THE RELATIVE BIAS IN SAMPLING ESTIMATES OF PRODUCTION AND DISEASE PARAMETERS OF CAGED ATLANTIC SALMON

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ABSTRACT

The relative selection bias in dip net sampling of caged Atlantic salmon was investigated at two different stages of the production cycle. The studies occurred at a large, intensively managed farm with three grow-out sites in the Bay of Fundy, New Brunswick, Canada. The first cross-sectional study involved 25 cages of one-sea-winter salmon at two sites which were being size-sorted for management purposes. A pre-grading sample was obtained by crowding a group of fish within their cage and collecting three to five at a time in a dip net. A systematic random sample was subsequently obtained from the same cages during the size-sorting procedure. Weight and length measurements were recorded for each fish by each sampling method. The estimated means for weight, length, and condition factor (Wt/Lng^3) from each sampling method were compared separately at each site by a paired t-test.

The mean fish weight estimated by the dip-net sampling method was significantly heavier (102 grams, $p=0.013$) than that estimated by the systematic random sampling method at one site. The dip sample mean fish length was significantly shorter (.82 cm, $p=0.0001$) at one site and significantly longer (0.21 cm, $p=0.044$) at the other site when compared to the random sample. Condition factor was significantly greater (0.099, $p=0.0003$) at one site only. Within individual cages, the dip sample estimates of means ranged from 145 grams lighter to 355 grams heavier, from 1.7 cm shorter to 0.7 cm longer, and from 0.04 units less to 0.26 units more for condition factor when compared to estimates from random samples.

Descriptive statistics were generated for site and cage-level size parameters using systematic random samples. At the site-level, the distributions of weight, length and condition were significantly different from normal except for weight at one site. The intraclass correlation coefficients for cage effect were highest for condition factor (maximum 0.237) and lowest for length (minimum 0.07).

The study farm experienced a slight increase in fish mortalities with a noticeable increase in the prevalence of visceral granulomatous lesions during the period between the first study and harvest. The sampling method comparison was repeated using weight, length, condition factor, and the prevalence of granulomatous lesions as outcomes for the second study. A subsample from each method was also obtained for an examination of the prevalence of subclinical infections with *Renibacterium salmoninarum* as defined by bacterial isolation on selective media.

Prevalence of granulomatous lesions at necropsy was significantly higher ($p<.05$) in dip samples than in systematic random samples. Lesions were also significantly associated with smaller fish in one cage ($p<.05$).

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ABBREVIATIONS USED IN THE TEXT

CF - condition factor

95% CI - 95 percent confidence interval

cm - centimeter

cm^3 - cubic centimeter

df - degrees of freedom

g - gram

kg - kilogram

L - liter

Lng - length

m - meter

MSB - mean square between

MSW - mean square within

n - number of observations

OR - odds ratio

p - probability of type I error

SD - standard deviation

SE - standard error of the mean

wt - weight

1. INTRODUCTION

1.1 Sampling Strategies

Planned samples from a population are frequently used to monitor production and disease levels at fish farms for production management and scientific purposes. Epidemiologic studies involve sampling in field situations to describe the frequency or distribution of variables within a population or to assess specific associations between factors and disease or production levels (Martin, Meek, and Willeberg, 1987). Sampling methods and selection of the correct sampling units are major components of the study design and have an important impact on the validity of extrapolations from the sample to the population (Kleinbaum, Kupper, and Morgenstern, 1982).

The basic unit around which a sampling procedure is planned and statistical analyses based is referred to as the unit of concern. The sampling unit refers to the members of the population from which a sample is selected. The size of the sampling unit can vary from the individual to an aggregate of individuals but is never smaller than the unit of concern. It is critical to maintain the distinction between the unit of concern and the sampling unit throughout the design and analysis of a study for results to be meaningful (Cornfield, 1978).

In aquaculture settings, the simplest aggregates of individuals are most frequently tanks, cages, raceways, or ponds. Identifying, treating and measuring

individual fish is rarely possible in an aquaculture production setting.

A frequently observed phenomenon in fish culture is the "cage-effect", or tank-effect, where large differences in response to environmental influences occur between apparently identical groups (Michel, Tixier, and Mevel, 1984). Cage effect can account for a large part of the association attributed to the factors being studied and result in erroneous conclusions. Since samples may be taken from several cages and involve hundreds or thousands of individual fish, the degrees of freedom in statistical analyses must be correctly identified for the study to be critically evaluated. When the cage is identified as the unit of concern, the use of the mean performance of a set of cages in statistical analyses is usually appropriate (Wohlfarth and Moav, 1991). The chance that even the smallest differences will be falsely identified as statistically significant can increase substantially if the degrees of freedom are based on the wrong unit (Whiting-O'Keefe, Henke, and Simborg, 1984).

Once the units of concern are chosen, the next consideration in a field study is the identification of the sampling units, the sampling method and its representativeness of the target population. There are two main types of sampling in field studies : non-probability and probability sampling. Non-probability sampling does not rely on formal random techniques to identify units and the probability of selection is unknown. In contrast, probability sampling schemes in which every member of the population has a known, non-zero probability of being included in the sample, draws a sample by some method of random selection

consistent with these known probabilities. The probability of selection is taken into account whenever estimates are derived from the sample (Snedecor and Cochran, 1980).

Non-probability sampling methods include judgement sampling, convenience sampling and purposive sampling (Martin, Meek, Willeberg, 1987). There is no way to determine the precision of the sample estimate when non-probability samples are used since the probability sampling formulae for the standard error of the estimate and confidence limits do not apply (Snedecor and Cochran, 1980). Although non-probability sampling often provides information quickly and cheaply and many investigators believe that such methods can be used to obtain representative units of the population, samples often provide biased estimates of target population parameters (Thrusfield, 1986; Martin, Meek, Willeberg, 1987). The magnitude of bias is not reduced by larger sample sizes when informal sampling procedures are used and, in fact, may give false confidence in the resulting estimates (Kelsey, Thompson, and Evans, 1986).

Confusion frequently arises in the scientific literature when the sampling technique is called "random" but the description of the method lacks specific detail. In many of these instances, the true sampling method is likely a convenience or haphazard method. In aquaculture settings, a true random sample requires innovative techniques which would warrant special consideration in the materials and methods section.

The most common convenience sampling technique employed in the

Atlantic salmon industry of Atlantic Canada involves crowding all or part of a population and then collecting individuals with a dip net (referred to as the *dip* or *dip-net* or *crowd and dip* method). The potential for bias exists in that, although the person dipping may not try to select certain fish, some fish may have characteristics which make them more easily caught by a dip net.

Another popular method is to collect individuals with a dip net during the administration of feed. This method is similar to estimating wild fish population characteristics from a sample caught by baited traps (Magnan, 1991) or anglers using baited hooks. The fish in these samples will be from the part of the population that is eating and, thus, more likely to be healthy and growing.

It is preferable to use probability samples unless it is not feasible or prohibitively expensive (Snedecor and Cochran, 1980). Probability sampling methods use formal, pre-determined random processes which prevent any selection influence by the investigator. Simple random and systematic random sampling are examples of probability sampling. Simple random sampling requires that a list of all members of the study population be identified prior to the study and then, using a random number table or computer-generated random number list (eg. Byers, 1991), the sample units are selected. Sampling by the simple random method is difficult to put into practice in aquaculture field studies since there are frequent errors in the estimation of the total cage population (Moring, 1989), studies often involve large populations, and identification of large numbers of individuals is difficult.

Systematic random sampling has many of the advantages of simple random methods but with fewer restrictions and easier application to aquaculture situations. Levy and Lemeshow (1980) give an excellent account of the specific details of systematic sampling. Briefly, a systematic sample is achieved by determining the desired interval, k, making a formal random selection of the starting point, j, within the first interval and then selecting the individuals corresponding to $j, j + k, j + 2k, j + 3k$, and so on, until the entire sampling frame is exhausted. The identification of individuals can be through a unique number or name on each animal or by the order of passage of the entire population past a point in single file.

The primary advantage of systematic random sampling over a simple random selection procedure is its practicality in field situations. However, if the characteristic being estimated is related to the chosen sampling interval or the order of the sampling frame, then estimates may be biased and the standard error inaccurate. For the purposes of this study, fish sizes are assumed to be in random order as the fish are removed from the cage by a pump. A systematic sample can be considered equivalent to a simple random sample under these assumptions (Levy and Lemeshow, 1980) and similar procedures can be used to calculate the variance of the means.

When the population is small and the interval does not end with the last individual, individuals that present after the last selected unit will not have the same opportunity of being included in the sample. This could introduce a bias if

the excluded part of the population tends to have different characteristics than the included part. This potential bias is of little concern if the population is large and the sampling interval is comparatively small (Martin, Meek, Willeberg, 1987).

1.2 Definition of Bias

The term *bias* can have different meanings depending on the scientific context. The statistical definition of the bias of an estimate is the average of the errors of the estimate. To determine the sampling bias, the investigator must take repeated samples of the same population and be able to identify individuals within the population (Snedecor and Cochran, 1980). Thorburn (1992) evaluated the randomness of samples collected by dip-net methods from rainbow trout in tanks using this type of method. It would be very difficult to evaluate the randomness of this sampling technique in a sea cage production system since repeated sampling procedures are stressful to fish and the identification of large numbers of fish is usually cost prohibitive.

Selection bias has also been defined as the distortion in the estimate resulting from the manner in which subjects are selected into the sample (Kleinbaum, Morgenstern and Kupper, 1981). Systematic error occurs when there is a difference between the actual parameter being estimated and the true parameter of interest (Kleinbaum, Kupper and Morgenstern, 1982). The systematic error definition will be used when referring to bias in this thesis.

1.3 Sampling Opportunities During the Atlantic Salmon Production Cycle

The production cycle of cultured Atlantic salmon, from egg to harvest, includes a minimum of 18 months in freshwater followed by 16 to 20 months in seawater. Very few opportunities exist in this production cycle when the entire population is presented in such a fashion that all individuals can be sampled by a simple random or a systematic random method. However, there are certain production practices which seem to be adaptable to systematic random sampling.

Whenever the entire population is handled for some reason, systematic sampling can be considered. The first opportunity for such samples occurs in the freshwater hatchery when fish are transferred from fry units to larger tanks. Although it is not currently practised, fish could be forced rapidly through a channel in single file and systematically selected during the frequent grading procedures occurring in the hatchery. A large amount of mixing of fish from different tanks usually occurs and investigators should pay special attention to which population is sampled and with what time frame the sample is consistent (i.e. pre- or post-mixing).

Systematic samples might also be possible as the fish leave the hatchery to be transferred to sea cages. At this stage in the production cycle, the number of smolt purchased from the hatchery by the grower are estimates obtained one to two months previously. Smolt numbers are obtained by first calculating the average weight of each smolt in a small group and then dividing the total weight

of a group of fish by the average fish weight. The final counts are estimates which can have up to 15% error (Piper et al., 1986). The random error would be expected to have a mean of zero and, given that systematic error did not occur due to the sampling method, the growers will receive more fish in a group as often as they receive fewer fish from the same hatchery. However, the grower requires an accurate estimate of size and number of fish in each cage for appropriate management decisions regarding feed, mortality rates and transfers between cages. For these reasons, a complete count and a representative sample of fish at the point of transfer from hatchery to sea cages would be very useful.

At many sea cage sites in the Bay of Fundy, Atlantic salmon smolt are stocked at densities which will limit growth during the second growth season in sea water. The population of the original cage is reduced by connecting an empty cage to the heavily stocked cage and forcing about half of the population to swim through a channel to the empty cage. The channel is often deep (up to two meters) and wide (approximately two meters), resulting in the passage of several fish simultaneously. By altering the channel to permit single file passage, devising a method of collecting individuals, and passing all fish from the original cage into new cages, systematic samples would be possible at this point in the production cycle. Fish have usually grown from about 100 grams to over 500 grams during this time with a great potential for mortality due to smoltification failures and exposure to marine pathogens. Consequently, the farm records are likely to require revision of population numbers and biomass estimates following the first

six months in seawater.

Salmon may be available for systematic samples as they pass through a size-sorting apparatus. Larger farms tend to perform size-sorting procedures using fish pumps and automatic graders. Smaller farms often perform this task by visually assessing each fish collected with a dip net in the first autumn, and/or by harvesting early maturing fish during the second summer of sea growth using the same collection methods. The population prior to grading can be sampled as fish exit the cage while a second sample will be required at each of the cages after grading to estimate population indices of those cages. Any characteristics of the original cages will be obscured by the mixing of populations.

The final opportunity for collecting systematic samples occurs at harvesting. Many farmers tend to harvest only part of a cage at a time by removing larger fish and returning smaller individuals to the cage. These different harvesting practices would complicate the sampling procedure by requiring the early harvest of selected small fish if they were included in the sample. The entire cage population would need to be available for sampling even if the farmer reached the total desired harvest number part way through the cage.

As the aquaculture industry relies more and more heavily on health professionals to maximize growth and minimize disease, there is a greater emphasis on epidemiological studies to elucidate factors associated with disease and investigate production losses. Field studies become increasingly important because of their direct applicability to the fish farmers' experiences. Study design

and statistical analyses must be unbiased for the results to be of value to scientists and producers alike.

Many statistical methods used for hypothesis testing in epidemiology studies require that the outcome variables be sampled from normally distributed populations that have equal variance between groups (Glantz, 1987; Glass, Peckham and Sanders, 1972; Ricker, 1973). These assumptions have been infrequently tested by formal methods for the cage culture of Atlantic salmon. The samples collected in this study were of sufficient size to examine the variance of weight and length variables of one-sea-winter Atlantic salmon within cages as well as between cages.

The research presented in this thesis will attempt to identify some potential biases or weaknesses in current aquaculture field study methods so that more appropriate interpretation of these studies may be made. The crowd and dip sampling method will be examined for selection bias at two different stages of the Atlantic salmon production cycle: first, after one sea winter, and second, immediately prior to harvest. Convenience samples will be compared to systematic random samples for the estimates of cage mean weight, cage mean length and cage mean condition factor. The difference in disease prevalence by the two sampling methods will also be examined for harvested fish.

1.4 Sampling for Estimates of Bacterial Kidney Disease Prevalence

Although there has been considerable research emphasis on the causative agent of bacterial kidney disease, *R. salmoninarum*, its effect on the host tissues and its detection in experimental fish, there has not been nearly this emphasis on the risk factors of the disease or its effect on fish production. To the author's knowledge, there are no reports of population surveys which identify the prevalence of BKD with regard to the intrinsic host factors of individual fish from the same population. Host factors may distort the observed association between environmental factors and disease (Martin, Meek, Willeberg, 1987). Hence, investigations into the natural history of bacterial kidney disease and its transmission between populations may require analyses which control for these host factors.

For example, the host factors of size, sex and sexual maturity may influence the risk of diseases such as BKD. The presence of an association of individual fish size with BKD infection would be important knowledge for several reasons: fish infected with *R. salmoninarum* may have reduced growth rates compared to non-infected individuals, or smaller, less dominant fish may be more susceptible to BKD, or both may occur together. Since prevention of vertical transmission is an important part of various control strategies (Elliott, Pascho, and Bullock, 1989), differences in susceptibility to infection between sexes and maturity levels may limit their success.

There are many diseases associated, directly or indirectly, with the anatomic and/or physiological differences between sexes (Martin, Meek, Willeberg, 1987). Pascho, Elliott and Streufert (1991) provide screening data on the proportion of female spawners infected with *R. salmoninarum* compared to male spawners which, although not calculated in their report, represents a significant difference between sexes. "Randomly selected" female spawners had a 0.37 prevalence of infection (95% CI: 0.31, 0.42) compared to 0.21 for male spawners (95% CI: 0.16, 0.26). There are very few other reports which include the BKD prevalence stratified by sex. Since there have been reports of hatchery grading altering the sex ratios within smolt groups (Ritter et al., 1986), the role of sex as an intrinsic host factor may be important.

The final objective of this study will be to examine the association between some selected intrinsic host factors and the presence of bacterial kidney disease in market-sized Atlantic salmon. The host factors of interest will include fish size (measured by weight, length or condition factor), sex and sexual maturity.

2. RELATI[illegible]ELECTION BIAS IN ESTIMATING SELECTED PRODUCTION PARAMETERS AFTER ONE-SEA-WINTER

2.1 Introduction

Estimating parameters related to disease and production in aquaculture requires that the collection of representative samples be feasible at various times during the 36 to 40 month production cycle. Opportunities for simple random samples are very infrequent in Atlantic salmon culture. Non-probability sampling, in the form of manual dipping of fish which have been crowded within their cage, is often the most practical selection technique available to aquaculturists. The randomness of various sampling techniques has been examined in freshwater tank situations (Thorburn, 1992; Seeger *et al.*, 1977) but sea cage sampling techniques have never been evaluated to determine if the sample is representative of the population within the cage.

The most commonly used sampling technique in sea cage salmon culture, the crowd and dip method, is executed by dividing the net pen and crowding a portion of the population to allow the manual dipping of several fish at a time. It is very difficult for the person catching the fish with the dip net to choose the size or appearance of the individuals in the sample. For this reason, many investigators have regarded this technique as a form of random sampling. However, by definition, it is a convenience sample (Martin, Meek, Willeberg, 1987).

Observations confirm at least one individual frequently escapes from the dip net as a group of fish is brought to the surface. Certain characteristics may be common to the fish that remain out of reach of the dip net or manage to escape capture distorting any dip net sample of the population. If the population parameters estimated from such a sample are related to the characteristics which affect the probability of being included in the sample, then the validity of these estimates becomes doubtful.

Simple random sampling yields unbiased samples where each member of the target population has an equal probability of being included in the sample (Lillenfeld and Lillenfeld, 1980). Knowledge of the true population size is required for simple random sampling (Levy and Lemeshow, 1980), but it is rarely available in fish farming. Frequent discrepancies occur between estimated and actual cage populations in Atlantic salmon culture due to inaccurate estimates of initial fish counts, escapes, predators, decomposed mortalities, or other unaccounted losses (Moring, 1989).

Other forms of probability sampling require that the probabilities of selection are known, but not necessarily equal. In contrast, non-probability sampling can give no objective assurances that potential biases have not entered into the method of selecting a sample (Lillenfeld and Lillenfeld, 1980). Procedures with unknown sampling probabilities have limited value in epidemiology since a statistical assessment of the accuracy and precision of the characteristics of the selected sample in representing the population can not be

made.

Systematic sampling, a form of probability sampling, approximates simple random samples if there is random ordering of individuals within the target population (Levy and Lemeshow, 1980). Systematic sampling has many advantages in aquaculture. Since sampling will continue throughout the reference population, this method requires that knowledge of the population size be approximate only. The sample size will reflect the actual total population size. Due to the constant interval between the selected individuals, samples can be selected quickly and without constant reference to a random number list.

Population mean weight and length are important indicators of the performance of Atlantic salmon stock both for the producer and for the epidemiologist. The producer requires the knowledge of average fish size and cage biomass to predict feed pellet size and daily feed consumption. Farm managers depend on these estimates for health monitoring and marketing strategy decisions. Fish health scientists use sample estimates of the mean weight and length to monitor responses to husbandry and disease control strategies. Mean weight and length were chosen as variables of interest in this study because of their importance to producers and scientists and also because there existed an opportunity to objectively measure large numbers of fish by both convenience and systematic samples.

Analysis of variance and linear regression are common statistical tests utilized by aquaculture scientists. Important to the correct interpretation of these

tests are the assumptions that the observations are drawn from normally distributed populations in which the variances are the same even if the populations means are different (Glantz, 1987; Ricker, 1973). The effects of non-normality alone are often slight but heterogeneous variances may seriously affect the level of significance (Glass, Peckham, and Sanders, 1972).

There are few published observational studies describing population frequency distributions of indices, such as weight and length, on a site or cage basis for salmon cultured in sea cages. Fish length often approximates a normal distribution in wild fishery sampling data (Ricker, 1979) but this has not been observed so readily in aquaculture situations. In populations of cage-reared channel catfish, there is a tendency for weight frequency distributions to be skewed to the right and for a few exceptionally large fish to occur (Konikoff and Lewis, 1974).

The variation of weight between individuals is often very large when compared to the mean weight of individuals within populations. In their early study of the effect of density on growth of cultured Atlantic salmon fry, Reftsie and Kittelsen (1976) report standard deviations of weight which almost equal the group mean. Huse et al. (1990) describe weights in five sea cages of Atlantic salmon as one of the outcomes for their examination of the effect of shading on growth, mortality and ectoparasite infestation. The standard deviation represents about 30% of the mean weight in their study using 1500 to 2200 gram fish. However, Reftsie and Kittelsen (1976) do not describe their sampling method and

Huse et al. (1990) used a convenience sampling method. Neither group reports an evaluation of normality within groups of fish or an examination of variance homogeneity between groups.

Weight and length frequency distributions are presented as histograms by Delabbio, Glebe and Sreedharan (1990). The study involves four groups of 100 fish each which makes it difficult to extrapolate their results to a cage population of 3000 fish or more. Their distribution appears unimodal and possibly skewed to the right. However, a formal description of each distribution, including skewness and kurtosis, is not provided nor are there any results of tests for normality given.

Size-selective mortality may contribute to the creation of non-normal distributions in cultured fish. Ottera (1992) reports a right skewed weight distribution in small groups of cod (*Gadus morhua* L.) surviving a growth experiment. Size-selective mortality in early sea life has been demonstrated in juvenile chum salmon as well (Healey, 1982).

Variation in body length between individuals has been investigated for wild stocks of spawning Pacific salmon (Beacham and Murray, 1985). Male salmon tend to be larger and more variable in length than females. If this difference was present in other populations, the frequency distributions for size within cages could be expected to vary depending upon the ratio of males to females within it.

The primary objective of this study was to test the null hypothesis that the convenience sampling technique, dipping from a crowded group, selected fish with the same mean weight, length and condition factor as a systematic sample of the

same population. If the null hypothesis was rejected, then the secondary objective included an examination of the consistency of the difference between cages and between sites at this farm. This sampling experiment was also designed to assess the feasibility of performing systematic samples in an Atlantic salmon sea cage production facility under field conditions.

Distribution normality and homogeneity of variance assumptions are important considerations in hypothesis testing. Thus, a final objective of this observational study was to use systematic random samples to describe the distributions of weight and length in several cage populations of Atlantic salmon reared in a production facility under similar environmental and management conditions.

2.2 Materials and Methods

2.2.1 Study and Target Populations

The reference population of interest for this study was the approximately 50 Atlantic salmon aquaculture sites on the Canadian side of the Passamoquoddy Bay in the Bay of Fundy which operate within a 20 kilometer radius of each other. There were another 20 sites raising Atlantic salmon located on the United States side of the same body of water to which the results could be generalized but these sites were not considered when selecting the study population.

The selection of the farm site for this study was a convenience sample from the Canadian sites of Passamoquoddy Bay. It was necessary that the study farm have a large number of cages from the same year class with a documented disease and production history. The co-operating farm was the first such farm contacted.

The study population consisted of one farm with three sites in operation and a total of approximately 100 cages of Atlantic salmon. All cages involved in the study were 12 m by 12 m by 6 m deep. Each site was separated from its closest neighboring site by 500 to 1500 meters of water with site 1 and site 3 being the farthest distance from each other.

All cages in the study contained salmon derived from smolt transferred in the spring of 1990. Smolt were normally stocked at 6000 per cage at densities of 0.5 to 0.75 kg/m^3. The density increased to about 5 to 7 kg/m^3 by late autumn of

1990 at which time the cage populations were routinely divided into two cages by swimming fish from one cage to another (empty) cage. Cage populations normally remained at 2500 to 3500 individuals until harvest in the autumn of 1991 or winter of 1992. The final stocking density may have reached 17 to 19 kg/m^3 by harvest.

Size-sorting of fish did not occur every year although the farm management elected to perform the procedure in 1991. The farm preferred to grade fish in the spring (usually late April to early May) after the smolt of the next year class had been transferred to seawater and the water temperature was between 5 and 8 °C. The stocking density in May of the second year in seawater (prior to size-sorting) was typically 6 to 8 kg/m^3.

2.2.2 Sample Size Determination

An independent sampling was performed on one-sea-winter salmon in July of 1989 by the farm staff. The sample size had been arbitrarily set at 100 fish from 25 cages for which weight and length measurements were recorded. A crowd and dip selection method was used. The standard deviation of fish weight among cages was 0.254 kg and for fish length it was 2.46 cm.

The distributions of weight and length observed in 1989 were expected to be similar to the distributions for 1991 fish. The true standard deviation was possibly larger if there was bias in the dip sampling technique. Table I demonstrates the various sample sizes required at different levels of type I and

type II error for different estimates of standard deviation for weight (page 45, Martin, Meek, Willeberg, 1987). A sample size of 131 was considered the lowest acceptable under these circumstances and a preferred sample size would be greater than 180 fish per cage.

The standard deviation of the difference between dip and random samples was unknown. Thus, it was difficult to estimate the minimum number of cages required in the study. It was decided to perform the two sampling methods on all cages available at the co-operating farm.

Table I

Summary of Sample Size
Calculations for Weight Within Cages

d[a]	α[b]	Power[c]	s[d]	n[e]	s[d]	d[a]	n[e]
.10	.01	.80	.25	146	.51	.10	608
.10	.05	.80	.25	98	.51	.10	405
.10	.01	.90	.25	186	.51	.10	769
.10	.05	.90	.25	131	.51	.10	542
.10	.01	.95	.25	224	.51	.10	924
.10	.05	.95	.25	163	.51	.10	673

[a] d = acceptable difference (kg)

[b] α = type I error

[c] Power = 1 - β

[d] s = standard deviation

[e] Formula used for determination of sample size (n) for *t*-test:

$$n = 2(Z_\alpha + Z_\beta)^2 s^2 / d^2$$

where Z_α=2.58 when α=0.01, Z_α=1.96 when α=0.05 and Z_β=1.65 when β=0.05, Z_β=1.28 when β=0.10, Z_β=0.84 when β=0.20

2.2.3 Sampling Protocol

The potential for heirarchal organization within groups of Atlantic salmon has been reported (Gunnes, 1976; Huntingford *et al.*, 1990) and its effect was taken into consideration in the sampling design of this study. The crowd and dip sample was taken prior to the systematic sample so that the heirarchy within each cage was undisturbed. As well, fish from different cages were likely to be mixed during the grading making it necessary to sample by the crowd and dip net method first. The systematic random sampling technique could be performed at any time and be expected to obtain similar samples.

Although grading may not be more than a moderate stress to salmon, repeated handling procedures can produce additive physiological stress responses (Flos, Reig, Torres, and Tort, 1988). Each time the cage was sampled, it was considered a stressful event for the fish. The time delay between samples in this study was never less than seven days to minimize adverse reactions by the fish and never more than 14 days to minimize growth between sampling events.

The order of cages to be sampled was dictated by the farm husbandry concerns of rising water temperature, site and cage location, and the order of planned size-sorting. The order of sites to be sampled was dictated by the results of the crowd and dip samples and similar concerns as listed for the order of cages. Originally, the farm planned to size-sort three sites with 11 cages at site 1, 20 cages at site 2, and 8 cages at site 3. A crowd and dip sample was taken from

each of 39 cages representing a total sample size of approximately 8,000 fish. However during the size-sorting procedure, the water temperature increased more quickly than expected (to 7.5°C) and technical problems with the fish pump necessitated that farm management cancel the size-sorting planned for the eight cages at site 3. For these reasons, only 31 cages at two sites were available for the systematic samples.

2.2.4 Selection by the Crowd and Dip-Net Method

The design of the net-pens (Figure 1) allowed the division of the water space into compartments but did not allow for the exact number of fish in each compartment to be controlled. Prior to crowding the fish, the central portion of the net was pulled to the surface to divide the population approximately in half. One half of the population was then crowded into approximately one-third of the space on one side of the central divider (Figure 2). The degree of crowding was limited by the necessity to minimize stress over the sampling period.

Once the fish were sufficiently crowded and the anaesthetic induction tank was ready, a staff member used one of two standard dip nets (circular opening of approximately 75 cm diameter, on a 3 meter handle) to transfer a group of fish from the net-pen to the anaesthetic induction. Personnel performing the strenuous task of dipping were not predetermined and so varied from cage to cage. The number of fish in each dip was dictated by the ability of the person

dipping to catch fish in the net, the crowding level in that cage and the request of management to transfer no more than five each time to minimize stress. As fish were dipped into the anaesthetic induction tank, the number of fish in the group was recorded by the record-keeper.

It was not possible to separate the fish into dipped groups identified by their order of removal from the cage since this would have necessitated five or more anaesthetic induction tanks. To identify the general order in which fish were dipped from the cage, the records included the order of transfer from the anaesthetic tank to the measuring station.

Once the measurements were recorded and the fish recovered from anaesthetic, the fish were returned to the side of the cage where sampling was not occurring (Figure 2). Hence, the sampling was non-replacement.

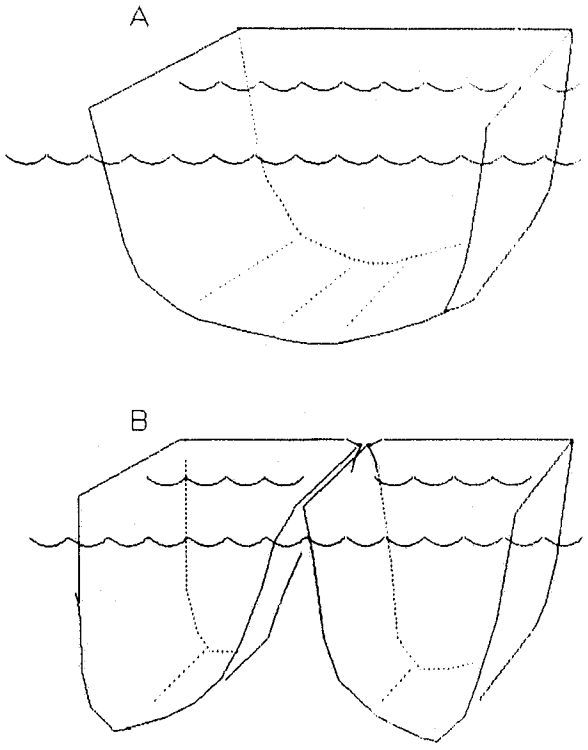


Figure 1. Schematic diagram of net pen design and division. The fish population was divided by pulling the central portion of the net to the water surface. A.) unaltered cage, and B.) cage divided.

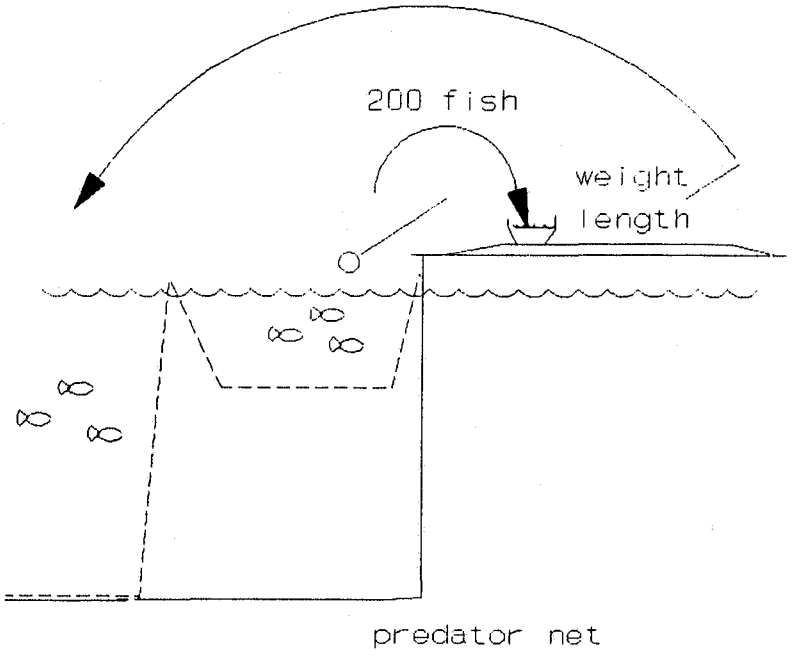


Figure 2. Schematic diagram crowd and dip sampling technique. The cage population was divided and fish were crowded into one end. About 200 fish were dipped from the group, anaesthetized, weighed and replaced.

2.2.5 Systematic Random Selection

At sites 1 and 2, the same cages as sampled by the crowd and dip method were re-sampled using a systematic random selection process. The systematic samples were taken seven to 14 days after the crowd and dip samples. The entire population of fish in the cage was crowded towards one side of the pen by lifting the net to the surface on one side and proceeding towards the center (Figure 3). At the center, a visual inspection assured that all fish were indeed located in one half of the cage. The net was then released except for the center where it was left attached to act as a barrier. In this manner, the cage was divided into compartments in which all of the cage population was located in one half and the other half was void of fish.

A fish pump (Silkstream, Innovac Technology, Richmond, B.C.) was used to transport fish from the cage to the top of the size-sorting apparatus, a change in elevation of three to four meters. The fish were gradually crowded towards the entrance of the fish pump as the numbers remaining in the cage decreased. The degree of crowding was controlled to reduce stress to the fish and still pump fish as rapidly as possible without large groups entering the pump simultaneously.

Prior to reaching the grading apparatus, all fish travelled through a de-watering box where fish passed over aluminum bars which allowed most of the water to drain away. Once through the de-watering box, the fish slid onto a flat one meter by one meter table at the entrance to the grading apparatus. Fish

could present in any orientation, but most frequently presented head or tail first. Fish would usually enter onto this table in rapid succession but in a manner amenable to counting individually. The passage of any body part under a support bar was used as the identification order for systematic sampling at which point the selected fish were removed from the table and re-directed to the measurement station.

Occassionally, fish did not have enough momentum to exit from the de-watering box on their own. This occurred more frequently when the fish were alone or in small groups. To prevent any selection process other than the order in which fish entered the area, assistance was not given at the de-watering box unless the fish was alone, in which case it was assisted and counted as the next fish.

The first fish (j) sampled from each cage was predetermined by a random number list generated by drawing a number from a hat. Depending on the estimated number of fish in each particular cage, every tenth, twelfth or fifteenth (i.e. kth) fish was selected after the initial randomly chosen fish (i.e. $j, j + 1k, j + 2k$, and so on), where j was less than or equal to k. A sample size of 200 or greater was attempted. Although error in estimates of the number of fish within a cage resulted in some between cage variation in the sample size, the randomness was preserved by continuation of the systematic sampling through the entire population within the cage

On occasion, the true order for systematic sampling was lost when fish exited too quickly from the de-watering box. When this occurred, the person

selecting the fish cleared away all fish on the table and sampled the next one to pass under the support bar. A quick judgement was then made as to the approximate number of fish missed and every fifth to eighth fish was selected until the deficiency was made up. Selection was never made from fish that were already on the table. All fish were identified by the order of entry onto the table. The selection of fish during disrupted counts occurred in less than ten percent of each sample.

The tasks of fish selection and weight/length measurements alternated between two people only, the author and a technician.

At this point, the fish were either selected for weight and length measurements (described in section 2.2.6) or they proceeded through to the size-sorting apparatus. The apparatus, constructed locally for the study farm, had four channels consisting of dividers attached to gradually widening bars. Small and medium fish passed through into respective compartments which lead to different cages through flexible tubes. The larger sized fish stayed atop the bars to pass through another flexible tube into a third cage. Fish were counted as they exited from each tube and entered their new cage. A total count for the pre-sorted cage was obtained by adding all graded fish and any mortalities removed at the pump or grader.

Re-introduction of the fish which were selected for weight and length measurments occurred at the top of the grader where they were not available for selection again. These fish would then be graded and counted in the same

manner as the non-selected fish. Fish that were injured by the pump were not treated differently with respect to sample selection. However, these fish were not returned to the grader unless there was a good chance for recovery. Pump mortality counts were maintained for the purposes of calculating total pre-sorted cage numbers.

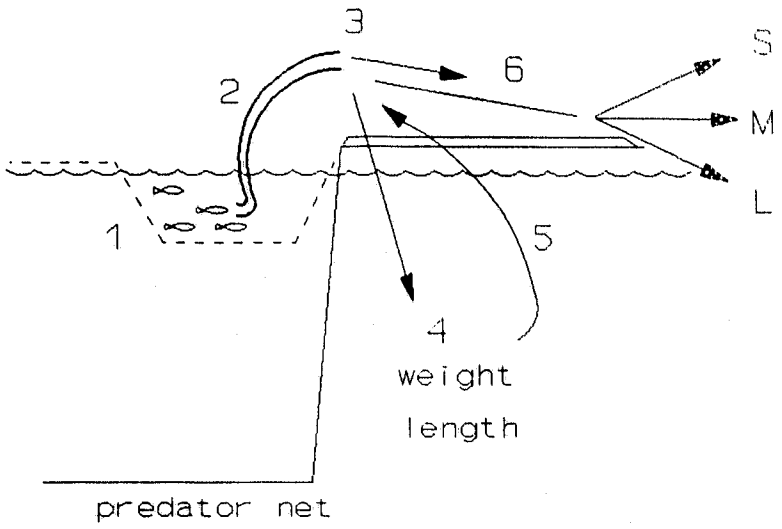


Figure 3. Schematic diagram of systematic sampling technique. All fish were crowded (1) towards the fish pump (2). They were systematically selected (3), anaesthetized for weight and length measurements (4), and recovered. They returned (5) to the grader to be sorted by size: small (S), medium (M), or large (L).

2.2.6 Anaesthetic Induction and Recovery

Fish sampled for weight and length measurements were handled in the same manner regardless of sampling method employed. An anaesthetic induction tank, consisting of one (or sometimes two) 1.5 meter by 1.5 meter by 1.5 meter deep plastic box(es), was freshly established with each new cage. Benzocaine, dissolved in acetone at a concentration of 28 grams per litre, was added to the water in amounts dictated by effect (5 to 10 mg/L) and mixed thoroughly.

Induction time, from entry into the anaesthetic bath to the point of lying still on a measuring board, was normally five to ten minutes. In the case of the crowd and dip net sampling, fish were added to the induction tank in groups as they were caught by the dip net at a rate approximating the rate of removal for measurement.

For the systematic random samples, the selection rate of fish intended for weight and length measurements was dictated by the speed with which fish were pumped to the size-sorting apparatus. On six occasions during the random sampling procedure, technical difficulties arose with the generator or weigh scale whereby the induction tank was becoming dangerously overcrowded and the selected fish were at excessive risk of anaesthetic death. Systematic sampling was discontinued and fish already in the anaesthetic induction tank were re-routed past the measurement station and into recovery. The incompleteness of a systematic sample was noted on the record forms for those particular cages.

2.2.7 Measurements

Length measurements were done on a custom-made trough. The device was a 15 centimeter diameter, sagitally-cut polyvinylchloride (PVC) pipe with lines drawn every one centimeter on the inside curve. The total length was 90 centimeters. Three such troughs were utilized, one for each site. There was no exchange of measurement apparatus between sites, thus minimizing the possibility of disease transfer. The same trough was used for the measurements of dip net and random samples at the same site so as to avoid the introduction of a systematic measurement error between samples.

Fish weights were measured using the length measuring device placed on top of a wooden trough made to fit on top of the weigh scale. The same wave compensating scale (SS-J80 SeaGoing Scale, Microweigh Industries, Vitchburg, VA, USA), which measured to the nearest ten grams, was used for all measurements (dip net and systematic random samples).

2.3 Statistical Methods

2.3.1 Comparison of Cage Means by Different Selection Methods

All individual fish weight and length measurements were entered into the database management system DBase III Plus (Ashton-Tate, Torrance, CA, USA, 1985). The selection method, site and cage identification number and dip number for exit from the anaesthetic tank were used to identify individual fish. Initial data entry errors and obvious outliers were identified by examining extreme values for weight or length. Only cages with complete data from both selection methods were used in any further analyses.

Statistical analyses were performed on the SAS statistical software package (SAS Institute Inc., Box 8000, Cary, NC, USA, 1985).

Condition factor (C.F.) was calculated using the Fulton-type formula:

$$C.F. = \frac{WT}{LNG^3} \times 100$$

where WT was the weight of individual fish in grams and LNG was the length of individual fish in centimeters (Anderson and Gutreuter, 1983). Data entry errors and outliers were again identified by examining for extreme condition factor values since this index takes into account the relationship between weight and length.

Descriptive statistics for weight, length and condition factor were calculated

on a cage-level basis for each cage and for each method. A new data set containing the site and cage identification and cage mean values for weight, length and condition factor and their respective standard deviations was created.

The distributions of cage and site variables were examined for departures from normality. Visual inspection of the stem-and-leaf plots and normal probability plots was made at each step as an informal assessment of distribution normality. The null hypothesis that the values of each fish-level variable were a random sample from a normal distribution was tested for each cage and each site. Fish-level variables within individual cages were examined for departures from normality distribution assumptions by calculating the Shapiro-Wilk statistic, W, which is the ratio of the best estimator of the variance to the usual corrected sum of squares estimator of the variance (SAS Procedures Guide for Personal Computers, Version 6 Edition, 1985). After grouping individuals from each cage together by site, the distributions were examined for normality violations by the Kolomogorov D statistic (SAS Procedures Guide for Personal Computers, Version 6 Edition, 1985). All transformations performed on the variables were also assessed for normality in similar fashion.

The Shapiro-Wilk statistic, W, was used to assess the probability of rejecting the normal distribution hypothesis. The Levene's median test (Glantz and Slinker, 1990) was used to test the equal variance assumption of weight, length and condition factor variables among cages.

A paired t-test (Glantz, 1987) was used to test the null hypothesis that the

crowd and dip sample estimate of the mean for cage *j* at site *k* was estimating the same population parameter as the systematic random sample estimate of the mean for cage *j* at site *k*. The paired t-test determines the probability that the absolute value of the difference between the crowd and dip sample estimate and the systematic random sample estimate of the cage mean was greater than zero due to chance alone (Cody and Smith, 1991). The null hypothesis was tested separately at each site for each of the three variables : weight, length and condition factor. The degrees of freedom were calculated using the number of cages compared.

Although the group of fish within cages was the smallest unit examined, individual fish were used as the units in a separate analysis of the influence of sampling method on the estimates of cage parameters to demonstrate the statistical impact of choosing the incorrect units for analysis. A two-way analysis of variance was used to assess the effect of sampling method while controlling for the effect of cage and to permit an examination of the interaction between sampling method and cage.

For each sampling method, the distributions of weight, length, or condition factor within cages were classified as to their violation of normality assumptions using the Shaprio-Wilk statistic, W, at a significance level of $p<.05$. Agreement between the two sampling methods for violating these assumptions was assessed by the kappa statistic, the level of agreement beyond chance (Martin, Meek, Willeberg, 1987).

The comparison of sampling methods was also made through examination of the probability of obtaining outliers for each method. The total number of fish with values greater or lesser than 1.96 standard deviations away from their cage means, for a given method, was calculated within cages. These fish would correspond to the extreme five percent of the population distribution within each cage for a particular variable if that variable were distributed normally.

The systematic random sample was used to estimate the cage mean and a 95% confidence interval (C.I.) for that estimate. The systematic sample was assumed to be taken from a random ordering of the fish sizes as the fish passed from the pump to the size-sorting apparatus. Under this assumption, the systematic sample was considered equivalent to a simple random sample (Levy and Lemeshow, 1980) and, thus, permitted the use of similar procedures to calculate the variance of the means.

Subsequent samples from the same cage should have yielded estimates of the cage mean outside the 95% C.I. for the mean five percent of the time if the selection criteria were not different. The crowd and dip sample estimates of the cage mean were then compared to the corresponding cage 95% C.I. estimates for the mean determined by the systematic random sample.

2.3.4 Calculation of Intraclass Correlation Coefficients

The intraclass correlation coefficient, δ, was estimated by r using the

following equation:

$$r = \frac{(s_b^2 - s_w^2)}{(s_b^2 + (n-1)\, s_w^2)}$$

where s_b^2 was the variance between cages, s_w^2 was the variance within cages, and n was the mean number of fish sampled per cage (Snedecor and Cochran, 1980). Using the General Linear Models (GLM) procedure in the SAS statistical software package (SAS Institute Inc, Version 6.04), the component of variance attributed to the cage effect was determined for each of the outcomes: weight, length and condition factor. The cage was considered a random effects variable in the partitioning of the deviations from the model using the cage as the only predictor. Mean squared deviation terms for the effect of cage and the mean square error remaining as error in the model were used for the s_b^2 and s_w^2 values in the calculation of the intraclass correlation coefficient as described previously. Analyses were performed separately by site.

2.4 Results

2.4.1 Characteristics of the Study Populations by Site

All of the three sites at the study farm were subjected to the crowd and dip sampling technique in May of 1991. The third site was not size-sorted due to a farm management decision unrelated to this study. Since only two sites were size-sorted, the comparison of sampling techniques could only be attempted at these sites.

Twenty-five cages were sampled by the two different methods: 11 at site 1 and 14 at site 2. Six additional cages at site 2 were available for sampling by both methods but, due to technical difficulties with the fish pump or weigh scale power supply, the data were incomplete and not appropriate for the comparison.

The total study population from which samples were taken by both sampling methods was 70,821 fish in 25 cages. The mean number of salmon per cage was 2833. One site had 31,045 fish in 11 cages (mean of 2822 per cage) and the second site had 39,776 fish in 14 cages (mean of 2841 per cage).

The total number of fish at sites 1 and 2 that were anaesthetized and measured for weight and length comparisons was 11,382. Crowd and dip samples resulted in 6564 fish being measured, including 1251 fish from 6 cages which were not sampled by the random method. The 5313 dipped fish eligible for the comparison of sampling methods was 7.5 percent of the study population and

consisted of 2399 (7.7%) from site 1 and 2914 (7.3%) from site 2. Systematic random samples from all 25 cages accounted for 6069 observations (8.6% of the population) consisting of 3019 fish (9.7%) from site 1 and 3050 fish (7.7%) from site 2.

The data from cages which had pump or mechanical failures occur during the systematic sampling procedure was not considered valid unless all of the population in the cage had an equal chance of being selected. In one case, 88% of the cage's population had been size-sorted when the pump failed and the remaining 369 fish were transferred to another cage without being available for systematic selection. Since the last 369 fish may have had different attributes than the first part of the population, the data from this cage were not used for any comparisons.

The mean number of fish sampled by the crowd and dip method was 218 per cage (minimum 209, maximum 237) at site 1 and 208 per cage (minimum 201, maximum 216) at site 2. At site 1, the mean number of fish per cage subjected to systematic random samples was 274 (minimum 225, maximum 328) and the mean was 218 (minimum 181, maximum 290) at site 2.

The data from the systematic random samples was used to calculate the best estimates of mean weight and determine the type of distribution of the populations within each site (Levy and Lemeshow, 1980). The convenience samples were not used for this purpose since they contained an unknown amount of bias and, thus, it would be inappropriate to calculate standard errors.

Although, the mean fish weight was almost 450 grams heavier and mean lengtn six centimeters longer at site 2 compared to site 1, the mean condition factor was slightly greater at site 1 (Table II). The frequency distributions were similar in both sites (Figures 4, 5 and 6) with weight at site 1 being the only variable which was not significantly different from normal (Table II: Kolmogorov D statistic 0.014, $p>.15$). However, visual inspection of the weight and condition factor frequency distributions revealed the general shape of a normal distribution. Length was slightly skewed to the left. Although both of the weight and length means were significantly different ($p<.01$) between sites, the condition factor was not significantly different ($p>.05$) between sites.

Table II

Site-Level Descriptive Statistics for Fish Weight, Length, and Condition

	WEIGHT[a]	LENGTH[b]	CONDITION[c]
Site 1			
n[d]	3016	3018	3016
Mean	1611.9	51.47	1.155
S.E.[e]	7.64	0.07	0.002
S.D.[f]	419.4	3.82	0.127
Skewness	0.004	-0.791	0.570
Kurtosis	-0.018	1.408	4.005
D:Normal[g]	0.014	0.104	0.054
Prob>D[h]	>.15	<.01	<.01
Site 2			
n[d]	3048	3049	3048
Mean	2057.7	57.49	1.073
S.E.[e]	7.70	0.06	0.002
S.D.[f]	427.6	3.58	0.137
Skewness	-0.169	-1.194	0.589
Kurtosis	-0.033	3.673	5.613
D:Normal[g]	0.024	0.129	0.052
Prob>D[h]	<.01	<.01	<.01

[abc] Weight (kg), Length (cm), Condition ($WT/LNG^3 * 100000$)
[d] n refers to number of observations
[ef] S.E. is standard error of the mean; S.D. is standard deviation
[g] D:Normal is Kolmogorov D statistic for distribution normality
[h] Prob>D is probability of a larger D statistic ($p<.05$ is significant)

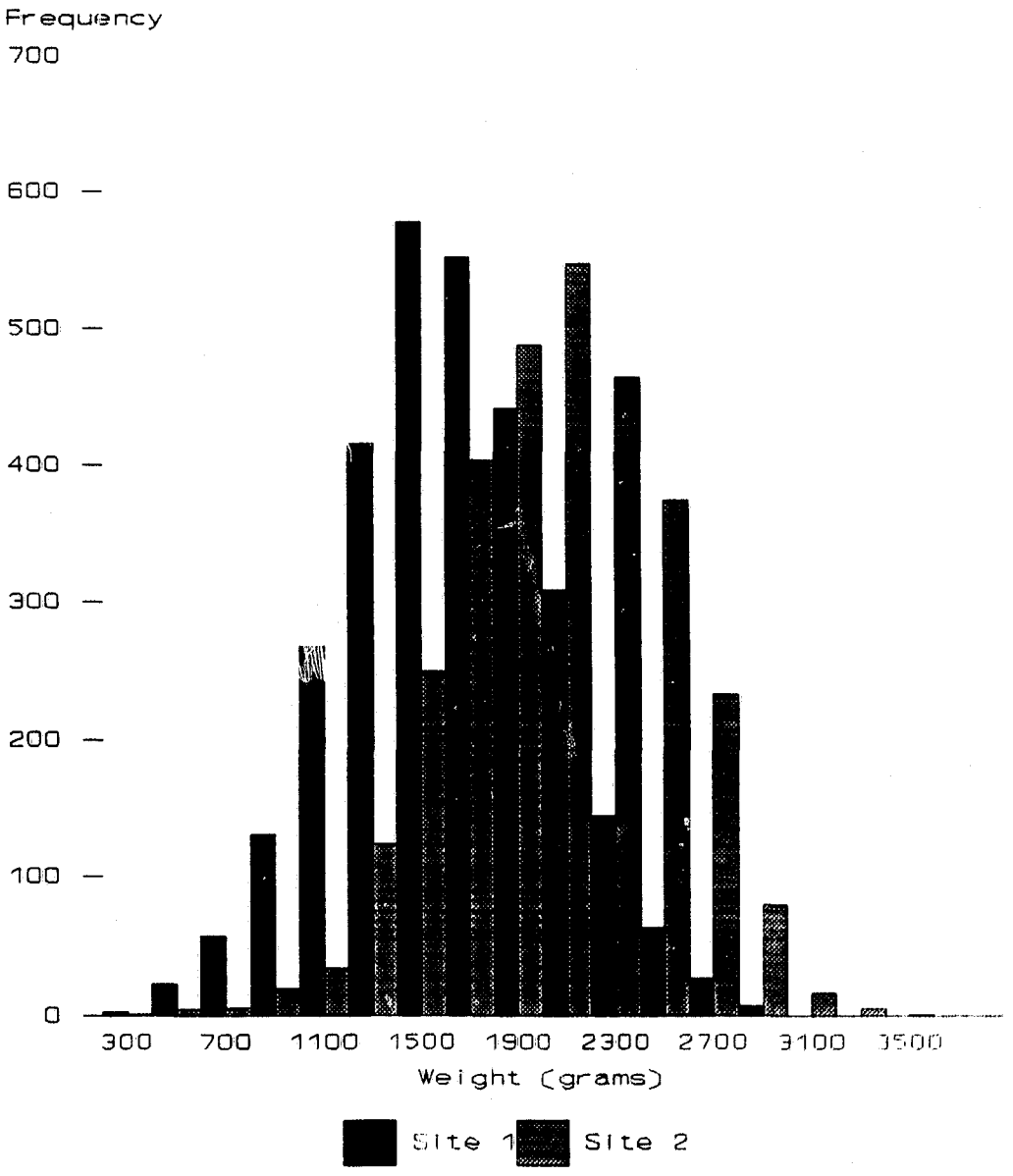


Figure 4. The frequency distribution of fish weights obtained by systematic random samples after one-sea-winter of growth at two sites.

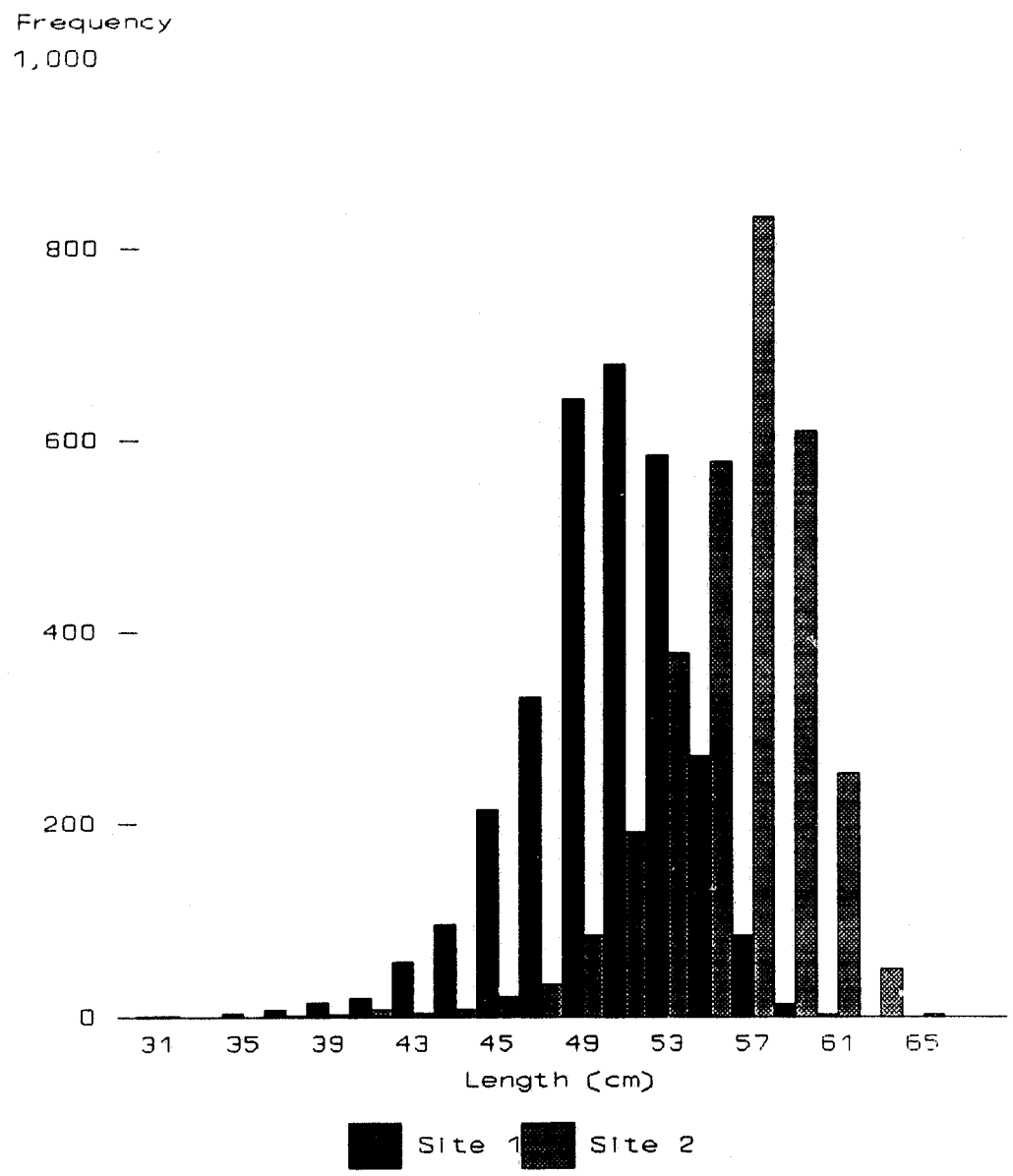


Figure 5. The frequency distribution of fish length obtained by systematic random samples after one-sea-winter at two sites.

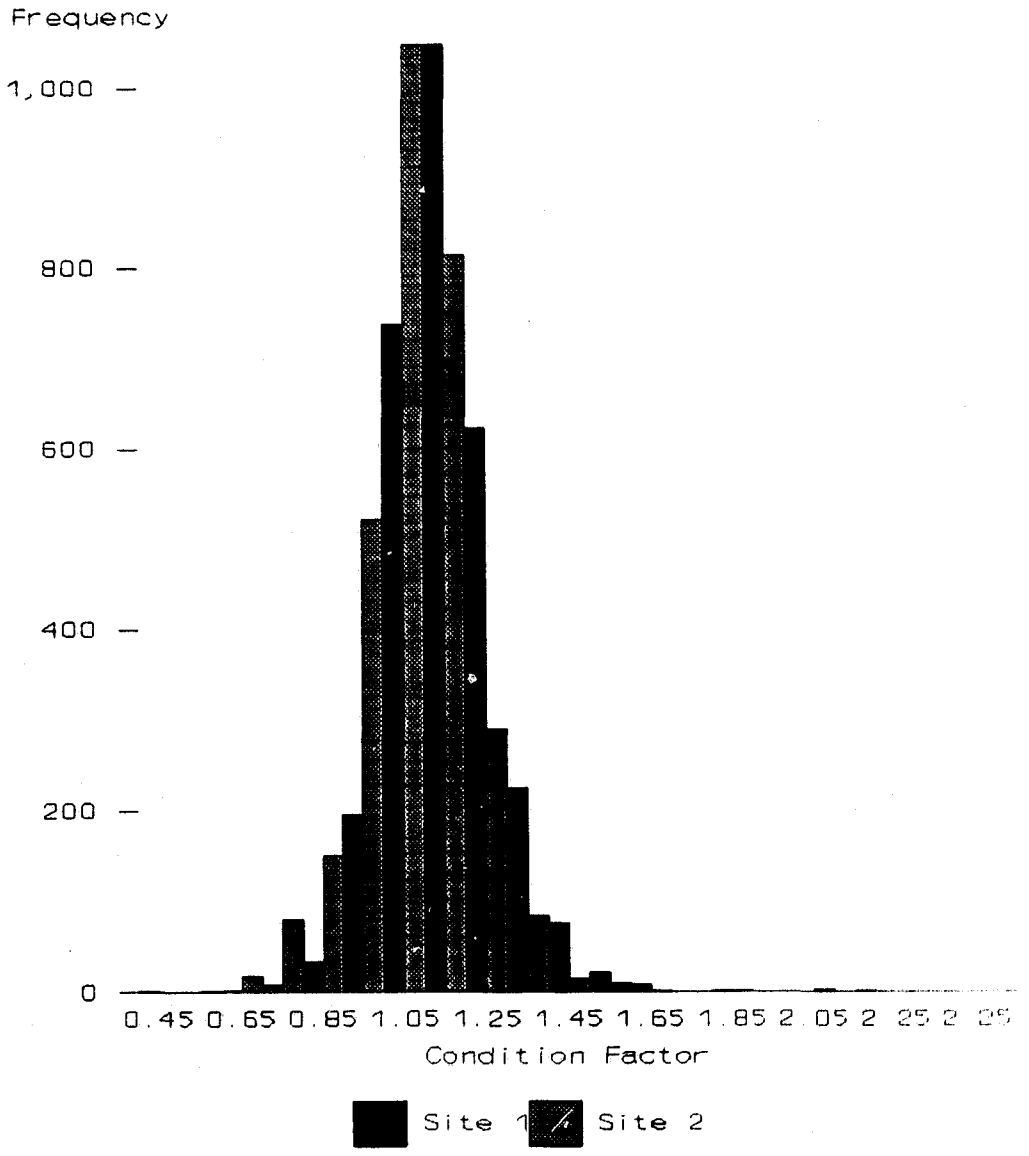


Figure 6. The frequency distribution of fish condition factor obtained by systematic random samples after one-sea-winter at two sites.

2.4.2 Comparison of the Crowd and Dip Sampling Technique to a Systematic Random Sampling Technique

A paired t-test was used to compare the difference between the crowd and dip sample estimate of the mean for cage *i* and the systematic random sample estimate of the mean for cage *i* controlling for the site. Dip samples of the cage populations were considered representative when the difference between estimates of the cage means by convenience samples and estimates of the same cage means by true random samples were not significantly different from zero.

The crowd and dip sampling method at site 1 over-estimated the cage mean weight by 38.5 grams ($p=0.088$) and cage mean length by 0.21 cm ($p=0.044$) when compared to the systematic random sampling method (Table III). At site 2, the cage mean weight was estimated to be 101.8 grams heavier ($p=0.013$) and 0.82 cm shorter ($p=0.0001$) by the convenience sample. The condition factor was not significantly different when estimated by either method at site 1, but was 0.099 g/cm^3 higher ($p=0.0003$) in the dip samples at site 2.

The variances of weight, length and condition were not homogeneous among cages when tested by the Levene's median test ($p<.01$). Variable transformations, including square, cube, square root, cube root, reciprocal, and exponential, were unsuccessful in stabilizing variance without introducing major problems with the normality of the distributions. The largest ratio of residual variances within groups was not more than a factor of 3.5 which was considered

acceptable for the robust methods of analysis of variance (Glantz and Slinker, 1990).

A two-way analysis of variance was performed to examine the effects of cage, sampling method and the interaction of sampling method and cage on the weight of individual fish within each site. Similar analyses also examined the effect of these factors on the length and condition of fish at each site. The means of the variables weight, length, and condition were significantly (weight: $p<0.001$, length: $p<0.05$, and condition: $p<0.001$) affected by the sampling method at both sites even after controlling for the effects of cage (Tables IV and V). The interaction term was also significant for all three variables at both sites except for length at site 1.

Systematic random samples were used to estimate the population mean and the 95% CI for the estimate. The crowd and dip sample estimates of the cage mean were then examined to determine if they exceeded the 95% CI for each cage. At site 1, dipped estimates of cage means exceeded the 95% CI 64% of the time for weight, 27.3% for length, and 72.7% for condition factor. Dipped estimates of cage means at site 2 were 71.4% outside the 95% CI for weight, 64.3% for length, and 92.9% for condition factor.

Each of the 25 cages was considered separately to examine for departures from normality within the cage populations using the Shapiro-Wilk statistic, W (Tables VI and VII). The systematic samples yielded 11 cages for weight, 23 for length and 20 for condition factor, that departed from normality assumptions

($p<.05$). With respect to distribution normality, dipping from crowded groups agreed with systematic samples in 18 of the 25 (72%) cages for the variable of weight. The agreement, kappa (Martin, Meek, Willeberg, 1987), for departure from normality was 0.44 beyond chance between the two samples for the weight measurements. There was 92% agreement for length measurements since only two cages were classified as normal by the systematic samples compared to zero cages by the dip method. Condition factor had the same classification 20% of the time which resulted in a kappa of -0.119.

The probability of obtaining fish that were in the extreme five percent of the population (that is, greater than 1.96 standard deviations from the mean) was similar for either sampling method. A noticeable trend was not evident for any of the variables weight, length, or condition factor.

Table III

Comparison of Sampling Differences with Cage as the Unit of Concern

Variable	Site[a]	n[b]	Dip[c]	Syst[d]	Diff[e]	%[f]	S.E.[g]	p
WT[h]	1	11	1650.4	1611.9	38.5	2.4	20.3	.0875
	2	14	2159.5	2057.7	101.8	4.9	35.3	.0128
LNG[h]	1	11	51.68	51.47	0.21	0.4	0.09	.0440
	2	14	56.67	57.49	-0.82	1.4	0.14	.0001
CF[h]	1	11	1.167	1.155	.0115	1.0	.0111	.3251
	2	14	1.172	1.073	.0987	9.2	.0201	.0003

[a] Analyses presented separately by site

[b] n is the number of cages used in analysis

[c] Dip is the mean value for dip samples

[d] Syst is the mean value for systematic samples

[e] Diff is mean difference $(\overline{x}_{DIFF})$: $\overline{x}_{DIFF} = \frac{\sum (\overline{x}_{i(dip)} - \overline{x}_{i(syst)})}{n}$

where *i* is cage, *dip* is dip sample, and *syst* is systematic sample

[f] % refers to Diff percent of systematic mean

[g] S.E. is standard error of the mean difference

[h] WT is weight (g), LNG is length (cm), CF is condition factor $(WT/LNG^3 * 100)$

Table IV

Effect of Sampling Method and Cage
on Estimates of Mean Weight, Length,
and Condition at Site 1
(Two-way Analysis of Variance)

Dependent Variable	Predictor Variable	DF[a]	F	p
WEIGHT	Cage	10	79.5	.0001
	Method[b]	1	12.3	.0005
	Cage*Method	10	3.4	.0002
	Full Model	21	40.5	.0001
LENGTH	Cage	10	42.3	.0001
	Method[b]	1	4.4	.0361
	Cage*Method	10	0.82	.6130
	Full Model	21	20.8	.0001
CONDITION	Cage	10	94.9	.0001
	Method[b]	1	11.7	.0006
	Cage*Method	10	11.2	.0001
	Full Model	21	51.1	.0001

[a] DF is degrees of freedom
[b] Method is sampling method (Dip or Systematic)

Table V

Effect of Sampling Method and Cage on Estimates of Mean Weight, Length, and Condition at Site 2 (Two-way Analysis of Variance)

Dependent Variable	Predictor Variable	DF[a]	F	p
WEIGHT	Cage	13	72.6	.0001
	Method[b]	1	96.5	.0001
	Cage*Method	13	11.9	.0001
	Full Model	27	45.0	.0001
LENGTH	Cage	13	59.0	.0001
	Method[b]	1	90.3	.0001
	Cage*Method	13	2.8	.0006
	Full Model	27	33.8	.0001
CONDITION	Cage	13	47.6	.0001
	Method[b]	1	1186.1	.0001
	Cage*Method	13	51.0	.0001
	Full Model	27	100.0	<.0001

[a] DF is degrees of freedom
[b] Method is sampling method (Dip or Systematic)

Table VI

Normality of Weight, Length, and Condition Distributions by Dip and Random Sampling Methods at Site 1

	p values[a]					
	Weight		Length		Condition	
Cage	Dip	Random	Dip	Random	Dip	Random
113	.578	.017	.000	.000	.805	.001
126	.091	.116	.000	.000	.006	.000
129	.562	.453	.000	.001	.000	.000
131	.832	.163	.000	.000	.332	.000
132	.078	.184	.001	.000	.042	.081
133	.204	.006	.000	.000	.000	.000
134	.403	.217	.009	.000	.484	.000
135	.576	.257	.000	.000	.282	.000
136	.366	.461	.000	.000	.063	.645
137	.978	.947	.000	.000	.000	.039
140	.611	.345	.000	.000	.997	.000

[a] **Values refer to the probability of obtaining a Shapiro-Wilk statistic, W, equal or less than that found for the cage distribution. Small probabilities indicate departure from normality.**

Table VII

Normality of Weight, Length, and Condition Distributions by Dip and Random Sampling Methods at Site 2

	p values[a]					
	Weight		Length		Condition	
Cage	Dip	Random	Dip	Random	Dip	Random
202	.190	.000	.000	.000	.000	.000
204	.000	.000	.000	.000	.627	.000
208	.000	.000	.000	.408	.754	.000
210	.001	.000	.000	.000	.127	.000
214	.066	.014	.000	.000	.060	.000
215	.000	.498	.000	.000	.002	.268
216	.000	.000	.002	.000	.243	.000
217	.009	.024	.000	.000	.007	.000
218	.224	.126	.000	.102	.095	.000
219	.000	.056	.000	.000	.551	.164
220	.005	.019	.000	.000	.048	.000
221	.001	.415	.000	.000	.030	.100
222	.000	.000	.000	.000	.000	.000
224	.245	.411	.000	.000	.147	.000

[a] **Values refer to the probability of obtaining a Shapiro-Wilk statistic, W, equal or less than that found for the cage distribution. Small probabilities indicate departure from normality.**

2.4.3 Consistency of the Cage to Cage Difference Between Crowd and Dip Samples and Systematic Random Samples

The difference between crowd and dip sample estimates of the cage mean weight and the systematic random sample estimates of the same mean varied considerably between cages at the same site. The difference ranged from 63.1 grams lighter to 171.6 grams heavier in the dip sample at site 1 (Figure 7). At site 2, only one cage had a mean weight which was lighter when estimated by the dip sample. In the remaining 13 cages the dip sample estimate of the mean weight was heavier than the random sample estimate. The greatest difference detected at either site was 356.8 grams heavier in dip sample which occurred at site 2.

The estimates of cage mean length also varied considerably between cages at the same site (Figure 8). Four of eleven cages at site 1 had dip samples with mean lengths lower than random sample means while all cages at site 2 were shorter on dip samples. The difference in cage mean length ranged from 0.17 cm shorter to 0.68 cm longer in dip samples at site 1 and from 1.71 cm to 0.13 cm shorter at site 2.

The cage mean condition factor ranged from 0.038 g/cm^3 lower to 0.080 g/cm^3 higher in the dip samples at site 1 (Figure 9). All dip sample estimates of cage mean condition factor were higher than the random sample estimates at site 2. The smallest difference was 0.008 g/cm^3 and the largest was 0.258 g/cm^3.

The intraclass correlation coefficients were estimated from the systematic sample data for each variable at each site (Table VIII). The coefficients were generally highest for condition factor, followed by weight and then length. All coefficients were higher in site 2 than in site 1.

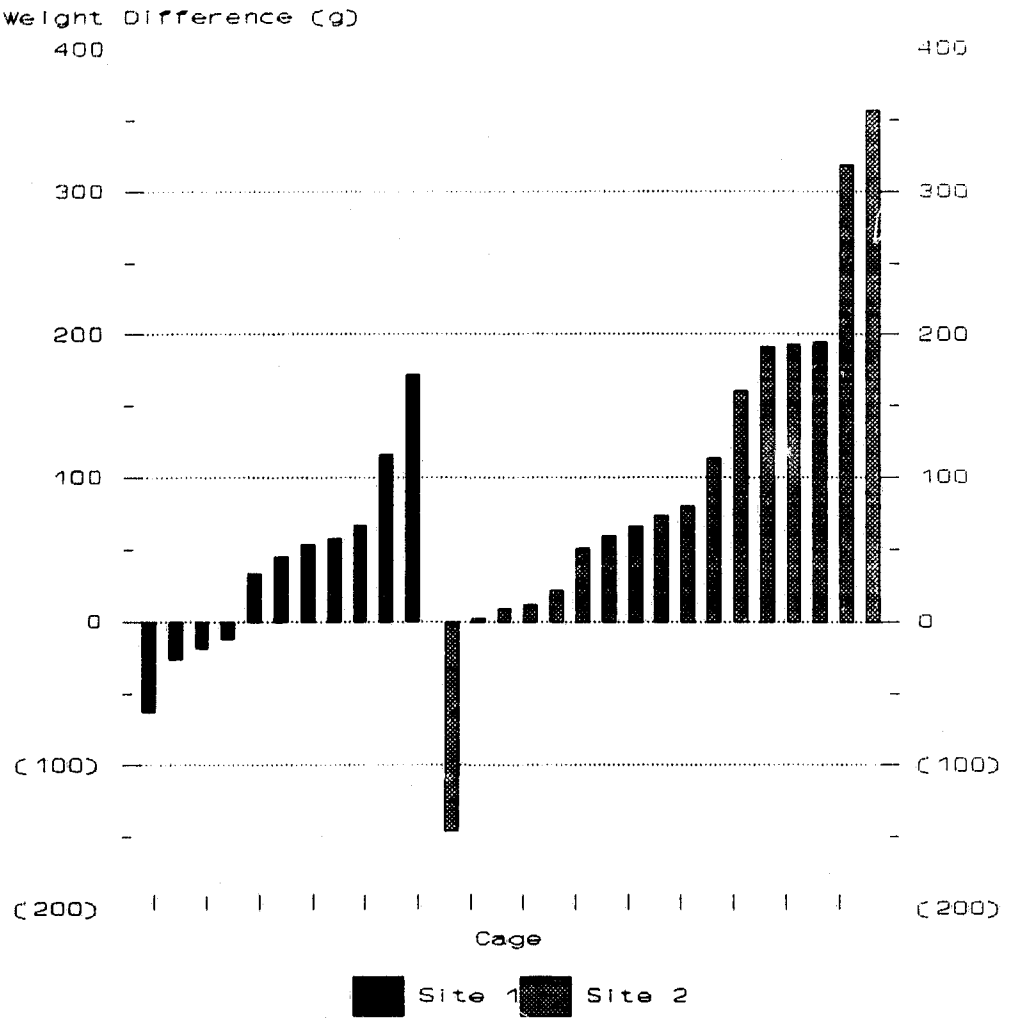


Figure 7. Difference (grams) between mean weight estimated by the dip sample and mean weight estimated by the systematic sample. Each bar represents one cage. All estimates are based on 180 fish or more.

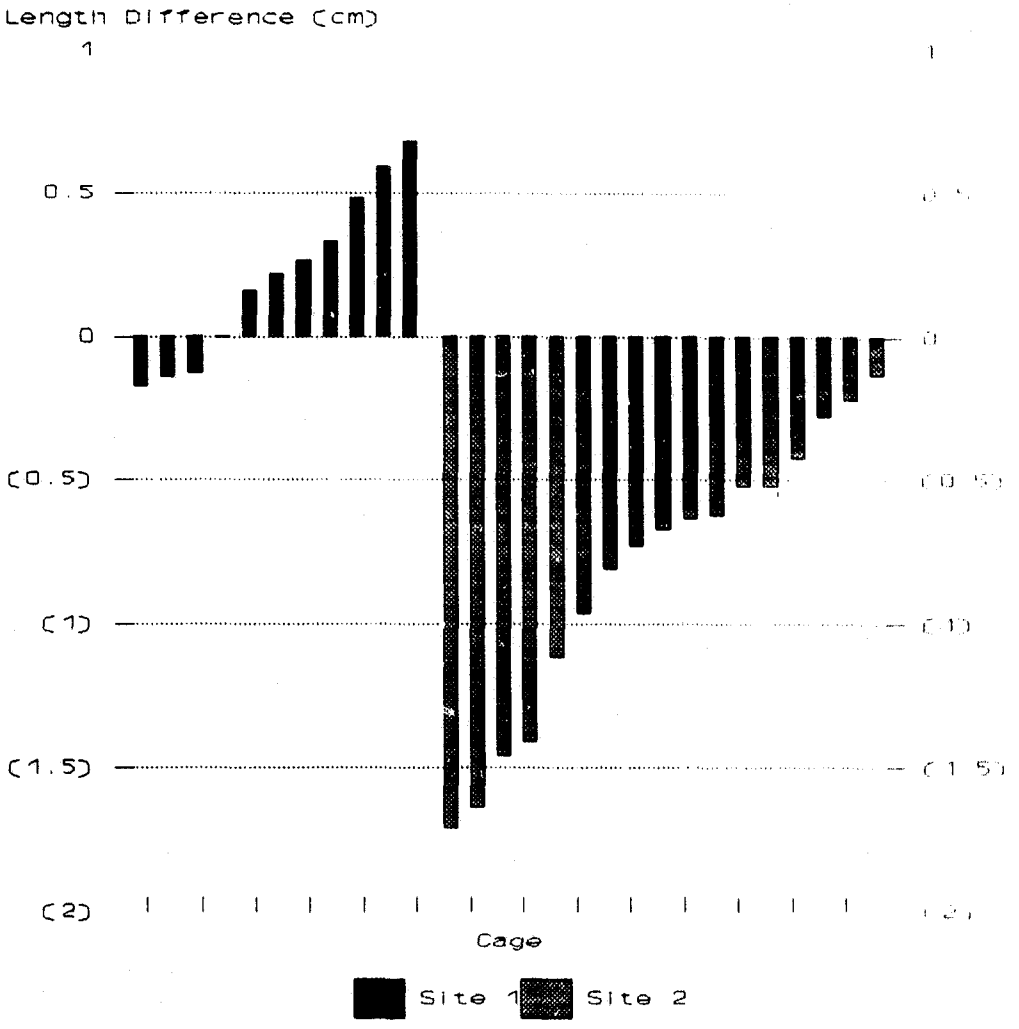


Figure 8. Difference (cm) between mean length estimated by a dip sample and mean length estimated by a systematic sample. Each bar represents one cage. All estimates are based on 180 fish or more.

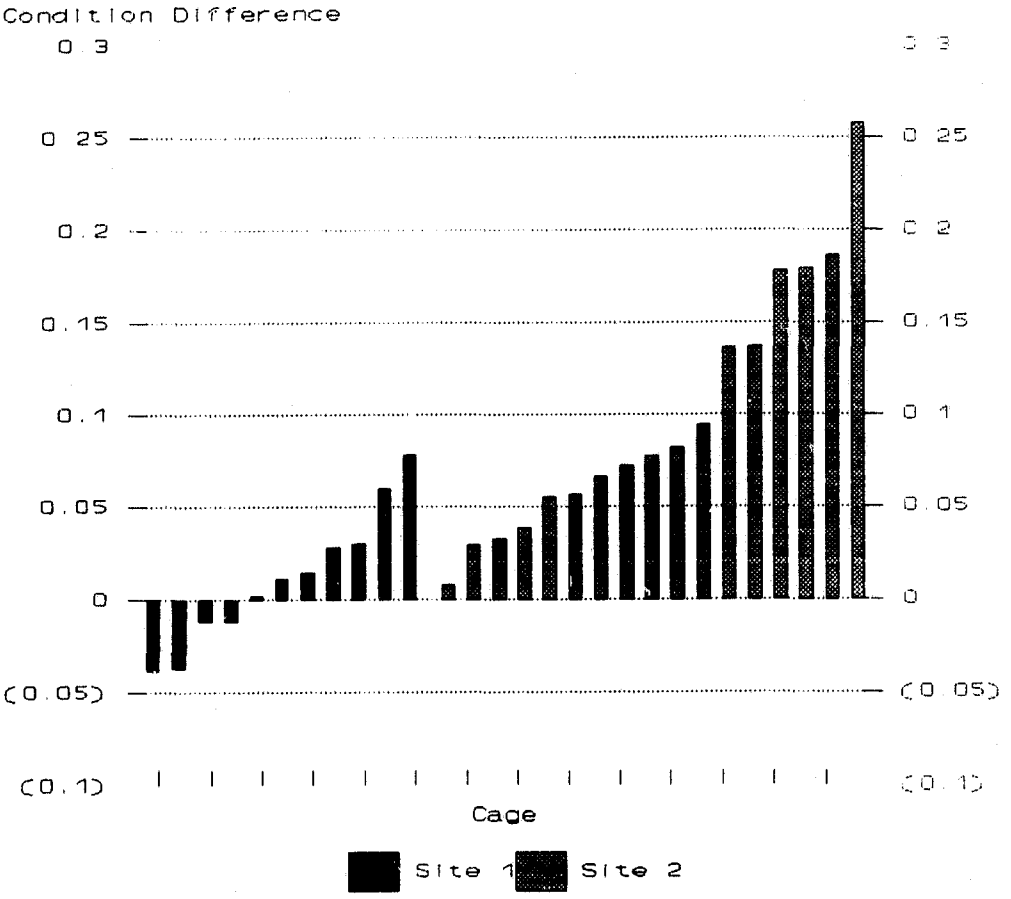


Figure 9. Difference between mean condition factor estimated by dip sample and mean condition factor estimated by systematic sample. Each bar represents one cage. All estimates are based on 180 fish or more.

Table VIII

Intraclass Correlation Coefficients[a]

Estimated from Systematic Samples

	WT	LNG	CF
Site 1	0.125	0.071	0.142
Site 2	0.148	0.122	0.237

[a] The intraclass correlation coefficient (ρ) is estimated by the following equation:

$$\hat{\rho} = \frac{MS_B - MS_W}{MS_B + (m-1)MS_W}$$

where MS_B is the *mean square between* cages and MS_W is the *mean square within* cages derived from the analysis of variance with cage as a random effect, and m is the mean number of fish sampled per cage.

2.5 Discussion

2.5.1 Limitations of Dip Net Samples

The primary objective of this study was to determine if the commonly used convenience sampling technique, dipping from a crowded group, estimated the same cage mean parameters as a systematic random sample of the same population of Atlantic salmon in sea cages. The convenience samples estimated significantly different cage mean parameters of weight, length and condition factor when compared to a systematic random sample at one or both sea cage sites in the study. One site had more pronounced differences than the second site indicating a site to site variation in selection biases.

The method of sampling employed in this study included a pre-selection of fish by dividing the cage in half (see figure 2, page 16). There was no reliable method to determine if there was any selection bias in the way in which the population was divided at that time. A selection bias present in the initial division of the cage population into two groups may have accounted for all or part of the final differences observed between selection methods. In theory, selection bias at two stages of the dip net sampling procedure may have been in opposite directions and resulted in a final selection of individuals whose mean size was closer to the true mean.

Only weight, length and condition factor were used in this comparison since

these were of interest to the farm management, convenient and relatively easy to measure indices in large numbers of fish and did not require lethal sampling. Other fish attributes may be more or less responsible for the inclusion of fish in a convenience sample of this type. Weight and length may act as confounders for a true association between some other factor, such as disease, and the increased probability of being included in a dip sample. A confounder is a factor for the outcome under study whose control will reduce or completely correct a bias when estimating the true factor-outcome relationship (Kleinbaum, Kupper and Morgenstern, 1982). Although weight and length had an apparent association with the probability of being included in the convenience sample in this study, the association may not exist if the true selection factor could be identified and controlled for in the analysis.

The major disadvantage of using multiple t tests to compare weight, length or condition factor relates to the fact that with more frequent assessment of different indices, the greater the chance of concluding a difference exists when it does not (Glantz, 1987). The indices of interest were highly correlated with each other and, therefore, it was decided to treat each size index as a separate statistical analysis.

Systematic random sampling occurred seven to 10 days after the dip net samples were obtained. Although feed was withheld for up to two or three days before and after any sampling event, feeding was not discontinued completely and, therefore, some fish growth was possible. Change in size of fish between sampling

events could not be measured directly nor could adjustments for growth be included in the analysis. If growth had occurred, the estimates of the mean weight using systematic samples would have been lighter immediately after the dip net samples and, thus, magnify the apparent difference in estimates.

The populations within cages at this study farm had distributions which were slightly skewed to the left for both weight and length. This result suggests that the majority of the population grew at a similar rate while a small proportion remained on a slower growth curve. This fact should be considered when farms are making decisions regarding size-sorting of fish. The increased mixing that accompanies the sorting of fish into several size groups disrupts the cage heirarchy and increases the potential exposure of fish to new pathogens. If creating uniform sized groups while minimizing the spread of infectious disease is the goal at this farm, then the most gain may actually result from removing only the smallest fish from the group and allowing the rest of the fish to remain as a single unit.

It is interesting to note that the dip net samples agreed with systematic random samples frequently when the length was not normally distributed but agreed very infrequently when the distribution of condition factor was not normal (Tables VI and VII, pages 53 and 54). The agreement beyond chance for predicting normality of weight distributions was moderate (kappa of 0.44). Hypothesis testing requires that the sample be evaluated for significant differences from normality within cages which, according to the results in this study, are not reliably predicted for condition and possibly weight using convenience sampling

methods. The departure from normality observed in these populations did not seem to be excessive and may not be important for robust statistical methods.

On a cage level basis, large sample sizes (180 individuals or more) were used to improve the precision of the estimated cage parameters (Glantz, 1987). Since many statistical analyses involved the cage as the unit of concern and the difference between the estimated means was the outcome evaluated, the estimate of the cage mean weight and length required precise estimates in order to minimize the possibility that differences observed between convenience and systematic samples were due to poor precision at the cage level.

Larger samples of individuals per cage indirectly influences the power of the test of significance by improving the precision with which cage-level means are estimated (Koepsell et al., 1991). Many farms rely on estimates based on sample sizes of 50 fish or less to make management and disease control decisions. A greater difference between dipped and systematic sample estimates of cage mean weight, length and condition factor would be expected as the sample size was reduced.

Although a different age-group and different rearing conditions were involved, the results agreed with the findings of Seeger et al. (1977) that there exists a selection bias for length when dipping a sample from crowded groups. They evaluated the precision and accuracy of repeated sampling using various sized scoop nets in salmon maintained in troughs and suggest that average length is underestimated by scoop net samples due to larger fish more easily avoiding

capture. Larger fish were defined as fish of greater length which neglects the weight or condition of the fish as factors of capture probability. When the weight was taken into account in the larger, one-sea-winter fish of this study, heavier fish with higher condition were preferrentially selected by the dip net.

Thorburn (1992) provides evidence that dip-netting crowded rainbow trout from small tanks can yield random samples while both dip-netting fish at the water inflow and netting them from a disturbed group are biased sampling methods. The difference between the rearing systems of the previous two studies, that is the trough system of Seeger et al. (1977) versus the small square or circular tanks of Thorburn (1992), may account for the discrepancy in results. Since the population dynamics of hatchery tanks are likely quite different from that of grow-out sea cages, comparisons of the previous two systems to the rearing conditions of this study are difficult. The level of crowding within groups may have been higher in Thorburn's experiment. As well, the sampling techniques used in the comparisons made by Thorburn (1992) are not easily reproduced in a sea cage production system.

There are some less frequently employed sampling techniques at sea cage facilities which were not evaluated in this study. Dip-netting fish during feeding has been occasionally used to obtain samples. This method has some similarities to sampling by anglers using a baited hook. An obvious potential source of bias with this technique is that the fish must be eating to be captured and be included in the sample. Fish which are eating will tend to be healthier and larger than fish

which are not eating and cause the smaller, sicker fish to be under-represented in the sample.

Convenience samples are occasionally obtained during certain group processing events. Size-sorting and harvesting events are examples of opportunities for sampling. Non-probability sampling at these times is likely prone to the same selection biases as at any other time.

In this study, the direction of the difference in length estimates between dipped samples and systematic random samples varied depending on the site: significantly shorter in one site and significantly longer in the other site. The magnitude of the difference was small (0.2 and 0.8 cm for site 1 and site 2 respectively). Fish condition indices frequently employ length or a transformation of length in their calculations (Bolger and Connolly, 1989). Biased estimates of mean length, even small biases, are important when examining the condition indices of cage populations.

There were significant interactions between cage and sampling method for estimates of mean weight, length (Site 2 only), and condition. The presence of this interaction indicated that the estimate of mean weight was influenced by the sampling method in different ways depending on the cage being sampled. This may have resulted from different staff members from cage to cage, the fish within the cage reacting differently towards the procedure, or a combination of factors which were not controlled in the analysis.

A secondary objective of this study, once a difference between sampling

methods had been detected, was to evaluate the consistency of the difference between cages at the same site and between sites at this farm. Since every farm would likely have different sampling techniques and different factors influencing the selection pressures, a correction factor would, at best, be site or farm specific. The results of this study suggest that the magnitude and direction of selection bias present in this form of non-probability sampling was not predictable between sites. Due to factors changing within a site over time, the bias would probably vary between sampling events at the same site as well.

The study farm was performing a size-sorting procedure on one-sea-winter Atlantic salmon for management purposes which provided an opportunity for the systematic random samples. These probability samples would not have been available otherwise. Few farms in the Bay of Fundy perform this type of size-sorting since most do not have access to the equipment or number of personnel required for such an intensive management practice. Due to the very fact that the study farm had the ability to size-sort large numbers of fish mechanically, the fish population and management practices at this farm may not be typical of farms in the area. The study farm managed their populations of fish such that the variation of weight and length within cages tended to be very small compared to the typical farm in the Bay of Fundy (Thorburn, written communication, 1992). The maximum potential difference in estimates of cage mean parameters was therefore less at this particular farm than may have been possible at another farm with more within-cage variation. Although an industry-wide system for disease or

production monitoring for comparison of the study farm to the industry does not exist, the overall health and productivity of the study farm may differ from other farms. Thus, the ability to generalize the results to farms outside the study farm may be limited.

2.5.2 Unit of Concern

The definition of the unit of concern was an important consideration in this study since the results changed dramatically depending on the unit used. Failure to make the distinction between individuals versus aggregates as units of concern often renders the results of little or no value (Martin, Meek, Willeberg, 1987). The importance of using aggregates of individuals to investigate health management problems of other food animal species has been demonstrated in the veterinary literature (Martin et al., 1982; Martin, Meek, Willeberg, 1987). Koepsell et al. (1991) recognized in human community-based health studies that contolled evaluation of designs which target interventions towards entire communities must involve a comparison of a group of study communities with a group of control communities. Substantially exaggerated claims of statistical significance may result if the data are analyzed as if individuals had been randomized to treatment (Cornfield, 1978).

Aquaculture field studies rarely randomize to the level of the individual fish due to the great difficulty in identifying and observing individuals through time.

Few reports from such studies, however, analyze the data based on the group. Although the pen of fish is the smallest unit which can be followed through time, even that distinction can be confused by fish transfers into and out of the pen.

The two-way analysis of variance was used to demonstrate the difference in conclusions when the individual is used as the unit of concern rather than the group. Although the effect of cage and the interaction of cage and sampling method were controlled for in the analysis, the sampling method appeared much more significant an influence on the estimates of cage means when the individual, rather than the cage, was considered as the unit of analysis. For example, estimating cage mean condition factor at site 1 was not significantly different ($p=0.325$) between sampling methods when the cage was identified as the correct unit but was highly significant ($p=0.0006$) in the analysis at the fish level.

Although this is not a novel concept, it is a good example of the impact of choosing the incorrect unit can have on the interpretation of the results. Other statistical methods which use the cluster as the unit of randomization are possible (eg. Donner and Donald, 1987). Together with any potential biases in favour of the publication of significant differences (Szklo, 1991), the incorrect identification of the experimental unit could lead to misleading assumptions regarding husbandry and disease control strategies in aquaculture. Group-based studies of disease (Williams et al., 1981; Koepsell et al., 1991) and field trials of new vaccines or disease prevention strategies (Rothman, 1986) are vulnerable to misinterpretation due to comparing at the individual level but randomizing at the

group level.

The aquaculture literature contains many conclusions based upon the use of the individual as the unit in the analyses when the initial randomization involved groups. For example, mean length and maturation rates were found significantly different between groups of sea caged Atlantic salmon reared under natural light and groups reared under continuous light conditions (Kråkenes *et al.*, 1991). This conclusion may illuminate certain basic characteristics in the biology of fish growth and sexual maturation and cause the farmer to consider implementing light control as a managment strategy. However, the conclusions were based upon a two-way analyses of variance with randomization at the cage level and analysis at the individual level. The study reported comparisons of 976 fish versus 902 fish but, in actuality, involved four treatment cages versus four control cages.

The cage-effect described by Michel, Tixier, and Mevel (1984) may be responsible for a large proportion of the differences between groups and conclusions based on analyses at the fish level become weak. This cage effect has, thus far, been suspected but not quantified. The intraclass correlation coefficients calculated from systematic random samples (see Table VIII, page 60) represent a quantification of this cage effect at this farm.

The total variation of all observations may be partioned into two components: the variation between subjects within a class of the study variable and the variation among classes of the study variable (Kelsey, Thompson, Evans,

1986). In this particular case, the total variation may be divided into the variation between individuals within a cage and the variation among cages. The intraclass correlation coefficient is the proportion of the total variance that is associated with the class, or cage, to which it belongs (Snedecor and Cochran, 1980). The intraclass correlation coefficient increases, from 0 towards 1 in absolute terms, as the variation between cages increases relative to the variation within cages (Kleinbaum, Kupper, and Morgenstern, 1982).

Within the cage, each individual's response to environmental insults is dependent to some degree on the population to which it belongs. If slower growth of fish is regarded as an independent response to the environment, then slow growth should be randomly distributed within cages and the intraclass correlation coefficient related to size should not be different from zero. In this study, the intraclass correlation coefficients varied from seven to almost 24 percent.

The cage had a substantial influence on the size of fish within it. There are many possible reasons for this cage influence. Smolt were transferred to sea cages at different mean weights varying from 80 grams to 130 grams. The variance of weight, and presumably length and condition factor, was quite small at transfer since all fish were graded in the hatchery within the two months prior to transfer. However, the magnitude of the variance as a smolt is impossible to calculate since only one group of fish is weighed and enumerated for the determination of the mean. The impact of smolt weight on growth potential is difficult to study under

field conditions since there are frequent transfers of fish with unknown characteristics between populations throughout the production cycle. In addition, a simple method of accurately sampling the population between additions is lacking.

The study farm reduces stocking densities after 6 to 10 months in seawater by forcing about half of the population of one cage into an empty cage through a channel. This form of cage-splitting may select fish with similar growth-related characteristics to swim through the channel and result in two fairly homogeneous cages. Increasing the homogeneity within the cages would increase the intraclass correlation coefficient.

There are many environmental factors which could potentially influence the variation of growth between cages. The location of the cage at the site may have better or worse water quality. Certain cages may receive preferrential feeding and health monitoring if they are conveniently located within the site. Although the management endeavours to provide consistent, high quality care to all cages, net defouling procedures and other husbandry considerations may have different criteria for action depending on their location. Many husbandry or environmental conditions at sea cage sites, such as weather and tidal currents, may influence the growth of fish but remain beyond the control of the farmer. The greater the tendency for environmental factors to affect individuals within a group similarly and between groups differently, the greater will be the intraclass correlation coefficient.

Growth homogeneity within cage populations is probably also influenced by the collective response to disease (Yorke et al., 1979; Anderson and May, 1985) and vaccination (Halloran et al., 1991) as observed in other animals and humans. The smallest fish in cages whose population is more resistant to disease would tend to grow more quickly than the smallest fish in cages whose population is less resistant to disease. Thus, two individuals in the same relative position with respect to their cage's size distribution are more like the population within their cage than the population of small fish in general.

2.5.3 Implications of Biased Samples

Mixing of fish at the study farm occurred following a reduction of stocking density 6 to 10 months after sea transfer and during the size-sorting procedure performed after one winter in seawater. At the study farm, stocking density within each cage typically began at less than 1 kg/m^3 (at smolt transfer) and reached 6 to 10 kg/m^3 by late autumn at which time the cage population was divided into approximately half. At the time of splitting the pens, the populations were frequently supplemented with 200 to 1000 fish from other pens so that the total was at the desired level. Reduced stocking density (Refstie and Kittelsen, 1976; Refstie, 1977; Holm, Refstie and Bø, 1990) and size-sorted groups (Abbott and Dill, 1989; Baardvik and Jobling, 1990) are associated with improved growth rates. However, the mixing which occurs with both of these procedures causes great difficulty in tracking groups of fish through the production cycle. As the distinction between cages decreases, the smallest aggregate of individuals becomes increasingly larger to the point where the simplest aggregate is the site or farm.

An attempt was made to retrospectively examine the study farm's records for smolt characteristics and husbandry factors that might be associated with the variability of growth obtained from the random samples at size-sorting. However, there was a loss of distinction of most of the cages which made the investigation of growth factors meaningless in this study. It is suspected that a similar contamination of recorded data may be common to other farms in Atlantic

Canada and elsewhere. The current practices of mixing groups of fish emphasizes the need for reliable sampling methods to estimate disease and production levels at different points during the production cycle of Atlantic salmon.

The difference between dipped samples and systematic samples was not large in this study. The three to five percent difference observed in weight estimates would not make very much difference to the producer if the purpose of the sampling was to make market predictions. However, many epidemiology studies examine the difference on production outcomes between two interventions and rarely will the difference in growth be greater than 10 percent. Selection bias will affect the sample estimate to an unknown degree and a portion of the difference could be attributed to the uncertainty of the sampling method. To monitor changes over time, the cage parameters will require repeated sampling which will compound the problem.

Clinical trials involving new chemotherapeutics require the ability to sample fish at regular intervals for the estimate of mean fish weight and variability, as well as for estimates of disease prevalence and antibiotic residues. Feed trials to compare growth are frequently based upon convenience samples and subject to the same selection biases as disease studies. The precision of the estimates remains unknown unless probability sampling methods are employed.

The greatest danger with convenience sampling lies in the inability to assess the precision of estimated parameters and that the probability of inclusion in a sample varies between cages and between sites. Suggested treatment or disease

intervention strategies based upon studies involving convenience samples require large differences in the outcomes for the presence of an effect to be trusted. Small to moderate differences are suspicious due to the unknown influence of selection method.

3. FACTORS ASSOCIATED WITH THE PROBABILITY OF INCLUSION IN A SAMPLE OF ATLANTIC SALMON FROM CAGES AT HARVEST - A Pilot Study

3.1 Introduction

Differences between the convenience sampling technique, known as dip sampling, and a true random sampling method have been observed previously (see Chapter 2). The previous study involved only fish which were in sea cages for one year and all were in the range of 1.5 to 3.0 kg in weight. There was a site-specific magnitude of selection bias and a difference in mean weight of 100 to 150 grams. To investigate the potential for selection bias in cages containing larger fish, this study was performed on cages of Atlantic salmon as they were being harvested. The mean weight and length of fish within the cages were compared to the means obtained by systematic random samples of the same cage.

Although it has not been investigated previously, subclinical disease was considered a potentially strong influence on the probability of being selected in a dip net sample of caged Atlantic salmon. It has been frequently observed that populations of fish which are clinically ill have reduced appetites and less vigour in pursuit of feed pellets (Roberts and Shepherd, 1986). A subclinical infection at the cage level may lead to a high prevalence of subclinically infected individuals and/or a low prevalence of clinically infected individuals and continue to appear as

a relatively healthy group. Dip net sampling for disease prevalence from such a population might be expected to produce one of three scenarios: (a) infected fish, unable to escape capture in the dip net as readily as their healthy cohorts, would be over-represented in the sample causing the apparent prevalence to be higher than the true prevalence; (b) healthy fish, by displacing sick fish to less accessible areas of the cage, would cause the less robust fish to be under-represented in the sample and the apparent prevalence to be lower than the true prevalence; or (c) disease status would not affect selection probability. This study was an attempt to investigate the influence of disease on the probability of inclusion in a dip sample using gross necropsy lesions and isolation of *Renibacterium salmoninarum* as measures of subclinical disease.

The harvest represented an opportunity to obtain a systematic random sample without added stress to the fish and without an economic loss of fish product to the farmer. The added benefit of sampling during this event was the availability of necropsy examinations and tissue collection for subclinical disease prevalence determination without sacrifice of fish. BKD lesions have been observed during abattoir inspections of farms where clinical BKD was absent (Midtlyng, 1991).

Although individuals within the cage population continue to become infected and can die during the last few months prior to harvest, the majority are expected to be clinically healthy. Consequently, abattoir fish tend to be overlooked as a source of data for disease investigation. Chronic diseases with

low case fatality rates tend to have a higher prevalence in older animals due to the fact that the opportunity for exposure to an infectious agent is prolonged. Most lesions found during routine slaughter inspection are chronic and can indicate economically important problems (Martin, Meek, Willeberg, 1987). For these reasons, it was decided to use slaughter inspection as a source of data for this study.

Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum*, is a chronic disease in which the affected cages can exhibit extended periods of low mortality rates in seawater (Fryer and Sanders, 1981; Bruno, 1986). Infections with *R. salmoninarum* can apparently proceed asymptomatically for long periods (Warren, 1983; Bullock and Herman, 1988). These characteristics make the use of samples from slaughtered fish appropriate for investigations concerning asymptomatic BKD in Atlantic salmon groups in which BKD is present.

There are several methods of identifying subclinically infected individuals in a population. Since *R. salmoninarum* infected fish may have no recognizable symptoms, screening tests applied to all individuals in a sample would assist the classification of infected versus non-infected. The identification of *R. salmoninarum* infected fish will be limited by the test sensitivity which is defined as the proportion of the diseased individuals that test positive (Martin, Meek, Willeberg, 1987). Poor test sensitivity will increase the number of individuals that are falsely identified as disease negative. Conversely, poor test specificity, the proportion of animals that do not have the disease of interest and test negative

(Martin, Meek, Willeberg, 1987), will increase the number of individuals falsely identified as disease positive. Since valid estimates of diagnostic test sensitivity and specificity are prerequisites for estimation of disease prevalence (Martin, 1984) and since much of this information remains unknown for BKD tests (eg. Armstrong et al., 1989), estimates of true prevalence of *R. salmoninarum* infection remain difficu. to define.

Growth of bacteria on culture media, with confirmatory testing of cultured colonies, is generally accepted as one of the few pathognomonic tests for identification of BKD-positive individuals, that is, the test specificity approaches 100 percent. Culture has been observed to yield false negative results (Gudmundsdóttir, Helgason and Benediktsdóttir, 1991) but, without another gold standard for comparison, determination of the true sensitivity is very difficult.

Although many surrogate tests for subclinical BKD exist, many of which presumably have high sensitivities (i.e. a low number of false negatives), an unknown proportion of false positives in the group of test positives would complicate the interpretation of observed associations between factors of interest and diseased fish. Since bacterial culture on a selective media (with subsequent confirmatory testing of colonies) is essentially free of false positive results, it was decided that culture was the diagnostic test with which to screen the population sample. In this study, the minimum true prevalence of BKD infection, as defined by the positive culture of *R. salmoninarum*, was one outcome of interest.

Case-finding strategies identify individuals with certain symptoms or

characteristics which have a higher probability of being disease-positive and can then be tested using more specific diagnostic methods (Sackett, Haynes, and Tugwell, 1985). Fish which have visible granulomatous lesions most likely have a higher probability of being positive on culture than a random selection of fish from the population. Gudmundsdóttir, Helgason and Benediktsdóttir (1991) present results which compare visible lesions to culture. If a positive culture is considered the gold standard for BKD-positive fish, then their results provide an estimated maximum sensitivity of 42% for necropsy diagnosis of BKD (i.e. out of a possible 104 culture-positive fish, 44 fish had visible lesions). Culture may have falsely identified some fish as negative causing the number of fish in the lesion-negative, culture-positive classification to be falsely low and, thus, the culture sensitivity to be higher than it should have been. Since necropsy examination of large numbers of fish was more feasible than screening the same number of fish by bacterial culture, this study used necropsy examination and scoring for granulomatous lesions as another method to screen a large sample of the population for bacterial kidney disease.

There has been very few reports of host factors associated with the prevalence of infection with *R. salmoninarum*. Within cages, the fish have individual characteristics, such as sex, size, or immune status, which may make them more susceptible to disease. Characteristics like fish size may also be considered a result of disease status since an infection may weaken the individual and reduce their competitive ability within the group. Knowledge of

characteristics associated with bacterial kidney disease may permit a better understanding of disease transmission factors, the effects of BKD on production, or optimum case-finding strategies in populations with low disease prevalence. Using the BKD screening results from this observational study, the association between individual host factors and the BKD infection status was assessed.

The primary objective of this study was to test the null hypothesis that dip net sampling and systematic random sampling selected pen-reared, market-sized Atlantic salmon with equal probability with respect to individual fish size and BKD status. The secondary objective was to examine the associations between individual fish characteristics and the presence of either granulomatous lesions at necropsy or the presence of culturable *R. salmoninarum*.

3.2 Materials and Methods

3.2.1 Study and Target Populations

The target population for this study was the Atlantic salmon aquaculture sites in the Bay of Fundy, New Brunswick, Canada. The selection of the farm site was by convenience since this farm had been involved in a previous study on selection bias at a different stage of the production cycle. The study farm had management practices which included harvesting salmon throughout the year. Although this was not a prerequisite for this study, it was necessary for a more feasible sampling schedule.

The population size within cages was close to 3000 at the time of grading in May, 1991. The populations were not mixed between grading and harvest. The only changes were due to mortalities and possibly to seal attacks which might not be accounted in the records due to unknown total numbers eaten. The final cage population was between 2500 and 3200 fish in pens which were 12 m by 12 m by 6 m deep.

There was a slight increase (less than 0.3 % per day) in mortality rates in three of the cages during June and July of 1991 with lesions that resembled bacterial kidney disease (BKD) in a small proportion of the carcasses retrieved by divers. Subsequent identification of the agent responsible for BKD, *R. salmoninarum,* in a subsample of mortalities was made by indirect fluorescent

antibody test (IFAT) and western blot techniques by another laboratory. Identification of *R. salmoninarum* was made by a similar fashion in several of the other cages containing the same year class but whose mortality rates were not considered higher than normal for that time of year. The mortality rates of all cages, including the cages which had slightly increased mortality for a short period, returned to expected rates until the time of harvest. None of the cages involved in this study were subjected to any antibiotic therapy through the seawater phase of production.

3.2.2 Study Design and Sampling Protocol

The coordination of the sampling and the harvest schedules was an important component to this study. The farm routinely harvests an entire cage of fish before beginning the next cage. However, the number harvested from a cage on any particular harvest day varies with marketing demands. This means that one cage may be harvested over a period of seven to 10 days depending on the number removed, the starting population of fish in the cage, and the number of days per week that the farm is harvesting.

Fish were selected at the cage site and transported to the processing plant with the remainder of the harvest fish. Following necropsy, tissue samples were collected at the processing plant in St. George, New Brunswick, and transported to the Atlantic Veterinary College in Charlottetown, Prince Edward Island for

bacteriological processing. The travel time between St. George and Charlottetown, a distance of more than 400 kilometers, often exceeded five hours. Although the farm frequently harvested from the same cage on consecutive days, it was not usually possible to collect, transport and process tissue samples more frequently than every third day. As a consequence, only cages which were not harvested on consecutive days were considered eligible for the study.

The farm consisted of three sites within close proximity to each other and cages were chosen for harvest from any of the three sites depending on fish weight. All cages sampled were located at one site only. Whenever the farm decided to change sites for harvesting, the study was delayed until the harvest schedule returned to the study site.

The general sampling and slaughter procedures are schematically presented in Figure 10. Prior to any fish being harvested from the study cage, a seine net was used to collect about 500 to 800 salmon at one side of the cage. The seine net was pulled out of the water gradually to crowd the salmon sufficiently for capture with a dip net. A dip net approximately one meter in diameter with a three meter handle was used to collect one to three fish each time. Once caught, they were placed directly into the system normally used for harvesting fish but kept separate from other harvested fish for identification purposes. The total number of fish sought for the dipped sample was 150 fish.

The routine slaughtering procedure at this farm was not altered for this study (see Figure 10). Fish were transported by dip net or by fish pump (Trans-

Vac or Silkstream pumps, Innovac Technology, Richmond, B.C.) to a large, curved-bottom tank containing crushed ice in seawater. During the study, the farm purchased a new fish pump (Silkstream) which altered the study design slightly to accomodate the different system. The principles of the slaughter were the same for both pumps.

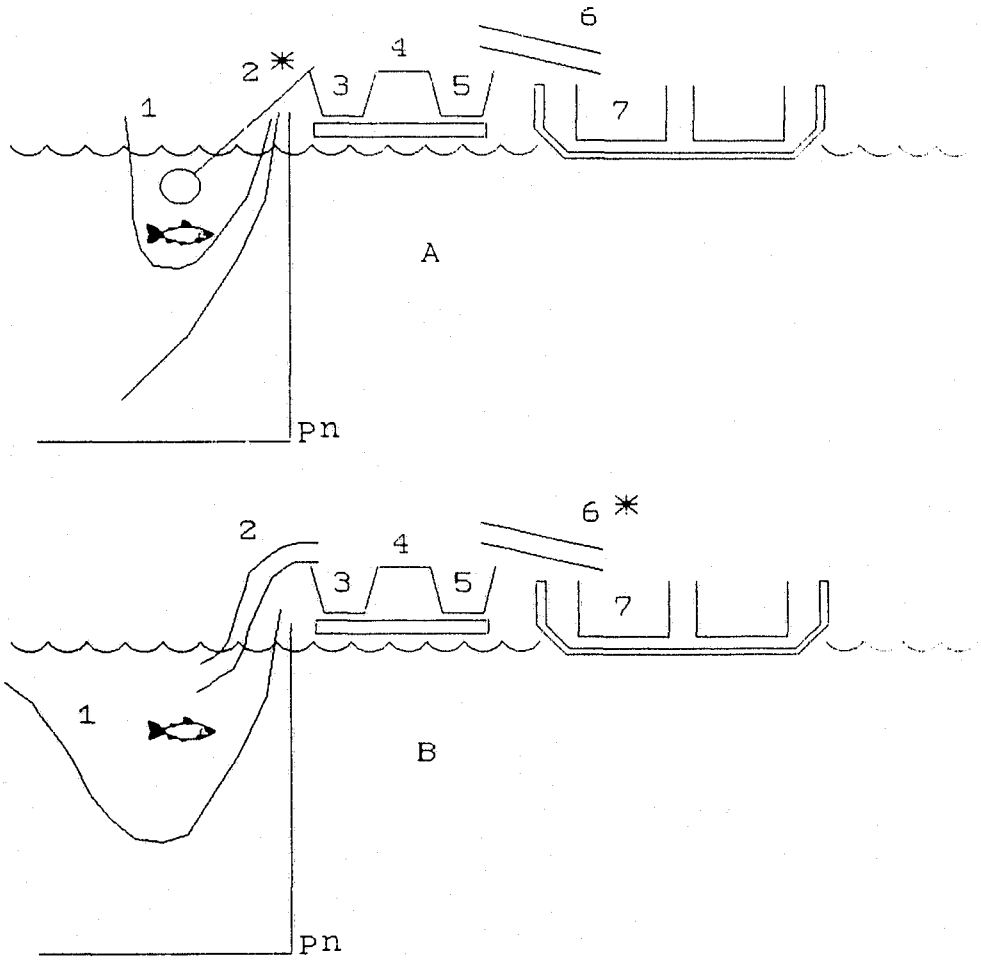


Figure 10. Schematic diagram of sampling procedure during slaughter at the farm site (A: dip sampling procedure, B: systematic sampling procedure). 1. Crowding fish by seine net (in A) or decreased pen volume (in B). 2. Transfer of fish to harvest platform (dip net in A, pump in B). 3. CO_2 and ice tank. 4. Site of gill arch transections. 5. Exsanguination tank. 6. Transfer of slaughtered fish to barge. 7. ice boxes for transport of carcasses to processing plant. * indicates selection site for sample, pn = predator net.

While the fish were in the ice tank, carbon dioxide was bubbled through the ice bath as an anaesthetic. Once the fish became less active, a group of 20 to 40 fish were transported to an adjoining table where the gill arches were transected. At this point, the fish slid into another ice bath to exsanguinate. Each fish remained for five to 15 minutes in the anaesthetic tank and an additional five to 15 minutes bleeding in the ice bath before transfer to an insulated box (1.5 m by 1.5 m by 1.0 m deep) containing crushed ice. Boxes, containing 125 to 150 fish each, were covered and transported by boat to a truck waiting on shore. The boxes of fish would arrive at the processing plant within two to three hours of being removed from the cage.

The systematic sampling technique occurred throughout the harvest of the cage. As the fish passed from the floating slaughter platform to the transport boxes on a nearby boat, they tended to travel in single file. At this point, the fish were counted by the order of passage through the chute and selected systematically after beginning with a random number in the first group. The sampling interval varied between cages depending on the estimated total population of the cage. Since a final sample size of 150 - 200 fish was desired, a sampling interval of every twelfth to fifteenth fish was used.

All of the fish in the systematically selected sample were placed in a separate box with crushed ice and transported at the same time as the rest of the harvested fish. Once at the processing plant, the box containing the sample was set aside for fish measurements and tissue collection. Due to the nature of the

harvest routine, one cage might be harvested over a period of six to eight hours which meant that the systematic sampling procedure continued for the same length of time. The final box of sampled fish would arrive with the last group of boxes delivered to the processing plant.

At the processing plant, a subsample of fish from each sampling method was required to identify the individuals on which a more intense disease screening would be performed (Figure 11). Fifty fish were to be included in this subsample. This sample size was chosen to maintain reasonably precise estimates of disease prevalence while remaining within the time constraints imposed by the processing plant.

There were two sampling procedures occurring simultaneously: one sample to make a comparison on the basis of production parameters and necropsy findings and the other sample to compare dip versus systematic samples on the basis of culture results. The sample size was different because of the time constraints involved with tissue sampling from large numbers of fish. Tissues for bacterial culture were collected from all of the fish involved in the subclinical disease screening sample and tissues were also preserved in formalin when any individual had gross lesions at necropsy. In the larger sample of less intensely examined fish, tissues were collected for bacterial culture and formalin preservation only if fish were abnormal on necropsy. If a fish did not have visible lesions on brief necropsy, then the carcass was returned to the processing plant with no further diagnostic work performed.

To obtain representative subsamples from each larger group of fish already sampled at the cage site, a systematic sampling method was employed at the processing plant. The sampling interval was every fourth fish for systematically sampled groups and, thus, the total sample size varied depending on the actual total number of fish in the cage. The sampling interval used to obtain the subsample from the dipped group of fish varied according to the total number sampled divided by 50 and rounding.

All fish, regardless of sampling method, were identified with a uniquely numbered tag secured through the mouth and one gill. The tag numbers were recorded prior to tagging the fish. The sampling interval for the subsamples referred to the order appearing on the record form so that the person selecting fish from the total sample could not influence the selection of fish for the subsample.

The fish destined for an intensified disease screening were placed into one box containing crushed ice, regardless of the sampling method employed. All of the remaining sampled fish were placed into a separate ice box, also without regard to selection method. Consequently, there were two ice boxes containing mixtures of fish from the two sampling methods: one box for detailed necropsy and tissue collection and the other box for brief necropsy only. Although each fish was identified by a recorded gill tag number, the category of sampling method to which a fish belonged was not immediately available to the investigators during procedures from this point onward.

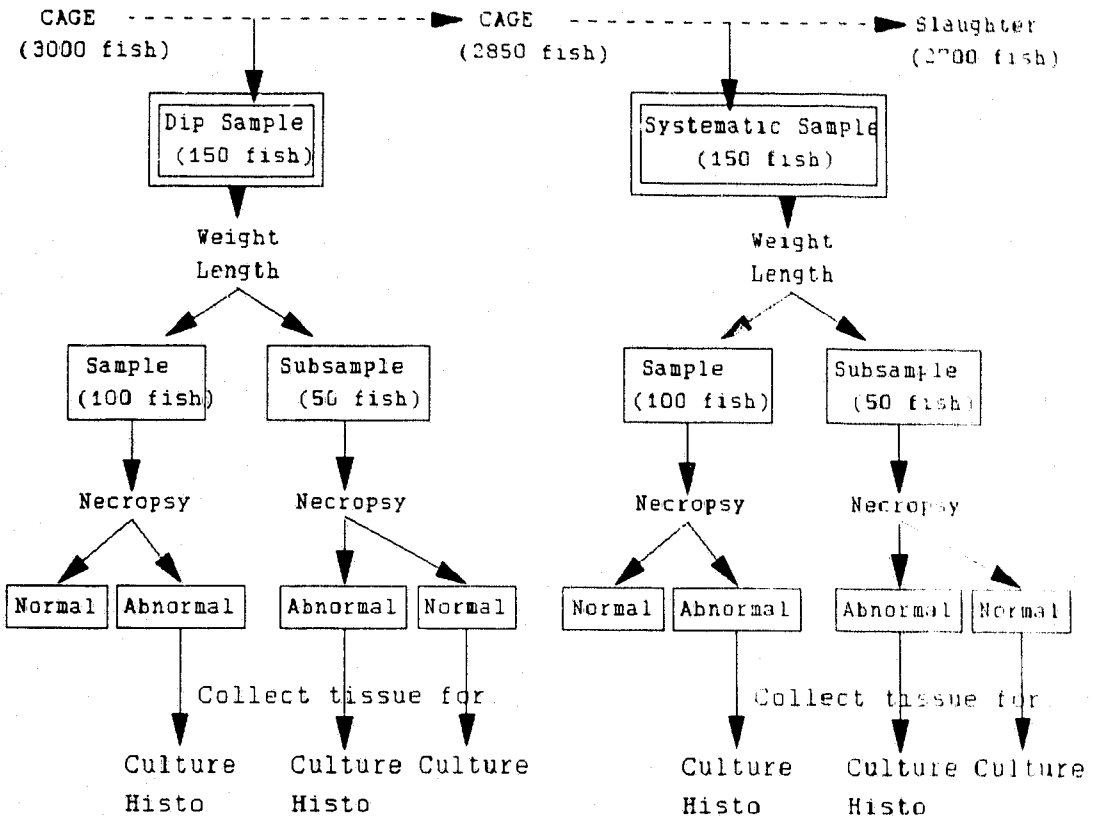


Figure 11. Schematic diagram of the sampling protocol. Dip and systematic samples were selected at the farm site, systematic selection of subsamples occurred at the processing plant.

3.2.3 Fish Examination and Tissue Sampling

Following the placement of a uniquely numbered gill tag but prior to any other procedures, all fish were weighed using a stationary scale (Weigh-Tronix, Advek, Moncton, N.B.) at the processing plant. Weights were recorded to the nearest one-hundredth of a pound and converted to kilograms. Total length to the nearest centimeter was obtained by placing the fish on a flat surface and measuring, with a flexible fiberglass tape, from the end of the snout to the most caudal point of the fish.

Fish were placed onto a flat table which was wiped cleaned between every necropsy using 70% ethanol. All instruments were wiped clean, soaked in 70% ethanol and passed through a flame between each step of the necropsy and tissue collection procedures. With the head of the fish oriented to the left, a ventral midline incision was made starting at the anus and proceeding cranially to the cardiac region using a stainless steel roe knife (ball pointed knife of the type used by the processing plant). Using rat-toothed forceps, the abdominal wall was lifted to permit visual inspection of the viscera (except the kidney) and the presence or absence of granulomatous lesions was recorded onto a microcassette recorder. The culture number and corresponding gill tag number, as well as the sex, sexual maturity and any gross abnormalities, were also recorded at this time.

A scoring system was utilized to assess the presence of granulomatous lesions in the viscera of fish examined grossly. All fish had a brief necropsy

performed and the kidney, spleen, liver, gonad, and heart were given a score of zero or one corresponding to the presence of any granulomatous lesions. A score of zero was given to tissue which had no visible lesions. Abnormalities, other than granulomatous lesions, were noted but not scored. The fish which were part of the culture-based comparison and fish which were abnormal in the necropsy-based comparison were treated in the same manner from this point onward. All fish which were part of the necropsy-based comparison but received scores of zero were returned to the processing plant and not investigated any further.

Tissues were collected for the isolation of *R. salmoninarum* using the following sampling procedure. Two to five gram portions of the spleen and liver (avoiding the gall bladder) were removed aseptically and placed into separate sterile, plastic collection bags (Whirl-pak) which were pre-labelled with consecutive culture numbers and tissue specification. When granulomatous lesions were present, the lesions were included in the tissue collected. If lesions were observed in other tissues apart from spleen, liver or kidney, then those tissues were aseptically sampled as well.

The viscera was held to one side and the swim bladder removed to expose the renal capsule. Using aseptic technique, the renal capsule was removed along its entire length. Three pieces of kidney tissue (head, middle, and tail kidney), two to four grams each, were removed and placed into the same sterile collection bag. Lesions, if present, were included in the collected tissue.

Tissue specimens were sealed in their collection bags and placed on

crushed ice in a cooler for transport to the Atlantic Veterinary College. Tissues were stored in this manner for a maximum of 18 hours prior to processing for culture.

Tissue samples were collected for histological examination whenever lesions were observed. Small samples (less than 5 mm thick) of spleen, liver, heart, gonad, and three regions of kidney were collected into one tissue bag containing formalin. One lesion in any organ resulted in the collection of all tissue samples for histology.

3.2.4 Tissue Processing for Culture

The procedure used to isolate *Renibacterium salmoninarum* from tissue samples was similar to that used by Gudmundsdóttir, Helgason, and Benediktsdóttir (1991) and is briefly presented here. At the Atlantic Veterinary College, tissue specimens were individually weighed. About 10 ml of cold, sterile peptone saline (0.1% peptone and 0.85% NaCl) was added to each gram of tissue and the bag resealed. A Stomacher Lab-Blender (Model 400, Seward Medical, London, England) was used to homogenize the tissue in peptone saline for 30 to 90 seconds and then the contents were transferred to plastic 50 ml centrifuge tubes. The homogenate was centrifuged (TJ-6 Centrifuge, Beckman Instruments, Fullerton, CA, USA) for 20 minutes at 2200*g* while at 4C. The supernatant was discarded, the pellet resuspended in cold peptone saline at a 1:1 ratio by volume

(visually estimated) and then thoroughly mixed on a vortex blender.

A sterile, polyester fiber-tipped, disposable applicator (Becton Dickson, Rutherford, NJ, USA) was used to transfer some of the homogenate to selective kidney disease media (Austin, Embley, and Goodfellow, 1983). Agar plates were divided into two sections, or three if necessary, by a line drawn on the bottom of the plastic to signify the tissue of origin. The homogenate was streaked onto the plate using a sterile platinum inoculating loop. The media plates were then sealed using parafin wax to minimize moisture loss. The plates were incubated at 15C in plastic bags and examined periodically over the next 20 weeks.

Two microscope slides were smeared with the disposable applicator used to inoculate the agar. Smears were permitted to air dry and then one slide was heat-fixed by passing through a flame three times while the other slide was fixed in acetone for 10 minutes or more. Microscope slides were stored for future staining and examination.

3.2.5 Identification of Colonies Grown on Selective Kidney Disease Media

Examination of media plates started at four weeks and continued until 20 weeks post-inoculation. Colony morphology was described and portions of all colonies were transferred to two microscope slides. On each of the glass slides, a small portion of the colony was added to a drop of sterile saline and emulsified. Each was permitted to air dry and then one slide was heat-fixed by passing

through a flame three times and the other slide was acetone-fixed for 10 minutes or more.

The heat fixed slides were Gram stained using standard methods. Gram stained slides were examined under oil immersion to determine the presence or absence of Gram positive, small (less than about 3 μm long) bacilli. The examination was performed without knowledge of the colony morphology or other results.

The acetone-fixed slides and *R. salmoninarum* positive control slides were stained by the indirect fluorescent antibody technique (IFAT). Each smear was covered with rabbit anti-BKD serum at a concentration of 1:40 (serum : sterile phosphate buffered saline [PBS]) and placed in a moisture chamber for 30 minutes or more. Following rinsing of each slide with PBS (Bacto-FA Buffer, Difco Laboratories, Detroit, MI, USA), slides were soaked in PBS for 10 to 15 minutes and then gently blotted dry. Goat anti-rabbit immunoglobulin conjugated with fluorescein isothiocyanate (FITC) and rhodamine counterstain (mixture of 0.2 ml FITC IgG, Cappel, Organon Teknika Corporation, West Chester, PA, USA, + 0.2 ml Bacto-FA Rhodamine Counterstain, Difco Laboratories, Detroit, MI, USA, + 9.6 ml PBS) was used to cover each smear and then the slides were placed in a moisture chamber for 30 minutes or more. Each slide was then rinsed with a freshly made carbonate-bicarbonate buffer solution consisting of 10.6 g of Na_2CO_3 (JT Baker, Phillipsburg, NJ, USA) in 200 ml of distilled water mixed with 33.6 g of $NaHCO_3$ (JT Baker, Phillipsburg, NJ, USA) in 800 ml of distilled water. Slides

were soaked in this solution for 10 to 15 minutes and then gently blotted dry. Coverslips were mounted with Bacto-FA Mounting fluid (pH 7.2, Difco Laboratories, Detroit, MI, USA) and slides were stored in the dark at 4°C until examined. All slides were examined within four days of IFA staining.

An Epi-Fluorescent microscope (Axioplan, Carl Zeiss, Inc., Germany) was used for the examination of IFA slides. The presence or absence of positive staining cells which were similar in shape and size to the *R. salmoninarum* cells on the positive control slides was determined without knowledge of colony morphology or other results.

The isolation of *Renibacterium salmoninarum* was confirmed if the colonies were small, smooth and cream coloured and bacteria were both gram-positive and positive on IFAT.

3.2.6 Histopathology of Lesion Positive Fish

Tissue samples which were stored in formalin since necropsy were transported to the Atlantic Veterinary College for routine histopathology. Tissues were trimmed, embedded in parafin wax, and cut to 6 μm thin sections by standard histologic technique. Three thin sections of each block of wax were transferred onto glass slides and stained with either haemotoxylin and eosin, Gram, or Periodic Acid Schiff stains. Slides from each fish were identified alphabetically so that the identity of the fish was not known at the time of light

microscopic examination.

The hematoxylin and eosin stained slides were examined to confirm the presence of granulomatous lesions which were compatible with a previous infection with *R. salmoninarum*. To confirm the original diagnosis, all slides were re-examined by a pathologist who did not have prior knowledge of the first observer's findings.

In a separate examination of each case, Gram stained lesions were examined for the presence of Gram-positive bacteria which resembled a case known to be BKD-positive. To detect bacteria which can be difficult to see on routine H and E stained slides, Gram and PAS stained slides were also used to assist in the diagnosis (Ferguson, 1989). Two different observers examined the slides independently and equivocal slides re-examined. The presence or absence of Gram-positive bacteria was recorded for identification of lesions which were more highly suspect of active BKD.

3.3 Statistical Methods

3.3.1 Comparing Sampling Methods

Weight (in kilograms) and length (in centimeters) measurements of each fish in the larger sample were entered into the data managing software package, dBase3 Plus (Ashton-Tate Inc.) along with the corresponding information of cage identification, fish tag number, sex, sexual maturity, and a necropsy-based scoring of lesions. Descriptive statistics and t-tests were obtained by analysis using SAS (SAS Version 6.1, 1992, SAS Institute Inc., Cary, NC, USA). Logistic regression was performed using the analytical software Statistix (Statistix Version 4.0, 1992, Analytical Software, St. Paul, MN, USA).

Since the fish in the dipped sample were not available to be included in the systematic sample due to non-replacement sampling, a random selection of one in 15 dipped fish was made following the sampling procedures. These fish were then included in the analysis as both dipped and systematically sampled fish.

The large samples intended for less intense diagnostic evaluation were used to test the hypothesis that dip sample estimates of the prevalence of gross lesions at necropsy were the same as systematic sample estimates of lesion prevalence. Odds ratios and associated chi-square test of significance were calculated for the two sampling methods. The hypothesis that dip sample estimates of mean weight were the same as systematic sample estimates of mean weight within cages, using

the Student's t-test. Similar hypotheses regarding estimates of mean length and condition were tested with the same sample. The smaller subsamples were subjected to similar t-tests to determine if the subsample varied from the larger sample, but otherwise were not used to make any conclusions regarding the difference between sampling methods for weight, length or condition.

Fish in the subsample collected for the intense diagnostic examination were classified as positive or negative for *R. salmoninarum* (described previously) and the influence of sampling method on the number of culture positive fish was examined by computing the odds ratio and its associated chi-square test statistic. The sex and sexual maturity of individuals were only recorded for this subsample and not for the larger samples. The odds ratio and associated chi-square were used to assess the association between sampling method and sex within this sample.

3.3.2 Association of Individual Fish Characteristics with Disease Outcomes

T-tests were applied to the differences in weight, length, or condition of fish grouped by *R. salmoninarum* isolation, the presence of granulomatous lesions at necropsy, sex, and sexual maturity. All analyses were performed separately by cage and fish from the two sampling methods were combined following the sampling method comparison.

The statistical software Epi Info (Version 5, 1990, USD Inc., Stone

Mountain, GA, USA) was used to analyze cross-tabulations of categorical data. The following two way tables were assessed: sex and sexual maturity by the presence of lesions on brief necropsy, by the presence of gram-positive bacteria in lesions observed at brief necropsy, and by the isolation of *R. salmoninarum* from fish with lesions. The measure of association used in the analysis was the odds ratio and the chi-square test was used for significance testing. Cages were analyzed separately.

At the individual fish level, logistic regression coefficients were calculated using gross lesions at necropsy as the dependent variable and weight, length or condition factor as the independent variable of primary interest. Sampling method and the interaction of sampling method with weight were included in the logistic regression equation. A similar logistic regression was performed using isolation of *R. salmoninarum* as the dependent variable. The probability that an individual fish would be diagnosed BKD positive was described by the logistic function:

$$\hat{P}_{BKD}(x) = \frac{1}{1 + e^{-(\beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 x_2)}}$$

where β_0 was the logistic regression coefficient of the constant, β_1 was the coefficient of the dependent variable term: weight, length or condition factor, β_2 was the coefficient of the sampling method term (x_2=0 for dip, x_2=1 for systematic sampling), and β_3 was the coefficient of the interaction term (Glantz

and Slinker, 1990). To test whether coefficients differed significantly from zero, the ratio of the observed value of each coefficient divided by its associated standard error was compared to the standard two-tailed t distribution. The appropriateness of the logistic regression model was assessed using the Hosmer-Lemeshow goodness of fit test (Glantz and Slinker, 1990).

3.4 Results

3.4.1 Description of the Study Population

There were two cages which were complete in terms of data collection for the two types of sampling methods at harvest (Table IX). The sample sizes were reduced based on immediate farm concerns regarding time delays and personnel requirements. Hence, one cage had a dip sample size of only 69 fish for reasons beyond the control of the investigator.

Based upon the systematic samples, in which sample sizes were similar between cages (n_A = 173 versus n_B = 174), both cages had similar mean weight but the fish in cage A were 4.5 cm longer in mean length. As a result, the mean condition factor was substantially lower in the cage with the longer fish (0.97×10^5 in cage A compared to 1.15×10^5 g/cm^3 in cage B). Both cage populations had weight distributions which were not significantly different from normal using the Shapiro-Wilk test statistic (SAS Procedures Guide for Personal Computers, Version 6 Edition, p350). Length and condition factor distributions were significantly different from normal but generally bell-shaped with only slight skewness.

The prevalence of granulomatous lesions in the systematic samples of fish in cage A was 3.5% (95% CI: 0.7%, 6.2%) and 0.6% (95% CI: 0.03%, 1.7%) in cage B (Table IX). Gram-positive bacteria resembling *R. salmoninarum* were

observed in six (46%) of the 13 fish with granulomatous lesions regardless of sampling method in cage A and in two (29%) of the seven fish examined in cage B (one fish with granulomatous lesions did not have a histology sample taken). All systematically sampled fish which were lesion-positive, determined on the basis of any organ having visible granulomatous lesions, had diffuse kidney lesions. In addition to kidney lesions, one of the seven lesion-positive fish had splenic lesions and three had hepatic lesions. Nineteen of the 20 lesion positive fish examined had histological granulomatous lesions which were compatible with, but not necessarily due to, infection with *R. salmoninarum*. Nephrocalcinosis lesions were also observed in 25% of the histological samples.

The subsamples of fish taken for intense diagnostic purposes were used to determine the prevalence of subclinical infection, sex ratios and proportion of mature fish within a cage (Table X). The prevalence of positive *R. salmoninarum* cultures in the systematic subsamples, was 7.3% in cage A (95% CI: 0.1%, 15.3%; $n_A = 41$) and 7.7% in cage B (95% CI: 0.1%, 16.1%; $n_B = 39$). Cage A contained 56.1% females (95% CI: 40.9%, 71.3%) compared to 52.6% females in cage B (95% CI: 36.7%, 68.5%). Early sexual maturity occurred in 4.9% of the systematic sample in cage A (95% CI: 0.1%, 11.5%) and in 5.3% of cage B (95% CI 0.1%, 12.4%).

Table IX

Descriptive Statistics of Fish Obtained by Two Sampling Methods

	Sampling Method			
	Dip		Systematic	
	Mean	SD[a]	Mean	SD[a]
Cage A:	(n = 69)		(n = 173)	
WT (kg)	4.48	0.629	4.51	0.691
LNG (cm)	76.8	3.36	77.2	4.08
CF[b] (g/cm^3)	0.986	0.082	0.975	0.074
	Prev[c] (95% CI)		Prev[c] (95% CI)	
Lesions	0.101	(.030,.172)	0.035	(.007,.062)
	Mean	SD[a]	Mean	SD[a]
Cage B:	(n = 160)		(n = 174)	
WT (kg)	4.574	0.815	4.428	0.676
LNG (cm)	73.34	4.154	72.65	3.754
CF[b] (g/cm^3)	1.150	0.112	1.149	0.095
	Prev[c] (95% CI)		Prev[c] (95% CI)	
Lesions	0.044	(.012,.076)	0.006	(.001,.017)

[a] SD refers to standard deviation
[b] CF refers to fish condition factor ($WT/LNG^3 * 100000$)
[c] PREV refers to prevalence proportion
PREV = number of fish with lesions / n

Table X

Descriptive Statistics of Subsamples Obtained by Two Sampling Methods

	Sampling Method: Dip		Sampling Method: Systematic	
	Mean	SD	Mean	SD
Cage A:	(n = 52)		(n = 41)	
WT (kg)	4.51	0.571	4.48	0.606
LNG (cm)	76.8	3.19	77.2	3.23
CF (g/cm^3)	0.992	0.083	0.968	0.053
	Prev (95% CI)		Prev (95% CI)	
Lesions[a]	0.077	(.004,.149)	0.073	(.001,.153)
R.s.[b]	0.106	(.022,.190)	0.073	(.001,.153)
Female	0.519	(.394,.644)	0.561	(.409,.713)
Mature[c]	0.000	(.000,.056)	0.049	(.001,.115)
Cage B:	(n = 54)		(n = 39)	
WT (kg)	4.541	0.769	4.322	0.654
LNG (cm)	73.30	3.31	72.18	3.66
CF (g/cm^3)	1.144	0.102	1.143	0.093
	Prev (95% CI)		Prev (95% CI)	
Lesions[a]	0.019	(.001,.054)	0.026	(.001,.075)
R.s.[b]	0.130	(.040,.219)	0.077	(.001,.161)
Female	0.585	(.452,.718)	0.526	(.367,.685)
Mature[c]	0.076	(.004,.147)	0.053	(.001,.124)

[a] Lesions refer to gross granulomatous lesions at necropsy
[b] *R.s.* refers to confirmed isolation of *R. salmoninarum*
[c] Mature refers to sexually mature at necropsy

3.4.2 Comparing Sampling Methods for Estimates of Weight, Length, Condition Factor, and Necropsy Lesions

Within each cage, dip net samples selected fish with any lesions significantly (X^2_A=4.33, p=0.04; X^2_B=5.19, p=0.02) more frequently than systematic samples (Table XI). When fish were classified as lesion positive only if they had diffuse or cystic lesions in their kidneys (Table XII), the difference in cage A was not significant(X^2_A=0.05, p=0.82) but remained significant in cage B (X^2_B = 4.13, p=0.04).

3.4.3 Comparing Sampling Methods for Disease and Early Sexual Maturation Estimates

Two by two contingency tables were used to assess the odds ratio relating fish which had positive *R. salmoninarum* cultures to sampling method within each cage (Table XIII). Neither cage had odds ratios which differed significantly from unity. A similar analysis of the influence of sampling method on the occurrence of visible lesions in the fish pre-selected for further diagnostic follow-up also failed to demonstrate any significant difference between methods. In addition, there was no significant influence of sampling method on the proportion of females nor on the proportion of sexually maturing fish in either cage.

3.4.4 Individual Fish Characteristics Associated with Isolation of *R. salmoninarum* or Granulomatous Lesions

The association between individual host characteristics, such as size and sex, and the isolation of *R. salmoninarum* or the presence of gross lesions at necropsy were evaluated in both the larger samples and the subsamples selected from these larger samples. Certain information, specifically sex and sexual maturity, were only available from the subsamples and, therefore, comparisons were occasionally limited to these groups of fish.

There was no significant difference between fish in which *R. salmoninarum* was isolated and fish in which the bacteria was not isolated with respect to weight, length, or condition factor (Table XIV). The difference between the mean size variables of the fish with gross lesions at necropsy and fish without lesions was also non-significant in the sample of fish selected for the purpose of culture. However, the male fish in cage A were significantly heavier than the female fish in this sample.

The frequency distributions of the various size parameters as they relate to the classification status of *R. salmoninarum* isolation on selective kidney disease media are presented in Figures 12, 13 and 14. There was a slight tendency to isolate bacteria less frequently in the longer salmon of cage A, otherwise, *R. salmoninarum* tended to be isolated quite evenly throughout the size distribution in both cages.

The size of fish with gross granulomatous lesions was compared with the size of fish without lesions using the larger sample of fish which were subjected to necropsies and weight and length measurements. The weight of fish with lesions was significantly different from those without lesions in cage B ($T = 2.81$, $p = 0.005$; Table XV) and approaching significance in cage A ($T = 1.71$, $p = 0.089$). Except for a marginal difference observed for fish length in cage B ($T = 1.67$, $p = 0.096$), all other size parameters were not significantly different between lesion positive and lesion negative fish.

The frequency distributions (Figures 15, 16 and 17) exhibited some noticeable trends. Although lesions occasionally appeared in larger fish, they tended to be more frequent in fish at the lower end of the distributions. This was particularly true in cage B where the largest fish were rarely lesion positive and the variation in the population size parameters was generally greater.

The predictor variables of weight, length or condition factor were used in separate logistic functions to examine the association between fish size and the probability of being classified as lesion positive, while controlling for the effect of sampling method. Depending on the cage, each of the size variables was significantly associated with the presence of granulomatous lesions at necropsy (Table XVI). In general, larger fish had lower predicted probabilities of having gross lesions than did smaller fish.

Table XI

Contingency Tables of Gross Lesions at Necropsy by Sampling Method

Cage A:

Method	Lesions observed at necropsy: Negative	Positive	Total	Apparent Prevalence
Dip	62	7	69	0.101
Random	167	6	173	0.035
Total	229	13	242	0.054
	Odds Ratio:	0.32	(95% CI[a]: 0.09, 1.12)	
	Uncorrected X^2:	4.33	(P=0.04)	

Cage B:

Method	Lesions observed at necropsy: Negative	Positive	Total	Apparent Prevalence
Dip	153	7	160	0.044
Random	174	1	175	0.006
Total	327	8	335	0.024
	Odds Ratio:	0.13	(95% CI[a]: 0.01, 1.04)	
	Uncorrected X^2:	5.19	(p=0.02)	

[a] **Cornfield 95% confidence limits for odds ratio**

Table XII

Contingency Tables of Severe Kidney Lesions by Sampling Method

Cage A:

	Diffuse kidney lesions:			Apparent
Method	Negative	Positive	Total	Prevalence
Dip	67	2	69	0.029
Random	167	6	173	0.035
Total	234	8	242	0.033
	Odds Ratio:	1.20	(95% CI: 0.21, 8.98)	
	X^2:	0.05	(P=0.82)	

Cage B

	Diffuse kidney lesions:			Apparent
Method	Negative	Positive	Total	Prevalence
Dip	154	6	160	0.038
Random	174	1	175	0.006
Total	327	8	335	0.024
	Odds Ratio:	0.15	(95% CI: 0.01,1.27)	
	X^2:	4.13	(p=0.04)	

[a] **Cornfield 95% confidence limits for odds ratio**

Table XIII

Contingency Tables of *R. salmoninarum* Isolation by Sampling Method

Cage A:

	R. salmoninarum isolated			Apparent
Method	Negative	Positive	Total	Prevalence
Dip	47	5	52	0.096
Random	38	3	41	0.073
Total	85	8	93	0.086
	Odds Ratio:	0.74	(95% CI[a]: 0.13, 3.95)	
	Uncorrected X^2:	0.15	(P=0.69)	

Cage B:

	R. salmoninarum isolated			Apparent
Method	Negative	Positive	Total	Prevalence
Dip	47	7	54	0.130
Random	36	3	39	0.077
Total	83	10	93	0.108
	Odds Ratio:	0.56	(95% CI[a]: 0.10, 2.69)	
	Uncorrected X^2:	0.66	(p=0.42)	

[a] **Cornfield 95% confidence limits for odds ratio**

Table XIV

Mean Weight, Length, and Condition of Fish Grouped by *R. salmoninarum* Isolation, Presence of Lesions, or Sex (Subsample for Detailed Examination)

	R.s.		Lesion		Sex	
Variable (CAGE)	Neg (n)	Pos (n)	Neg (n)	Pos (n)	Male (n)	Female (n)
$Weight_{(A)}$	4.51 (85)	4.27 (8)	4.51 (86)	4.29 (7)	4.62[z] (43)	4.39[y] (50)
$Weight_{(B)}$	4.44 (83)	4.54 (10)	4.44 (91)	5.03 (2)	4.48 (42)	4.42 (51)
$Length_{(A)}$	77.1 (85)	76.3 (8)	77.1 (86)	76.4 (7)	77.6[y] (43)	76.5[y] (50)
$Length_{(B)}$	72.8 (83)	73.4 (10)	72.8 (91)	76.0 (2)	73.1 (42)	72.6 (51)
$CF_{(A)}$	.983 (85)	.962 (8)	.983 (86)	.960 (7)	.987 (43)	.977 (50)
$CF_{(B)}$	1.143 (83)	1.149 (10)	1.144 (91)	1.143 (2)	1.137 (42)	1.149 (51)

[y] Significantly different at $p<.10$
[z] Significantly different at $p<.05$

n is number of fish in group, weight is in kilograms, length in centimeters, and CF is condition factor ($WT/LNG^3*100000$)

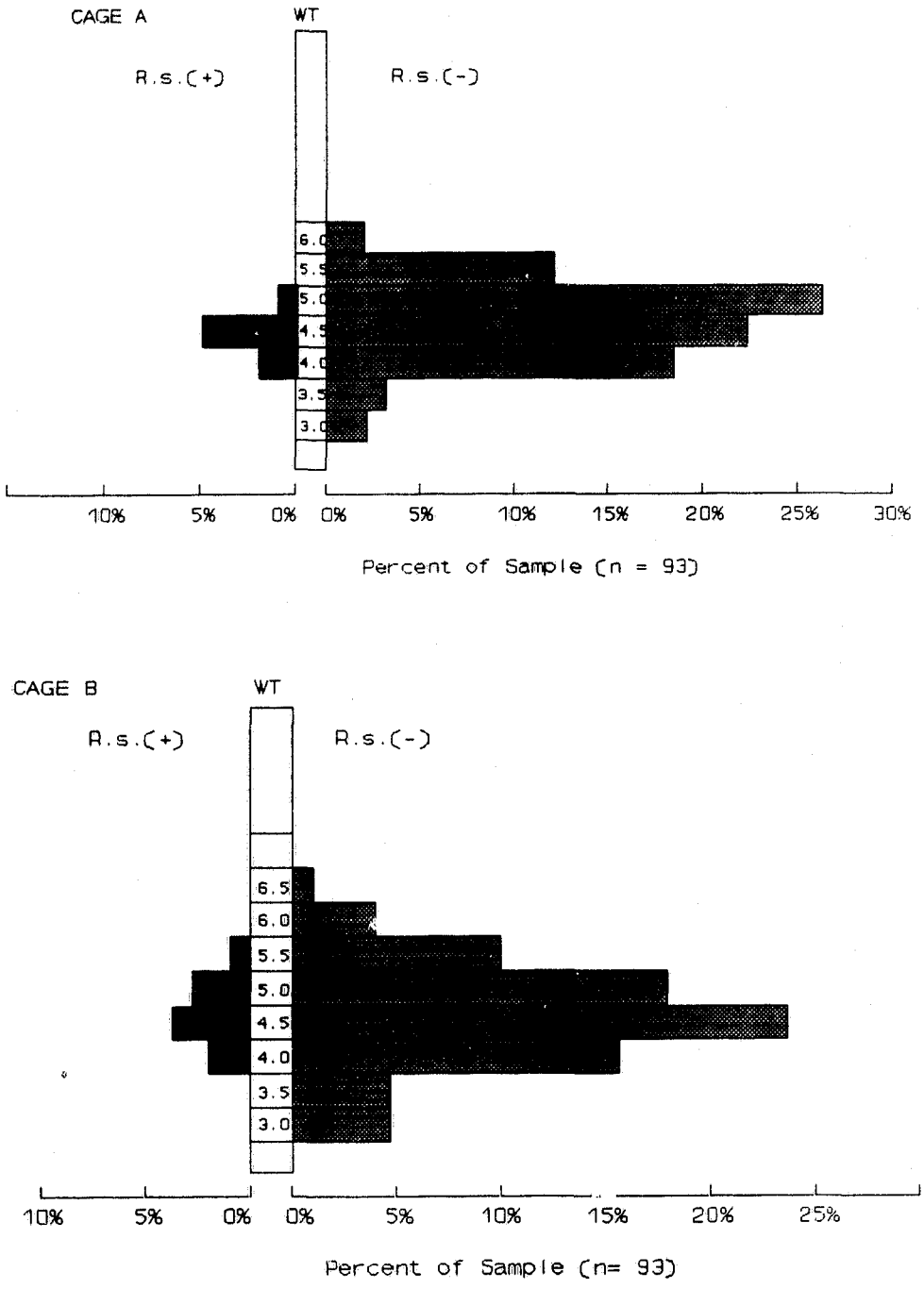
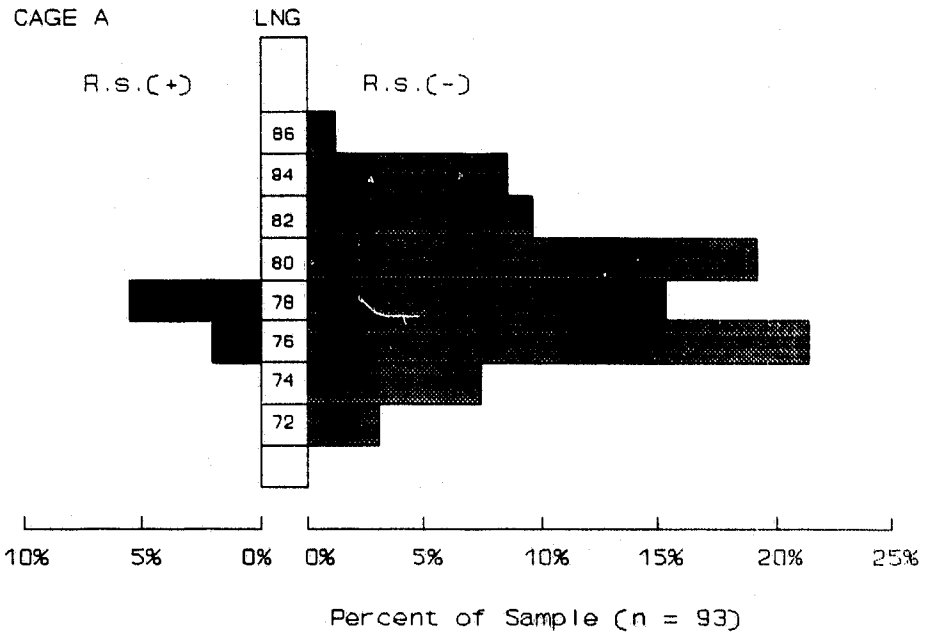


Figure 12. The frequency distributions of fish weight (kg) related to the isolation of *R. salmoninarum* (R.s.) on selective kidney disease media.



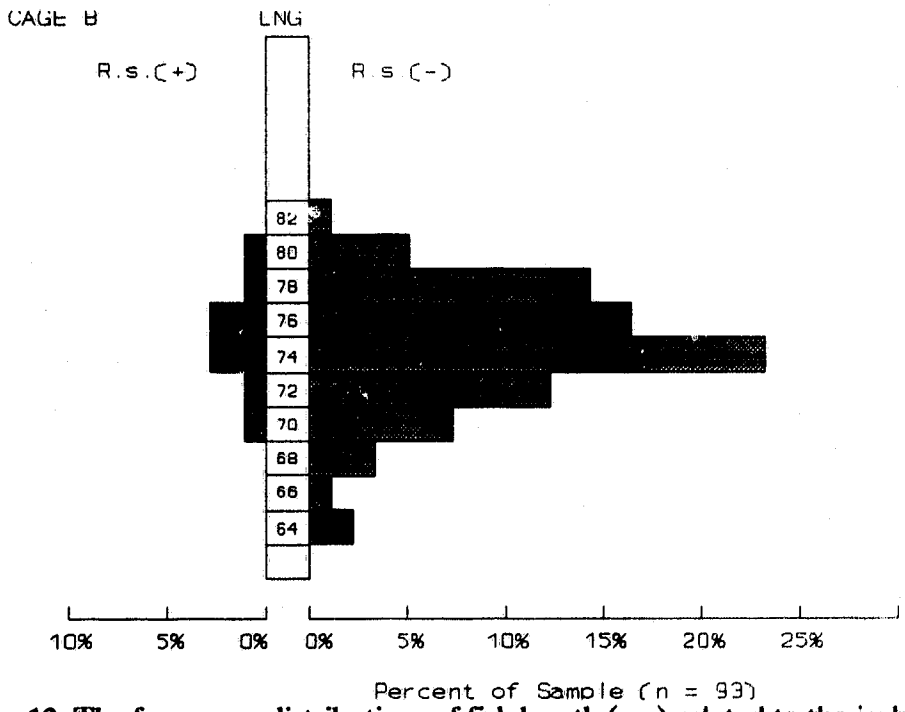


Figure 13. The frequency distributions of fish length (cm) related to the isolation of *R. salmoninarum* (R.s.) on selective kidney disease media.

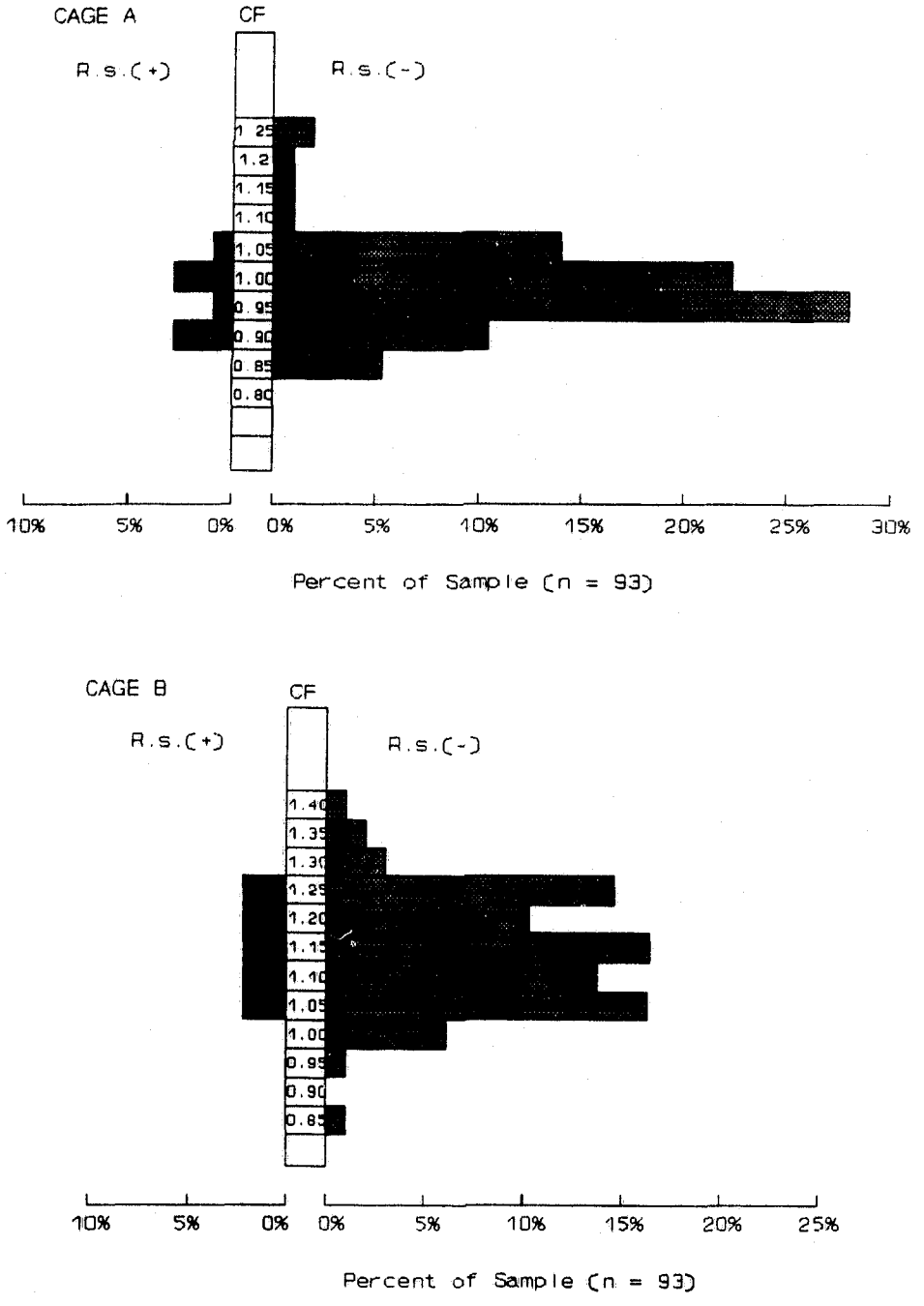


Figure 14. The frequency distributions of fish condition factor ($WT/LNG^3*100000$) related to the isolation of *R. salmoninarum* (R.s.) on selective kidney disease media.

Table XV

T-Test Comparisons of Fish Weight, Length, and Condition for Fish Grouped by Lesion Presence or Absence (Examination of Entire Sample)

	Lesion Negative			Lesion Positive			
$Variable_{(CAGE)}$	Mean	SE[a]	n[b]	Mean	SE[a]	n[b]	p
$Weight_{(A)}$	4.52	.044	229	4.19	.194	13	.089
$Weight_{(B)}$	4.50	.043	327	3.71	.397	8	.005
$Length_{(A)}$	77.1	.258	229	76.0	0.961	13	.312
$Length_{(B)}$	72.8	.309	327	69.5	2.196	8	.096
$CF^{c}_{(A)}$	0.980	.005	229	0.946	.023	13	.126
$CF^{c}_{(B)}$	1.146	.007	327	1.079	.040	8	.128

Weight is in kilograms, Length in centimeters

[a] SE refers to standard error of the mean

[b] n refers to number of fish in group

[c] CF refers to condition factor ($WT/LNG^3*100000$).

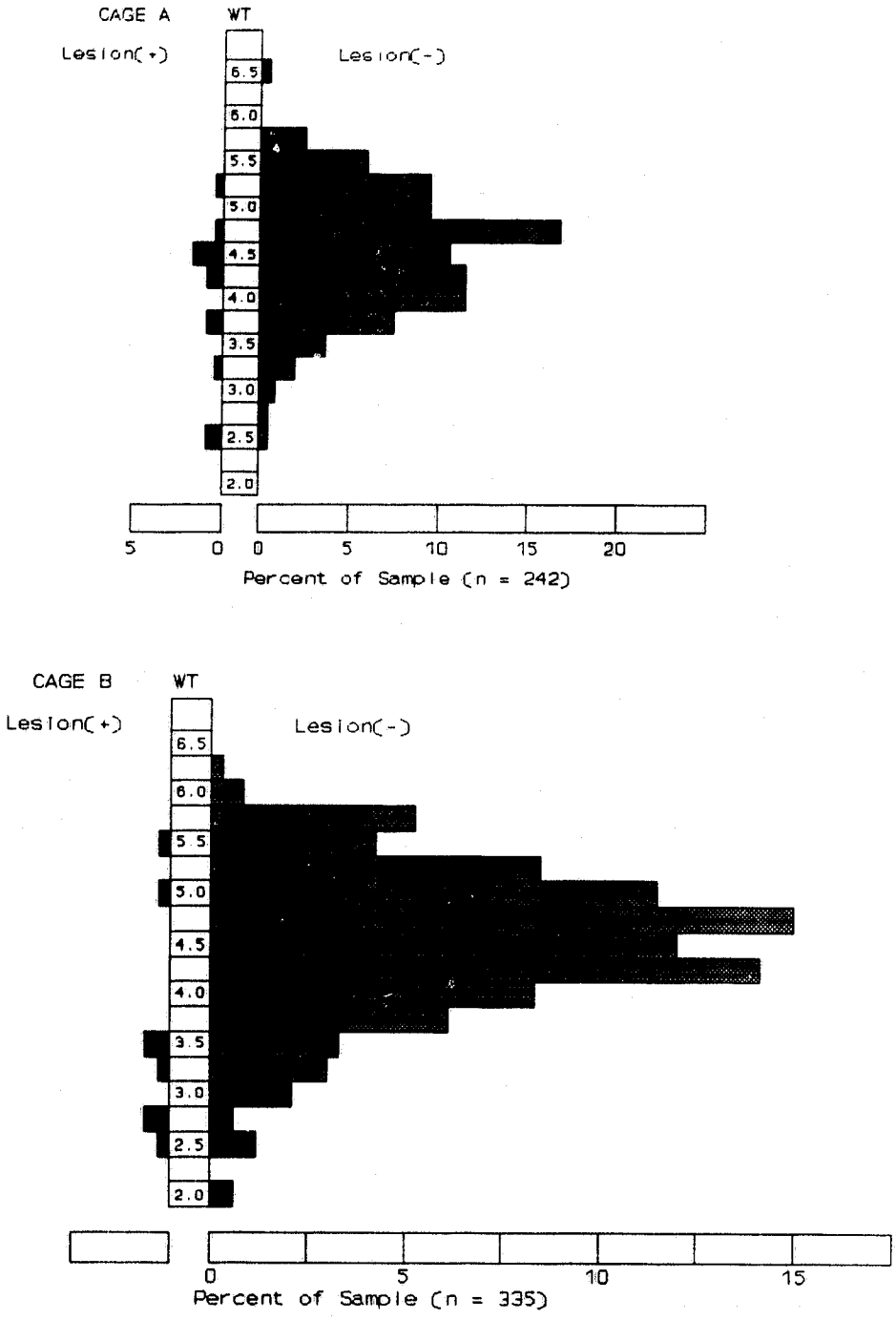


Figure 15. The frequency distributions of fish weight (kg) related to the presence (+) or absence (-) of granulomatous lesions at necropsy.

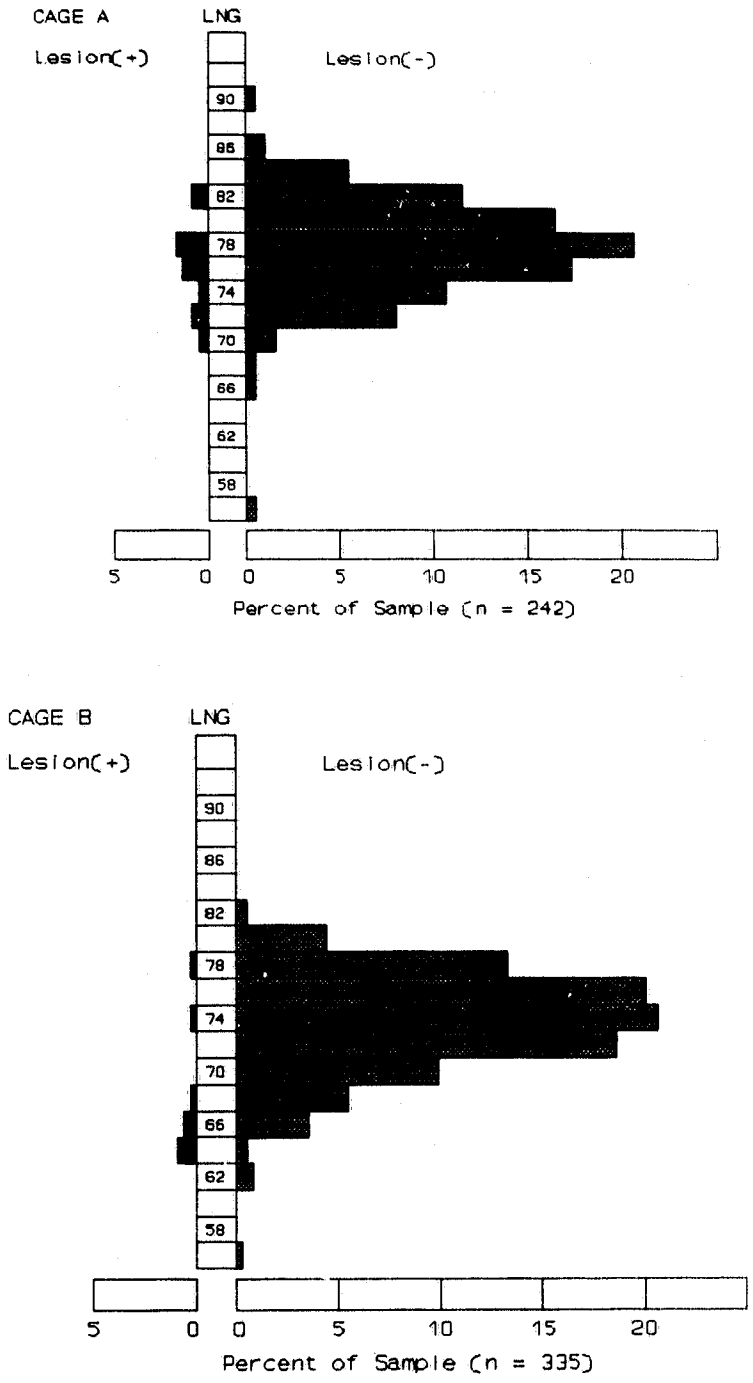


Figure 16. The frequency distributions of fish length (cm) related to the presence (+) or absence (-) of granulomatous lesions at necropsy.

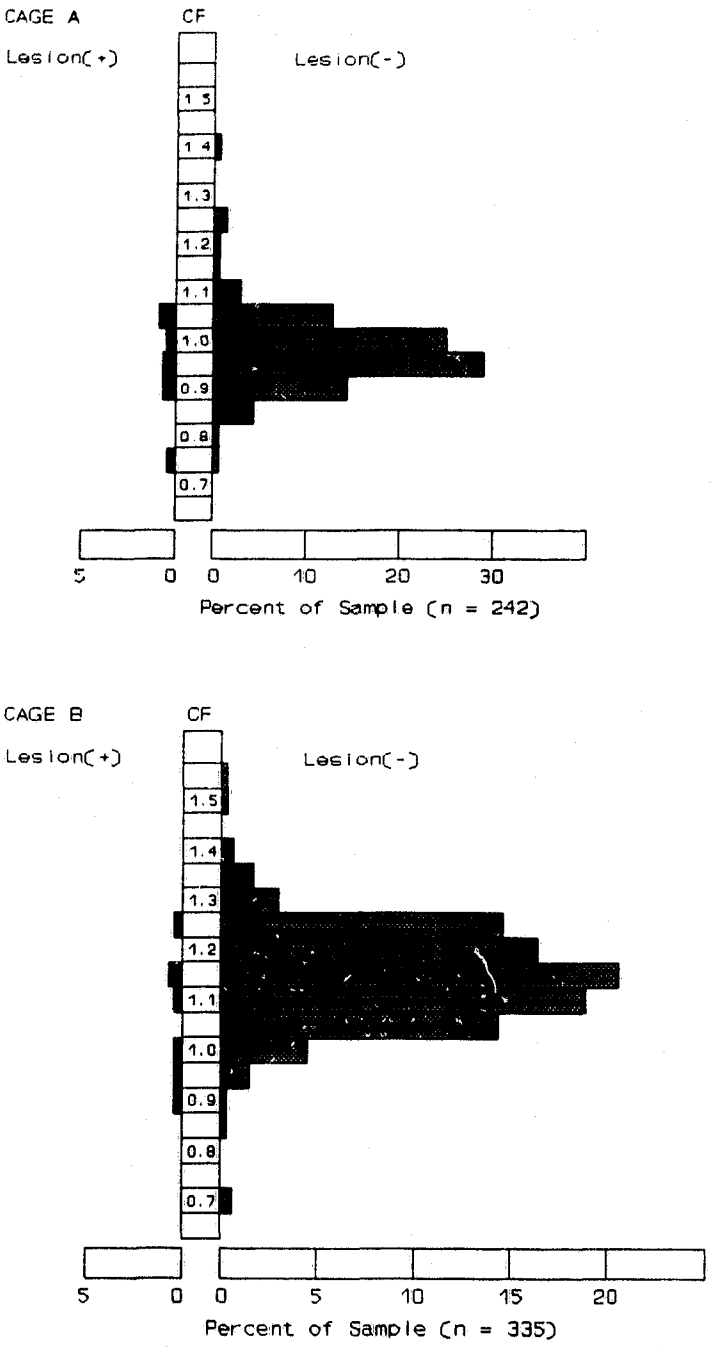


Figure 17. The frequency distributions of fish condition factor ($WT/LNG^3*100000$) related to the presence (+) or absence (-) of granulomatous lesions at necropsy.

Table XVI

Logistic Regression Coefficients for Prediction of Gross Lesions at Necropsy

Dependent Variable = Abnormal on Brief Necropsy

	Coefficients[a]						H-L[b]
	β_0	p	β_1	p	β_2	p	p
Cage A: (n = 242)							
Weight	0.950	0.605	-0.717	0.089	-1.162	0.046	0.743
Length	3.130	0.568	-0.069	0.333	-1.134	0.049	0.555
Condition	4.526	0.249	-6.919	0.091	-1.214	0.037	0.699
Cage B: (n = 333)							
Weight[c]	2.310	0.151	-1.316	0.002	-19.80	0.046	0.534
Length	10.68	0.033	-0.193	0.007	-64.78	0.089	0.969
Condition	2.755	0.383	-5.231	0.069	-2.008	0.058	0.532

[a] Coefficients refer to the following logistic function:

$$\hat{P}_{BKD}(x) = \frac{1}{1+e^{-(\beta_0+\beta_1 x_1+\beta_2 x_2+\beta_3 (x_1 x_2))}}$$

where $\hat{P}_{BKD}$ refers to the predicted probability that a fish of weight = x (or length or condition) will be diagnosed positive for BKD. The p value in the table refers to the error in rejecting the null hypothesis: $\beta=0$. β_0 is constant, β_1 is coefficient for size variable, β_2 is coefficient for sampling method ($x_2=0$ for dip, $x_1=1$ for random), β_3 is coefficient for method*size interaction.

[b] H-L refers to Hosmer-Lemeshow Goodness of Fit test.

[c] Logistic equation had a significant interaction term: β_3 = 3.814 (p=0.042)

3.5 Discussion

3.5.1 Randomness of Dip Net Samples Obtained at Harvest

To evaluate the possible selection bias from dip net samples for different outcomes, a two phase sampling protocol was devised. Salmon were selected by the two sampling methods in sufficient numbers to allow a reasonable estimate of weight and length parameters and then a subsample of this larger sample was obtained for the more intense diagnostic evaluation necessary for subclinical disease prevalence estimates. It may be more practical to consider the simultaneous sampling studies as two separate studies: one in which a larger sample was taken for production estimates and brief necropsies, and another in which a smaller sample was taken for subclinical disease prevalence.

There was no evidence in the larger sampling study to suggest that the convenience sampling method provided biased estimates of mean weight, length or condition factor of market-sized Atlantic salmon. The market fish used in this study had been size-sorted five to six months prior to the harvest and may account for the normal distributions observed in the harvested salmon. Normal distributions and relatively small size variability in the market-sized fish may contribute to the apparent lack of differences between sampling methods.

Brief necropsies were performed on all of the fish sampled by either method regardless of the intended diagnostic follow-up. It was evident from this

comparison that the dip net samples tended to significantly over-estimate the prevalence of visible lesions on brief necropsy in each of the cages studied. All of these lesions were not necessarily attributable to bacterial kidney disease. However, since the fish were usually mixed in the same box after tagging and the individuals performing the necropsy were blinded to the classification of sampling method, any errors in detection of gross lesions were expected to be randomly distributed between experimental groups. Consequently, the number of fish misclassified as positive for visible lesions should be similar and not affect this assessment. In addition, subsequent histological examination revealed that all had lesions which were granulomatous in nature and were compatible with, but not necessarily due to, a previous episode of BKD.

The sample size used in this study was relatively small since only two cages were sampled successfully. The analysis was performed on each cage separately to give results relevant to that particular cage since each group had a different prevalence of BKD. If each cage was a random sample of cages from the general population of cages of interest and the objectives were to examine selection bias at the cage level, then the results represent a sample size of two and clearly lack the power to make meaningful conclusions regarding the equivalence of the two sampling methods at the cage level.

Although the estimate of the prevalence of culture positive fish was higher in the dip net sample than in the systematic sample for both cages (45% higher in cage A and 69% higher in cage B), there was no significant difference between

sampling methods within cages. A larger number of cages in the sample would be necessary to provide better evidence for rejecting the null hypothesis that the two sampling methods were sampling the same population.

3.5.2 Disease Surveillance in Fish Processing Plants

Three trials were performed in late August and early September of 1991, prior to initiating the study. The systematic random sampling technique was applied to a small number of fish during the harvest and the necropsy technique was performed on the sample. Various tissues were collected, transported to the Atlantic Veterinary College in Prince Edward Island and processed for isolation of *R. salmoninarum*. In total, tissues from 71 salmon were included in the bacteriological practice runs and were excluded from any analysis involving a comparison of sampling methods.

Due to the fact that this study was performed in a production facility with many employees being paid to process the fish, there were always strict time restrictions on the tissue sampling procedure. There was no opportunity to sample fish prior to the harvest which meant that the fish to be processed and the fish to have necropsies arrived simultaneously. When the company completed the processing of all of the fish from the cage of interest except the study fish, the necropsy and tissue collection had to be abandoned and the unexamined fish returned to the system. Since the necropsy and tissue collection often took more

time than the processing plant required to eviscerate, grade and pack a load of fish, there was frequent loss of a portion of fish from the study. There was no way to determine the effect which this loss caused on the estimate of the prevalence of lesions or bacterial infection. As a consequence, the samples which were incomplete were not used for any sampling comparisons.

It is important to note the magnitude of the sampling protocol used in this study. As a pilot study, it was designed to assess the feasibility and need of this type of comparison at this stage in the production cycle of Atlantic salmon. The harvest represented an opportunity to sample multiple tissues from numerous fish for estimates of disease prevalence within cages. The difficulties encountered due to the constraints of production are serious considerations in this type of field study but many are surmountable with efficiency and adequate numbers of technical assistants.

3.5.3 Factors Associated with Bacterial Kidney Disease

In a cross-sectional study such as this one, associations between suspected factors and disease can be assessed but the time sequence of events cannot be determined (Kelsey, Thompson, and Evans, 1986). For this reason, the conclusions regarding any association of disease and host characteristics made in this study must be interpreted with caution. A further limitation of this study is the fact that only two cages were examined and associations observed in these two

cages may be unique to these cages or to this farm. Within these two cages, for example, there were only eight fish in total which were sexually mature. The difference between infection prevalence of the mature fish compared to the immature fish could not be examined since an infected mature fish was not obtained in this small sample.

There was no significant difference in the subclinical infection rate obtained by the bacterial culture of *R. salmoninarum* from asymptomatic fish between males (mean 0.072) and females (mean 0.109). Using the formula provided by Martin, Meek, and Willeberg (1987, p.45) for sample size calculations in observational studies, a sample in the order of 1350 individuals per sex would be necessary to detect the difference observed in this study with 80% probability of declaring a significant difference correctly (i.e. type II error of 0.20). The sample size was 93 fish per cage, clearly not sufficient to assess small differences between the sexes in this situation. However, larger differences have been observed between males and females in populations with higher BKD prevalence (Pascho, Elliott, Streufert, 1991).

The phenomenon of sex ratios differing between weight classes has been presented with respect to hatchery grading practices (Ritter, et al., 1986) where female fish were proportionately more abundant in the large size classes. The proportion of females in the study sample of market-sized Atlantic salmon was not significantly different from 50% in either cage. This conclusion was based on the results from only two cages and may not represent the true state in all sea cages.

Due to mixing of fish from different cages, it was impossible to determine the original weight of the smolt which became this final group. Thus, it was not possible to assess the influence of grading practices in the hatchery on the final sex ratios at harvest.

There have been attempts to associate the mean values of various organismic indices and autopsy-based assessments of health with the presence of disease in a population (Goede and Barton, 1990; Novotny and Beeman, 1990) but these studies did not examine the association between the diagnosis of disease with these indices at the individual level. In this observational study, gross lesions identified at necropsy were significantly associated ($p<.05$) with the weight, length and condition of the fish at the individual level in one cage and with weight ($p<.10$) in the other cage (Table XVI). The observed association between weight, sampling method and the presence of lesions indicates that lesions are more prevalent in fish which have been caught in the dip net and more prevalent in fish of lower weight. The presence of a significant interaction suggests that the influence of the two factors are not independent and that the association between weight and lesion presence varied between sampling methods. Since fish weight and length were highly correlated, a model containing both variables together was not attempted. The purpose of performing the logistic regression with all three size variables (weight, length, and condition) was to investigate associations using size measurements easily collected by producers and scientists.

The association between fish weight and lesion presence may indicate that

fish with lower weight are more susceptible than heavier fish to BKD infection or that BKD infection causes the fish to grow more slowly. If the prevalence of BKD was low, as in these cages, and the mortality rate was unaffected by BKD, then the expected distribution of weight in the population would be skewed to the left for chronic BKD cases. Since both cages had a normal weight distribution, either the mortality rate was higher in smaller fish or BKD did not affect the distribution for some other reason.

The observed distribution of lesion-positive fish within the cage distributions for size may have been caused by the chronic nature of BKD. Any size of fish may be at similar risk of becoming diseased but with time the diseased fish grow more slowly and approach the lower end of the weight distribution. If these fish represent the more chronic infections and also have a higher fatality rate, then their deaths would remove their contribution to the size distribution of the population. Consequently, the tendency for BKD positive fish to be smaller is counteracted by the tendency for the BKD positive fish to die prior to harvest.

Fish are considered to have subclinical BKD when they have no external symptoms but are diagnosed positive for the bacteria *R. salmoninarum* (Evelyn, Ketcheson, and Prosperi-Porta, 1981). There was no conclusive evidence in this study for an association between size measurements and isolation of *R. salmoninarum* from individual fish. However, the frequency of bacteria-positive fish tended to be less at higher weights and lengths in one cage.

Culture on selective media may not identify all of the true infections with

this bacteria and, thus, reduce the sample size of the apparently infected group below that required for an accurate assessment of this association. If there was a true association such that smaller fish had higher prevalence but only half were correctly identified as positive, then the smaller, false-negative fish would be included with the true negatives and reduce the mean fish size of the true uninfected group. False-positive diagnosis was not a problem since bacterial isolation has very high specificity.

In summary, the presence of gross lesions compatible with infection with *Renibacterium salmoninarum* was significantly associated with individual fish weight, length or condition factor depending on the cage studied. In general, smaller fish had higher clinical BKD prevalence than did larger fish. Subclinical infection with BKD, as defined by the isolation of *R. salmoninarum*, was not associated with any of the individual fish size parameters although larger fish tended to have lower prevalence of the organism. Sex was not associated with subclinical BKD infection and sexual maturity was too infrequent to assess any associations. The sample sizes within cages were relatively small to make conclusions regarding any small differences between the sexes.

4. GENERAL DISCUSSION

This thesis describes an investigation into the potential for selection bias when the crowd and dip net sampling methods are used to collect samples of Atlantic salmon from sea cages. Dip samples were obtained from 25 sea cages at two sites in the Bay of Fundy, New Brunswick, Canada, immediately prior to a size-sorting procedure. The estimated means of weight, length and condition factor at the cage-level were compared to the corresponding estimated means from systematic random samples taken during the size-sorting. Two primary objectives were accomplished: the evaluation of a convenience sampling method (dip sampling) with the random sampling method as a gold standard, and the description of site-level and cage-level weight, length and condition factor using random samples.

The dip sampling method tended to significantly over-estimate the mean fish weight at one of the two sites. The difference in estimates amounted to over 100 grams (4.9% of mean weight) at the site in which fish had a mean weight of 2058 grams and a standard deviation of 419 grams. Length was significantly over-estimated at one site by 0.2 cm (< 0.5% of mean) and under-estimated at the other site by 0.8 cm (1.4% of mean length). Dip samples significantly over-estimated the condition factor at one site by 0.099 ($g*10^2/cm^3$; 9.2% of mean) and did not significantly differ from the systematic samples at the other site.

All estimates of the difference between dip samples and systematic random

samples were highly significant ($p<.001$) when individual fish were considered the unit for analysis. Cage-level analyses of the same comparisons resulted in non-significant differences (even at $p<.10$) in some instances, demonstrating the danger of accepting conclusions based upon ill-defined sampling or analytic units. Inappropriate statistical methodology concerning the units used within the study, a problem which has been identified in community-based human medical studies (Cornfield, 1978), appears to be prevalent in the aquaculture scientific literature.

Using random samples to describe cage-reared Atlantic salmon populations within and between cages has rarely, if ever, been performed. The cage-effect, which represents the amount of dependence one individual's response has on the response of another individual within the same group, was described in general terms by Michel, Tixier, and Mevel (1984). However, the amount of variation due to the cage-effect had not been quantified. Within the two sites studied, the proportion of weight variation due to the cage in which a fish belonged was 0.12 at one site and 0.15 at the other site. The cage-effect accounted for up to 24% of the variation in fish condition factor in the cages studied, while length variation was least influenced by the cage (seven to 12%). Thus, the fish were partially dependent on the group to which they belonged for their response to the factors influencing growth.

There are several important implications of the previously mentioned results to epidemiologic studies involving Atlantic salmon in sea cages. In field studies in which the goals are to estimate the effect of a particular treatment

compared to a control group, the analysis must account for the cage-effect. Because they were part of a more successful group, fish in a treated group may differ in growth rate (or response to disease) compared to the control group regardless of the treatment effect. Since an individual fish within a cage will come into contact with other individuals within the same cage but remains separated from individuals belonging to other cages, the probability of disease transmission within a cage (or unit) is greater than outside the cage (or unit) (Halloran *et al.*, 1991). Thus, incorrectly assuming independence between individuals of the same cage can lead to spurious conclusions and a false level of statistical significance. Biases towards the publication of significant results (Szklo, 1991; Dickersin, Min, and Meinert, 1992) lead to an over-representation of reports with falsely significant results and an under-representation of falsely non-significant results. Aquaculture production and disease management should be based upon critical evaluation of the scientific literature.

Although the weight and length of a fish may influence the probability of that fish being included in a dip sample, other factors may be involved. Each group of fish within different cages probably reacts differently to the dip sampling procedure and the procedure itself is impossible to reproduce exactly between cages or at the same cage. For these reasons, the comparison of fish between cages and between sites becomes very inexact when dip samples are used to estimate the means.

To investigate the potential for disease to affect the sampling probability, a

second study was performed on fish as they were being sent to slaughter. Dip samples, obtained prior to the slaughter of the cage of fish, were used to estimate weight, length, condition, granulomatous lesion prevalence, and the proportion of fish in which *R. salmoninarum* was isolated. These were compared to the same estimates obtained through a systematic random sample at the time of slaughter. Although the size of fish appeared similar, the prevalence of granulomatous lesions at necropsy was significantly higher in the dip samples. Due to the small sample size of two cages, it was difficult to make general conclusions about the performance of the sampling methods between cages.

There was no evidence in this sampling study of market-sized fish to suggest that the convenience sampling method provided biased estimates of mean weight, length or condition factor. Population distributions appeared different in the market salmon compared to the one-sea-winter (1SW) salmon. The 1SW groups had distributions which often differed significantly from normal and were frequently skewed to the left. The market fish used in this study, although originating from the same 1SW groups of the previous study, were size-sorted and grown for another five to six months prior to harvest. The size-sorting may have accounted for the more normal distributions observed in the harvested salmon. As well, normal distributions and less size variability in the market-sized fish may have contributed to the apparent lack of differences between sampling methods.

The bacterial kidney disease prevalence at the two cages used in this study appeared to be less than 10%. Selection bias due to subclinical BKD presence

within a cage may require a threshold level for there to be any noticeable effect on the probability of a fish being included in the dip sample. Future investigations of the potential effect of disease prevalence on sampling probabilities could be performed at selected sites where the prevalence of BKD is higher, such as British Columbia (eg. Brown, Albright, and Evelyn, 1990) or Scotland (eg. Bruno, 1986). Although not examined in this study, other diseases could be included in the screening of sampled fish to determine their influence on selection probability.

Mortalities related to disease status in the months immediately prior to slaughter may have decreased the apparent prevalence of disease determined post-slaughter. Most diagnostic tests are only useful after a period of disease incubation which results in the disease prevalence being under-estimated when using a single sample. For these reasons, investigations into chronic disease prevalence at slaughter should include disease screening on pre-slaughter mortalities. Lacking this information, this study provided an estimate of the minimum prevalence of bacterial kidney disease.

Culture on selective media was chosen as the diagnostic test for subclinical infection with *R. salmoninarum* since reliable interpretation of a positive result was possible. A negative result on culture was considered an unreliable indication of the true status since host or test factors may have contributed to the inability to grow the bacteria. Although commonly used as screening tests, fluorescent antibody-based tests were not used in the comparison of sampling methods due to the poor repeatability (Armstrong, *et al.*, 1989), and the poor ability to detect a

BKD-positive fish (Pascho, *et al.*, 1987). Due to the general lack of substantiation for positive results on diagnostic tests other than culture, only culture was used to diagnose the minimum true prevalence of infection with *R. salmoninarum* in this study.

In conclusion, opportunities for obtaining true random samples in cage culture of Atlantic salmon are very infrequent. Fish within cages are not independent in their responses to the environment or to disease and, therefore, the cage-effect must be considered in the study design and analysis. Correct identification of unit of concern is crucial for the conclusions to be meaningful. The comparison of fish from different cages is fraught with bias when the convenience sampling method, crowd and dip, is used to estimate the cage mean of the index of interest.

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