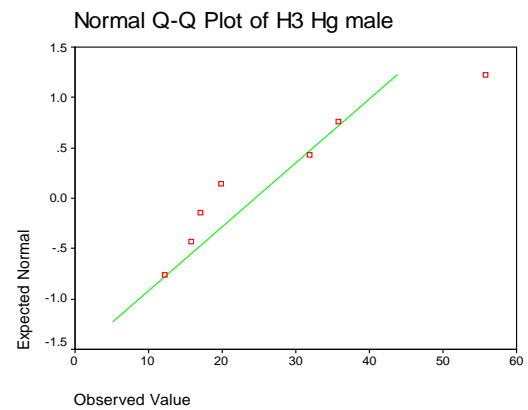
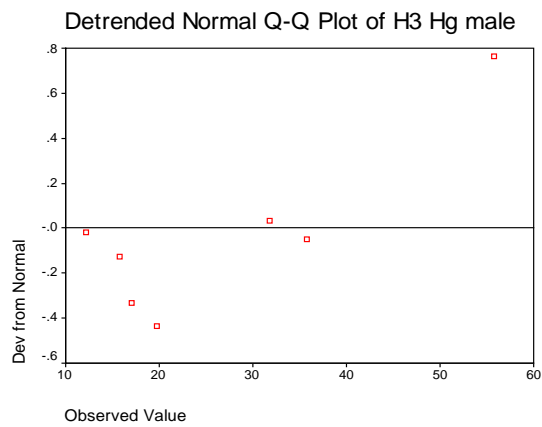
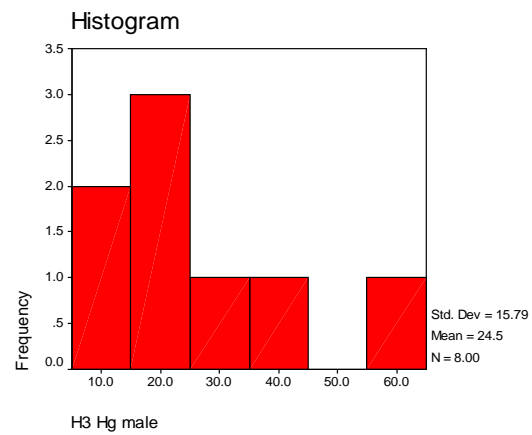
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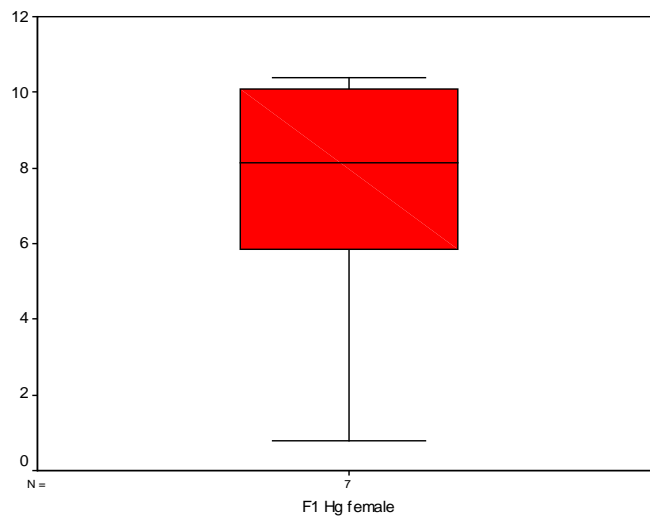
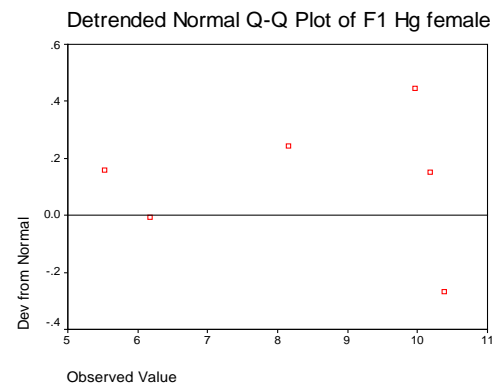
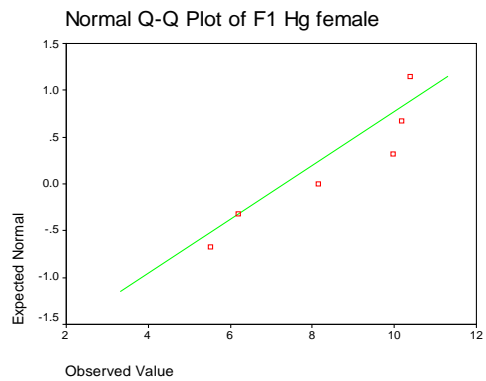
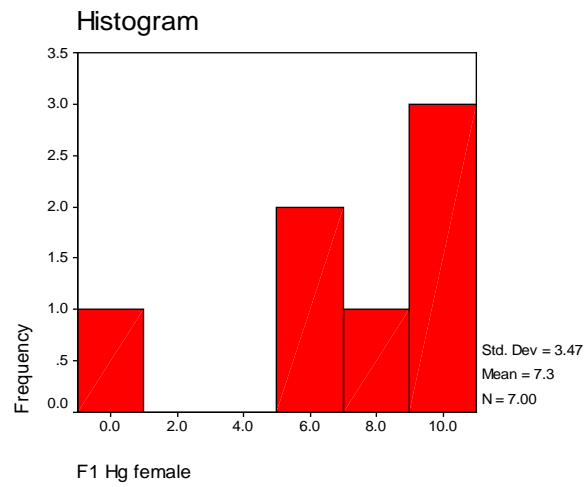
Hind Limb skin-off Hg male



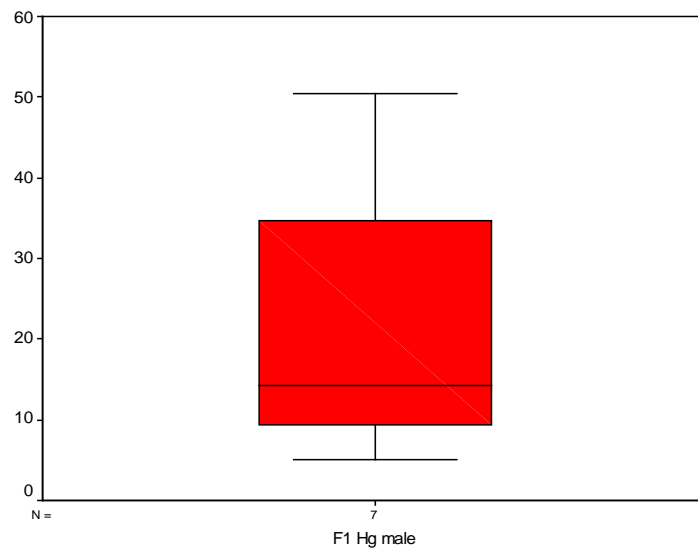
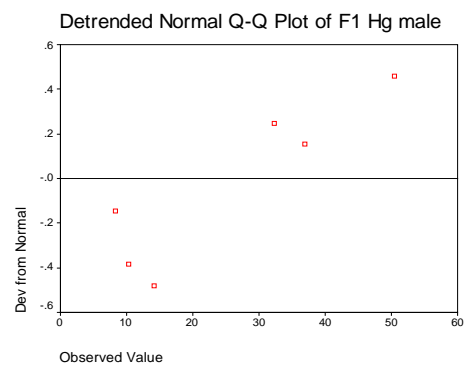
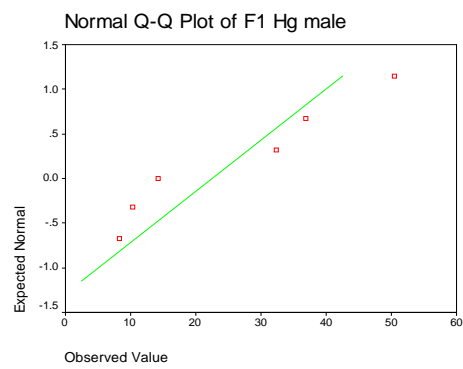
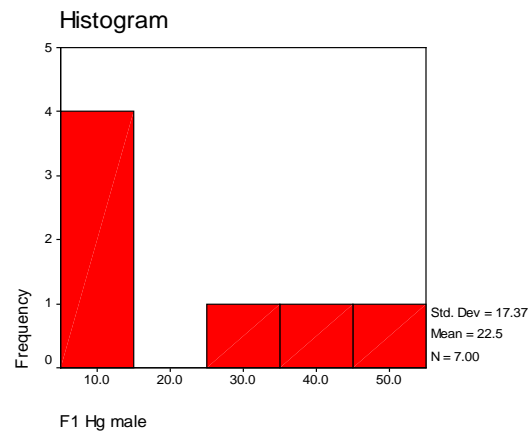
Hind Limb skin-on Hg male



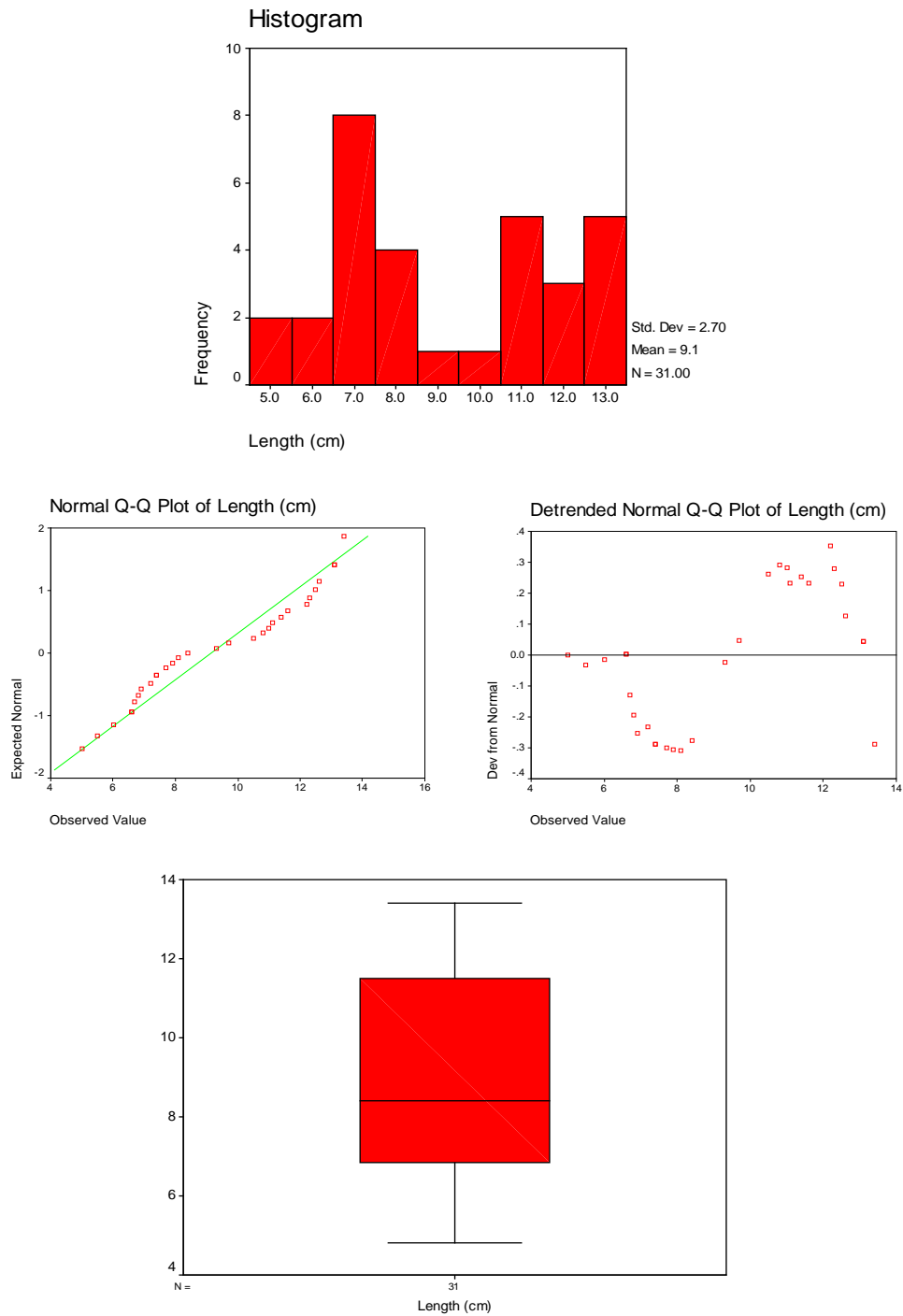
Front limb Hg female



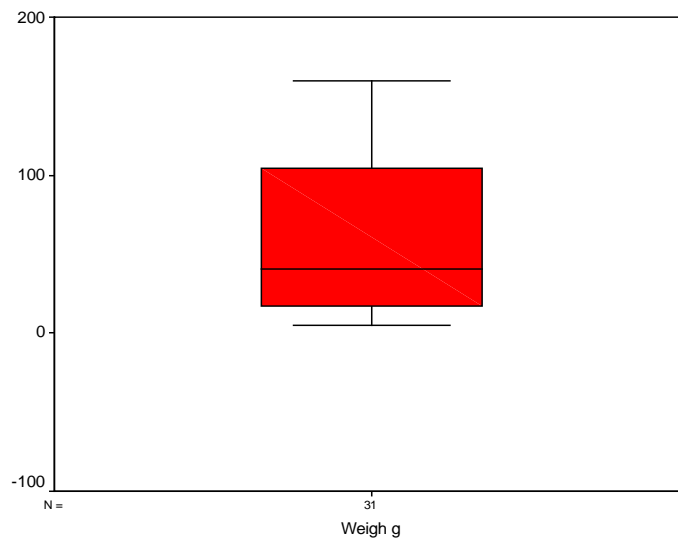
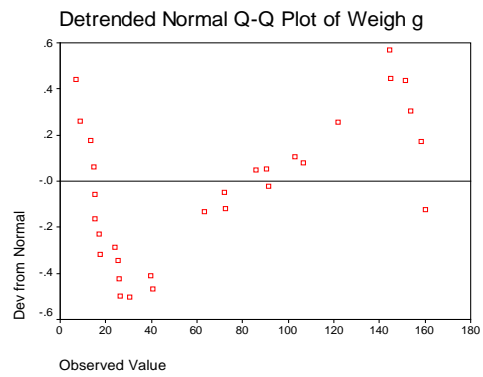
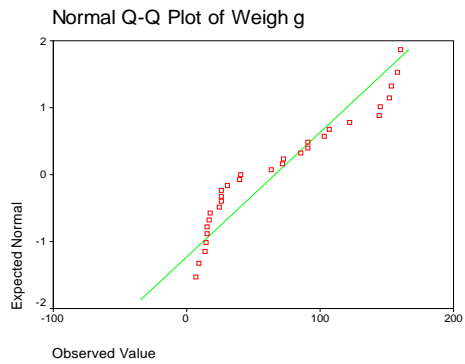
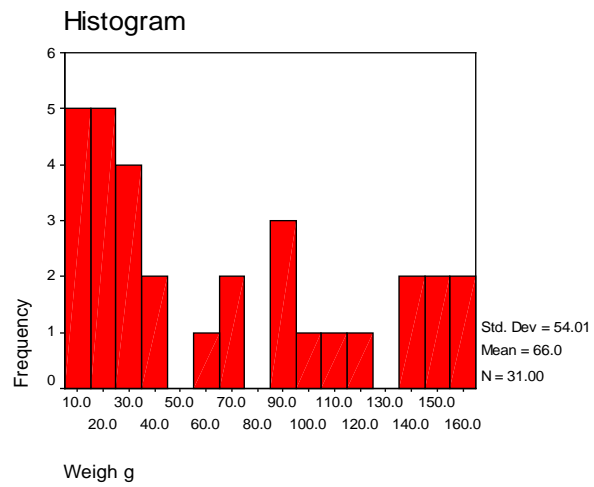
Front Limb Hg male



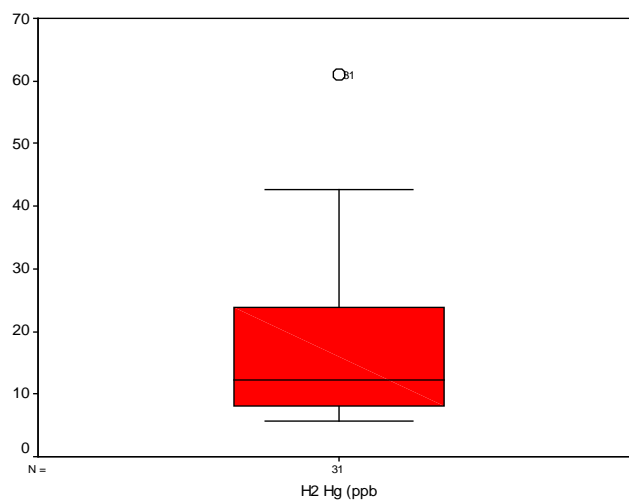
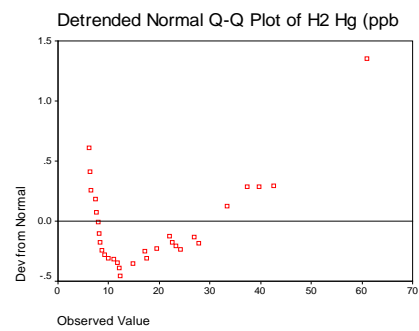
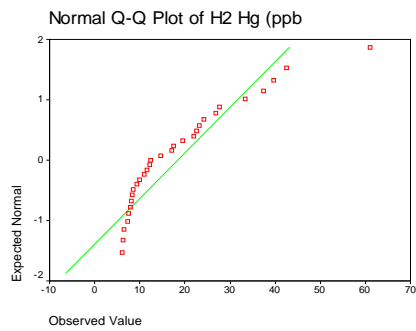
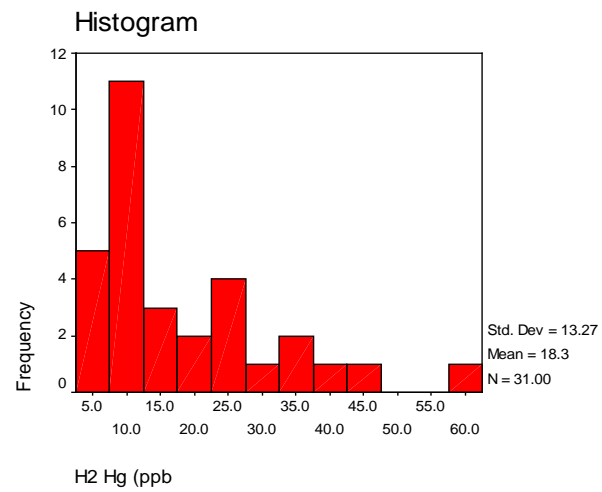
Length (cm)



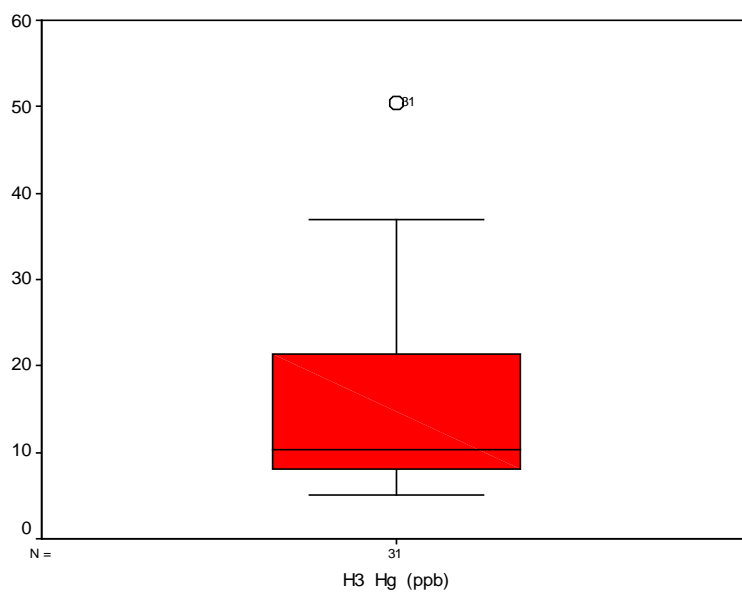
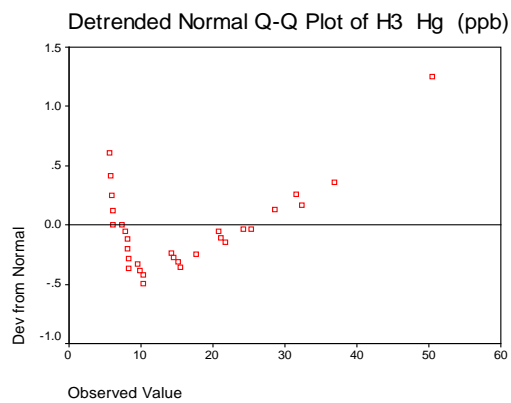
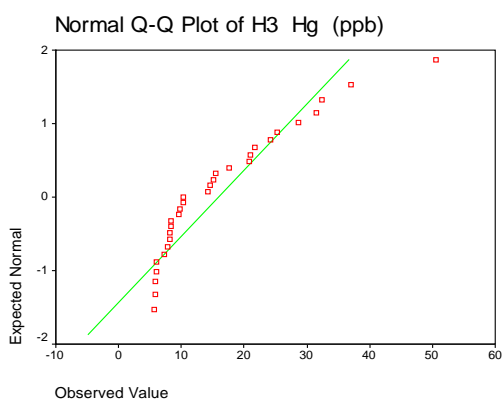
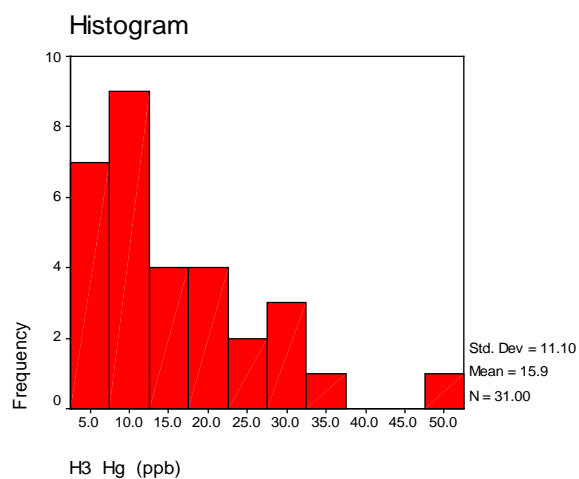
Weigh (g)



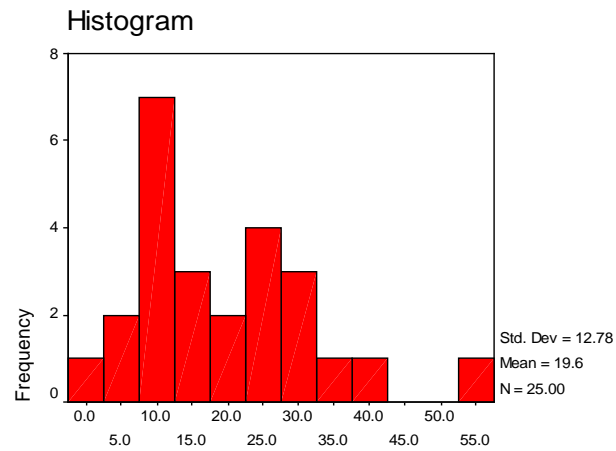
Hind leg skin-off Hg (ppb)



Hind limb skin-on Hg (ppb)



Font limb Hg (ppb)



F1 Hg (ppb)

