Investigation of Weight Loss in Pacific cod (*Gadus macrocephalus*) Due to Exsanguination

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ABSTRACT

Pacific cod (*Gadus macrocephalus*) are an important groundfish species harvested by longline vessels in the Bering Sea and Aleutian Islands. Exsanguination, or bleeding, is an important processing step at the time of capture to obtain high product quality, including freshness and the visual appearance of fillets. The National Marine Fisheries Service, Alaska Region, is responsible for assessing fishery catch, which is generally a round weight estimate collected prior to any processing, including exsanguination. However, longline vessels present a special situation because exsanguination is required prior to weighing, which may create a bias in round weight estimates due to lost blood weight. This study examines weight loss due to exsanguination by sampling Pacific cod at time intervals of 30 seconds, 1:00 minute, and 1:30 minutes after landing. Study results show that, on average, Pacific cod generally bleed the most during the first 30 seconds with smaller volumes in later time periods. Moreover, the rate of bleeding is dependent on whether the cod is alive at the time of bleeding, whether the cod was bleeding from a wound prior to exsanguination, and the size of the Pacific cod. On average, Pacific cod lost 0.66% of their body weight in 30 seconds and about 1% after 1:30 minutes.

CONTENTS

INTRODUCTION

The freezer-longline fleet in Alaska consists of catcher processor vessels that fish primarily for Pacific cod in the Bering Sea and Aleutian Islands (BSAI) management area. On December 22, 2010, the Longline Catcher Processor Subsector Single Fishery Cooperative Act (Act) was signed by President Obama (Senate Bill 1609). In brief, the Act authorizes freezer-longline vessels participating in the BSAI area directed Pacific cod fishery to form a single cooperative and requires the National Marine Fisheries Service (NMFS) to promulgate regulations supporting cooperative formation, including provisions for catch accounting.

The Freezer-Longline Conservation Cooperative (FLCC) comprises all the vessels fishing the freezer longline catcher processor allocation of Pacific cod in the BSAI management area. As a result, the cooperative is able to fish Pacific cod as an exclusive allocation, which allows a slower fishing pace and improved coordination between vessels. Because the cooperative has fine scale control over both target and non-target catch, resulting in opportunities to manipulate catch data, monitoring requirements were increased. A well-designed monitoring program addresses potential biases, but also provides timely accounting of catch that does not interfere with product quality requirements. Trawl fisheries in the BSAI management area addressed these needs by weighing all species on motion compensated flow scales. A similar monitoring methodology can be applied to the FLCC with some important distinctions.

Trawl catcher processors participating in quota programs in the North Pacific are required to weigh all catch on flow scales (Figure 1) prior to processing. These scales are advantageous in that they offer timely, accurate, and precise accounting without impeding the flow of fish into the factory. After the catch is weighed, at-sea observers take species composition samples which allow NMFS to estimate what percentage of the weighed catch consists of each species. The

estimated species composition is extrapolated to the total catch weight and is used to calculate the total amount of quota species.

On freezer-longliners, NMFS and the industry are proposing to weigh Pacific cod on flow scales similar to those used on trawl vessels (Figure 1). However, the approach used on trawl vessels requires modification for freezer-longliners because observers collect species composition samples prior to catch being weighed and discards are not always brought onboard these vessels. In addition, freezer-longline vessels need to exsanguinate fish quickly for product quality reasons and this most effective if quickly conducted after capture and the fish is still alive (Valdimarsson et al. 1984, Botta et al. 1986, Huss 1995). The reduction in weight due to blood loss potentially compromises efforts to obtain an unbiased weight estimate from a flow scale. Weighing of whole Pacific cod occurs upstream from the bleeding station on most freezer longline vessels. However, this location is unsuitable for a flow scale (Figure 2) because it is exposed to weather and there is little room between the fish stripper (a mechanical method of removing a hook from fish) and the bleeding station.

There are two alternative locations for flow scales on a freezer-longliner: (1) after the bleed tank or pan; or (2) directly after the bleeding station, but before the bleed tank or pan. Exsanguination of Pacific cod prior to weighing occurs at both locations. Location 1 allows complete bleed-out of Pacific cod and results in a product recovery rate (PRR) for bled fish being applied to the round weight (RW). The current bled PRR is 0.98 (product code 03 in Table 3 to 50 CFR 679), which corresponds to a 2% loss from RW. Pacific cod weighed at location 2 are incompletely bled, and may not reflect the 0.98 PRR due to the short bleeding time prior to weighing.

This project evaluates potential bias from exsanguination of Pacific cod if they are weighed prior to the bleed out tank (location 2). The study examines the influence on blood loss due to cod size, activity level, gaffing injury, and bleeding time. Study results should not be applied to the bled PRR (NMFS product code 03) because the methodology does not examine the continued blood loss that occurs in a bleed-out tank or pan. Evaluation of the NMFS PRR would require controlling for fish storage and handling conditions, such as whether the fish are "bled-out" in flowing seawater, on a pan, and bleed-out time as well as gear soak time (Sigler et al. 2007). Further, representative samples would be needed from the entire freezer-longline fleet to characterize vessel effects and seasonal fishing behavior, which this study does not address.

METHODS

Methods for this project were derived from studies on fallow deer (Mulley et al. 2010) and rainbow trout (Tretsven and Patten 1982). These studies directly weighed blood loss rather than weighing the animal before and after exsanguination. This methodology controls for variation extrinsic to blood lost such as slime and swallowed water.

This project was conducted on the F/V *Bristol Leader*, a 167 ft freezer-longliner that fishes for Pacific cod under the FLCC. Sampling occurred in the Bering Sea between July 14, 2010, and July 19, 2010.

Pacific cod were sampled using a randomly selected start time during haul retrieval and a systematic selection of one cod every 30 minutes. The 30 minute sampling time interval was selected to allow the sampler time to conduct sampling duties. At-sea observer sampling was conducted during the sampling period and had priority over the study because of its importance

to the daily management of the fishery. Therefore, samples for this study were not taken concurrently with at-sea observer sampling and some fish otherwise selected could not be sampled. Samples were taken throughout a 24-hour period, with lulls in sampling resulting from an 8-hour break for the vessel to set gear and to avoid conflicts with the at-sea observer sampling.

Pacific cod were sampled immediately after they were brought onboard the vessel. The fish were either gaffed aboard and removed from the line by the fish stripper or retrieved using a pole net if the fish dropped off the line before reaching the vessel. On rare occasions a Pacific cod could not be retrieved using a pole net, resulting in the next Pacific cod on the line being sampled. A note was made about whether the fish was pole netted or losing blood from the fish stripper or gaff wound. For each sampled Pacific cod, the following information was collected:

1. The RW of the Pacific cod was estimated using a platform scale (Marel M2000 60 kg capacity × 0.02 kg) that was tested daily using 10 kg, 25 kg, and 50 kg test weights.
2. The length of the cod was measures to the nearest centimeter.
3. The activity level of the cod was estimated using three qualitative categories: active and moving, alive but not active, and dead.

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The Pacific cod were exsanguinated using a standard method employed by the freezer-longline fleet. This method involves using a serrated knife to cut just inside of the collar and across the gills and to the back bone. Immediately after being cut, the fish were held over one gallon Ziploc freezer bags and blood was collected at the following time intervals: 30 seconds, 1:00 minute, and 1:30 minutes. Each fish was held by the sampler so that the blood dripped

directly into the sample bag. Precisely weighing small volumes of blood is logistically difficult while at-sea, so this collection procedure allowed blood weight to be measured later in a laboratory setting. After each blood sample was completed, the Pacific cod was weighed again on the platform scale.

The bags of sampled blood were sealed and placed in a -32° C freezer. Samples were transported frozen and stored in a -21° C freezer. Prior to weighing at the laboratory, the bags of blood were thawed, wiped down, and weighed on a laboratory scale (Ohaus c305 300g × 0.1g). The tare weight for the bags was determined by weighing bags that were from the same lot used during sampling.

STATISTICAL ANALYSIS

Statistical analysis was carried out to identify potential covariates that influence blood loss as well as the amount and variability of blood loss during the study period. The analysis of covariates relied on modeling methods that were used to evaluate covariates. Total blood loss over time was evaluated using summary information, statistical tests, and bootstrapping techniques. Detailed descriptions of these methods follow.

Several model structures were examined because the statistical relationship between RW and blood loss (Y) was unknown. A linear least squares (lm) provides a simple Gaussian error structure for residuals, while more complex error structures were evaluated using a linear mixed-effect (lme) and generalized least squares (weighted least squares; GLS) modeling techniques. Goodness of fit for these models was compared using the Akaike Information Criterion (AIC; Akaike 1974), graphical residual analysis, and t-tests to determine covariate significance. These

models are not nested and, as a result, log-likelihood ratio tests are not appropriate for comparison across models and are not provided.

The lm and GLS used the same covariate structure in the model formulation. The following covariates were included in the model:

- RW of the Pacific cod prior to the exsanguination sampling period;

- whether the Pacific cod was bleeding prior to exsanguination (B);

- the Pacific cod's activity level (A), which could take on an active, inactive, or dead value; and

- an interaction effect between Pacific cod activity and whether the Pacific cod was bleeding.

The lm model was fit using the "stats" package in R-Cran (R Core Development Team 2010) and used Gaussian (normal) error structure on the error (ε) component such that:

$$Y_i = \alpha + RW_i + B_i + A_i + A_i : B_i + \varepsilon_i \ \ where \ \varepsilon_i \sim N(0,\sigma^2)_,\qquad(1)$$

where α the intercept term and $N(0,\sigma^2)$ indicates a Gaussian distribution of the errors (ε_i), with a mean of 0 and variance (σ^2). The activity factor levels compared dead and inactive Pacific cod with active Pacific cod. The GLS model used a fixed Gaussian variance structure to allow for a larger residual spread with larger RW values:

$$\varepsilon_i \sim N(0,\sigma^2 * RW_i)_.\qquad(2)$$

The lme retained the covariates from equation 1 and the fixed variance formulation from equation 2, but treated activity (A) as a normally distributed random intercept effect. Both the

mixed effect and GLS model were fit using restricted maximum likelihood estimation described in Zurr et al. (2009), for example. The "nlme" (Pinheiro et al. 2010) package in R-Cran was used to fit the GLS and lme models

A generalized additive model (GAM) was also used to assess whether a linear model appropriately characterized the data (Wood 2004). The GAM used the same covariates described in equation 1, but with the addition of a thin plate regression spline smoothing function applied to the Pacific cod RW covariate (Wood 2003). The GAM model was fit using the R-Cran "mgcv" package (Wood 2004).

Comparisons between the time periods of 30 seconds, 1:00 minute, and 1:30 minutes were made using a two-tailed paired t-test and a Wilcoxon signed rank test. The null hypothesis for both tests was that there was not a significant difference in blood lost between each time period. Tests for significance were evaluated at the 95% confidence level. The paired t-test is robust against departures from normality, but the Wilcoxon sign-rank test (a non-parametric test) is also provided. The Wilcoxon sign-rank test has 95% of the detection power compared with the paired t-test, but it is useful for confirming whether a sample distribution may influence results from the t-test statistic (Zar 1999). Finally, 95 percentile intervals of the sample median were calculated for median weight loss as a proportion of body mass and total blood loss weight. Percentile intervals were calculated using a bias corrected bootstrap (Rizzo 2008).

RESULTS

A total of 127 out of 134 Pacific cod sampled were included in the study results. The first five Pacific cod sampled in the study were excluded because of design changes that discontinued weighing periods greater than 2 minutes and 2 Pacific cod were excluded due to sampling error.

The daily number of Pacific cod sampled ranged from 5 Pacific cod on July 14, to 32 Pacific cod on July 18, with the other days ranging between 17 and 29 Pacific cod sampled per day (Figure 3). The size distribution of Pacific cod ranged between 0.51 kilograms (kg) and 12.45 kg, with a positively skewed distribution reflected by mean and median weights of 3.26 kg and 2.56 kg, respectively (Figure 4).

The GLS model with a fixed variance structure had the best fit as indicated by it having the lowest AIC statistic. The AIC for the GLS model with a fixed variance was 972.80, which was less then both the lm (1021.34) and the lme models (998.59). The lm model had a large amount of heterogeneity that resulted in residual values increasing for larger RW values of Pacific cod. This heterogeneity can lead to incorrect variance estimates and interpretation of statistical parameters. The GLS with a fixed variance structure incorporated heterogeneity into the model and corrected residual error problems present in the lme model. The variance structure resulted in both the quantile-quantile and standardized residual plots of the GLS showing no large patterns in the residual error (Figure 5).

The GLS model showed an increasing trend in blood loss as the size of the Pacific cod increased (Figure 6). This result was expected because larger fish have greater blood volume, with the intercepts showing differences depending on the activity condition of the Pacific cod and whether it was bleeding prior to capture. The model underestimated blood loss for "active" Pacific cod between 4 kg and 8 kg. This was investigated using a GAM with a thin plate regression spline (Figure 7). The model explained 79% of the overall deviance and had an estimated degree of freedom (edf) of 5.32 for the RW smoothing function. The high smoothing function edf indicates non-linearity for Pacific cod weights greater than 5 kg, which had poor sampling coverage and high variability, particularly for fish 7 kg and larger. Further, the authors

are not aware of a biological reason for blood loss to be less for fish between 5 kg and 8 kg. The non-linearity could indicate blood being lost during collection (not weighed), but we could not find evidence of this systematic sampling problem occurring. For these reasons, along with the AIC information, the non-linear hypothesis was rejected and the GLS considered the best model form.

Significant covariates at the 95% level (alpha = 0.05) for the GLS model were the RW of the Pacific cod, whether the Pacific cod was dead as compared with an active Pacific cod, and whether the Pacific cod had been bleeding prior to sampling (Table 1). The intercept parameter and whether Pacific cod were active was not significant. Of the significant covariates, a positive relationship was observed between RW and blood loss, and a negative relationship between active or inactive Pacific cod in comparison with dead Pacific cod (i.e., dead Pacific cod tended to bleed less). However, a sample size of only 8 dead Pacific cod with limited size ranges severely limits statistical inferences to the harvested population.

There were 21 Pacific cod that were bleeding prior to the exsanguination method being employed for the study, and these fish were included in the model as a factor. The causes for the pre-study blood loss were hook injury due to the fish stripper breaking the fish's jaw or pulling the hook out of the fish's mouth and gaff injury. The location of the gaff wounds varied, with some occurring in the Pacific cod's eye, one occurring on the Pacific cod's throat, and most in the head region of the Pacific cod. Modeling results suggest that Pacific cod bleeding prior to being sampled had less overall sampled blood loss. The coefficient value for bleeding Pacific cod was -8.88 and was statistically significant (t=-3.27, p=0.0014; Table 1). Interaction effects between blood and activity level were not included in the final model due to a lack of statistical significance and influence on the model fit.

Blood loss was greatest during the first 30 seconds after exsanguination, with blood amounts thereafter much lower. The mean and median blood loss during the first 30 seconds was 21.71 g and 17.60 g, respectively (Table 2). The 30 second period corresponded with a median percent body weight loss of 0.66%, with a lower 95 percentile of 0.59% and an upper of 0.73%. Mean blood loss amounts for the 1:00 minute and 1:30 minutes periods had similar means of 6.40 g and 4.31 g, respectively. Blood loss as percentage of body weight during these periods was 0.20% (1:00 minute) and 0.14% (1:30 minutes). Summation of blood loss across all periods resulted in a cumulative mean blood loss of 32.41 g that corresponded to approximately 1% of the median RW for Pacific cod.

Statistical differences in blood loss between time periods were evaluated by activity level using a two-tailed paired t-test. The groups designated as inactive and active were combined based on the previously discussed GLS regression analysis not showing statistical difference between the groups. Test results showed statistically significant different levels of blood loss (alpha= 0.05) between all periods for non-dead Pacific cod and between the 30 second period and all other periods for dead Pacific cod (Table 3). The 1:00 minute and 1:30 minutes period for dead Pacific cod did not have significantly different levels of blood loss (t=1.85, p=0.105). All significant results showed mean blood loss amounts to be lower in later periods, demonstrating that Pacific cod bled most during the initial 30-second period. However, only 8 fish were sampled in the dead activity category and thus statistical inferences are likely not robust. This is especially important considering the marginally significant t-values at 30 seconds versus the 1:00 minute and 1:30 minutes periods.

There was evidence that some of the differences in blood loss between time periods had small deviations from the normal distribution. Results from the paired t-test produced similar p-

values and significance (alpha= 0.05) as the non-parametric Wilcoxon paired rank test. Paired t-tests are robust against departures from normality and thus this result is not unexpected given the data distribution.

DISCUSSION

The highest volume and rate of blood less occurred during the initial 30-second period; blood continued to be lost at a lower rate in later periods. The majority of vessels would likely place flow scales as close to the bleeding station as possible due to space constraints on scale location (Alan Kinsolving pers. comm.). This placement would result in most vessels weighing Pacific cod within 30 seconds of bleeding. Pacific cod from this study bled most during the first 30 seconds; however, a finer temporal resolution was not sampled. In addition, other factors including the RW of the Pacific cod, whether the Pacific cod was bleeding due to the fish stripper or gaff wound, and the activity of the Pacific cod also influenced bleeding.

The study did not assess whether sampled Pacific cod and associated exsanguination characteristics were typical for the entire freezer-longline fleet. For example, obtaining a large sample size even on a single trip was difficult. During the sample trip, 48 magazines of gear (1,200 hooks per magazine) were hauled per day and between 100 and 150 cod were caught on each magazine of gear, yet due to logistical constraints only 134 cod could be sampled. For example, results suggest a larger number of dead or wounded Pacific cod would lower the average amount of blood loss, whereas a preponderance of large Pacific cod would result in a greater volume of blood loss. Even the location of the gaff wound likely influences the amount of blood lost. Thus, these attributes varying throughout the fleet would influence the bias on RW associated with bleeding.

Although the study cannot be extrapolated to the entire fleet, it provided important information about exsanguination patterns for Pacific cod. For example, the most extreme observations of blood loss were 1.4% in the first 30 seconds and 2% after 1:30 minutes. Overall there was low variation in the percentage of RW blood loss, with 95 percentiles ranging from 0.90% to 1.1%. The range of blood loss also incorporates potential variation in the time between when Pacific cod are bled and they pass over the flow scale. The study demonstrated a significantly lower rate of blood loss after the initial 30 second period, with low variation in lost weight. This suggests that the exsanguination time prior to weighing will play a significant role in the magnitude of the RW bias due to bleeding. This potential bias is reduced by roughly one third if the lag period is 30 seconds or shorter, which is the configuration most vessels are likely to have.

SAMPLING CHALLENGES AND FUTURE STUDY

Prior to disembarking to conduct the study, none of the sampling gear made it to F/V *Bristol Leader* because of flight schedule weather delays. The sampling gear included six three gallon buckets for holding the sample bags, jumbo Whirl Pak bags for collecting blood, several knives, and rags for wiping down the Pacific cod. Although the study was successful with available equipment, it may have been easier to collect the blood samples using buckets and sealable plastic bags that are wide enough to contain the head of a large Pacific cod. A larger collection apparatus may have allowed for a larger sample size by increasing sampling efficiency between trial periods.

The bleeding cut used by the freezer-longline fleet created challenges for collecting blood samples. For larger Pacific cod that lost a large volume of blood, it was difficult to ensure all the

blood made it into the bag. Also, when moving the Pacific cod between the sample periods it is possible the some drops of blood could have been lost. In order to address this situation in the future, a system could be constructed to suspend the Pacific cod above the collection bags and move the collection bags under the Pacific cod during the selected sample periods. In addition, the exsanguination cut is large enough that the heart muscle is occasionally cut out. The weight of the heart was included in the samples because this tissue loss is representative of the Pacific cod prior to going over the flow scale. The loss of the heart muscle may represent vessel-specific variation due to the person doing the bleeding. This type of variation was not captured in this study.

Many vessels will place the bleeding station within a few seconds of the flow scale so bled Pacific cod may go over the scale in shorter time than the 30 second interval. Temporal detail less than 30 seconds was not collected nor could a model be fit to determine projected blood loss during that interval. Future studies could select smaller time increments, which could be collected at 10 second intervals while remaining logistically practical. The greater temporal detail may also allow a model describing blood loss over time and the relationship between blood loss and the other covariates described in this study.

In conclusion, this study provides information about the rate of blood loss that Pacific cod experience based on a set of covariates. The time period from the initial cut is the most important covariate, and will have a substantial influence on the amount of blood loss, particularly in the first 30 seconds after the initial cut. Further study would be useful for a more robust extrapolation of these results to the entire freezer-longline fleet due to the limited sampling frame in this study and nature of the covariates. These covariates include activity of

Pacific cod, frequency of Pacific cod bleeding due to wounds, size distribution of Pacific cod, and variability in cutting techniques.

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Figure 1. -- Example of motion compensated flow scale used by trawl catcher processors
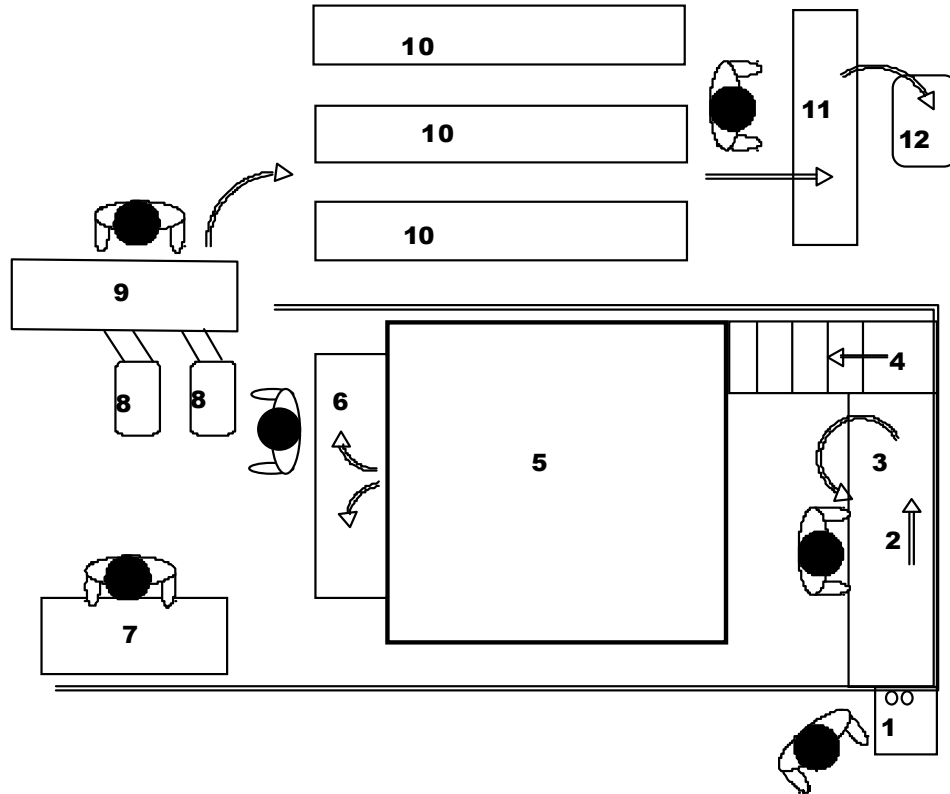
participating in quota programs in the North Pacific.

Figure 2. -- Generic layout of a freezer-longline factory and flow of fish into the factory. The numbers correspond to activity locations: (1) Fish are removed from the longline as they come on board by fish-stripper or roller man; (2) Fish are cut by the bleeder, and this is also generally the location where at-sea observers sample catch; (3) Un-retained catch is discarded. Catch is also discarded by the roller man at location 1 and small amounts are discarded inside the factory at locations 6, 9, and 11; (4) Fish enter the incline belt to bleed tank; (5) The bleed tank; (6) Fish flow out of bleed tank into a shallow pan; (7) The at-sea observer work area; (8) The fish are decapitated by machines; (9) Fish are gutted and placed on plate freezer pans; (10) Plate freezers where fish are "flash frozen"; (11) Frozen fish are glazed and packaged; (12) Bagged fish are stored in freezer hold.
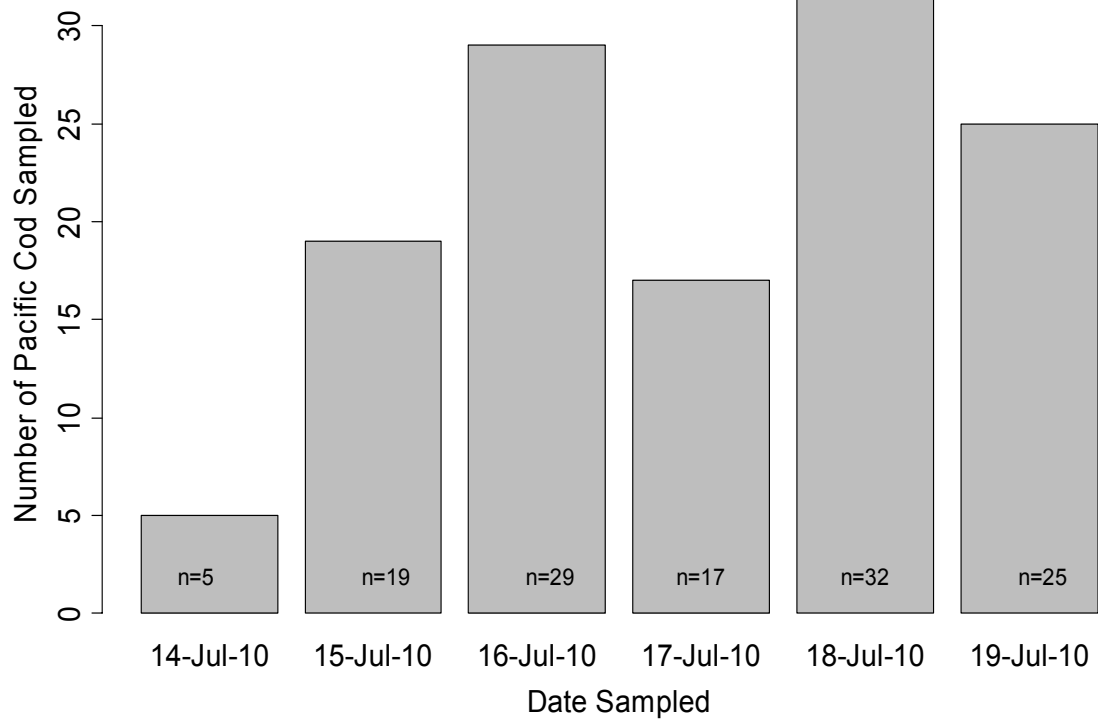
18

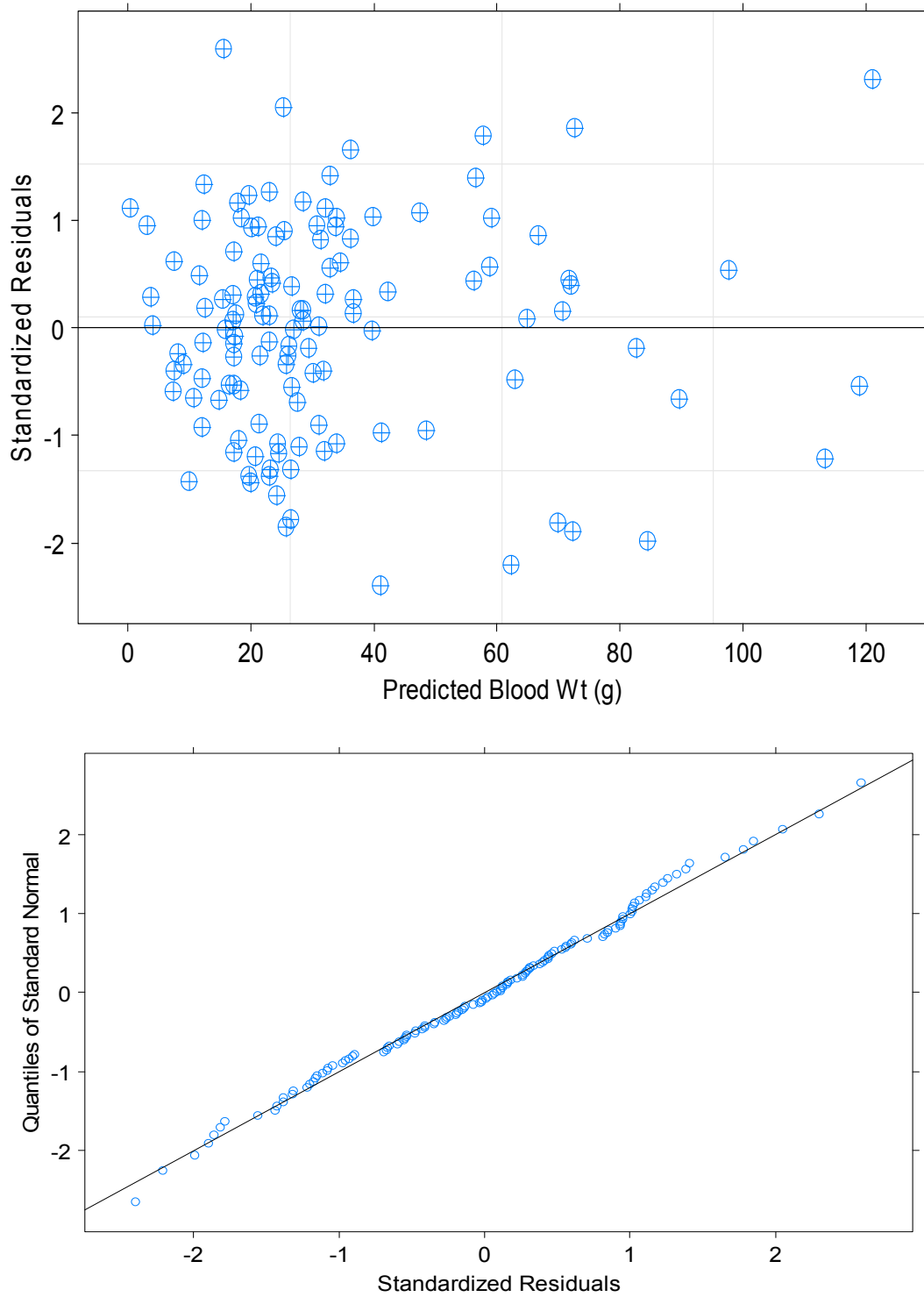Figure 3.-- Distribution of the number of Pacific cod that were sampled each day during the study period.

Figure 4. -- GLS goodness of fit plots. Top panel shows standardized residuals and predicted

weight of blood loss. Bottom panel shows quantile-quantile plot of the GLS fit.
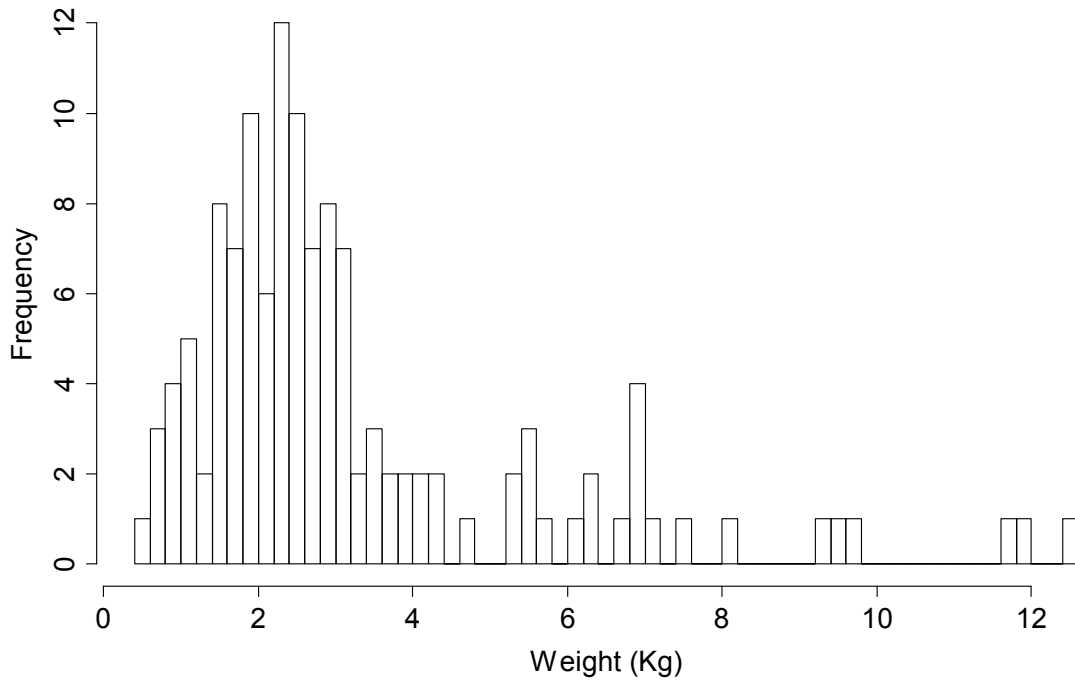
Figure 5. -- Distribution of sampled Pacific cod weights during the study period. Frequency bins correspond to 0.2 kg increments. GLS goodness of fit plots.
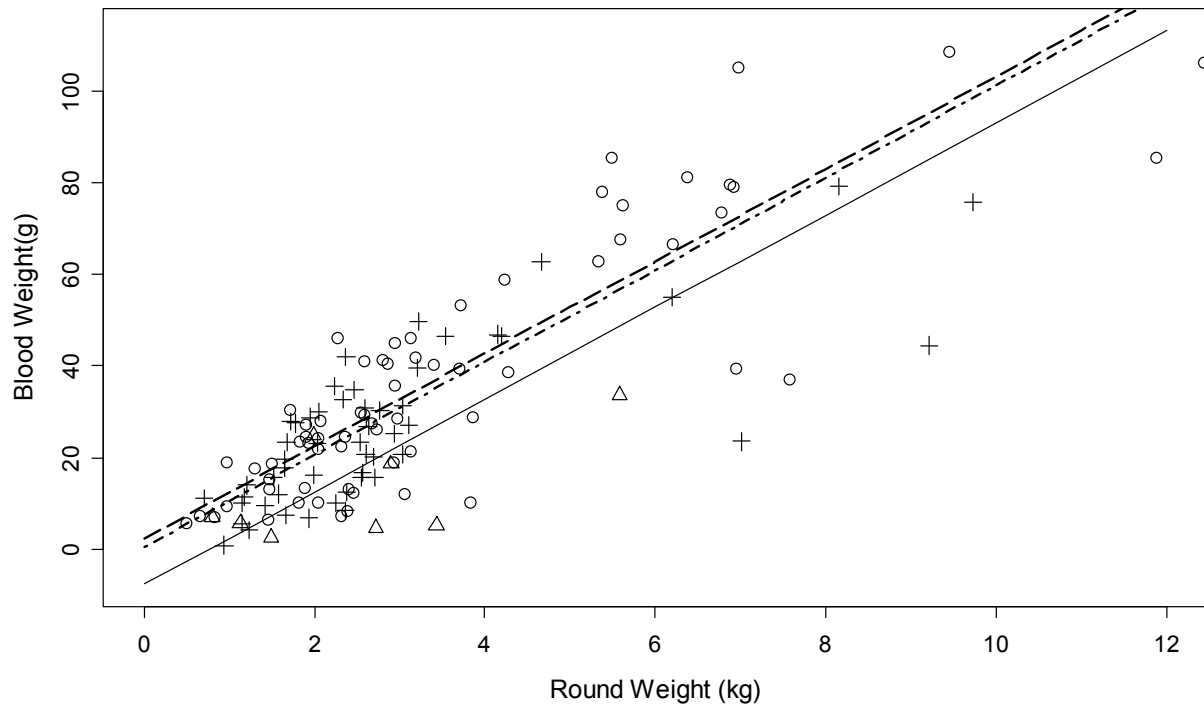
Figure 6.-- GLS model fits comparing blood loss with the RW of Pacific cod. The dashed model
fit line represents Pacific cod designated as "active", the dot-dashed line represents
Pacific cod designated as "inactive", and the solid line represents dead Pacific cod.
Raw data overlay the model fits and symbols indicate Pacific cod activity levels,
where circles represent active, crosses represent inactive, and triangles indicate dead
Pacific cod. The prediction dataset assumes Pacific cod were not bleeding prior to
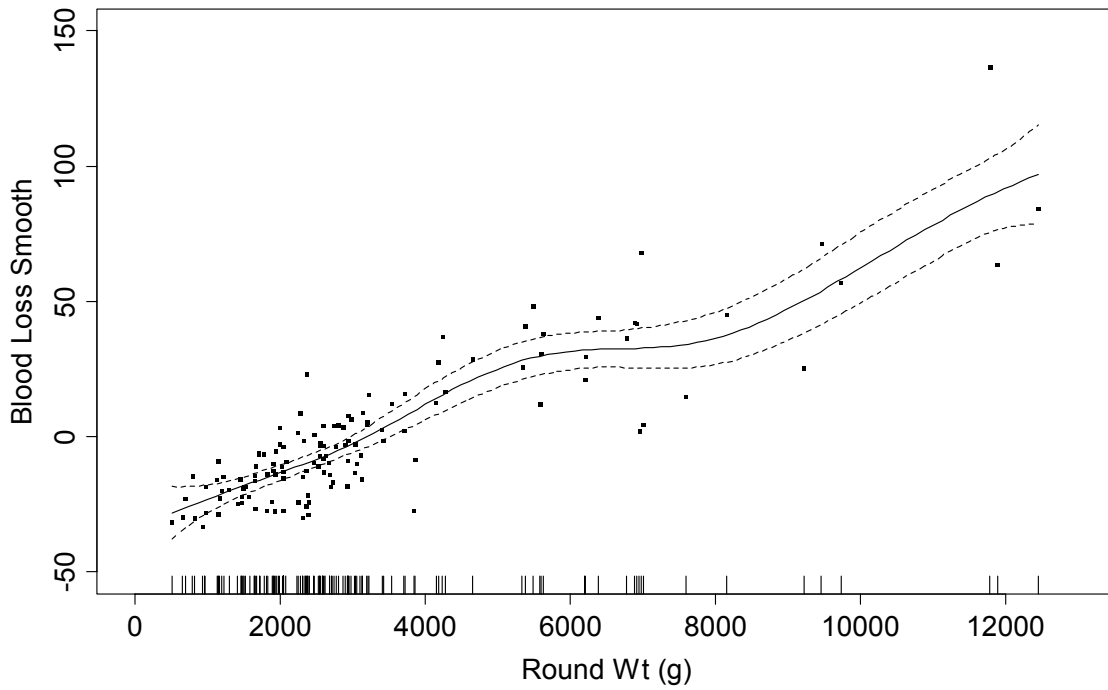being sampled.

Figure 7. -- Estimated smoother for the generalized additive model. The solid line is the

estimated smoother and the dotted lines depict the estimated 95% confidence bands.

The rugplot on the x-axis shows the relative sample density of Pacific cod by RW.

The raw data are shown as points relative to the smoothing function.

Table 1.-- Summary statistics of GLS model describing the relationship between total blood

loss, fish weight, and activity level. * denotes statistical significance at alpha=0.05.

Bleeding = yes indicates that the fish was bleeding prior to the sampling protocol

being applied.

	Coefficient	St. error	t-value	p-value
Intercept	2.32	1.91	1.21	0.23
Fish Wt (g)	0.0097	0.0006	16.27	<0.001*
Dead	-9.94	3.52	-2.83	0.006*
Not Active	-2.50	1.90	-1.31	0.19
Bleeding=yes	-8.88	2.71	-3.27	0.0014

Table 2. -- Summary statistics for blood weight in each measurement period. Note % blood

weight (BW) is the weight of the blood divided by the round weight. Bootstrapped

median 95 percentile intervals (bias-adjusted) are shown as Lower (L-95) and Upper

(U-95). Intervals do not include variance from scale error.

Time (minutes)	Sample type	Mean	Median	Minimum	Maximum	L-95	U-95
0:30	Wt (g)	21.71	17.60	0.30	125.80	14.30	19.80
	% BW	0.66	0.66	0.03	1.43	0.59	0.73
1:00	WT (g)	6.40	4.90	0.40	34.00	4.20	5.40
	% BW	0.20	0.20	0.03	0.66	0.18	0.22
1:30	WT (g)	4.31	3.10	0.10	24.00	2.50	3.60
	% BW	0.14	0.13	0.01	0.55	0.11	0.14
All	WT (g)	32.41	25.30	0.80	173.60	21.80	28.70
	% BW	0.99	1.03	0.09	2.01	0.90	1.11

Table 3. -- Within-group paired t-test (two-tailed) comparison for blood loss weight (g) by time period and activity. Group activity is indicated by "A" for not dead and "D" for dead.

Time (minutes)		1:00 (A)	1:30 (A)	df (A)	1:00(D)	1:30(D)	df (D)
	Diff	16.03g	18.22g		4.49g	5.23g	
0:30	t-value	11.75	12.41	118	2.47	2.40	7
	p-value	<0.001	<0.001		0.043	0.048	
	Diff	NA	2.18g		NA	0.74g	
1:00	t-value	NA	7.47	118	NA	1.86	7
	p-value	NA	<0.001		NA	0.105	